Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jul - Sept 2014 / v.16 / n.3 / 285-290

http://dx.doi.org/10.1590/1516-635x1603285-290

Performance of Broilers Submitted to High CO_2 Levels During Incubation Combined with Temperature Fluctuations at Late Post-Hatch

Author(s)

Fernandes JIM[I]
Bortoluzzi C[I]
Esser AFG[I]
Contini JP[I]
Stokler PB[I]
Faust D[I]

[I] Federal University of Parana, Campus Palotina, 85950-000, Parana, Brazil

Mail Address

Corresponding author e-mail address:
Jovanir Inês Müller Fernandes
Laboratory of Poultry Experimentation, Federal University of Paraná - Campus Palotina, Rua Pioneiro, 2153, Jd. Dallas, Palotina – Parana, CEP: 85.950-000, Tel (55+44-3211-8525)
E-mail: jimfernandes@ufpr.br

Keywords

Cyclic variation of temperature, Heterophil/Lymphocyte ratio, hypercapnia.

Submitted: August/2013
Approved: June/2014

ABSTRACT

Incubation represents around 1/3 of the life cycle of broilers of modern strains; therefore, the appropriate management of this period is crucial to ensure the quality of the neonate. An experiment evaluated the effect of carbon dioxide concentrations during incubation on the live performance, heart morphology, and differential leukocyte count in the blood of broilers submitted to fluctuating temperatures between 35 and 42 days of age. In total, 2,520 fertile eggs were distributed according to a completely randomized design with four CO_2 concentrations (4,000; 6,000; 8,000 and 10,000 ppm) during the first ten days of incubation, after which all eggs were incubated at the same CO_2 level (4,000 ppm). After hatching, male chicks were placed in the experimental broiler houses, and at 35 days of age, 25 birds from each level of CO_2 were separated and placed in cages to study the effect of cyclic temperature variations up to 42 days of age on their live performance, heterophil:lymphocyte ratio, and heart morphology. At 42 days of age, blood was collected for hematology and two birds per replicate were sacrificed and evaluated for ascites score and heart and liver relative weights. Heart morphology was assessed by analyzing digital images. In this experiment, hypercapnia during incubation and fluctuating temperatures during the growout did not affect mortality, ascites score, heart and liver relative weights, or heart characteristics ($p>0.05$). However, heterophill:lymphocyte ratio increased ($p<0.05$) with increasing carbon dioxide concentrations during incubation. It was concluded that high CO_2 levels during incubation did not influence the resistance of broilers to fluctuating temperatures during the last week of age.

INTRODUCTION

Genetic selection in the poultry industry has produced incredibly fast-growing broilers, and this is already observed during embryonic development. In order to achieve the highest productivity and production efficiency, it is essential to fully understand the requirements of the developing embryo during incubation (De Smit *et al.*, 2008).

Since the development of the first incubation machine, commercial hatcheries of the early 20th century underwent many changes that improved their efficiency and reliability. More recently, single-stage incubation was introduced. In single-stage incubators,all eggs within the incubator are set at the same developmental phase (same embryonic age). In this type of incubation system, labor costs are reduced, and the equipment can be cleaned and disinfected at the end of each hatching cycle in order to minimize microbial contamination risks (Hulet, 2007). Furthermore, single-stage incubators can precisely meet temperature, relative humidity, and CO_2 concentration requirements of the embryo during the different phases of embryonic development (Bennett, 2010). These conditions will create the ideal environment for embryonic development (Everaert *et al*., 2007).

It has been demonstrated that different CO_2 levels may play a role in the embryo development (Walsberg, 1980; De Smit *et al*., 2006; Everaert *et al*., 2008). High CO_2 levels early in incubation, similar to situation in nature, may have beneficial effects. Several recent studies investigated the effect of hypercapnia, i.e. high CO_2 concentrations, on broiler performance (Buys *et al*., 1998; De Smit *et al*., 2006; Everaert *et al*., 2008). An increase in CO_2 during early incubation is reported to improve hatchability, accelerate pre- and postnatal growth, and hatching time (De Smit *et al*., 2006; Tona *et al*., 2007).

Although these results are promising, it is necessary to understand the best level and period to apply this management practice (De Smit *et al*., 2006), and understand the physiological changes induced by hypercapnia. Everaert *et al*. (2007) evaluated the use of high CO_2 levels during the second half of incubation and did not find any differences in the live weight of 7-d-old chicks. However, other studies showed that high CO_2 levels during the first 10 days of incubation increased embryo body weight and early hatchability (De Smit *et al*., 2008). Therefore, it is believed that the best age of CO_2 exposure is during the formation of chorioallantoic membrane (CAM) (from 5 to 11 days of incubation), resulting in increased CAM vascularity and better oxygen transport capacity in later stages (Druyan etet *al*., 2012).

This period of critical hypoxia may trigger structural changes in the lungs and heart, predisposing birds to ascites (Decuypere *et al*., 2000). Consequently, the incubation period may potentially improve broiler growth, metabolism and health. For instance, Buys *et al*. (1998) considered that embryos exposed to higher CO_2 levels during incubation may have lower incidence of ascites during the rearing phase.

