

Effects of different egg turning frequencies on incubation efficiency parameters

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ABSTRACT This study aimed to evaluate the effects of different egg turning frequencies on incubation efficiency parameters. Nine hundred sixty brown fertile eggs, with an average weight of 52.20 ± 0.85 g, from 38-week-old CJD (Carijó Pesadão) breeder hens were randomly distributed among 4 treatments before incubation. Each treatment corresponded to a turning frequency, being 24 (control), 12, 6, or 3 times per day, at an angle of 45° , until day 18 of incubation. The experimental design was a randomized complete block design with 4 treatments. Analysis of the incubation parameters was based on

6 replications per treatment. The eggs that were turned 12, 6, and 3 times per day exhibited a decrease in hatchability of the fertile eggs of 6.61, 15.51, and 19.70%, respectively, when compared with the control group ($91.84 \pm 2.73\%$). With a decrease in turning frequency, there was a gradual increase in early (2.84 ± 1.89 to $14.31 \pm 1.82\%$) and late (3.57 ± 1.39 to $8.05 \pm 1.24\%$) mortality rates. An egg turning frequency of 24 times per day during incubation provided high hatchability rates. In contrast, the turning frequencies of 12, 6, and 3 times per day showed significant losses in hatchability.

Key words: broiler breeder, embryo mortality, hatching result, turning frequency, viability

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INTRODUCTION

The profitability of incubators is determined by monitoring their physical environment. Thus, providing the ideal conditions for embryonic development, during artificial incubation, is crucial to improve the productivity and economic index (Decuyper et al., 2001; Bergoug et al., 2013). Turning eggs, for example, is a physical parameter that can affect the success of incubation and the quality of chicks (Moraes et al., 2008). This parameter plays a key role in embryonic growth (Yoshizaki and Saito, 2002) because it supports the absorption and metabolization of the albumen and yolk nutrients in the embryo (Eycleshymer, 1906) and prevents embryo adherence to the inner shell membrane (Romanoff, 1960). In addition, understanding the effects of egg turning on embryo physiology, such as accumulation of proteins in the amniotic fluid, increased vascularized area, and gas exchange (Wilson, 1991; Pearson et al., 1996), is important for artificial incubation.

Egg turning involves several aspects such as turning frequency (Wilson, 1991). Commercial setters usually operate with a turning frequency of 24 times per day until the 18th D of incubation (Freeman and Vince, 1974). However, in some studies, the setters were programmed to turn the eggs at a frequency of 12 times per day during the incubation process (Almeida et al., 2015; Morita et al., 2016; Zhong et al., 2018). For example, in a study by Leandro et al. (2000), 3 commercial incubators equipped with an automatic egg turning system were used to turn eggs every 2 h. In this sense, there is still no standardization by the manufacturers of incubators and research centers with regard to the turning frequency.

Few studies have evaluated the effects of a turning frequency of 12 times per day on the incubation yield. For example, in a study by Robertson (1961a), no significant difference was shown between the hatchability of eggs turned 12 and 24 times, although a mean difference of 3.25% was obtained to the detriment of 12 times. According to the author, although no statistical support was available, it probably constitutes a real difference, and from a practical point of view, it would be advantageous to turn eggs 24 instead of 12 times. Other studies have evaluated the use of fewer turning frequencies (8, 6, 4, 3, 2, and 1 time per day) during incubation (Insko and Martin, 1933; Kaltofen and Ubbels, 1954; Robertson,

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1961b; Abiola et al., 2008). The researchers noted that the highest turning frequency used resulted in better incubation results. Optimum turning frequency has been demonstrated to be 96 times daily (Wilson, 1991; Elibol and Brake, 2003), although 24 times daily has been accepted as the most practical under commercial circumstances, owing to the relatively small differences between 24 and 96 times (Freeman and Vince, 1974).

Considering the lack of research related to the turning frequency and the absence of standardization by the manufacturers of incubators and research centers, we hypothesize that better knowledge about the effects of turning frequency, during incubation, will lead to adequate and efficient physical environment control, thus allowing improvements in the production index for both industry and poultry producers. Therefore, the objective of this study was to evaluate the effects of different egg turning frequencies (24, 12, 6, and 3 times per day) on incubation efficiency parameters.

MATERIALS AND METHODS

This study was approved by the Animal Use Ethics Committee of the University of Brasília (CEUA-UnB), protocol no: 61/2019.

