Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.4 / 001-008

http://dx.doi.org/10.1590/1806-9061-2023-1810

Original Article

Effect of Hypercapnia During Incubation and Broiler Breeder Age on Development of the Gastrointestinal Tract in Embryos and Hatchlings

■Author(s)

Souza LFA[I] https://orcid.org/0000-0002-0142-664X
Almeida EA[II] https://orcid.org/0000-0001-7244-9745
Silva LA[III] https://orcid.org/0000-0001-6273-3240
Nogueira BGR[IV] https://orcid.org/0000-0001-9300-2723
Espinha LP[V] https://orcid.org/0000-0002-7571-3000
Lunedo R[VI] https://orcid.org/0000-0001-6347-1764
Furlan RL[III] https://orcid.org/0000-0001-9549-0329
Macari M[III] https://orcid.org/0000-0002-6850-7145

[I] Federal Rural University of Pernambuco, Department of Animal Sciences, Recife, Brazil.
[II] Federal University of Mato Grosso, Institute of Agrarian and Environmental Sciences, Sinop, Brazil.
[III] São Paulo State University, Department of Animal Morphology and Physiology, Jaboticabal, Brazil.
[IV] University of Western São Paulo, Presidente Prudente, Brazil.
[V] Cobb-Vantress Brazil, Guapiaçu, Brazil.
[VI] Master Agroindustrial, Videira, Brazil.

■Mail Address

Corresponding author e-mail address
Lilian Francisco Arantes de Souza
Departamento de Zootecnia, Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros, s/n, Dois Irmãos, Recife – PE – 52171900 – Brasil.
Phone: 5516 981191168
Email: lilian.arantes@ufrpe.br

■Keywords

Carbon dioxide, hatching, intestinal mucosa, intestine, poultry.

Submitted: 02/May/2023
Approved: 06/September/2023

ABSTRACT

Exposure to increasing concentrations of CO_2 in the first 10 days of incubation may have effects on the development of bird cardiac and respiratory organs. Moreover, the age of breeders can influence hatching performances. This study aimed to investigate the effect of exposure to increasing concentrations of CO_2 in the first 10 days of incubation on the morphophysiological development of the digestive system of embryos and chicks from broiler breeders aged 31 and 41 weeks. A total of 860 fertile eggs from the Cobb strain were distributed in a completely randomized design, in a 2 x 2 factorial arrangement, with 2 different gaseous environments (Control (C) – no increase in CO_2 concentration and, Hypercapnia (CO_2) – a gradual increase in CO_2 concentration until reaching 1% on the 10th day) and 2 different broiler breeder ages (31 and 41 weeks). Half of the eggs were obtained from 31-week-old breeders, and the other half from 41-week–old breeders. Compared to the control group, incubation in an atmosphere with 1% CO_2 led to an increase in villus heights in the duodenum, jejunum, and ileum of the embryos, with a reduction in villus density in the same segments. Chicks from 41-week-old broiler breeders showed higher villus heights in the duodenum, jejunum, and ileum at day 1 post-hatching, and lower villus density at 7 days. It was concluded that the incubation of fertile eggs in hypercapnia conditions could positively affect the small intestine of embryos and post-hatch chicks.

INTRODUCTION

Despite the great technological development already achieved in artificial incubation, some aspects related to the gaseous environment are still being investigated to determine epigenetic effects and increase the efficiency of the process (Decuypere & Bruggeman, 2007; Druyan *et al.*, 2018; Okur *et al.*, 2022). Previous studies have shown that a gradual increase in CO_2 concentrations up to the first 10 days of embryonic development positively impacts incubation, resulting in reduced hatching time, improved hatchability, reduced embryonic mortality and changes in bird development during and after hatching (De Smit *et al.*, 2006, 2008; Bruggeman *et al.*, 2007; El-Hanoum *et al.*, 2019; Kroetz Neto *et al.*, 2023).

Exposure to increasing concentrations of CO_2 in the first 10 days of incubation induces a reduction in albumen pH, stimulates the vascularization of the chorioallantoic membrane, and increases the levels of thyroid hormones and corticosterone (Bruggeman *et al.*, 2007; Tona *et al.*, 2007; De Smit *et al.*, 2006, 2008; Verhoelst *et al.*, 2011; El-Hanoum *et al.*, 2019). Fernandes *et al.* (2017) demonstrated the effects of hypercapnia in the first 10 days of incubation on the density of

blood vessels in the chorioallantoic membrane, with an increase in the relative weight of the liver and changes in the morphological aspects of the heart, such as an increase in the thickness of the right ventricular wall. CO_2 exposure did not affect chick body temperature and blood pressure (Rocha *et al.*, 2020), but reduced the ventilatory response in chicks after hatching (Szdzuy & Mortola, 2008). Although many studies show effects on different systems, few researches, if any, describe the effect of hypercapnia on the development of the gastrointestinal tract.

