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Original Article

Effect of Storage Time, Broiler Breeder Strain, and Age on Hatchability and First-Week Broiler Performance

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ABSTRACT

Managing fertile eggs and minimizing seasonal impacts is crucial in poultry farming. This study evaluated the effects of the storage time of fertile eggs, broiler breeder strain, and age on hatchability and first-week broiler performance. Twelve treatments were carried out in a factorial arrangement with two broiler breeder strains (Cobb MV and Ross 308AP), two breeder age intervals (between 30-40 weeks and between 50-60 weeks), and three egg storage times (4, 7 and 9 days). The Ross 308AP strain showed the highest hatchability (91.74%) at 30-40 weeks, while Cobb MV had the greatest weight gain (162.75g) from days 1 to 7 at 50-60 weeks. Longer egg storage affected weight gain at 7 days for Cobb MV. Nine-day storage reduced chick weight (45.46g) from breeders aged 50-60 weeks. Lower first-stage embryonic mortality was observed in Ross 308 AP (3.83%) in breeders aged 30-40 weeks (4.13%), and in eggs stored for 4 days (4.01%). The Ross 308AP had the greatest percentage of infertile eggs (10.25%), and breeders aged 50-60 weeks showed greater percentages of infertility (10.14%). The Cobb MV had lower second-stage embryonic mortality but higher percentages of discarded chicks. Hatchability and first-week broiler performance depend on storage time, breeder age and strain.

INTRODUCTION

Chick quality is one of the main parameters used in poultry farming, with qualitative and quantitative methods being used to assign reference measures such as mortality, weight, and visual scores (Ishaq *et al*., 2015). Well-managed fertile eggs reflect on chicks' health quality at hatch, leading to dry navels and no residual yolk or lesions on the limbs (Franco *et al*., 2019). After collection at the farm and transportation of the fertile eggs to the hatchery, several aspects must be considered during incubation, such as breeder age and health status, thus avoiding the mixing of chicks at birth. Considering these aspects, storing fertile eggs for different periods is necessary to ensure the homogeneity and quality of one-day-old chicks (Schmidt *et al*., 2012). For these reasons, artificial incubation is a fundamental process for modern poultry farming and has a relationship with the use and performance of the animals (Franco *et al*., 2019).

Broiler performance is mainly influenced by the strain (Lara *et al*., 2008; Marcato *et al*., 2010; Tona *et al*., 2010), as well as nutritional and environmental factors (Tona *et al*., 2004). However, other factors, such as breeder age and the storage time of fertile eggs can influence the embryo and one-day-old chick, impacting the growth potential of the birds. Cobb and Ross are the most widely produced broiler strains worldwide for meat production (Tona *et al*., 2010; Kpomasse

et al., 2021). Chicks derived from both strains have different growth trajectories, which are deduced from differences in physiological parameters (Tona *et al*., 2010). However, the complexity of each genetic line influences its development (Shim *et al*., 2012), and the exact differences that can optimize the performance of each line is not well understood, involving many factors such as temperature and humidity during incubation, feed energy density, light programs, among others.

The effect of storage time on hatchability and its effect on progeny differs between studies. According to Elibol & Brake (2008), fresh fertile eggs have a greater hatching rate than stored eggs, while Nasri *et al*. (2020) observed that eggs stored for up to 12 days are not affected if properly stored. Tona *et al*. (2004) showed that after three days of storage, fertile eggs already suffer negative effects on hatching and chick quality, as well as requiring a longer incubation period, mainly due to the increase in albumen pH during storage.

Breeder age also has an impact on fertility, hatchability, and embryonic mortality, resulting in worse egg quality (Alsobayel *et al*., 2012). As there is seasonality in the production of hatching eggs and the consumption of chicken meat, the supply of hatching eggs fluctuates greatly throughout the year. Therefore, we hypothesized that storage time on fertile eggs, broiler breeder strain, and age, isolated or integrated, affect hatchery and first-week performance traits in broiler chicks. Thus, this study aimed to evaluate the effects of the storage time on fertile eggs, broiler breeder strain, and age on hatchery and first-week performance traits in broiler chicks.

MATERIALS AND METHODS

Ethical approval

Research on animals was conducted according to the determinations of the institutional committee on animal use (437/2021).

