Abanico Veterinario. January-December 2020; 10:1-10. http://dx.doi.org/10.21929/abavet2020.39
Original Article. Received: 25/08/2020. Accepted: 28/11/2020. Published: 20/12/2020. Code:2020-73.

Effect of incubator carbon dioxide level on embryonic development and hatching parameters in broiler chicken

Efecto del nivel de dióxido de carbono de la incubadora sobre el desarrollo embrionario y parámetros de eclosión en pollo de engorda

Prado-Rebolledo Omar* [ID], Castellano-Ortega José [ID], Ruíz-Ramírez Johnatan [ID], Zepeda-Batista José [ID], García-Casillas Arturo [ID]**

Facultad de Medicina Veterinaria y Zootecnia, Universidad de Colima. México. *Autor responsable: Prado-Rebolledo Omar. Kilómetro 40, Carretera Colima-Manzanillo. Crucero de Tecomán, Colima. C.P. 28100. **Author for correspondence: García-Casillas Arturo. omarpr@ucol.mx, jcastellanos4@ucol.mx, jruiz7@ucol.mx, jzepeda15@ucol.mx, cesargarciacasillas@hotmail.com

ABSTRACT

Oxygen (**O_2**) and carbon dioxide (**CO_2**) are vital gases for the embryo during the incubation process, its level is essential at pipping, to evaluate the effect of incubator carbon dioxide level on embryonic development, hatching parameters, and post-hatch growth of broiler, humidity loss, hatchability, weight of chicken, size of chicken, blood glucose, hematocrit and plasma proteins were measured. A total of 600 eggs from commercial breeding Cobb 500 41 weeks, were selected by weight from 65 to 70 g, were distributed on two incubators. A machine was kept at 4000 ppm and the other to 3000 ppm CO_2. A 2 x 2 factorial design was used. The hatchability was better to 3000 ppm of CO_2 and egg weight of 65 g chicken egg; the chicken was heavier with eggs of 70 g, to more ppm of CO_2 reduction in the loss of humidity, was observed over a large chicken, blood glucose levels were not affected, but the values of plasma protein were less than 3000 ppm CO_2. Improved hatching parameters at lower ppm of CO_2 during the incubation process.
Keywords: incubation, carbon dioxide, embryo, gases.

RESUMEN

El oxígeno (**O_2**) y el dióxido de carbono (**CO_2**) son gases vitales para el embrión durante el proceso de incubación, su nivel es imprescindible en el momento del picaje, con la finalidad de evaluar el efecto del nivel de dióxido de carbono de la incubadora sobre el desarrollo embrionario, los parámetros de eclosión y el posterior crecimiento del pollo de engorda, se midió la pérdida de humedad, incubabilidad, peso del pollo, tamaño del pollo, glucosa sanguínea, hematocrito y proteínas plasmáticas. Un total de 600 huevos de reproductora comercial Cobb 500 de 41 semanas, se seleccionaron por peso de 65 y 70 g, se distribuyeron en dos máquinas incubadoras. Una máquina se mantuvo a 4000 ppm y la otra a 3000 ppm de CO_2. Se utilizó un diseño factorial 2 x 2. La incubabilidad fue mayor a 3000 ppm de CO_2 y peso de huevo de 65 g; el pollo más pesado fue con huevo de 70 g, a mayor ppm de CO_2 menor pérdida de humedad, a menor ppm de CO_2 se observó un pollo más grande, los niveles de glucosa no se afectaron, pero los valores de proteínas plasmáticas fueron menores a 3000 ppm de CO_2. Se mejoran los parámetros de eclosión al bajar las ppm de CO_2 durante el proceso de incubación.
Palabras clave: incubación, dióxido de carbono, embrión, gases.