Another important factor is the age of the broiler breeder, which affects the weight of eggs and chicks, albumen quality (Tona *et al.*, 2004), the development of the embryo (Peebles *et al.*, 2001), and the conductance of the shell (Peebles *et al.*, 1987). These and other variables can also be affected under hypercapnia

Considering:

i. that embryonic development is characterized not only by the growth of organic systems in isolation, but rather by an interaction between them;

ii. that the embryonic environment can modulate the development of such systems (Burggren *et al.*, 2016) and;

iii. the angiogenic effect on the chorioallantoic membrane of hypercapnia during early incubation (Verhoelst *et al.*, 2011);

this research was developed to test the hypothesis that exposure to CO_2 in up to 10 days of incubation would stimulate the development of the gastrointestinal tract of embryos and post-hatch chicks.

MATERIAL AND METHODS

Experimental design

Research on animals was conducted according to the standards of the Institutional Committee on Animal Use (Protocol 025330/12).

This study was conducted in Jaboticabal, São Paulo, Brazil (21°14'05" South latitude, 48°17'09" West longitude, average altitude of 615.01 m).

A total of 860 Cobb500® eggs (430 from 31-week-old breeders and 430 from 41-week–old breeders) obtained from a commercial hatchery (Globoaves, Itirapina, São Paulo, Brazil) were weighed (60.0 and 66.5 g, for broiler breeders aged 31 and 41 weeks, respectively), numbered, and distributed in a completely randomized design in a 2 x 2 factorial arrangement with 5 replications of 43 eggs. The factorial arrangement includes 2 incubator gaseous environments and 2 breeder ages. The eggs were equally distributed in 2 vertical incubators equipped with humidity and temperature controllers (Premium Ecológica, Belo Horizonte, Minas Gerais, Brazil), in which there was either no increase in CO_2 concentration (Control, C), or a gradual increase in CO_2 concentration until reaching 1% on the 10th day (Hypercapnia, CO_2), as shown in Figure 1. The increase in CO_2 concentration was obtained through the gradual injection of CO_2 in the incubator. The incubators' CO_2 concentration was monitored using a computerized system and CO_2 sensors (Vaisala GMW21D, Waarloos, Belgium).

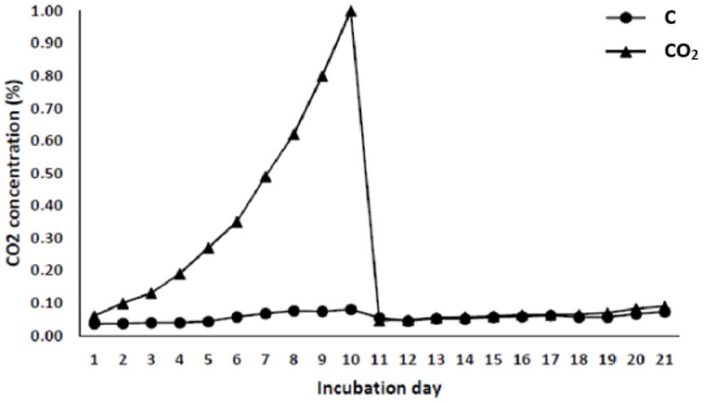

Figure 1 – CO_2 concentrations (%) in the incubators. (C: control and CO_2: increase in CO_2 concentration up to 1% in the first 10 days of incubation).

Incubator temperature and air humidity were maintained at 37.5°C and 60%, respectively, until 21 days, and eggs were turned 45° every hour until the 18th day of incubation. The two incubators were maintained under normal ventilation conditions, with openings open throughout the incubation. After hatching, the chicks were housed in cages (1.0 m wide x 0.60 deep x 0.40 m high) in an air-conditioned chamber with a temperature of 32 °C and a continuous light program until 7 days of age. The diet contained 22.48% crude protein and 2960 kcal of metabolizable energy/kg, following the recommendations of Rostagno *et al.* (2011).

Egg Weight Loss, Hatchability, Chick Body Weight, and Chick Quality

Egg weight loss was calculated as EWL (%) = $[(W_0 - W_F)/ W_0] \times 100$, where W_0 is egg weight at setting and W_F is egg weight at the 18th day of incubation. Hatchability was calculated as the percentage of fertile eggs set that hatched. After hatching, chicks were weighed, and chick quality was evaluated according to Tona *et al.* (2003).

Allometric Growth of Digestive System Organs

The allometric growth of digestive system organs was evaluated in embryos at 16, 18 and 20 days of incubation, and in chicks at 1, 4, and 7 days after hatching (after fasting for 12 hours), using five embryos and five chicks from each treatment at each age. Embryos and chicks were euthanized by decapitation and the digestive system was separated into yolk sac, proventriculus, gizzard, intestine, and liver, with each organ being washed and weighed. The body weight of the embryos and chicks was obtained by discounting the weight of the yolk sac from the total weight of the live bird. The relative weight (%) of each organ was obtained through the ratio between the weight of the organ and the body weight of the embryo or chick (discounting the yolk sac).