Experimental treatments

Fertile eggs from two broiler breeder strains, Cobb MV and Ross 308AP, were used. Eggs were obtained from breeders with two different age intervals, between 30 and 40 weeks, and 50 and 60 weeks. The progenies received the same vaccine program in the rearing and production phases. After collection, the eggs were allocated in plastic trays for 84 eggs and stored in a proper setter trolley. The eggs were fumigated with paraformaldehyde, at a dosage of 6 g/m³ in the room. After total volatilization of the product, the eggs remained in contact with the disinfectant for 15 minutes. At the end of the disinfection process, the setter trolleys with the eggs were stored in a climate-controlled room at a temperature of 20°C, with relative humidity between 50 and 70%, and then sent to the broiler hatchery, located in Lages, Santa Catarina, Brazil (latitude 27°49'0" south, longitude 50°19'35" west, and altitude of 916 meters).

In the hatchery, the fertile eggs were stored until incubation for 4, 7, and 9 days after oviposition in a room with adequate climate control, at a temperature between 18 and 21°C, and relative humidity between 50 and 70%. Twelve experimental treatments were carried out in a factorial arrangement, with two strains of broiler breeder hens (Cobb MV and Ross 308AP), two breeder ages (between 30-40 weeks and between 50-60 weeks), and three storage times of fertile eggs (4, 7 and 9 days). One thousand and eight fertile eggs were used in each treatment, being distributed in trays containing 84 eggs. Each tray of 84 eggs was identified and considered an experimental unit, totaling 12 repetitions per treatment in the hatchery.

Incubation

The eggs were incubated in trays of 84 eggs in multistage machines (CASP, model CM 125, Brazil) with a capacity of 20,736 eggs per load. The trays were randomly distributed in the hatchery to occupy all possible positions and heights inside the machine (i.e. front, middle, and back; bottom, middle and top). A dry bulb temperature of 37.5°C and wet bulb humidity of 29.5°C were used in the hatchery. At 12 days of incubation, ovoscopy was performed to account for infertile eggs and early mortality by diagnosing embryos. At 19 days of incubation, the eggs were transferred and vaccinated *in ovo* with Marek, Bouba, and Gumboro vaccines. Contaminated and broken eggs during the process were counted. After being vaccinated, the eggs were transferred to hatching trays and placed in the hatchers for another 48 hours, and chicks were collected at 21 days post-incubation.

The hatchability was measured as the total number of hatched chicks divided by the total number of hatched fertile eggs for each treatment, with fertile eggs counted through the embryo diagnosis of each tray. The embryo diagnosis consisted of breaking all the eggs that did not result in hatched chicks and evaluating them through a careful analysis. Eggs

without signs of fertilization were considered infertile, and thus made up the percentage of infertile eggs over the total number of incubated eggs. Embryo mortalities were classified into Phase 1 – first phase mortality: embryos with death between 1 to 4 days of incubation; Phase 2– second phase mortality: embryos with death between 5 to 8 days of incubation; Phase 3 – third phase mortality: embryos with death between 9 to 17 days of incubation; Phase 4 – fourth phase mortality: embryos with death between 18 to 21 days of incubation. The parameters of eggs pipped live and pipped dead were also evaluated during the process of opening unhatched eggs. Eggs where the chick had already broken the eggshell and was still alive were considered pipped live eggs, while those that had pipped (and were already dead) were accounted for as eggs pipped dead.

Chicks

The hatched chicks were sexed, classified, and allocated in plastic boxes with 100 chicks per box, respecting animal welfare standards. The classification evaluated the individual quality of each chick, observing dry down, good healing of the navel, locomotion characteristics, absence of beak lesions, as well as physical anomalies that could compromise the development of the chick, according to Tona *et al*. (2003).

Only male chicks were used for performance evaluation. They were weighed individually, with a minimum weight of 40.05 grams and a maximum of 47.37 grams. All of them received spray vaccination for Chicken Infectious Bronchitis (Massachusetts strain).

After vaccination, the pens with chicks were identified according to each treatment and kept in rooms with a controlled environment, with temperatures between 22 and 28°C and relative humidity between 50 and 70%. Subsequently, they were sent to the experimental farm in an air-conditioned truck, maintaining the aforementioned temperature and relative humidity parameters. Individuals classified as discarded chicks were sacrificed following animal welfare standards, using a high-speed macerator.

