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ANIMAL PRODUCTION

Pre-incubation storage time and in ovo injection with maltodextrin on Pekin duck incubation parameters

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ABSTRACT: The objectives of this study were to assess the effects of i) pre-incubation storage time of Pekin duck eggs on incubation parameters and ii) different levels of *in ovo* injection with maltodextrin on Pekin duckling weight. The study was divided into two experiments using hatching eggs of Cherry Valley SM2 hens with egg-laying ages between 31 and 40 weeks. In experiment I, 8,820 eggs were subjected to different periods of pre-incubation storage (one-seven days). For experiment II, 120 eggs weighing between 75 and 85 g were selected and inoculated with 250 µL of 0.75% saline solution with different concentrations of maltodextrin treatments (0%, 1.5%, 3.0%, and 4.5%). In relation to pre-incubation storage time, eggs stored for one day had lower hatching and hatchability rates and higher duckling mortality rates than eggs stored for longer periods ($P < 0.05$). *In ovo* injection with 3.0% maltodextrin in 0.75% saline solution significantly increased the hatching weight of Pekin ducklings (53.62 g) compared to that by other study treatments ($P < 0.05$). Therefore, Pekin duck eggs produced between 31 and 40 weeks of life may be stored between two and seven days without affecting hatchery productivity parameters. The hatching weight of Pekin ducks may be improved with *in ovo* injection with 3.0% maltodextrin in 0.75% saline solution.
Key words: *Ana boschas*, hatchability, hatchery, maltodextrin, weight gain.

Efeito do tempo de estocagem pré-incubação e adição de maltodextrina *in ovo* sobre os parâmetros de incubação em marrecos de Pequim

RESUMO: Os objetivos deste estudo foram i) avaliar a influência do tempo de estocagem pré-incubação dos ovos de marrecos de Pequim sobre os parâmetros de incubação, e ii) avaliar o efeito de diferentes níveis de inclusão da maltodextrina *in ovo* sobre o peso inicial dos pintinhos de marrecos de Pequim. O estudo foi dividido em dois experimentos, onde foram utilizados ovos incubáveis de matrizes Cherry Valley SM2 com idade de postura entre 31 e 40 semanas. No experimento I 8.820 ovos foram submetidos a diferentes períodos de estocagem pré-incubação (um a sete dias). Para o experimento II, foram selecionados 120 ovos com peso entre 75 a 85 gramas, sendo inoculado 250 µL de solução salina 0,75% associada à maltodextrina em diferentes concentrações nos tratamentos (0%, 1,5%, 3,0% e 4,5%). Em relação ao tempo de estocagem pré-incubação, os ovos estocados por um dia apresentaram menores percentuais de eclosão e eclodibilidade e maior percentual de descarte ao nascimento quando comparado com os ovos estocados por mais tempo ($P < 0,05$). A inoculação de 3,0% maltodextrina em solução salina 0,75% *in ovo* resultou em maiores peso ao nascimento dos pintinhos (53,62g), diferindo dos demais tratamentos avaliados ($P < 0,05$). Portanto, ovos de marrecos produzidos entre as 31 e 40 semanas de vida das aves podem ficar estocados entre dois a sete dias em sala de estocagem sem influenciar os parâmetros de produtividade do incubatório e a inclusão de 3,0% de maltodextrina associada a solução salina 0,75% *in ovo* melhorou o peso ao nascimento de marrecos de Pequim.
Palavras-chave: *Ana boschas*, eclodibilidade, ganho de peso, incubatório, maltodextrina.

INTRODUCTION

Duck farming includes rearing ducks for various purposes, such as egg, meat, feather and *Foie Gras* production. The Pekin duck (*Anas boschas*) is the most bred and consumed breed worldwide (RUFINO et al., 2017). Fertilized Pekin duck eggs generally undergo a pre-hatching delay period during which they are stored below physiological zero, defined as 21 °C to prevent embryonic development (BAGLIACCA et al., 2005; WALSTRA et al., 2010).

Pre-incubation storage is commonly used in hatcheries to accumulate the number of eggs sufficient to fill incubator trays and to anticipate periods of high market demand (FASENKO et al., 2001; NAZARENO et al., 2014). Pre-incubation egg storage time may decrease hatchability after one day under commercial delayed hatching conditions (FASENKO et al., 2001). However, long pre-incubation storage periods of Pekin duck eggs affect egg weight, eggshell weight, and albumen height and viscosity (LIU et al., 2022), possibly delaying

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embryonic development and causing embryonic malformations, in addition to increasing cellular necrosis (BAGLIACCA et al., 2005; SÖZCÜ & IPEK, 2018). Furthermore, nutrients such as amino acids, fatty acids, nucleotides, sugars, and vitamins are degraded, whereas those such as ammonia, biogenic amines, and some aromatic substances are produced in duck eggs stored for long periods, affecting egg quality (LIU et al., 2022).