Intestinal Light Microscopy

Samples from the duodenum were collected in the proximal descending portion of the duodenal loop, from the jejunum, posterior to the distal portion of the duodenal loop, and from the ileum, in the region cranial to the cecum. The samples were collected from 5 birds of each treatment at 16, 18, and 20 days of incubation, and at 1, 4, and 7 days after hatching, the same birds used in the evaluation of organ allometric development. The samples were fixed in Bouin's solution, dehydrated in an increasing series of ethanol (70%, 80%, 90%, and 100%), and included in paraffin. Five histological sections with 5 micrometers each were made per sample, and they were stained with hematoxylin and eosin. The histological sections' images were obtained using an image capture system (Leica QWin; Leica Imaging Systems, Wetzlar, Germany).

The analyzes were carried out using the Image J® software (Rasband, 2004). The height of villi in the first stage was measured according to Uni *et al.* (2003), at 16, 18, and 20 days in embryos, and at 1, 4, and 7 days in chicks, with 30 readings/intestinal segment.

Intestinal Scanning Electron Microscopy

The density of intestinal villi was evaluated at 20 days of incubation, and at 1, 4, and 7 days after hatching in 5 birds of each treatment, using scanning electron microscopy in the same regions of the small intestine mentioned previously. The samples were processed according to the methodology described by Maiorka *et al.* (2003), in the same birds used in the evaluation of organ allometric development and intestinal light microscopy. Villus density per area was expressed as the number of villi/mm^2.

Statistical analysis

The results are presented as means with the standard error of the mean. All statistical analyses were performed using the SAS software (SAS Institute, 2001). The data were analyzed using Analysis of Variance (ANOVA), and in case of significant differences, the means were compared by the Tukey test at 5% probability (p<0.05).

The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijk},$$

in which Y_{ijk} is the response variable measured for different incubator gaseous environments (i) and breeder ages (j) in replication k, μ is the general constant, αi is the fixed effect of incubator gaseous environments, β_j is the fixed effect of breeder ages, $\alpha \times \beta)_{ij}$ is the interaction between incubator gaseous and breeder ages, and ε_{ijk} is the random error term.

Comparisons were made after verifying the homogeneity of the variance between treatments using the Levene test (Petrie & Watson, 2006), and the normality using the Cramér-von Mises test (Sprent, 1989).

RESULTS

Data analysis showed no interaction between hypercapnia and broiler breeder age for all parameters analyzed. Table 1 shows data on egg weight loss, hatchability, chick weight and quality score, with no difference between control and CO_2 treatments (p>0.05). For breeder ages, the lowest egg weight loss (p=0.0498) and highest chick weight (0.0007) were for breeders aged 41 weeks. Exposure to CO_2 did not influence the weight of the yolk sac and the relative weight of the digestive system organs, with only an increase in embryo weight being observed at 16 days of incubation (p=0.011) with incubation in hypercapnia, with no observed effect after hatching (data not shown).

In comparison to 31-week-old broiler breeders, eggs from 41-week-old broilers resulted in higher body weight (p=0.036, 0.047, and 0.024, respectively) and yolk weight (p<0.001, 0.003, and 0.037, respectively) at 16, 18 and 20 days of incubation. Moreover, eggs from 41-week-old broiler breeders resulted in a higher chick weight 1, 4, and 7 days after hatching (p=0.002, <0.001 and 0.005), and a higher relative liver weight

Table 1 - Average values of egg weight loss (EWL, %), hatchability (%), day-old chick body weight (CBW, g), average chick quality score (CQE) and chicks with quality score=100 (100CQE, %), in eggs from 31- and 41-week-old breeders incubated without increase in CO_2 concentration (C) and with gradual increase in CO_2 concentration until reaching 1% on the 10th day (CO_2).

Factors	EWL	Hatchability	CBW	CQE	100CQE
CO_2 concentration					
C	9,61	90,06	42,57	97,32	77,50
CO_2	9,48	92,38	43,09	97,73	79,50
Breeder age (weeks)					
31	9,95 a	92,13	41,35 b	98,00	78,50
41	9,14 b	90,31	44,31 a	97,05	78,50
SEM*	0,40	1,94	0,94	0,61	3,98
p value					
CO_2	0,7515	0,2565	0,4758	0,5058	0,6437
Breeder age	0,0498	0,3680	0,0007	0,1343	1,0000
CO_2 x Breeder age	0,7730	0,6466	0,9772	0,4466	0,4897

*SEM: Standard error of the mean.
Means followed by different letters differ according to Tukey's test at 5% (p<0,05).

at 1 day after hatching (p=0.013), when compared to eggs from 31-week-old broiler breeders (Figure 2).