Experimental farm

The chicks were housed in an experimental farm located in the city of Içara, Santa Catarina, Brazil (latitude 28°41'00.1" south, longitude 49°12'58.8" west, and altitude of 31 meters), where the climate is mesothermal, humid type (Cfa) according to the classification of Köppen & Geiger (1928), with an annual average temperature of 25°C. The farm consisted of a conventional 60x12m building, with fans, nebulizers, heating, hanging feeders, and nipple drinkers. Rice husks were used as poultry litter.

Three thousand eight hundred and forty chicks were allocated in 96 pens measuring 1.8x1.8m, with a capacity for 40 birds/pen and eight replications per treatment. Feed and water were provided *ad libitum*. The birds received a pre-starter diet (from 1 to 7 days of age) with 23.33% crude protein and 2.950 kcal/kg of metabolizable energy, consisting of milled corn (54%), soybean meal (39%), corn oil (1.59%), limestone (1.15%), and mineral-vitamin premix (4.26%), formulated to meet the nutritional requirements of the rearing phase.

The birds were individually weighed on arrival at the farm and at the end of the first week of life using a bench scale, with a graduation of 0.5g. Weight gain was calculated by subtracting the final live weight at 7 days of life from the weight on arrival at the farm. The weight multiplication at 7 days was calculated by dividing the value of the weight at 7 days by the weight on arrival at the farm. The number of dead birds was counted daily from the housing up to seven days of age in each pen, and used to calculate the mortality rate, dividing the total number of dead birds by the number of housed birds.

Experimental design and statistical analysis

The experimental design was completely randomized with twelve treatments in a 2 x 2 x 3 factorial arrangement, with two broiler breeder strains (Cobb MV and Ross 308AP), two breeder ages (between 30-40 weeks and between 50-60 weeks), and three storage times of fertile eggs (4, 7 and 9 days), with twelve replications for hatchery variables and eight replications for performance variables.

Hatchery and performance traits data were tested for normal distribution and residue homogeneity using the Shapiro-Wilk and Levene tests, respectively. Parametric data were analyzed using the MIXED procedure in a model that included strain, age, and storage time as a fixed effect and tray as a random effect. The random tray effect was only considered for the hatchery data. Interactions between strain, age, and storage time were tested. Means were compared using Tukey's test. Using Akaike's information criterion, the variance components (VC) structure was found to be the best model for the residual covariance structure. The following statistical model was used:

$$Y_{ijklm} = \mu + B_i + A_j + S_k + BA_{ij} + BS_{ik} + AS_{jk} + T_l + \varepsilon_{ijklm}$$

where Y_{ijklm} represents dependent variables; μ is the overall mean of the observations; B_i is the fixed effect of the breeder strain (i = Cobb MV and Ross 308AP); A_j is the fixed effect of the breeder age (j = between 30-40 weeks and between 50-60 weeks); S_k is the fixed effect of the storage time (K = 5, 7 and 9 days); BA_{ij} is the interaction between strain and age; BS_{ik} is the interaction between strain and storage time, AS_{jk} is the interaction between age and storage time; T_l is the random effect of the tray (l = 1 to 12); and ε_{ijklm} is the random residual experimental error.

Nonparametric data were analyzed using the NPAR1WAY procedure (Kruskal Wallis), and means were compared using the DSCF test. The following statistical model was used:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} represents dependent variables; μ is the overall mean of the observations; T_i is the fixed effect of the treatment (strain, age, or storage time); and ε_i is the random residual experimental error in Y_{ij} observations.

Analyzes were performed using the SAS software (Statistical Analysis System Inst. Inc., Cary, NC, version 9.3), and differences were considered statistically significant when p<0.05.

RESULTS

The effects of the interactions (strain × age, strain × storage, age × storage, and strain × age × storage) and the main factors (strain, age, and storage) on hatchery and broiler performance traits are shown in Table 1.