In the production system of Pekin ducks, the transition from embryo to the post-hatching stage is a critical period for Pekin ducklings due to energy catabolism (CHEN et al., 2010). Generally, birds endure considerable fasting times that compromise performance and immunity (BOTTJE et al., 2010). In addition, the first post-hatching week accounts for 17% of the total growth period and approximately 10% of final body weight gain (LILBURN, 1998; CHEN et al., 2010), directly affecting the development of the birds.

The embryo ingests amniotic fluid from the 15th day of hatching delay (KLASING, 1998); therefore, *in ovo* nutrition, which consists of supplying exogenous nutrients to the amnion of the avian embryo, signals a potential development in poultry. Various studies have shown that *in ovo* injection of carbohydrates increases viability and weight gain, reduces mortality, boosts the immune system, improves intestinal function in poultry after hatching; therefore, improving productive performance and meat yield (CHEN et al., 2010; GAAFAR et al., 2013; ZHANG et al., 2016; RETES et al., 2018).

Among the different carbohydrates used in *in ovo* nutrition, maltodextrin, resulting from partial hydrolysis of starch, is highly promising. *In ovo* injection with maltose and dextrin may increase the intestinal surface area, jejunal activity, digestion capacity, and body weight gain during early development (TAKO et al., 2004; ABOUSAAD et al., 2017). In addition, maltodextrin can be easily found in the form of powder and concentrated solution.

Several studies have assessed the effect of pre-incubation storage time on embryonic development as well as hatching delay and parameters in chickens (FASENKO et al., 2001, DYMOND et al., 2013) and Pekin ducks (SÖZCÜ & IPEK, 2018; PEREIRA et al., 2021). Several studies have demonstrated the benefits of *in ovo* injection in chickens (TAKO et al., 2004; ZHANG et al., 2016; RETES et al., 2018) and ducks (CHEN et al., 2010; GAAFAR et al., 2013), but no study has assessed the effects of *in ovo* injection with maltodextrin on Pekin ducks.

The objectives of this study were to assess the effects of i) pre-incubation egg storage time on the percentage of hatchability, hatching rate, percentage of contaminated eggs, interrupted development, hatching delays, as well as early and late embryonic and duckling mortality rates, and of ii) different levels of *in ovo* injection with maltodextrin on the hatching weight of Pekin ducklings.

MATERIALS AND METHODS

Storage, incubation, and hatchery process

Two experiments were performed at the Duck Farming Experimental Hatchery of the Catarinense Federal Institute (*Instituto Federal Catarinense* – IFC; Araquari Campus), located in the state of Santa Catarina, in the south region of Brazil (26°22'12" south latitude, 48°43'20" west longitude and 9 m above sea level).

The eggs used in this study were laid by Pekin hens of the Cherry Valley SM2 duck breed. Eggs were collected daily at 06:30 am and transported to the hatchery where they were subjected to selection management (identification of cracked or deformed eggs), manual washing with potable chlorinated water, and disinfection by fumigation with quaternary ammonium. Eggs were then separated in incubation trays with a capacity of 126 eggs each, and stored for up to seven days in a room with a temperature between 19 and 21 °C and humidity between 70% and 75%. Before incubation, the eggs were transferred to a preheated room at a temperature between 28 and 30 °C, where they remained for approximately 8h to ensure a gradual transition in temperature until incubation, thus avoiding heat shock.

In both experiments, the eggs were incubated in a multi-stage incubator (Petersime® Belgium), at 45°, while turning every 60 min. The temperature was maintained at 37.5 °C and relative humidity at 65%, with controlled ventilation. The incubation time was 25 days. On the 10th day, early candling was performed to select eggs with embryonic development, identifying and discarding infertile and contaminated eggs, as well as fertile eggs with interrupted development. On the 25th day of incubation, the eggs were transferred to a hatchery (Petersime®, Belgium), at a temperature of 36.5 °C and 70% relative humidity, where they were deposited in boxes suitable for hatching; subsequently discarding contaminated eggs and eggs with interrupted development again. At birth, on the 28th day of incubation, Pekin ducklings with abnormal development and poorly healed navels were discarded.

Experiment I

A total of 8,820 hatching eggs of Cherry Valley SM2 hens with an egg-laying age between

31 and 40 weeks were subjected to pre-incubation storage periods of 1, 2, 3, 4, 5, 6 and 7 days, thus storing 1,260 eggs per treatment. For each week of production, trays with 126 eggs, labeled according to their storage time during the pre-incubation period, were used for each treatment.