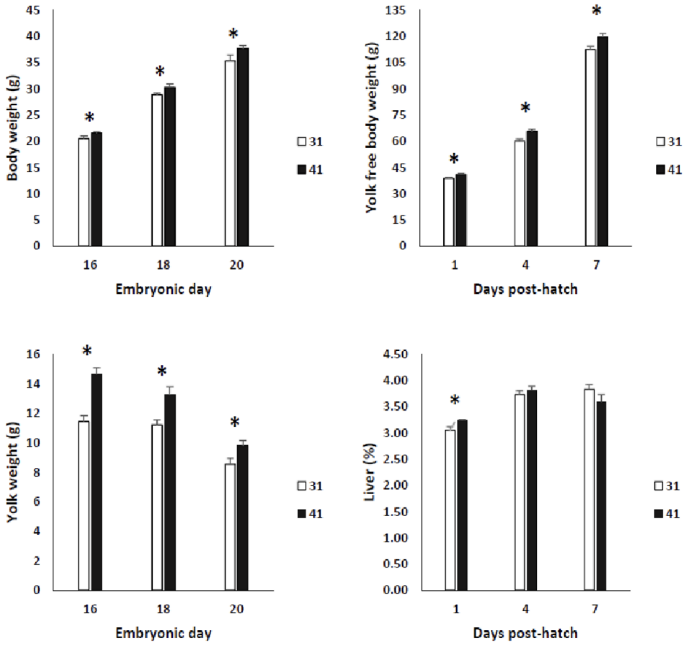

Figure 2 – Effect of broiler breeder age (31 and 41 weeks) on the weight of embryos and yolk at 16, 18 and 20 days of incubation and weight of chicks free of the yolk sac and liver at 1, 4 and 7 days after hatching. Asterisks indicate a significant difference between groups (p<0.05).

Incubation at 1% CO_2 resulted in an increase in the height of the intestinal villi in the duodenum at 20 days of incubation (p<0.001) and 1 day after hatching (p=0.001), and at 16 days of incubation in the jejunum (p<0.001) and ileum (p=0.026) (Figures 3, 4 and 5). Villus of embryos and chicks from 41-week-old breeders showed higher height in the duodenum at 20 days of incubation (p=0.001) and 1 day after hatching (p=0.018). In the jejunum, increased height of villi was observed at 1 day after hatching (p=0.008), and in the ileum at 1 and 7 days after hatching (p=0.025 and 0.022, respectively) for chicks from 41-week-old breeders (Figures 3, 4 and 5).

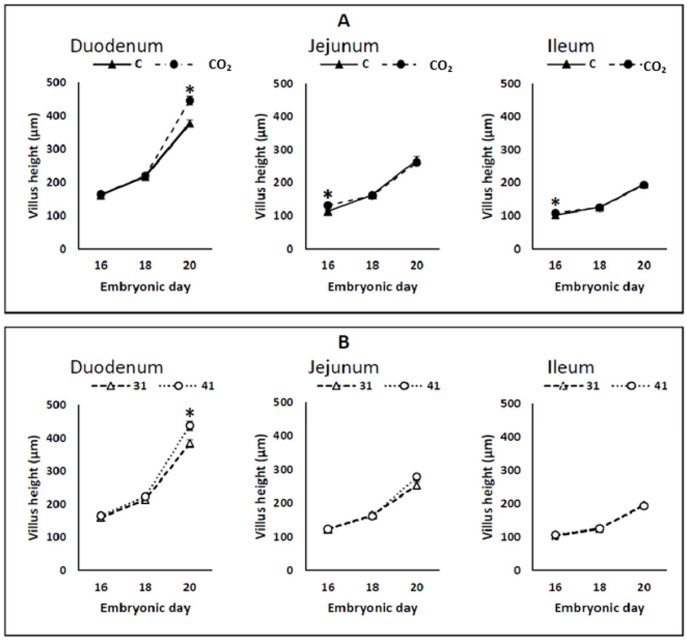

Figure 3 – Effect of incubation in control (C) and increase in CO_2 concentration up to 1% in the first 10 days of incubation (CO_2) (A) and effect of breeder age (31 and 41 weeks) (B) on height of the villi in the duodenum, jejunum and ileum of embryos at 16, 18 and 20 days. Asterisks indicate a significant difference between groups (p<0.05).

Villus density was also influenced by incubation in hypercapnia. In comparison to the control group, the group incubated in 1% CO_2 showed lower villus density in the duodenum in chicks 1 day after hatching (p<0.001). In the jejunum, this effect was observed in embryos with 20 days (p=0.015) of incubation and in chicks at 4 and 7 days after hatching (p=0.004 and 0.015, respectively). In the ileum, the lowest density was observed in embryos with 20 days of incubation

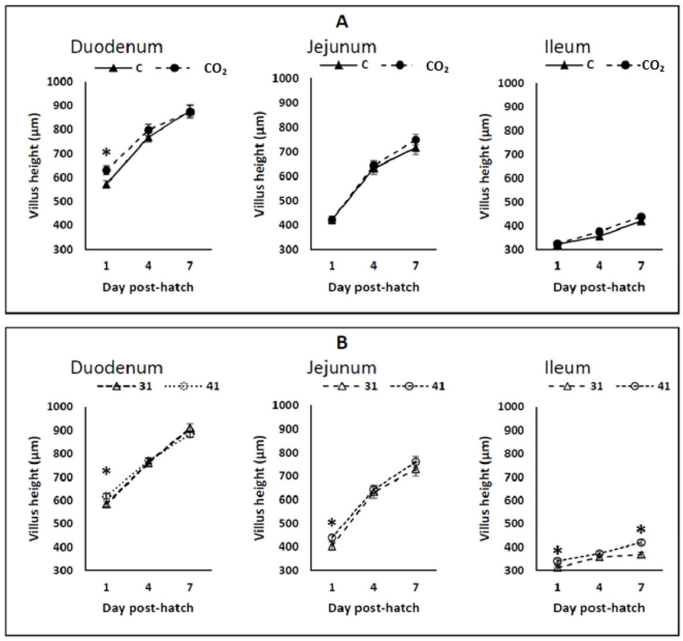

Figure 4 – Effect of incubation in control (C) and increase in CO_2 concentration up to 1% in the first 10 days of incubation (CO_2) (A) and broiler breeder age (31 and 41 weeks) (B) on the height of the villi in the duodenum, jejunum and ileum of chicks at 1, 4, and 7 days after hatching. Asterisks indicate a significant difference between groups (p<0.05).