ABBREVIATIONS

ED	embryonic development	AS	ascites syndrome
CO_2	carbon dioxide	RH	relative humidity humedad relativa
pO_2	partial pressure of oxygen	O_2	oxygen
pCO_2	partial pressure of carbon dioxide		
CO_2			

INTRODUCTION

Embryonic development (**ED**) depends in the first instance on the pores of the shell that allow the diffusion of oxygen (**O_2)** and carbon dioxide (**CO_2**) between the external environment of the egg and the embryo's blood (Cordeiro and Hincke, 2016). This gas exchange develops through the chorioallantoic membrane (John, 2017), which is supplied by blood vessels and whose function is similar to the placenta of mammalian fetuses (Koyama and Tennyson, 2016). The primary function of the respiratory system is to transport **O_2** and **CO_2**, between the environment and the tissues (D'Alba *et al.*, 2017); therefore, respiration is regulated to meet metabolic demands, supplying O_2 and eliminating **CO_2** (Okur, 2019). The partial pressure of oxygen (**pO_2**) and the partial pressure of carbon dioxide (**pCO_2**) in the air chamber are a stimulus for the embryo to perform the pecking (Deeming, 2016).

Gildersleeve and Boeschen (1983) carried out an experiment with turkey eggs, where they added CO_2 levels higher than atmospheric concentrations, in order to stimulate ED during the beginning of the incubation period, although ED was stimulated, no differences in hatchability between eggs incubated in ranges of atmospheric CO_2 to 1% CO_2 during the first 2 days of incubation, so that the temperature of the shell, together with the concentration of CO_2, affects the body weight of the chick at birth (Maatjens *et al.*, 2014a; Maatjens *et al.*, 2014b).

De Smit *et al* (2006) y De Smit *et al* (2008) modified the ventilation conditions, to raise CO_2 during the first 10 days of the ED, using two reproductive strains, 45 and 60 weeks old; with different levels of susceptibility to ascites syndrome (**AS**). The CO_2 levels resulted in 1 and 1.5%, so the ED10 of higher CO_2 levels resulted in higher absolute and relative body weight (to the egg weight) than the ED10 to ED18; they had accelerated growth, elevated levels of corticosterone and plasma T3, and higher pCO_2 in the air chamber; the hatching window was smaller in a time of 10 to 15 h, and the chick weight was greater with respect to normal ventilation. Likewise, García *et al* (2013) restricted ventilation during the first 10 days of the ED and found an improvement in the incubation parameters.

During the last phase of development, the chicken embryo varies its O_2 consumption with the ambient temperature (Deeming, 2016). During the 2nd phase of ED, once the embryo produces its own metabolic heat, it removes CO_2 in a range of 0.05 to 0.3% (D'Alba *et al.*, 2017), so it also depends on the diffusion of gases to through the pores (Mortola and

Labbe, 2005). Thus, a hypoxic condition can limit ED, limit beak and foot development, develop heart hypertrophy, changes in heart rhythm, pericardial and pulmonary edema, changes in hemoglobin and AS; since in the last stage of ED, the phase where the hypoxic condition occurs, the embryo consumes up to 60 % more O_2 (Burggren and Elmonoufy, 2017; John, 2017; Itani *et al.*, 2018). Another factor that can play an important role in ED is altitude; since shell decreasing conductance. At this stage, the embryo needs energy from anaerobic metabolism (Huang et al., 2017). Blood glucose and tissue glycogen reserves are what provide this energy, necessary for hatching (Fathollahipour *et al.*, 2018). It is known that there are differences in embryos metabolism with different ages and commercial lines, so if it is intended to optimize the incubation conditions, it is necessary to deepen their requirements (Huang *et al.*, 2017).

The objective of the present investigation was to determine incubation parameter effect with two levels of CO_2 and two egg weights.

MATERIAL AND METHODS

The experiment followed institutional and national guidelines for the care and use of animals; all procedures were approved by the Ethics Review Committee of the University of Colima. The study was carried out with 600 fertile eggs of commercial Cobb 500 breeder, 41 weeks old and weighing 65 and 70 g. The eggs were placed in two commercial single-stage incubators (HatchTech; Gildetrom 25.3905 TB., Veenendaal, Netherlands), with a capacity of 4800 eggs/each. The machines, which have a temperature sensor (± 0.1ºF), a humidity sensor [± 1% relative humidity (**RH**)] and a CO_2 sensor (± 100 ppm). The eggs were turned at an angle of 45° and then 90° every hour.