Nine hundred sixty brown fertile eggs, with an average weight of 52.20 ± 0.85 g, from 38-week-old CJD (Carijó Pesadão) breeder hens were randomly divided into 4 treatments before incubation. Each treatment corresponded to a turning frequency, being 24 (control), 12, 6, or 3 times per day at an angle of 45° until day 18 of incubation. All eggs were stored, before incubation, for 3 D at 70% relative humidity and at a temperature of 18°C to 21°C .

For incubation, 240 eggs per treatment were identified, weighed, and allocated into a single-stage setter (Luna 480, Chocmaster, Curitiba, Paraná, Brazil) with 6 incubation trays, and each tray had 40 eggs. All setters were tested and evaluated before their use to confirm homogeneity among them.

The setters were operated at an average temperature of 37.5°C (99.50°F) and relative humidity of 55%, during the first 18 D of incubation. In the incubation room, the average temperature and humidity were 21.67°C

(71.01°F) and 63.96%, respectively. These meteorological variables were monitored by 2 thermohygrometers (608-H1, Testo, Campinas, São Paulo, Brazil) to maintain the correct operation of setters.

At day 18 (432 h of incubation), the eggs were weighed again to calculate egg weight loss during incubation, and then, the setters were adjusted for the hatching period. From day 19 (456 h of incubation) on, the setters were operated at an average temperature of 36.6°C (97.88°F) and a relative humidity of 65%. During this period, the number of chicks hatched and their respective weights were recorded. After 21 D (504 h of incubation), the unhatched eggs were counted, opened, and evaluated to determine the amount of infertile eggs and the period of embryonic mortality, early (0–7 D), middle (8–18 D) and late (19–21 D).

The variables evaluated in this experiment included the egg weight loss (%), fertility rate (%), hatchability of set eggs (%), hatchability of fertile eggs (%), embryonic mortality (%), and chick yield (%).

The experimental design was a randomized complete block design with 4 treatments. Analysis of the incubation parameters was based on 6 replications per treatment, in which each tray of 40 eggs constituted a replicate. Data were subjected to regression (PROC REG) and analysis of variance (PROC GLM) using SAS Studio (University Edition). Means were compared using Tukey's test at a significance level of 5%.

RESULTS AND DISCUSSION

Initial egg weights ($P = 0.45$; coefficient of variation [CV] = 1.53%) and egg weights during transfer ($P > 0.59$; CV = 1.51%; Table 1) did not differ among treatments. The turning frequencies did not affect ($P = 0.67$; CV = 12.23%; Table 1) the percentage of egg weight loss during incubation, with values ranging from $11.04 \pm 1.05\%$ to $11.87 \pm 1.28\%$. According to Baracho et al. (2010), this parameter is mainly determined by temperature and humidity during incubation. Because all eggs were incubated under ideal conditions of temperature and humidity, significant differences were not expected.

Table 1. Egg weight before setting and during transfer, egg weight loss, chick weight, and chick yield according to the turning frequency.¹

Turning frequency (times/D)	Egg weight before setting (g)	Egg weight during transfer (g)	Egg weight loss ² (%)	Chick weight (g)	Chick yield ³ (%)
24	52.28 ± 0.82^a	46.51 ± 0.78^a	11.04 ± 1.05^a	35.84 ± 0.64^a	67.78 ± 0.74^a
12	52.18 ± 0.89	46.15 ± 0.71	11.55 ± 1.41	35.42 ± 0.77	67.88 ± 1.24
6	52.02 ± 0.71	46.04 ± 0.58	11.49 ± 1.96	35.34 ± 0.98	67.74 ± 1.63
3	52.31 ± 0.96	46.09 ± 0.88	11.87 ± 1.28	35.39 ± 0.80	67.67 ± 1.39
<i>P</i> value	0.45	0.59	0.67	0.40	0.80
CV (%)	1.53	1.51	12.23	2.26	1.94
R ² adjust	0.01	0.08	0.06	0.13	0.04

^aNo significant differences exist between means ($P > 0.05$).

Abbreviation: CV, coefficient of variation.

¹Results are expressed as mean \pm SD.

²Egg weight loss = (initial egg weight – egg weight during transfer)/initial egg weight \times 100.

³Chick yield (%) = (chick weight at the day of hatch/initial egg weight) \times 100.