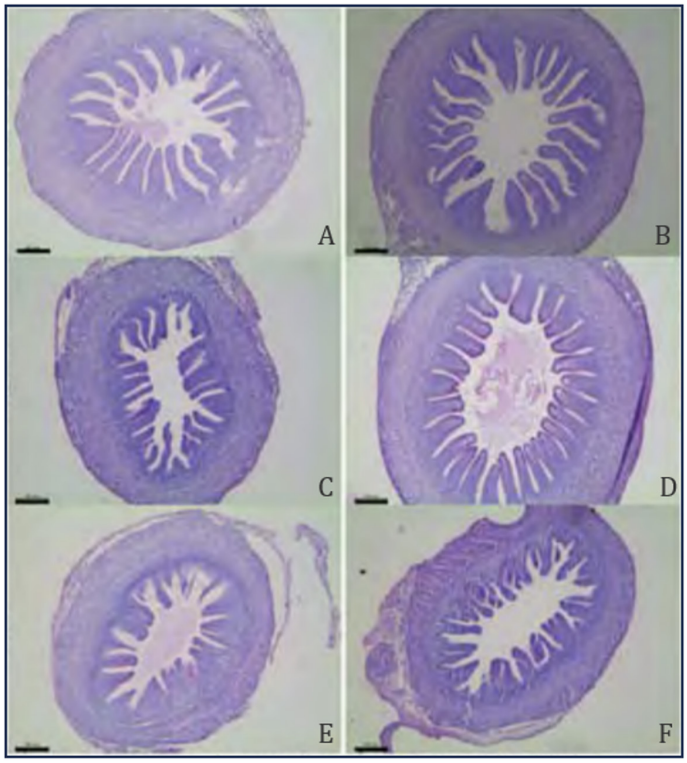

Figure 5 – Photomicrographs of cross-sections of intestinal segments of embryos at 16 days of incubation. A, C, and E represent the duodenum, jejunum, and ileum, respectively, of embryos incubated without increase in CO_2 concentration, and B, D, and F represent the duodenum, jejunum, and ileum, respectively, of embryos incubated with a gradual increase in CO_2 concentration until reaching 1% on the 10th day. (– = 100 µm).

(p=0.022) and in chicks on the 4th day after hatching (p=0.031) (Figure 6 and 7). Chicks from 41-week-old broiler breeders showed lower villus density in the duodenum, jejunum, and ileum at 7 days (p=0.014, 0.001, and 0.008) compared to chicks from 31-week-old breeders.

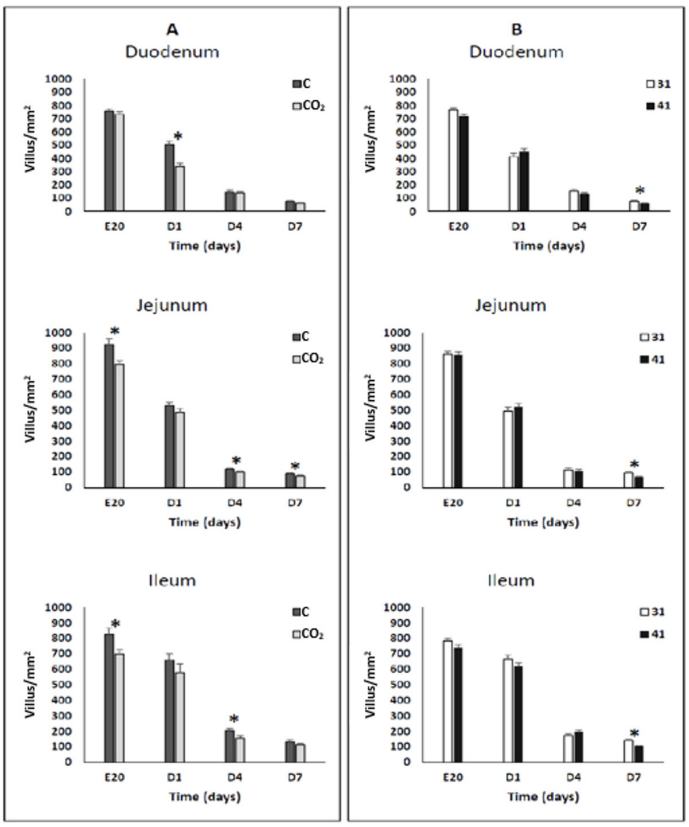

Figure 6 – Effect of incubation in control (C) and with an increase in CO_2 concentration up to 1% in the first 10 days of incubation (CO_2) (A) and of breeder age (31 and 41 weeks) (B) on villus density in the duodenum, jejunum and ileum of chicks at 1, 4 and 7 days after hatching. Asterisks indicate a significant difference between groups (p<0.05).