The air circulation was horizontal and laminar, through perforated radiators, which cause pressure differences, for better air distribution and a uniform flow through each egg mass, from top to bottom and from front to back. The same machines functioned as hatchers, and in both, the routine management of an incubator plant was carried out, from the reception of the egg to the removal of the hatchery machines.

Experimental design

The experiment was established as a completely randomized design, with a 2x2 factorial arrangement, with 2 concentrations of CO_2 from the incubator (3000 vs. 4000 ppm), and 2 egg weights (65 and 70 g); the treatments were divided into 2 subsequent groups with 2 treatments per group. Previously, a selection was made where broken, dirty, microfractured, deformed and out-of-weight eggs were discarded. The eggs were identified individually, with an indelible ink marker on the wide surface where the air chamber is located. The incubator machines were kept at the same temperature per stage throughout the incubation process; 37.8 °C during day 0, 37.6 °C from day 1 to 8, 37.5 °C from day 9 to 11 and 37.2 °C from day 12 to 21. The RH was kept at 50% throughout the

incubation process. The CO_2 concentration was monitored throughout the incubation process, to verify that it remained within the ranges established in the research protocol. The response criteria were individual weight of the eggs before incubation and during the transfer on day 18, to determine by weight difference the moisture loss, hatchability, body weight and size (measurement from the peak to the middle finger without considering the nail).

Blood parameter measurements

Twenty embryos were randomized for use in determining blood parameters. Blood was extracted from the jugular vein of the embryos or chickens, with a 1 mL syringe and a 30 gauge needle, and collected in heparinized tubes. Subsequently, blood was drawn in a heparinized capillary (150 µL) and immediately presented to a blood gas analyzer (GEM Premier 3000; Instrumentation Laboratory., Lexington, Massachusetts), to determine the glucose, hematocrit and plasma protein response criteria. .

Statistical analysis

The data were processed using the statistical program (SAS, System, v. 8.2, Cary, NC). The distributions of means and residuals were examined to verify the assumptions of the model. The hatchability, chick weight, moisture loss, chick size, glucose, hematocrit and plasma proteins were analyzed by sampling time; using general linear regression (PROC GLM), with 2 CO_2 concentrations, 2 egg weights and their interaction as class variables. For all parameters, chick was considered as the experimental unit. A Tukey multiple comparison test was performed, when the group effect was found to be significant (P <0.05). The data expressed in percentages were transformed to the arc-sine proportion for analysis.

RESULTS

In table 1, it is observed that the highest percentage of hatchability was with the concentration of 3000 ppm of CO_2, and with 65 g in egg weight. The CO_2 levels considered in the experiment did not affect the weight of the chicken; therefore, the treatments with 70 g eggs obtained heavier chickens. The lowest percentage in moisture loss with respect to CO_2 concentration was recorded at 4000 ppm and without statistical differences (P <0.05), between eggs with 65 and 70 g. The largest size of the chicken with respect to the CO_2 concentration was observed at 3000 ppm and without statistical differences between eggs with 65 and 70 g.

Table 1. Effects of 2 incubator carbon dioxide concentrations (3000 and 4000 ppm) and 2 egg weights (65 and 70g) on hatchability, chick weight, moisture loss, chick size, glucose, hematocrit and proteins plasma (n = 20 per CO_2 × egg weight)

Factor	Hatchability (%)	Chicken weight (g)	Moisture loss (%)	Chicken size (cm)	Glucose (mg/dL)	Hematocrit (%)	Plasma proteins (g/dL)
CO_2 (ppm)							
3000	88.10[a]	46.53	10.19[a]	19.44[a]	200.05	33.60	2.43[b]
4000	86.40[b]	47.00	8.70[b]	18.44[b]	188.85	36.80	2.71[a]
Mean square error	2.77	2.91	2.59	0.22	373.53	12.05	0.17
Egg weight (g)							
65	88.10[a]	44.99[b]	9.49	18.83	195.20	34.60	2.56
70	86.40[b]	48.54[a]	9.41	19.06	193.70	35.80	2.59
Mean square error	2.77	2.91	2.59	0.22	373.53	12.05	0.17
CO_2 (ppm) x egg weight (g)							
3000 x 65	88.60	44.13	10.51	19.44	195.60	34.30	2.34
3000 x 70	87.60	48.93	9.87	19.45	204.50	32.90	2.53
4000 x 65	87.60	45.85	8.47	18.22	194.80	34.90	2.78
4000 x 70	85.20	48.16	8.94	18.67	182.90	38.70	2.65
P-value							
CO_2	0.03	0.38	<.0001	<.0001	0.07	0.006	0.04
Egg weight	0.03	<.0001	0.62	0.13	0.80	0.28	0.82
CO_2 x egg weight	0.36	0.02	0.0007	0.15	0.09	0.02	0.23