The turning frequencies had no positive or negative effect on chick weight ($P = 0.40$; $CV = 2.26\%$) and chick yield ($P = 0.80$; $CV = 1.94\%$; Table 1). Chick weight is the most widely used indicator for the assessment of one-day-old chick quality (Tona et al., 2004). According to Tullett and Burton (1982), 99.47% of the variation in the initial chick weight is related to the initial egg weight, egg weight loss during incubation, and weight of the shell and residues at hatch. Therefore, the result of the present study is consistent because the initial egg weight and the egg weight loss between treatments were statistically similar.

The chick yield is a simple method of checking whether incubation time and parameters (mainly temperature and humidity) are corrects, with ideal values of 67 to 68% (Aviagen, 2011). In the present study, all treatments showed chick yields classified as “ideal,” that is, chicks with an ideal yield, when housed in a farm, will be more active, will have ideal weight, and will be ready to eat and drink water (Aviagen, 2011). This result was expected because all eggs were incubated under the same temperature and humidity conditions.

Fertility (%) was not significantly different among the treatments ($P = 0.13$; $CV = 3.89\%$; Table 2). The fertility rate mean in this study was $91.64 \pm 3.17\%$. All eggs were obtained from broiler breeders of the same flock that received the same management in the barn; so, differences among treatments were not expected.

The hatchability of the set eggs ($P < 0.0001$; $CV = 4.04\%$; $y = 66.27 + 1.79x - 0.03x^2$) and the hatchability of the fertile eggs ($P < 0.0001$; $CV = 3.74\%$; $y = 66.90 + 2.16x - 0.05x^2$) were significantly different among treatments (Table 2). The eggs that were turned 12, 6, and 3 times per day exhibited a decrease in hatchability of the fertile eggs of 6.61, 15.51, and 19.70%, respectively, when compared with the control group ($91.84 \pm 2.73\%$). In this study, the reduction of the percentages of early and late mortality explains the best hatchability of the set eggs and fertile eggs observed for eggs turned 24 times per day. Insko and Martin (1933) observed that the decrease in mortality associated with increased turning frequency occurred mainly

at the typical mortality peaks observed from 1 to 3 D and 17 to 21 D of incubation.

Corroborating the results of the present study, Kaltofen (1955) observed a significant improvement in the hatchability of eggs that were turned 24 times a day until the 18th D of incubation compared with eggs turned 3 and 8 times a day. In a study by Robertson (1961a), although the author found no significant effect on the hatchability of eggs turned 12 and 24 times a day, he observed that eggs turned 12 times a day were 3.25% less likely to hatch.

There was a significant effect of the treatments on early ($P < 0.0001$; $CV = 22.12\%$; $y = 20.13 - 1.75x + 0.04x^2$) and late mortality ($P = 0.02$; $CV = 31.02\%$; $y = 10.18 - 0.35x + 0.006x^2$) (Table 2). However, there were no significant effects of the treatments on the occurrence of mortality in the intermediate phase ($P = 0.11$; $CV = 38.69\%$). According to Boleli (2013), the main causes of mortality during this period are due to inadequate climatic factors (temperature and humidity) during incubation, egg contamination, and vitamin deficiencies such as those of vitamin B2, B5, and D. Because all eggs were incubated under controlled temperature and humidity and they were obtained from the same broiler breeder flock that received the similar feeding and nutritional management, we did not expect to find significant differences among treatments for this variable.

With decrease in turning frequency, there was a gradual increase in early (2.84 ± 1.89 to $14.31 \pm 1.82\%$) and late (3.57 ± 1.39 to $8.05 \pm 1.24\%$) mortality rates. Consistent with this result, some authors (Kaltofen and Ubbels, 1954; Kaltofen, 1955) observed that turning eggs 24 times a day resulted in lower mortality than with less frequent turning. Conversely, Elibol and Brake (2006a) observed that eggs turned 24 times per day at a 35° angle did not differ significantly in mortality compared with eggs turned 96 times per day at the same angle.