There was an interaction between breeder strain and age on the hatching percentage of fertile eggs (p=0.0112), 1-day weight (p=0.0014), 7-day weight (p=0.0002), and weight gain from 1 to 7 days (p=0.0042) (Table 2). The greatest hatching percentage of fertile eggs was observed in the Ross 308AP strain, in the breeder age between 30-40 weeks (91.74%). The Ross 308AP strain also showed a difference in the hatching percentage of fertile eggs for different breeder ages of the broiler breeders (30-40 and 50-60 weeks), while this effect was not observed in the Cobb MV strain. Chick weights at 1 and 7 days were lower for the Ross 308AP strain for breeder ages between 30-40 weeks, with 40.79g and 174.88g, respectively. The greatest weights of chicks at 1 and 7 days were observed in the Cobb MV strain with the breeder ages between 50-60 weeks, 46.51g and 208.67g, respectively. The greatest weight gain from 1 to 7 days (162.75g) was observed in the Cobb MV strain with breeder ages between 50-60 weeks.

Table 1 – Summary of the effects of interactions and main factors on hatchery and performance traits of broiler chicks.

Trait	Mean	SEM	*p*-value						
			Breeder strain	Breeder age	Storage time	Breeder strain × Breeder age	Breeder strain × Storage time	Breeder age × Storage time	Breeder strain × Breeder age × Storage time
Fertile hatching (%)	88.94	0.368	0.0138	0.0022	0.1850	0.0112	0.8335	0.3162	0.1963
Phase 1 (%)	4.94	0.247	<0.0001	0.0001	0.0236				
Phase 2 (%)	1.01	0.128	0.0004	0.4806	0.6944				
Phase 3 (%)	1.05	0.111	0.7971	0.4404	0.8926				
Phase 4 (%)	2.23	0.166	0.8516	0.0144	0.9630				
Pipped live (%)	0.43	0.060	0.8920	0.4123	0.6480				
Pipped dead (%)	0.16	0.037	0.2394	0.7700	0.1099				
Discarded chicks (%)	1.28	0.138	0.0448	0.8657	0.5820				
Infertile (%)	6.50	0.605	<0.0001	<0.0001	0.8231				
Mort. 1st week (%)	0.68	0.128	0.9535	0.1942	0.1239				
W1d (g)	43.83	0.235	<0.0001	<0.0001	0.0037	0.0014	0.1681	0.0203	0.1996
W7d (g)	193.07	1.301	<0.0001	<0.0001	0.1920	0.0002	0.0009	0.0286	0.6948
WG1to7d (g)	149.52	1.138	<0.0001	<0.0001	0.2261	0.0042	0.0005	0.0644	0.5105
Multip Weight (g)	3.40	0.016	<0.0001	0.1014	0.3505	0.0663	0.0054	0.0270	0.2106

p-Value: probability; SEM: standard error of the mean; Phase 1: first-phase mortality: embryos with death between day 1 and day 4 of incubation; Phase 2: second-phase mortality: embryos with death between day 5 and 8 of incubation; Phase 3: third-phase mortality: embryos with death between day 9 and 17 of incubation; Phase 4: fourth-phase mortality: embryos with death between day 18 and 21 of incubation; Mort. 1st week: first week mortality of chicks; W1d: Weight of one-day-old chicks; W7d: weight of chicks at 7 days of age; WG1to7d: weight gain of chicks between the first and seventh day of life; Multip Weight: multiplication of the initial weight of chicks at 7 days of age; values in bold are significant (p<0.05).

Table 2 – Effect of the interaction between strain of broiler breeder hens and breeder age on hatching of fertile eggs and performance of broiler chicks.

Breeder strain	Breeder age		Mean	SEM	*p*-value
	30-40	50-60			Breeder strain × Breeder age
Fertile hatching (%)					
Cobb MV	88.25^{Ba}	87.88^{Aa}	88.06	0.484	0.0112
Ross 308AP	91.74^{Aa}	87.82^{Ab}	89.78	0.488	
Mean	90.00	87.85			
SEM	0.488	0.484			
Weight of one-day-old chicks (g)					
Cobb MV	42.75^{Ab}	46.51^{Aa}	44.63	0.077	0.0014
Ross 308AP	40.79^{Bb}	45.28^{Ba}	43.03	0.077	
Mean	41.77	45.90			
SEM	0.077	0.077			
Weight of 7-day-old chicks (g)					
Cobb MV	195.29^{Ab}	208.67^{Aa}	201.98	0.533	0.0002
Ross 308AP	174.88^{Bb}	194.17^{Ba}	184.52	0.527	
Mean	185.08	201.42			
SEM	0.527	0.533			
Weight gain of chicks between the first and seventh day of life (g)					
Cobb MV	152.54^{Ab}	162.75^{Aa}	157.65	0.551	0.0042
Ross 308AP	134.00^{Bb}	148.79^{Ba}	141.40	0.551	
Mean	143.27	155.77			
SEM	0.551	0.551			