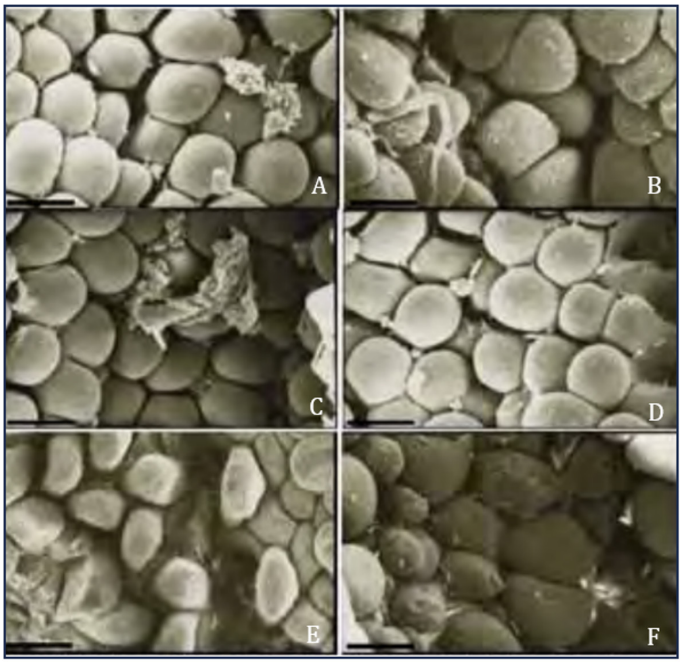

Figure 7 – Photomicrographs of the surface of intestinal segments of embryos at 20 days of incubation. A, C, and E represent the duodenum, jejunum, and ileum, respectively, of embryos incubated without increase in CO_2 concentration, and B, D, and F represent the duodenum, jejunum, and ileum, respectively, of embryos incubated with a gradual increase in CO_2 concentration until reaching 1% on the 10th day. (– = 50 µm).

DISCUSSION

Incubation with increased CO_2 concentration during the early stages can improve hatchability (De Smit *et al.*, 2006, 2008; El-Hanoun *et al.*, 2019) or not affect it (Bruggeman *et al.*, 2007, Fernandes *et al.*, 2016; Kroetz Neto *et al.*, 2023). In our study, no improvement in hatchability was observed with hypercapnia, regardless of the breeder age, showing that the effect of incubation in hypercapnia on hatchability can be influenced by other factors, such as genetics (De Smit *et al.*, 2008). Most research on incubation in hypercapnia so far has used non-ventilated incubators (De Smit *et al.*, 2006, 2008; Fernandes *et al.*, 2016). These can influence humidity conditions, resulting in differences in egg weight loss. In our work, incubators were kept ventilated and there was no difference in egg weight loss. Chick weight and quality were not influenced by incubation in hypercapnia, in line with other studies with a pattern of hypercapnia similar to ours (De Smith *et al.*, 2006, 2008; Fernandes *et al.*, 2016; Kroetz Neto *et al.*, 2023).

Total embryo weight (16th day incubation) in hypercapnia was similar to the findings reported in the literature (De Smit *et al.*, 2006, 2008; Tona *et al.*, 2007). However, regarding the relative weight of the gastrointestinal tract organs, no statistical differences were found when comparing the embryos and chicks from the control and hypercapnia groups. The exception was the lower relative weight of the liver observed in embryos incubated in hypercapnia at 16 days of age. Studies confirm the absence of an effect of incubation in hypercapnia on the relative weight of various organs such as heart, liver, gizzard, intestine, spleen, bursa and lungs (De Smit *et al.*, 2008; Maatjens *et al.*, 2014; Tong *et al.*, 2015; Fernandes *et al.*, 2017). Thus, in this study, the findings show that the weight of the organs of the gastrointestinal tract is also not influenced by incubation in hypercapnia.

Despite the lack of effects on the relative weight of the intestine, the increase in CO_2 concentration determined a change in villi height. The higher the villi, the lower the density per square area, indicating higher surface absorption. The effects of incubation in hypercapnia on villi development may be associated with increases in plasma T3 and corticosterone concentrations, as evidenced by De Smit *et al.* (2006). Such hormones accelerate the rate of embryo development (Decuypere *et al.*, 1991). These hormones are involved in the growth and functional maturation of various organs in embryos of avian species, including the intestine (Black, 1978). There have been reports of a relation between thyroid hormones and cell differentiation in the intestine of chick embryos (Moog, 1961).

The effects on villi development of CO_2 during incubation at a 1% concentration could also be attributed to the angiogenesis stimulus that occurs in conditions of hypercapnia. Verhoelst *et al.* (2011) reported higher vascularization of the chorioallantoic membrane in conditions of hypercapnia, supporting the idea that villi growth can be affected by increasing oxygen supply. Vasculogenesis occurs at the beginning of embryonic development; thus, the effect on intestinal villi development seems to be dependent of blood vessels and oxygen supply (Pardanaud *et al.*, 1989; Risau, 1997).