[a,b] The least squares means followed by different superscripts within a column and factor are significantly different (P <0.05).

In Table 1, it is observed that the combination between CO_2 concentrations and egg weights did not show differences within the group (65 and 70 g, respectively); but it occurred between groups, with the lowest chicken weight ($P < 0.05$), in the treatment that combines 3000 ppm of CO_2 with 65 g eggs. Regarding chicken size, the result with the largest size was observed in the combination of 3000 ppm of CO_2, with eggs of 65 and 70 g. Blood glucose concentration did not show differences in both factors. The highest percentage of hematocrit with respect to CO_2 concentration was recorded at 4000 ppm and without statistical differences ($P < 0.05$), between eggs with 65 and 70 g. The highest concentration of plasma proteins with respect to the concentration of CO_2, was recorded at 4000 ppm. The result with the highest percentage of hematocrit was recorded in the combination of 4000 ppm of CO_2, with eggs of 70 g.

DISCUSSION

Hatchability (it is the ability of the egg to hatch) improves with a lower concentration of CO_2 (Fathollahipour *et al.*, 2018). In the present work, it was found that the lower concentration of CO_2 improves hatchability; so the integrity of the shell plays an important role for gas exchange. Then, when the embryo reaches day 18 of incubation, lung respiration begins, where thyroid hormone plays an important role in the lung surfactant development (Hamidu *et al.*, 2018). The process of pulmonary respiration contributes O_2 to the chicken embryo (Deeming, 2016). However, the lung respiration maturation without having the shell intact, and with the increase in the metabolic demand for O_2 at the end of incubation, cause an increase in pCO_2 and a decrease in pO_2 in the air chamber, (Flores-Santin *et al.*, 2018), activating the birth trigger mechanism (Ramachandran and McDaniel, 2018). So the normal ED of the chicken depends on the air changes that take place through the chorioallantoic membrane (John, 2017), which, together with shell pores, carries out the exchanges of O_2 and CO_2 between the blood and the environment (Deeming, 2016).

The shell is the main responsible for the difference between pCO_2 and water vapor, where the O_2 flow is affected by the shell and the inner membrane (Ramachandran and McDaniel, 2018); so it may be one of the main reasons why hatchability was not effective at a higher level of CO_2. In the same way, the conductance of the gas in the shell depends on the ratio of the area of the pore to its length; in the end, this relationship is equivalent to shell thickness (Cordeiro and Hincke, 2016).

In the present study, it was observed that by having a lower concentration of CO_2 in the environment, hatching was improved; since a hypoxic condition was not created at the end of the incubation process and pO_2 and pCO_2 in the air chamber were not affected. When embryos are subjected to hypoxic conditions during the incubation process, the body growth, beak and legs is inhibited; as mentioned (Burggren and Elmonoufy, 2017). In the experiment, chicken weight was not affected by the different CO_2 concentrations,

but the chickens that came from eggs with 70 g were heavier. In this regard, it is known that chicken weight is directly related to egg weight (D'Alba *et al.*, 2017). The chicken size was greater when the CO_2 concentration was 3000 ppm, these data suggest that if the embryos develop in environmental conditions with a low O_2 concentration, they lose weight during pecking and hatching (Ramachandran and McDaniel, 2018). Therefore, embryos in dense conditions of ambient O_2 gain weight. This implies that embryos in O_2-rich environmental settings are better metabolically prepared to initiate pecking and birth (Deeming, 2016).