p-value: probability, SEM: standard error of the mean, different uppercase letters in the column and different lowercase letters in the row differ by Tukey's test at 5% ($p<0.05$).

There was an interaction between the storage time of fertile eggs and the strain on the weight at day 7 ($p=0.0009$), weight gain from 1 to 7 days ($p=0.0005$), and weight multiplication ($p=0.0054$) (Table 3).

The Cobb MV strain with a 4-day storage time for fertile eggs showed the greatest weight at day 7 (204.88g). Increasing the storage time of fertile eggs in this strain affected weight gain at 7 days, while this effect was not observed in the Ross 308AP strain. The weight gain from 1 to 7 days was greater in the Cobb MV strain with a 4-day storage time of fertile eggs (160.69g). The 7-day storage time of fertile eggs affected weight multiplication in the Cobb MV strain, while this effect was not observed in the Ross 308AP strain. Weight multiplication at day 7 was greater in the Cobb MV strain with a 4-day storage time for fertile eggs, resulting in 3.57 times the initial weight. In this strain, the storage time of fertile eggs harmed weight multiplication at day 7, while this effect was not observed in the Ross 308AP strain (Table 3).

Table 3 – Effect of the interactions between strain of broiler breeder hens and storage time of fertile eggs on the performance of broiler chicks.

Breeder strain	Storage time of fertile eggs (days)			Mean	SEM	*p*-vaue
	4	7	9			Breeder strain × Storage time
Weight of 7-day-old chicks (g)						
Cobb MV	204.88^{Aa}	199.81^{Ab}	201.25^{Ab}	201.98	0.533	0.0009
Ross 308AP	183.31^{Ba}	185.00^{Ba}	185.25^{Ba}	184.52	0.527	
Mean	194.10	192.41	193.25			
SEM	0.657	0.646	0.646			
Weight gain of chicks between the first and seventh days of life (g)						
Cobb MV	160.69^{Aa}	155.50^{Ab}	156.75^{Ab}	157.65	0.551	0.0005
Ross 308AP	139.94^{Ba}	141.81^{Ba}	142.44^{Ba}	141.40	0.551	
Mean	150.31	148.66	149.59			
SEM	0.675	0.675	0.675			
Multiplication Weight						
Cobb MV	3.57^{Aa}	3.49^{Ab}	3.52^{Aab}	3.53	0.014	0.0054
Ross 308AP	3.23^{Bb}	3.28^{Bab}	3.33^{Ba}	3.28	0.014	
Mean	3.4081	3.3925	3.4288			
SEM	0.017	0.017	0.017			

p-value: probability, SEM: standard error of the mean, different capital letters in the column and different lowercase letters in the line differ by Tukey's test at 5% ($p<0.05$).

There was an interaction between the storage time of fertile eggs and breeder age on the weight at day 1 (p=0.0203), weight at day 7 (p=0.0286), and weight multiplication at day 7 (p=0.0270) (Table 4). The weight of one-day-old chicks was greater for breeders aged 50-60 weeks, and storage time of fertile eggs for 4 and 7 days (respectively 46.22g and 46.01g). The 9-day storage time for fertile eggs negatively affected the weight of one-day-old chicks from 50–60-week-old breeders (45.46g). Chick weight at day 7 was greater for breeders aged 50-60 weeks and was not influenced, at this breeder age, by the storage time of fertile eggs of 4, 7, and 9 days (200.82g, 201.44g, and 202.00g, respectively). Weight multiplication at day 7 was lower for chicks from breeders age 50-60 weeks and 4 days of storage time, resulting in 3.35 times the initial weight.

Table 4 – Effect of the interaction of breeder age and egg storage time on the performance of broiler chicks.