New (1957) and Deeming (1989) reported that the absence of egg turning in the first week of incubation can cause deleterious effects to the embryo, such as premature adherence of the chorion to the inner membranes

Table 2. Fertility, hatchability of set eggs, hatchability of fertile eggs, and embryonic mortality according to the turning frequency.¹

Turning frequency (times/D)	Fertility ² (%)	Hatchability of set eggs ³ (%)	Hatchability of fertile eggs ⁴ (%)	Early dead (%)	Mid dead (%)	Late dead (%)
24	93.00 ± 3.93^a	85.34 ± 2.30^a	91.84 ± 2.73^a	$2.84 \pm 1.89^{b,c}$	1.41 ± 0.87^a	3.57 ± 1.39^b
12	91.33 ± 1.96	78.34 ± 2.30^b	85.77 ± 3.05^b	6.22 ± 1.99^b	2.19 ± 0.73	$5.46 \pm 0.69^{a,b}$
6	90.67 ± 2.53	70.33 ± 3.31^c	77.60 ± 3.34^c	$12.45 \pm 2.05^{a,b}$	2.59 ± 0.83	$7.37 \pm 3.09^{a,b}$
3	91.56 ± 4.27	67.55 ± 5.82^c	73.75 ± 3.89^c	14.31 ± 1.82^a	2.92 ± 0.64	8.05 ± 1.24^a
<i>P</i> value	0.13	<0.0001 ⁵	<0.0001 ⁵	<0.0001 ⁵	0.11	0.02 ⁵
CV (%)	3.89	4.04	3.74	22.12	38.69	31.02
R ² adjust	0.23	0.81	0.84	0.81	0.25	0.38

^{a,b,c}Means with different superscript letters in columns differ significantly ($P < 0.05$).

Abbreviation: CV, coefficient of variation.

¹Results are expressed as mean \pm SD.

²Fertility (%) = (number of fertilized eggs/number of eggs set) \times 100.

³Hatchability of set eggs (%) = (number of hatched chicks/total number of set eggs) \times 100.

⁴Hatchability of fertile eggs (%) = (number of hatched chicks/total number of fertile eggs) \times 100.

⁵Quadratic response.

of the eggshell, hindering the normal development of the chorioallantoic membrane and consequently embryonic mortality. Contrarily, Wilson (1991) stated that the increase in late mortality due to egg turning is associated with an increased incidence of malpositioned embryos.

However, as weight loss, chick weight, and chick yield did not vary between treatments, it is suggested that the increase in early and late mortality with a decrease in the turning frequency can be associated with chorioallantoic membrane development and malpositioned embryos at hatching, respectively.

CONCLUSIONS

An egg turning frequency of 24 times per day during incubation provided high hatchability rates. In contrast, the turning frequencies of 12, 6, and 3 times per day showed significant losses in hatchability. Therefore, it is essential for research centers and incubator manufacturers to standardize the egg turning frequency to 24 times.