Hatchability was assessed as the ratio between the number of hatched ducklings and the total number of incubated fertile eggs. The hatching rate was calculated by dividing the number of hatched ducklings by the number of incubated eggs multiplied by 100. The percentages of contaminated eggs and eggs with interrupted development were determined in the first candling on the 10th day of incubation and on the 25th day in the transfer of eggs to the hatchery. Hatching delay was defined as fully formed ducklings unable to hatch by day 28 when the hatchery was opened. Eggs were assessed in storage to calculate the percentage discarded in early candling, transfer, and hatching, and determine early and late embryonic and hatching mortality rates, respectively.

The experimental design was completely randomized in a model that considered the pre-incubation storage time as a fixed effect, and temperature, humidity, and production week as random effects. Data normality was assessed using the Shapiro-Wilk test, followed by analysis of variance (ANOVA; procedure of general linear models (PROC GLM)), and means were compared using Tukey's test at a 5% significance level in software SAS (SAS Inst. Inc., Cary, NC, USA, v.9.4).

Experiment II

In total, 150 eggs from Cherry Valley SM2 hens at peak production (35 weeks) were incubated in this experiment. On the 22nd day of incubation, the eggs were weighed individually, and 120 fertile eggs weighing between 75 and 85 g were selected and randomly distributed among the experimental treatments.

Nutrient solutions developed for injection were prepared using sterilized 0.75% saline. In these solutions, the necessary amount of maltodextrin was added to final concentrations of 0%, 1.5%, 3.0%, and 4.5% (Table 1). The nutrient solutions remained in a water bath at 37 °C before injection into the eggs. For injection, the eggs were cleaned with alcohol (70%) in the air sac region and punctured with a sterile 21-gauge needle heated to 37.5 °C, coupled to a micropipette. Then, 250 µL of nutrient solution and maltodextrin was inoculated into the amniotic fluid at 23 days of incubation in each egg, according to the experimental treatment.

After the injection, the hole drilled in the shell was sealed with molten paraffin, and eggs were returned to the incubator. The injection process was performed in a room with an average temperature of 30 °C, and the time outside the incubator did not exceed 10 min. After 25 days of incubation, after injection, the eggs were transferred to the hatchery, at a temperature of 36.5 °C and 70% relative humidity. At hatching, on the 28th day of incubation, Pekin ducklings with abnormal development and poorly healed navels were discarded, and others were weighed individually.

The experimental design was completely randomized, with four treatments (0%, 1.5%, 3.0%, and 4.5% maltodextrin) and 30 replicates (eggs). The normality of the Pekin duckling weight data was assessed using the Shapiro-Wilk test, followed by ANOVA (PROC GLM), and means were compared using Tukey's test at a 5% significance level in SAS software (SAS Inst. Inc., Cary, NC, USA, v.9.4).

RESULTS

Experiment I

The temperature and relative humidity of the storage room and the production week did not affect the study parameters ($P > 0.05$). In relation to pre-incubation storage time, eggs stored for 1 day had lower hatchability percentages and hatching rates than eggs stored for 2, 3, 4, 5, 6 and 7 days ($P < 0.001$). The pre-incubation storage time also affected duckling mortality ($P < 0.001$). The highest duckling mortality rates were observed in eggs incubated for one day (36%), whereas the lowest rates were observed in

Table 1 - Description of experimental treatments for assessing the effects of *in ovo* injection with maltodextrin on incubation parameters of Pekin duck eggs.

Treatment	Number of eggs	Composition
T1 – Control	30	250 µL 0.75% saline solution
T2 – Maltodextrin	30	250 µL (1.5% maltodextrin in 0.75% saline solution)
T3 – Maltodextrin	30	250 µL (3.0% maltodextrin in 0.75% saline solution)
T4 – Maltodextrin	30	250 µL (4.5 % maltodextrin in 0.75% saline solution)

eggs stored for five days (8%). Regardless of pre-incubation storage time, the highest mortality rates were found at hatching (17.34%), followed by early candling (7.94%) and transfer (3.92%) embryonic stages (Table 2).

Experiment II

The concentration of maltodextrin injected *in ovo* positively affected the hatching weight of Pekin ducklings ($P < 0.05$). Egg injection with 3.0% maltodextrin in 0.75% saline solution significantly increased the hatching weight of ducklings (53.62 g) compared to the other treatments (Figure 1).