Breeder age	Storage time of fertile eggs (days)			Mean	SEM	*p*-value
	4	7	9			Breeder age × Storage time
Weight of one-day-old chicks (g)						
30-40	41.94^{Ba}	41.60^{Ba}	41.77^{Ba}	41.77	0.077	0.0203
50-60	46.22^{Aa}	46.01^{Aa}	45.46^{Ab}	45.90	0.077	
Mean	44.08	43.80	43.62			
SEM	0.095	0.095	0.095			
Weight of 7-day-old chicks (g)						
30-40	187.38^{Ba}	183.38^{Bb}	184.50^{Bb}	185.08	0.527	0.0286
50-60	200.82^{Aa}	201.44^{Aa}	202.00^{Aa}	201.42	0.533	
Mean	194.10	192.41	193.25			
SEM	0.657	0.646	0.646			
Multiplication Weight						
30-40	3.46^{Aa}	3.40^{Aa}	3.41^{Aa}	3.42	0.014	0.0270
50-60	3.35^{Bb}	3.38^{Aab}	3.44^{Aa}	3.39	0.014	
Mean	3.40	3.39	3.42			
SEM	0.017	0.017	0.017			

p-value: probability, SEM: standard error of the mean, different uppercase letters in column and different lowercase letters in row differ by Tukey's test at 5% (p<0.05).

There was an effect of breeder strain (p<0.0001), breeder age (p=0.0001), and storage time of fertile eggs (p=0.0236) on first-stage embryonic mortality (Table 5). The Ross 308AP strain showed lower first-stage embryonic mortality compared to the Cobb MV strain, with mean values of 3.83 and 6.07%, respectively. Breeders with ages between 30-40 weeks showed lower first-stage embryonic mortality compared to those between 50-60 weeks, with mean values of 4.13 and 5.74%, respectively. Eggs stored for 4 days (4.01%) showed lower first-stage embryonic mortality when compared to eggs stored for 9 days (5.62%).

There was an effect of strain on second-stage embryonic mortality (p=0.0004) and discard chicks (p=0.0448) (Table 5). The Cobb MV strain showed a lower percentage of second-stage embryonic mortality and a greater percentage of discarded chicks compared to the Ross 308AP strain, with mean values of 0.56 and 1.45%, and 1.45 and 1.10%, respectively.

Table 5 – Effect of breeder strain, breeder age, and egg storage time on hatchery traits and mortality of broiler chicks.

Trait	Breeder strain		Breeder age		Storage time			Mean	SEM	*p*-value		
	Cobb MV	Ross 308AP	30-40	50-60	4	7	9					
Phase 1 (%)	6.07	3.83	4.13	5.74	4.01^{b}	5.20^{ab}	5.62^{a}	4.94	0.247	<0.0001	0.0001	0.0236
Phase 2 (%)	0.56	1.45	0.92	1.09	1.23	0.82	0.98	1.01	0.128	0.0004	0.4806	0.6944
Phase 3 (%)	1.09	1.00	1.03	1.07	1.04	0.97	1.13	1.05	0.111	0.7971	0.4404	0.8926
Phase 4 (%)	2.20	2.25	1.94	2.51	2.19	2.14	2.34	2.23	0.166	0.8516	0.0144	0.9630
Pipped live (%)	0.42	0.44	0.49	0.37	0.47	0.46	0.36	0.43	0.060	0.8920	0.4123	0.6480
Pipped dead (%)	0.20	0.11	0.15	0.16	0.16	0.06	0.25	0.16	0.037	0.2394	0.7700	0.1099
Discarded chicks (%)	1.45	1.10	1.38	1.17	1.07	1.60	1.16	1.28	0.138	0.0448	0.8657	0.5820
Infertile (%)	2.76	10.25	2.87	10.14	7.81	6.43	5.27	6.50	0.605	<0.0001	<0.0001	0.8231
Mort. 1st week (%)	0.66	0.71	0.81	0.55	0.53	1.06	0.45	0.68	0.128	0.9535	0.1942	0.1239

p-Value: probability, SEM: standard error of the mean, Phase 1: first-phase mortality: embryos dying between day 1 and day 4 of incubation; Phase 2: second-phase mortality: embryos with death between day 5 and 8 of incubation; Phase 3: third-phase mortality: embryos with death between day 9 and 17 of incubation; Phase 4: fourth-phase mortality: embryos with death between day 18 and 21 of incubation; Mort. 1st week: first-week mortality of chicks; different capital letters on the line differed by the DSCF test at 5% (p<0.05).