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REFERENCES

- Abiola, S. S., A. O. Afolabi, and O. J. Dosummu. 2008. Hatchability of chicken eggs as influenced by turning. *Afr. J. Biotechnol.* 7:4310–4313.
- Almeida, V. R., V. S. Morita, S. Sgavioli, T. I. Vicentini, D. M. C. Castiblanco, and I. C. Boleli. 2015. Incubation temperature manipulation during fetal development reduces adiposity of broiler hatchlings. *Poult. Sci.* 95:316–324.
- Aviagen. 2011. How to measure chick yield. Accessed April 2019. http://en.aviagen.com/assets/Tech_Center/BB_Resources_Tools/Hatchery_How_Tos/02HowTo2MeasureChickYield.pdf.
- Baracho, M. S., I. D. A. Nääs, and A. C. S. Gigli. 2010. Impacto das variáveis ambientais em incubatório de estágio Múltiplo de frangos de corte (Impact of environmental variables in multi setter incubation in broiler's production). *Eng. Agríc.* 30:563–577.
- Bergoug, H., C. Burel, M. Guinebretièreg, Q. Tong, N. Roulston, C. E. B. Romanini, V. Exadaktylos, I. M. McGonnell, T. G. M. Demmers, R. Verhelst, C. Bahr, D. Berckmans, and N. Etteradossi. 2013. Effect of preincubation and incubation conditions on hatchability, hatch time and hatch window, and effect of post-hatch handling on chick quality at placement. *World's Poult. Sci. J.* 69:312–334.
- Boleli, I. C. 2013. Estresse, mortalidade e malformações: princípios básicos e implicações para o sucesso da incubação (Stress, mortality and malformations: basic principles and implications for successful incubation). Pages 202–277 in *Manejo da Incubação [Incubation Management]*. Facta, Jaboticabal, São Paulo, Brazil.
- Decuyper, E., K. Tona, V. Bruggeman, and F. Bamelis. 2001. The day-old chick: a crucial hinge between breeders and broilers. *World's Poult. Sci. J.* 57:127–138.
- Deeming, D. C. 1989. Characteristics of unturned eggs: critical period, retarded embryonic growth and poor albumen utilization. *Br. Poult. Sci.* 30:239–249.
- Elibol, O., and J. Brake. 2003. Effect of frequency of turning from three to eleven days of incubation on hatchability of broiler hatching eggs. *Poult. Sci.* 82:357–359.
- Elibol, O., and J. Brake. 2006a. Effect of egg turning angle and frequency during incubation on hatchability and incidence of unhatched broiler embryos with head in the small end of the egg. *Poult. Sci.* 85:1433–1437.
- Eycleshymer, A. C. 1906. Some observations and experiments on the natural and artificial incubation of the common fowl. *Biol. Bull.* 12:360–374.
- Freeman, B. M., and M. A. Vince. 1974. *Development of the Avian Embryo*. Chapman and Hall, London.
- Insko, W. M., and J. H. Martin. 1933. Effect of frequent turning on hatchability and distribution of embryo mortality. *Poult. Sci.* 12:282–286.
- Kaltofen, R. S. 1955. Hatching experiments at Beekbergen—turning the eggs. *World's Poult. Sci. J.* 11:204–209.
- Kaltofen, R. S., and P. Ubbels. 1954. On the turning of eggs in artificial incubation. *Anim. Breed. Abstr.* 22:253.
- Leandro, N. S. M., E. Gonzales, J.C.V. Varoli, Jr, M. M. Loddi, and T. S. Takita. 2000. Incubabilidade e Qualidade de Pintos de Ovos Matrizes de Frangos de Corte Submetidos a Estresse de Temperatura (Hatchability and Chick Quality of Broiler Breeder Eggs Submitted to Stress Due to Temperature). *Rev. Bras. Cienc. Avic.* 2:39–44.
- Moraes, T. G. V., J. M. Romão, R. S. C. Teixeira, and W. M. Cardoso. 2008. Effects of egg position in artificial incubation of Japanese quail eggs (*Coturnix japonica*). *Anim. Reprod.* 5:50–54.
- Morita, V., V. R. de Almeida, J.B. Matos, Jr, T. I. Vicentini, H. van den Brand, and I. C. Boleli. 2016. Incubation temperature during fetal development influences morphophysiological characteristics and preferred ambient temperature of chicken hatchlings. *PLoS One* 11:e0154928.
- New, D. A. T. 1957. A critical period for the turning of hen's eggs. *J. Embryol. Exp. Morphol.* 5:293–299.
- Pearson, J. T., M. A. Haque, P. C. Hou, and H. Tazawa. 1996. Developmental patterns of O₂ consumption, heart rate and O₂ pulse in unturned eggs. *Respir. Physiol.* 103:83–87.
- Robertson, I. S. 1961a. The influence of turning on the hatchability of hens' eggs. I. The effect of rate of turning on hatchability. *J. Agric. Sci. Camb.* 57:49–56.
- Robertson, I. S. 1961b. The influence of turning on the hatchability of hens' eggs. II. The effect of turning frequency on the pattern of mortality, the incidence of malpositions, malformations and dead embryos with no somatic abnormality. *J. Agric. Sci. Camb.* 57:57–69.
- Romanoff, A. L. 1960. The Extraembryonic Membranes in the Avian Embryo, Structural and Functional Development. Pages 1041–1140 in *MacMillan Company*, New York.
- Tona, K., O. M. Onafbesan, Y. Jegu, B. Kamers, E. Decuyper, and V. Bruggeman. 2004. Comparison of embryo physiological parameters during incubation, chick quality and growth performance of three lines of broiler breeders differing in genetic composition and growth rate. *Poult. Sci.* 83:507–513.
- Tullett, S. G., and F. G. Burton. 1982. Factors affecting the weight and water status of the chick at hatch. *Br. Poult. Sci.* 23:361–369.
- Wilson, H. R. 1991. Physiological requirements of the developing embryo: temperature and turning. Pages 145–156 in *Avian Incubation*. S. G. Tullett ed. Butterworth-Heinemann, London.
- Yoshizaki, N., and H. Saito. 2002. Changes in shell membranes during the development of quail embryos. *Poult. Sci.* 81:246–251.
- Zhong, Z., Y. Yu, S. Jin, and J. Pan. 2018. Effects of mixing eggs of different initial incubation time on the hatching pattern, chick embryonic development and post-hatch performance. *Peer J* 6:e4634.