DISCUSSION

Experiment I

The temperature and relative humidity in the storage room did not affect the hatchery parameters, possibly due to the low temperature variation in the storage room that ranged from 20 to 24 °C. The average temperature of 21.9 °C was close to the optimal zone of 21 °C (FASENKO et al., 2007) for arresting embryonic development (BAGLIACCA et al., 2005; FIUZA et al., 2006; WALSTRA et al., 2010).

The relative humidity must be maintained between 70% and 85% to avoid embryonic dehydration and condensation droplets on eggshells (GOMES et al., 2013). The average humidity in the egg storage room was within the recommended range at 83% but cycled between 60% and 95% humidity. Although, storage room humidity below 70% has been shown to increase water loss from the egg and consequently decrease embryo viability (OLIVEIRA & SANTOS, 2018), the large variation did not affect hatchability in this study.

The hatchability rate decreased with the increase in pre-incubation storage time due to changes in specific physical characteristics of the egg, which decrease albumen quality (DYMOND et al., 2013; LIU et al., 2022). As the pre-incubation egg storage period increases, energy production decreases, nutrient degradation increases, and the ability of the embryo to utilize nutrients decreases, potentially affecting tissue growth in later stages (CHRISTENSEN et al., 2002; LIU et al., 2022). In the present study, hatchability unexpectedly did not decrease with pre-incubation storage time increases, possibly due to the short storage period evaluated. In some studies with Pekin duck eggs, differences in hatchability were observed after 10 days of pre-incubation storage (SÖZCÜ & IPEK, 2018; PEREIRA et al., 2021).

In the present study, hatchability percentages and rates were lower in eggs incubated for one day of pre-incubation storage. Similar results were observed by NOGUEIRA et al. (2016), who reported 68.61% hatchability in eggs from heavy hens stored for one day. MACARI et al. (2013) highlighted that incubating eggs after laying reduces the hatching rate, requiring eggs be stored for at least 24h before incubation. However, short pre-incubation storage periods (three–seven days) help incubation as this period is necessary for gelatinization to occur, to reach an adequate pH, and for the formation of the air sac, which is essential for embryonic development (NASRI et al., 2020). Thus, storing eggs for an adequate period can improve their hatchability index (NASRI et al., 2020; PEREIRA et al., 2021).

Table 2 - Effects of pre-incubation egg storage time (ST), storage room temperature (T), humidity (H), and production week (PW) on the incubation parameters of Pekin ducks.

Parameters	----------Storage time (days)----------							SD	----------Pr>F----------			
	1	2	3	4	5	6	7		ST	T	H	PW
Hatchability, %	52^{b}	73^{a}	77^{a}	81^{a}	83^{a}	79^{a}	74^{a}	1.28	<0.001	0.36	0.71	0.32
Hatching rate, %	50^{b}	68^{a}	73^{a}	77^{a}	81^{a}	76^{a}	70^{a}	1.20	<0.001	0.42	0.98	0.51
Contaminated eggs, %	5.0	3.4	3.1	1.9	2.3	1.6	1.9	0.16	0.12	0.96	0.34	0.32
Interrupted development, %	3.8	3.2	4.2	3.6	4.5	4.5	7.2	0.35	0.09	0.89	0.73	0.84
Hatching delay, %	1.0	0.7	0.6	0.7	0.4	0.8	1.7	0.13	0.19	0.36	0.08	0.17
Early mortality, %	7.7	7.2	7.5	7.4	6.9	8.6	10.3	0.37	0.23	0.29	0.07	0.97
Late mortality, %	6.3	3.6	3.9	3.4	3.9	2.3	4.1	0.42	0.32	0.29	0.27	0.08
Hatching mortality, %	36^{a}	21^{b}	16^{bc}	12^{bc}	8^{c}	13^{bc}	16^{bc}	1.02	<0.001	0.36	0.80	0.13

SD: standard deviation, ST: pre-incubation egg storage time, T: storage room temperature, H: storage room humidity, PW: production week. Pr>F: probability. Different lowercase letters on rows indicate significant differences according to Tukey's test ($P < 0.05$).