There was an effect of breeder age on fourth-stage embryonic mortality (p=0.0144) (Table 5). Breeders with ages between 30-40 weeks showed lower fourth-stage embryonic mortality, with the mean value of 1.94%.

There was an effect of breeder strain (p<0.0001) and age (p<0.0001) on the percentage of infertile eggs (Table 5). The Ross 308AP strain had the greatest percentage of infertile eggs, with 10.25% infertility. Breeders with ages between 50-60 weeks also showed greater percentages of infertility, with 10.14% of infertile eggs.

DISCUSSION

In the hatchery routine, eggs are rarely incubated on the same day of oviposition, being stored for later incubation for at least one day in an appropriate room. Macari *et al*. (2013) highlighted that incubating eggs after laying reduces the hatching rate, requiring eggs to be stored for at least 24 hours before incubation. However, short pre-incubation storage periods (three-seven days) help incubation, as this period is necessary for gelatinization, for an adequate pH to be reached, and for the formation of the air sac, which is essential for embryonic development (Nasri *et al*., 2020).

Thus, egg storage time is an important factor to obtain adequate incubation and production indicators. In this study, the egg storage time of up to 9 days did not influence the hatchability of fertile eggs. However, Alsobayel *et al*. (2012) showed a negative impact on the hatching of fertile eggs derived from broiler breeders stored for 7 days or more when compared to eggs incubated on the same day of oviposition.

According to Yassin *et al*. (2008), storage of up to 7 days has a negative daily impact of 0.2% on total hatchability, which increases to 0.5% per day when the period exceeds 7 days. However, the same study showed that there was a difference in total hatchability between the three tested hatcheries, demonstrating that other factors such as embryonic mortality, fertility, strain, and egg management can influence the hatching of fertile eggs.

The management of fertile egg in storage has two important variables: temperature and humidity. The temperature directly influences the uniformity and homogenization of embryonic development. The recommendation for storage are temperatures below 21°C and relative humidity of the air between 70 and 90%, which helps avoids egg dehydration (Fasenko, 2007). Moreover, regarding handling time, an interval of 5 hours between oviposition and cold room storage of fertile eggs led to the best hatching rates (Fiuza *et al*., 2006).

Iqbal *et al*. (2016) demonstrated that breeder age influenced the fertility of eggs: the older the breeder, the greater the percentage of infertility, which was reported to be 7.11 and 11.44% for breeders with 30 and 60 weeks, respectively. Differing from what was observed in this study, Islam *et al*. (2007) showed that the Ross strain had lower mean percentages of infertility when compared to the Cobb 500 strain, with 8.12 and 8.89%, respectively. This difference may be related to genetic improvement during the inter-study period, but it demonstrates that breeder age affects fertility and may be related to the breeder strain.

The greatest percentage of fertile egg hatching and the lowest first-stage embryonic mortality was observed in the Ross 308AP line aged 30-40 weeks. In a study with the Cobb strain, Tona *et al*. (2010) reported that the greatest hatchability rates were in hens aged 40 weeks. Alsobayel *et al*. (2012) showed a better hatching result on fertile eggs for the Cobb strain, with 82.4% hatchability compared to Ross and Arbor Acres (78.0 and 71.2%, respectively). This can be explained by evolution or genetic improvement in the time between experiments. Furthermore, hatching rates with a breeder age between 30-40 weeks are mainly related to lower early and late embryonic mortalities (Gucbilmez *et al*., 2013). Gucbilmez *et al*. (2013) observed that the best hatching results of fertile eggs were in Ross 308 aged 38 weeks, and these results were enhanced by a different egg handling practices, with a short-term heating technique during the days of storage.