Modern broiler strains are selected for fast growth and high feed efficiency, and therefore, more oxygen is required to meet their high metabolic rate. A high incidence of subclinical heart disease was demonstrated in fast-growing broilers, as well as right ventricle hypertrophy (Baghbanzadeh & Decuypere, 2008). Therefore, as a consequence of the underdevelopment of the pulmonary and cardiovascular systems of these birds (De Smit *et al*., 2008), birds suffer ascites, with mortality occurring between 5 and 6 weeks of age, although the etiology dates back to the embryonic period (Coleman & Coleman, 1991). The adaptation to the environment depends on a mechanism called epigenetic adaptation. Chickens may be habituated to tolerate thermal stress during the pre-natal period by the epigenetic adaptation mechanism (Nichelmann *et al*., 2002).

The influence of early hypercapnia on post-natal thermal tolerance needs to be further studied. For this reason, the objective of this study was to evaluate different CO_2 levels during the first 10 days of incubation and its relationship with exposure to high temperature of broilers between 35 and 42 days of age on live performance, heart morphology, and blood leukocyte counts.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Poultry House from the Federal University of Paraná, Sector of Palotina, Palotina, state of Paraná, Brazil. All animal husbandry and biological material collection procedures were approved by the local Ethics Committee on the use of experimentation animals. In total, 2,016 fertile eggs were distributed according to a completely randomized design into four CO_2 incubation levels (4,000; 6,000; 8,000; or 10,000 ppm) in four single-stage incubators. Eggs were incubated in hatchery belonging to a poultry company of the state of Paraná, Brazil, and the incubators were not ventilated until CO_2 has reached the desired level. The other variables (temperature and O_2 level) followed the standards of the company. The different CO_2 levels were applied during the first ten days of incubation, after which all eggs were submitted to the same CO_2 level (4,000 ppm).

After hatching, 840 Cobb male chicks were randomly assigned to same treatments used in the incubation phase (four treatments) with seven replicates of 30 birds each. Feed and water were supplied *ad libitum.* Diets based on corn and soybean meal were formulated according to the chemical composition of feedstuffs and nutritional recommendations adopted by the company. A lighting program of 24 hours of light was applied during the entire experimental period.

At 35 days of age, 100 birds with aproximately 2,300 kg average weight were housed in metal cages under controlled temperature and humidity and distributed according to the CO_2 levels to which they were submitted during incubation, with five replicates of five birds each. Birds were submitted to 12 hours at 25° C, which was maintained during the night, and

12 hours at 32-35° C during the day. The thermal oscillation is one of the main environmental factors that is negatively correlated with ascites in broilers.

At 42 days of age, all birds in each experimental unit and feed residues were weighed for the determination of body weight gain, feed intake, and feed conversion from 35 to 42 days of age. Two birds by replicate (10 birds per treatment) were randomly selected, and their blood was collected for total and differential leukocyte counts in blood smears, as described by Campo and Dávila (2002). In this methodology, 100 leukocytes, including granular (heterophil, eosinophil and basophil) and non-granular (lymphocyte and monocyte) leukocytes, were counted and the heterophil/lymphocyte ratio was then calculated.

Birds were weighed and sacrificed. The heart and liver of each bird was collected and weighed. Hearts were cross-sectioned below the left and right atrioventricular valves and a second cross section was made at the stem of the papillary muscle. A cross-sectional image of a 4-mm section of each heart was digitally recorded using an image analysis software (Image Proplus 4.1). Heart right wall thickness (RWT), left wall thickness (LWT), ventricular septum (VS), and heart circumference (CH) were determined.

Data were submitted to analysis of variance, using SAS software package (GLM procedure) (SAS, 1998). The assumption of normality was previously checked. When CO_2 level effect was detected, a regression analysis was run, in which linear and quadratic polynomial models were analyzed. Models were selected by the fitted coefficient of determination (fit-R2); by the significance of regression and the lack of fitness (tested by the F test); by the significance of regression coefficients (tested by the F test). Maximum and minimum points of the quadratic model were estimated by derivation of the fitted coefficient of the regression. The significance level was set at 5% for all statistics procedures.

RESULTS AND DISCUSSIONS

Hypercapnia during incubation and cyclic environmental temperatures from 35 to 42 days of age had no effect on performance (Table 01), heart and liver relative weights, or on heart morphology measures (Table 02).

Table 1 – Effect of CO_2 concentrations during incubation and temperature fluctuations from 35–42 days of age on the live performance of broilers.

CO_2 levels	Feed conversion ratio,g/g	Feed intake, g	Weight gain, g
4,000 ppm	1.936	1116.9	578.8
6,000 ppm	2.397	1206.6	505.0
8,000 ppm	1.868	1113.1	601.2
10,000 ppm	2.079	1123.3	540.0
Mean	2.046	1136.4	561.1
Regression	NS	NS	NS
SEM	0.066	15.865	17.248

SEM: Standard Error Mean; NS: Not Significant

The fact that CO_2 levels did not significantly affect body weight gain, relative liver weight, or heart morphology in broilers suggests that the embryos exposed to high CO_2 levels during incubation coped better with the effects of temperature fluctuations. It was concluded that the hypercapnia during incubation does not influence these characteristics.