The observed results regarding villus height and villus density suggest that hypercapnia of up to 1% in the first 10 days of incubation may improve nutrient absorption capacity in the small intestine.

The hypothesis that exposure to CO_2 would affect the digestive system differently depending on the age of the broiler breeder has not been confirmed, since there was no interaction between the factors studied. This shows that the results obtained with hypercapnia are independent from the age of the broiler breeder. On the other hand, broiler breeder age influenced some studied parameters; for instance, the lower percentage of weight loss in eggs from older broiler breeders (Iqbal *et al.*, 2016). Embryos and newly hatched chicks presented higher weight and higher yolk weight when coming from eggs from older breeders as compared to young ones, confirming the results obtained by Bray & Iton (1962), that evidenced the positive correlation between egg weight and embryo weight, especially at the end of incubation.

El Sabry *et al.* (2013) observed higher villi height and villus surface area in the jejunum of chicks from old broiler breeders compared to young ones. Schaefer *et al.* (2006) also reported a higher villus surface area in all segments of the small intestine in birds from older broiler breeders, compared to young ones. These results suggest that chicks from older broiler breeders have a greater ability to absorb nutrients.

CONCLUSIONS

In conclusion, incubation in hypercapnia can affect morphological characteristics in the small intestine, by increasing villus height and reducing density, regardless of the age of the broiler breeder.

REFERENCES

Black BL. Morphological development of the epithelium of the embryonic chick intestine in culture: Influence of thyroxine and hydrocortisone. American Journal of Anatomy 1978;153(4):573-600. https://doi.org/10.1002/aja.1001530408

Bray DF, Iton EL. The effect of egg weight on strain differences in embryonic and postembryonic growth in the domestic fowl. British Poultry Science 1962;3(3):175-88. https://doi.org/10.1080/00071666208415472

Bruggeman V, Witters A, De Smit L, *et al.* Acid–base balance in chicken embryos(*Gallus domesticus*) incubated under high CO_2 concentrations during the first 10 days of incubation. Respiratory Physiology & Neurobiology 2007;159(2):147-54. https://doi.org/10.1016/j.resp.2007.04.013

Burggren WW, Santin JF, Antich MR. Cardio-respiratory development in bird embryos: new insights from a venerable animal model. Revista Brasileira de Zootecnia 2016;45(11):709-28. https://doi.org/10.1590/S1806-92902016001100010

De Smit L, Bruggeman V, Tona JK, *et al.* Embryonic developmental plasticity of the chick: Increased CO2 during early stages of incubation changes the developmental trajectories during prenatal and postnatal growth. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 2006;145(2):166-75. https://doi.org/10.1016/j.cbpa.2006.06.046

De Smit L, Bruggeman V, Debonne M, *et al.* The effect of non-ventilation during early incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. Poultry Science 2008;87(3):551-60. https://doi.org/10.3382/ps.2007-00322

Decuypere E, Dewil E, Kuhn ER. The hatching process and the role of hormones. In: Tullet SG, editor. Avian incubation. 2nd ed. London: Butterworth Heinemann; 1991 p.239-56.

Decuypere E, Bruggeman V. The endocrine interface of environmental and egg factors affecting chick quality. Poultry Science 2007;86(5):1037-42. https://doi.org/10.1093/ps/86.5.1037

Druyan S, Ruzal M, Shinder D, *et al.* Effects of low oxygen during chorioallantoic membrane development on post-hatch growing performance of broiler chickens. Poultry Science 2018;97(6):1961-7. https://doi.org/10.3382/ps/pey052

El-Hanoun A, El-Sabrout K, Abdella M, *et al.* Effect of carbon dioxide during the early stage of duck egg incubation on hatching characteristics and duckling performance. Physiology & Behavior 2019;208:112582. https://doi.org/10.1016/j.physbeh.2019.112582

El Sabry MI, Yalçın S, Turgay-Izzetoğlu G. Interaction between breeder age and hatching time affects intestine development and broiler performance. Livestock Science 2013;157(2-3):612-7. https://doi.org/10.1016/j.livsci.2013.07.012

Fernandes JIM, Bortoluzzi C, Schmidt JM, *et al.* Single stage incubators and Hypercapnia during incubation affect the vascularization of the chorioallantoic membrane in broiler embryos. Poultry Science 2017;96(1):220-5. https://doi.org/10.3382/ps/pew274

Iqbal J, Khan SH, Mukhtar N, Ahmed T, *et al.* Effects of egg size(weight) and age on hatching performance and chick quality of broiler breeder. Journal of Applied Animal Research 2016;44(1):54-64. https://doi.org/10.1080/09712119.2014.987294

Kroetz Neto F, Gonzalez E, Novaes G, *et al.* Beneficial impact of hypercapnic conditions during early incubation on broiler hatchability, embryo mortality and postnatal performance. Brazilian Journal of Poultry Science 2023;25(2):1-12. https://doi.org/10.1590/1806-9061-2022-1728