The storage of fertile eggs for more than 4 days influenced the initial embryonic mortality. Alsobayel *et al*. (2012) observed lower rates of early and late embryonic mortality in eggs with zero days of storage when compared to eggs with 7 and 14 days of storage, reporting total embryonic mortalities of 9.6, 17.7, and 31.5%, respectively. These alterations in embryonic mortality following a longer storage period may be associated with a reduction in egg quality, such as poorer albumen quality, mainly due to albumen pH changes that affect the quality of the chalaza and the vitelline membrane (Rocha *et al*., 2013). This fragility of both the chalaza and the vitelline membrane makes the embryo more exposed in the first days of incubation, increasing mortality rates. In addition, the longer the eggs are stored, the lower the yolk height-to-diameter

ratio. Over time, the vitelline membrane surrounding the yolk degrades, making it more liquefied (Rocha *et al.,* 2013).

The strain influenced the percentage of chicks discarded at birth, with the lowest percentage being observed in the Ross 308AP strain. However, the first-week mortality of chicks was not influenced by the breeder strain, breeder age, or the storage time of fertile eggs. This differed from Yassin *et al*. (2009), who observed effects of strain, age, and egg storage time. This difference between the studies can be explained by the use of farm data from different hatcheries, and different breeder batches without considering the hatchery and chick farm traits.

There was an interaction between breeder strain and age, and between the storage time of fertile eggs and the breeder age for first-day chick weight. The Cobb MV strain at 50-60 weeks of age had greater chick weight on the first day. The thinner eggshell of the Ross 308AP strain compared to the eggshell of the Cobb MV strain results in a greater weight loss of the embryos during the incubation period, reflecting on their birth weight (Nangsuay *et al*., 2015). Greater first-day chick weights were observed in breeders at 50-60 weeks of age with a storage time of up to 7 days. This can be explained by the fact that the longer the egg storage time, the more water the egg will lose, resulting in lower oxygen rates for the embryo and less development, due to the decrease in cell metabolism (Rocha *et al*., 2013).

Integrative effects between strain and breeder age, between strain and storage time of fertile eggs, and between breeder age and storage time of fertile eggs were observed for chick weight at 7 days. The Cobb MV strain aged 50-60 weeks had the greatest chick weight at 7 days. The greatest weight of chicks at 7 days was observed in the Cobb MV strain when fertile eggs were stored for 4 days. Despite having a lower first-week weight than the Cobb MV strain, the Ross 308AP strain did not have a negative impact on the storage time of fertile eggs.

The Cobb MV strain has a greater metabolism rate, evidenced by the greater heat production when compared to the Ross 308AP strain, not only during the incubation period but also during the first week of life development (Tona *et al*., 2010). This greater metabolism rate explains the faster development and greater weight gain at the end of the 7 days of life (Tona *et al*., 2010). Furthermore, the Ross 308AP strain has a lower amount of liver glycogen three hours after hatching (Nangsuay *et al*., 2015), which could explain its lower performance in the first days of life. However, the complexity of each genetic line influences its development (Shim *et al*., 2012), and the exact differences that can optimize the performance of each line is not well understood, involving many factors such as temperature and humidity during incubation, feed energy density, and light programs, among others.

CONCLUSIONS

The Ross 308AP strain showed greater hatching of fertile eggs at breeder age between 30-40 weeks, but did not differ from the Cobb MV strain when reproduction age increased.

Despite not influencing the hatching rate of fertile eggs, their storage time negatively impacts first-phase embryonic mortality and the weight of one-day-old chicks.

The Cobb MV strain shows greater chick weight at 1 and 7 days, and weight gain from 1 to 7 days when compared to the Ross 308AP strain.

Lower first-stage embryonic mortality was observed in Ross 308 AP, for breeder age between 30-40 weeks, and in eggs stored for 4 days. The Ross 308AP strain had the greatest percentage of infertile eggs, and breeders aged 50-60 weeks showed greater percentages of infertility. The Cobb MV strain showed a lower percentage of second-stage embryonic mortality and a greater percentage of discarded chicks.

These findings suggest that differences in hatchability and first-week broiler performance depend on storage time, and breeder age and strain.

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AUTHOR CONTRIBUTIONS

Conceptualization, ALDV, AA, AS, MWG, VMM and VP; methodology: ALDV, AS, AS, PVM, MWG, VMM, FM, IB, ES and VP; formal analysis, VP; project administration, ALDV, AS, AS, VMM and VP; resources, VP; writing – original draft preparation, ALDV; writing – review and editing, FM, IB CBT and VP. All authors have read and agreed on the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data is be available upon request.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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