In the study, chicken size was used as a quality criterion to determine the productive performance of the flock; on the other hand, the glycogen content in body tissues is important in the physiological requirement at the time of initiating the pecking (Maatjens *et al.*, 2014a; Maatjens *et al.*, 2014b). Similarly, it directly influences embryo survival; thus, in the study, the CO_2 conditions did not affect the blood glucose levels in the chickens, which may condition birds' vitality on the farm.

The hematocrit level showed a slightly higher numerical trend in the treatment with a higher concentration of CO_2, information similar to that reported by Scheele *et al.* (2003) who quantified elevated hematocrit values as a result of an increase in the erythropoietic response. The plasma protein concentration was lower when the CO_2 conditions were kept at 4000 ppm. These data suggest that in CO_2-rich environments, the erythropoietic response is used more (Ramachandran and McDaniel, 2018), and as a consequence the concentration of plasma proteins is reduced, as observed in the present study.

During chicken ED, commercial incubators handle concentrations of 4000 ppm of CO_2; but with the results obtained, it is suggested to lower said concentration to 3000 ppm, mainly to improve hatchability.

CONCLUSION

3000 ppm CO_2 concentrations improve hatchability, moisture loss and chick size; it also has low levels of plasma proteins.

CITED LITERATURE

BURGGREN WW, Elmonoufy NA. 2017. Critical developmental windows for morphology and hematology revealed by intermittent and continuous hypoxic incubation in embryos of quail (*Coturnix coturnix*). *PLoS One*. 12(9):e0183649. ISSN: 1932-6203. http://dx.doi.org/10.1371/journal.pone.0183649

CORDEIRO CM, Hincke MT. 2016. Quantitative proteomics analysis of eggshell membrane proteins during chick embryonic development. *Journal of Proteomics*. 130:11-25. ISSN: 1876-7737. http://dx.doi.org/10.1016/j.jprot.2015.08.014

D'ALBA L, Torres R, Waterhouse GIN, Eliason C, Hauber ME, Shawkey MD. 2017. What does the eggshell cuticle do? a functional comparison of avian eggshell cuticles. *Physiological and Biochemical Zoology*. 90(5):588-599. ISSN: 1537-5293. http://dx.doi.org/10.1086/693434

DEEMING DC. 2016. How does the bird-nest incubation unit work? *Avian Biology Research*. 9(2):103-113. ISSN: 1758-1559. http://dx.doi.org/10.3184/175815516X14567543242701

DE SMIT L, Bruggeman V, Tona JK, Debonne M, Onagbesan O, Arckens L, De Baerdemaeker J, Decuypere E. 2006. Embryonic developmental plasticity of the chick: Increased CO_2 during early stages of incubation changes the developmental trajectories during prenatal and postnatal growth. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 145(2):166-175. ISSN: 1095-6433. https://doi.org/10.1016/j.cbpa.2006.06.046

DE SMIT L, Bruggeman V, Debonne M, Tona JK, Kamers B, Everaert N, Witters A, Onagbesan O, Arckens L, De Baerdemaeker J, Decuypere E. 2008. The effect of nonventilation during early incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. *Poultry Science*. 87(1):551-560. ISSN: 0032-5791. https://doi.org/10.3382/ps.2007-00322

FATHOLLAHIPOUR S, Patil PS, Leipzig ND. 2018. Oxygen regulation in development: lessons from embryogenesis towards tissue engineering. *Cells Tissues Organs*. 205(5-6):350-371. ISSN: 1422-6421. http://dx.doi.org/10.1159/000493162

FLORES-SANTIN J, Rojas Antich M, Tazawa H, Burggren WW. 2018. Hematology from embryo to adult in the bobwhite quail (*Colinus virginianus*): Differential effects in the adult of clutch, sex and hypoxic incubation. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*. 218(1):24-34. ISSN: 0301-5092. http://dx.doi.org/10.1016/j.cbpa.2018.01.005

GARCÍA HJ, Juárez EMA, Córdova SL. 2013. Gradual increase of CO_2 during first stages of incubation with late change of O_2 partial pressure, modifies the hatch trajectory of broiler chicks. *Veterinaria México*. 44(1):1-16. ISSN: 0032-5791. http://veterinariamexico.unam.mx/index.php/vet/article/view/325