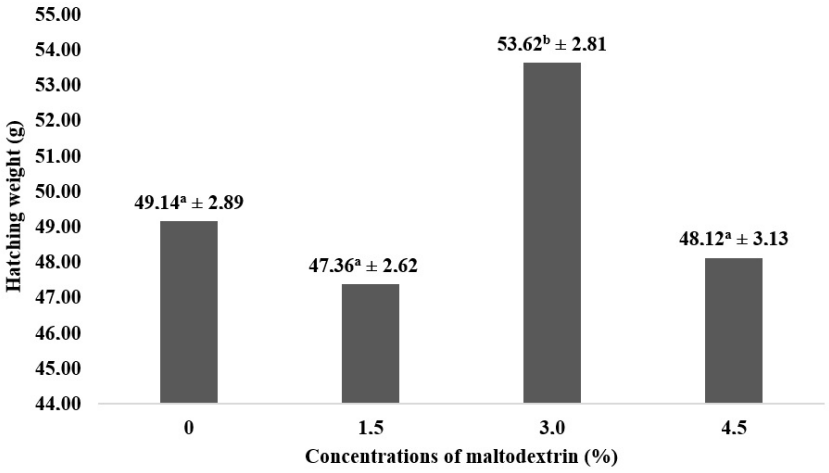

Figure 1 - Hatching weight of Pekin ducklings hatched from eggs inoculated with different concentrations of maltodextrin.

Previous studies have demonstrated that the egg-laying age of hens may also affect poultry hatchability (KIRK et al., 2007; NAZARENO et al., 2014; NASRI et al., 2020). In older hens, the maximum hatchability percentage is assessed with eggs incubated immediately after laying, whereas eggs laid by younger hens show higher hatchability rates when stored (KIRK et al., 1980). PEREIRA et al. (2021) observed an increased hatchability in eggs from hens with 52 weeks of age when incubated for up to two days after pre-incubation storage. In the present study, the hens were 31-40 weeks old and thus younger hens, which may explain the better hatchability results after one day of pre-incubation storage.

The lower hatchability percentage observed with one day of pre-incubation storage may also be attributed to multiple temperature shocks experienced in a period of only 24 h, such as the drastic decrease in temperature in the storage room, followed by transfer to the pre-incubation room within a few hours only and subsequent incubation. According to FIUZA et al. (2006), the internal temperature of eggs stabilizes only after 5h in the cold chamber, and the internal temperature of the eggs at the time of incubation must lie between 26 and 28 ºC. In this study, the eggs with a pre-incubation storage time of one day may not have stayed in storage long enough for temperature stabilization before undergoing a new pre-heating period, thereby negatively affecting the hatchability results.

A one-day increase in pre-incubation storage time may result in a one-hour addition to the incubation time and a one percent reduction in hatchability, in addition to impairing embryonic development (DECUYPERE & MICHELS, 1992). However, REIS et al. (1997) noted that this positive relationship between the increase in storage time and the increase in incubation time is not valid for eggs stored for a short period, as observed in hatchability.

Egg acclimatization before incubation is extremely important for reducing early embryonic mortality and minimizing the growth of microorganisms responsible for contaminating eggs, enabling eggs to adapt to future environmental conditions (WILSON, 1991), in addition to improving embryonic development and hatchability (DYMOND et al., 2013).

Eggs stored for one day had the highest duckling mortality rate and those stored for five days had the lowest rate. These results corroborated previous finding, which showed that a short pre-incubation storage time can be harmful (FASENKO et al., 2001) with significant losses in embryonic development after five days of storage (TONA et al., 2004). NOGUEIRA et al. (2016) assessed a high duckling mortality rate, which decreased with the increase in pre-incubation storage time.

Experiment II

In ovo supplementation or "early nutrition" during the incubation period improved and accelerated post-hatching duckling growth performance (CHEN et al., 2010; GAAFAR et al., 2013). Ducklings from eggs injected with 3.0% maltodextrin in 0.75% saline solution showed a higher hatching weight than ducklings from the other treatments. At an optimal

dose, maltose combined with dextrin has the ability to provide energy and increase jejunal villi, crypt depth, oxygen consumption and tissue area, thus enhancing development in one-day old ducklings (LEITÃO et al., 2014).

TAKO et al. (2004) reported that only 48h was enough to observe a higher villi width and surface area in the small intestine of ducklings treated with *in ovo* injection with maltose, sucrose, and dextrin than in those without *in ovo* supplementation, and that *in ovo* supplemented ducklings had a higher weight after hatching and during early development. These findings demonstrated the viability of using *in ovo* supplementation with maltodextrin for improving hatchery indices and hatching weight in Pekin ducks.

CONCLUSION

Pekin duck eggs laid by hens at 31-40 weeks of life may be stored for two-seven days, at suitable temperature and relative humidity, without harming hatchability. Injecting eggs with 250 µL of 3.0% maltodextrin in a 0.75% saline solution promotes hatching weight in Pekin ducklings.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the study design and manuscript writing. All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All animal procedures followed the rules of and were approved by the Animal Research Ethics Committee (*Comitê de Ética em Uso Animal* – CEUA), IFC, under protocol number 160/2016 (Experiment 1) and protocol number 222/2017 (Experiment 2).

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