Maatjens CM, Reijrink IAM, Molenaar R, *et al.* Temperature and CO_2 during the hatching phase. I. Effects on chick quality and organ development. Poultry Science 2014;93(3):645-54. https://doi.org/10.3382/ps.2013-03490

Maiorka A, Santin E, Dahlke F, *et al.* Posthatching water and feed deprivation affect the gastrointestinal tract and intestinal mucosa development of broiler chicks. Journal of Applied Animal Research 2003;12(4):483-92. https://doi.org/10.1093/japr/12.4.483

Moog F. The functional differentiation of the small intestine IX. The influence of thyroid function on cellular differentiation and accumulation of alkaline phosphatase in the duodenum of the chick embryo. General and Comparative Endocrinology 1961;1(5-6):416-32. https://doi.org/10.1016/0016-6480(61)90006-5

Okur N, Eratalar AS, Yiğit AA, *et al.* Effects of incubator oxygen and carbon dioxide concentrations on hatchability of fertile eggs, some blood parameters, and histopathological changes of broilers with different parental stock ages in high altitude. Poultry Science 2022;101(2):101609. https://doi.org/10.1016/j.psj.2021.101609

Pardanaud L, Yassine F, Dieterlen-Lievre F. Relationship between vasculogenesis, angiogenesis and haemopoiesis during avian ontogeny. Development 1989;105(3):473-85. https://doi.org/10.1242/dev.105.3.473

Peebles ED, Brake J. Eggshell quality and hatchability in broiler breeder eggs. Poultry Science 1987;66(4):596–604. https://doi.org/10.3382/ps.0660596

Peebles ED, Doyle SM, Zumwalt CD, *et al.* Breeder age influences embryogenesis in broiler hatching eggs. Poultry Science 2001;80(3):272-7. https://doi.org/10.1093/ps/80.3.272

Petrie A, Watson P. Statistics for veterinary and animal science. 2nd ed. Oxford: Blackwell Publishing; 2006.

Rasband WS. Image J. 2004 [cited in 2023 May 10]. Available from: http://rsb.info.nih.gov/ij/

Reis LH, Gama LT, Soares MC. Effects of short storage conditions and broiler breeder age on hatchability, hatching time, and chick weights. Poultry Science 1997;76(11):1459-66. https://doi.org/10.1093/ps/76.11.1459

Risau W. Mechanisms of angiogenesis. Nature 1997;386:671-4. https://doi.org/10.1038/386671a0

Rocha AC, Santos KM, Almeida AR, *et al.* Cardiorespiratory and thermal responses to hypercapnia in chickens exposed to CO2 during embryonic development. Respiratory Physiology & Neurobiology 2020;273:103317. https://doi.org/10.1016/j.resp.2019.103317

Rostagno HS, Albino LFT, Donzele JL, *et al.* Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3rd ed. Viçosa: UFV; 2011.

SAS Institute. User's guide(Reléase 8.2). Cary: SAS Institute; 2001.

Schaefer CM, Corsiglia CM, Mireles Jr. A, *et al.* Turkey breeder hen age affects growth and systemic and intestinal inflammatory responses in female poults examined at different ages posthatch. Poultry Science 2006;85(10):1755-63. https://doi.org/10.1093/ps/85.10.1755

Sprent P. Applied nonparametric statistical methods. London: Chapman and Hall; 1989.

Szdzuy K, Mortola JP. Ventilatory chemosensitivity and thermogenesis of the chicken hatchling after embryonic hypercapnia. Respiratory Physiology & Neurobiology 2008;162(1):55-62. https://doi.org/10.1016/j.resp.2008.04.001

Tona K, Onagbesan O, De Ketelaere B, *et al.* Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and chick posthatch growth to forty-two days. Journal of Applied Poultry Research 2004;13(1):10-8. https://doi.org/10.1093/japr/13.1.10

Tona K, Bamelis F, De Ketelaere B, *et al.* Effects of egg storage time and spread of hatch, chick quality, and chick juvenile growth. Poultry Science 2003;82(5):736-41. https://doi.org/10.1093/ps/82.5.736.

Tona K, Onagbesan O, Bruggeman V, *et al.* Non-ventilation during early incubation in combination with dexamethasone administration during late incubation: 1. Effects on physiological hormone levels, incubation duration and hatching events. Domestic Animal Endocrinology 2007;33(1):32-46. https://doi.org/10.1016/j.domaniend.2006.04.002

Tong Q, McGonnell IM, Roulston N, *et al.* Higher levels of CO2 during late incubation alter the hatch time of chicken embryos. British Poultry Science 2015;56(4):503-9. https://doi.org/10.1080/00071668.2015.1041097

Uni Z, Tako E, Gal-Garber O, *et al.* Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poultry Science 2003;82(11):1747-54. https://doi.org/10.1093/ps/82.11.1747

Verhoelst E, Ketelaere B, Decuypere E, *et al.* The effect of early prenatal hypercapnia on the vascular network in the chorioallantoic membrane of the chicken embryo. Biotechnology Progress 2011;27(2):562-70. https://doi.org/10.1002/btpr.569.