GILDERSLEEVE RP, Boeschen DP. 1983. The effects of incubator carbon dioxide level on turkey hatchability. *Poultry science*. 62(5):779-784. ISSN: 0032-5791. http://dx.doi.org/10.3382/ps.0620779

HAMIDU JA, Torres CA, Johnson-Dahl ML, Korver DR. 2018. Physiological response of broiler embryos to different incubator temperature profiles and maternal flock age during incubation. 1. Embryonic metabolism and day-old chick quality. *Poultry Science*. 97(8):2934-2946. ISSN: 1525-3171. http://dx.doi.org/10.3382/ps/pey089

HUANG S, Zhang L, Rehman MU, Iqbal MK, Lan Y, Mehmood K, Zhang H, Qiu G, Nabi F, Yao W, Wang M, Li J. 2017. High altitude hypoxia as a factor that promotes tibial growth plate development in broiler chickens. *PLoS One*. 12(3):e0173698. ISSN: 1932-6203. http://dx.doi.org/10.1371/journal.pone.0173698

ITANI N, Salinas CE, Villena M, Skeffington KL, Beck C, Villamor E, Blanco CE, Giussani DA. 2018. The highs and lows of programmed cardiovascular disease by developmental hypoxia: studies in the chicken embryo. *Journal of Physiology*. 596(15):2991-3006. ISSN: 1469-7793. http://dx.doi.org/10.1113/JP274111

JOHN NM. 2017. Structure and function of the shell and the chorioallantoic membrane of the avian egg: embryonic respiration. In: *The Biology of the Avian Respiratory System*. Springer. 219-247 p. ISBN: 978-3-319-44152-8. https://doi.org/10.1007/978-3-319-44153-5_9

KOYAMA T, Tennyson AJD. 2016. Respiratory pores on Ostrich *Struthio camelus* (Aves: Struthionidae) eggshells. *Advances in Experimental Medicine and Biology*. 923(1):51-55. ISSN: 0065-2598. http://dx.doi.org/10.1007/978-3-319-38810-6_7

MAATJENS CM, Reijrink IA, Molenaar R, van der Pol CW, Kemp B, van den Brand H. 2014a. Temperature and CO_2 during the hatching phase. I. Effects on chick quality and organ development. *Poultry science*. 93(3):645-654. ISSN: 0032-5791. http://dx.doi.org/10.3382/ps.2013-03490

MAATJENS CM, Reijrink IA, van den Anker I, Molenaar R, van der Pol CW, Kemp B, van den Brand H. 2014b. Temperature and CO_2 during the hatching phase. II. Effects on chicken embryo physiology. *Poultry science*. 93(3):655-663. ISSN: 0032-5791. http://dx.doi.org/10.3382/ps.2013-03491

MORTOLA JP, Labbe K. 2005. Oxygen consumption of the chicken embryo: interaction between temperature and oxygenation. *Respiratory physiology & neurobiology*. 146(1):97-106. ISSN: 1569-9048. http://dx.doi.org/10.1016/j.resp.2004.10.011

OKUR N. 2019. Effects of incubator carbon dioxide and oxygen levels, and egg weight on Broilers' hatchability of fertile eggs. *Brazilian Journal of Poultry Science*. 21(3). ISSN: 1516-635X. http://dx.doi.org/10.1590/1806-9061-2019-1038

RAMACHANDRAN R, McDaniel CD. 2018. Parthenogenesis in birds: a review. *Reproduction*. 155(6):R245-R257. ISSN: 1741-7899. http://dx.doi.org/10.1530/REP-17-0728

SAS. 2001. SAS/STAT User's guide. 8.2, v. SAS Institute Inc. Cary, NC.

SCHEELE CW, van Der Klis JD, Kwakernaak C, Buys N, Decuypere E. 2003. Haematological characteristics predicting susceptibility for ascites. 2. High haematocrit values in juvenile chickens. *British Poultry Science*. 44(3):484-489. ISSN: 0007-1668. http://dx.doi.org/10.1080/00071660310001598300