One of physical factor studied in the incubation process is the CO_2 level. The high metabolic rate of embryos of modern broiler strains requires ventilation of the incubator to remove the heat, which may lead to excessive removal of CO_2. However, recent studies questioned the need of high CO_2 levels during incubation. The assumed objective of providing high CO_2 levels is to allow the embryo to develop adaptive mechanisms in order to obtain good quality hatchlings and increase the resistance of broilers to stressors during the rearing period (Everaert *et al*., 2007; De Smit *et al*., 2008; Everaert *et al*., 2012).

Table 2 – Effect of CO_2 concentrations during incubation and temperature fluctuations from 35–42 days of age on relative heart weight (RHW) and relative liver weight (RLW), heart right wall thickness (RWT), left wall thickness (LWT), ventricular septum (VS) and circumference heart (CH) of broiler chicken.

CO_2 levels	RHW, %	RLW, %	RWT, µm	LWT, µm	VS, µm	CH, µm
4,000 ppm	0.44	1.79	2.03	7.23	7.28	69.37
6,000 ppm	0.45	1.71	1.86	6.92	7.42	70.80
8,000 ppm	0.44	1.88	1.99	7.07	7.83	67.80
10,000 ppm	0.44	1.75	2.03	6.77	7.41	69.13
Mean	0.45	1.77	2.01	7.00	7.52	69.77
Regression	NS	NS	NS	NS	NS	NS
SEM	0.011	0.033	0.076	0.104	0.132	0.641

SEM: Standard Error Mean; NS: Not Significant

Adaptation to environment conditions depends on a mechanism called epigenetic adaptation. Chickens can be conditioned to thermal stress tolerance during the incubation by the epigenetic adaptation mechanism (Nichelmann *et al*., 2002).

Several studies showed that a gradual increase of CO_2 levels from 7,000 to 15,000 ppm during the first 10 days of incubation in an airtight incubator accelerated embryonic development and improved hatchability (De Smit *et al*., 2008). Yet, many single-stage programs allow the damper to open in response to CO_2 levels at or above the set point and provide excessive amounts of fresh air that destabilize the thermal environment. This may interfere with physiological responses of broilers exposed to adverse temperature conditions. However, in the present study, no changes in the heart morphology that could be characterized as ascites were observed.

On the other hand, hypercapnia in initial phase of incubation may have a direct effect on the angiogenesis. Systemic acidosis, resulting from hypercapnia or induced by other factors, also increases angiogenesis (Everaert *et al*., 2008). The growth of the vascular network in the chorioallantoic membrane increases its O_2 diffusing in a sigmoid manner, parallelly to the change in O_2 uptake from the embryo (Dzialowski *et al*., 2002; Chan and Burggren, 2005). This leads to the hypothesis that early antenatal changes caused by temporal hypoxia may make natural critical hypoxia later during incubation less severe, and thereby reducing the sensitivity to ascites. Likewise, Decuypere *et al*. (1998) reported that broilers incubated at high CO_2 levels presented lower mortality due to ascites only during the first 2 weeks of age. Hassanzadeh *et al*. (2002) showed that eggs incubated at relatively high carbon dioxide concentration hatched earlier than those incubated at regular levels and the resulting broilers presented lower incidence of ascites during the rearing period.

Bahadoran *et al*. (2010) investigated the influence of hypoxia during the early stage of incubation and in a low altitude incubator until hatching on incidence of ascites in broiler chickens. The results indicated that early prenatal hypoxia due to high altitude may change the endocrine functions of embryos, enhance embryo growth, shorten the hatching window, and decrease the incidence of ascites in broiler chickens.

This was not confirmed in our study. However, there is little information regarding the effects of CO_2 levels during incubation on physiological response of broiler chickens exposed to fluctuating temperatures or to poor air quality (dust concentration, carbon dioxide levels, and oxygen levels). The incidence of ascites in broilers is also influenced by other factors. Broilers may not be able to adapt to the higher oxygen demands related to environmental stress. Additionally, if broilers with lung and/or heart capacity deficiencies encounter factors that interfere with oxygen transport, breathing ability, or cardiac output, they could become more susceptible to ascites (McGovern *et al*., 2001).

Relative to organ weights, our results are consistent with those obtained by De Smit *et al*., (2008), who did not find any significant differences in the heart weight of embryos exposed to high CO_2 levels (up to 7,000 ppm) during the first 10 days of incubation. Ascites in fast-growing broilers is usually associated with an increase in the weight, volume, and area of the right ventricle of the heart. When the right ventricle enlarges as a result of increasing blood pressure and increasing blood flow, the volume of blood the right ventricle can hold also increases. The ratio of right ventricle to total ventricle mass is a gross indicator of ascites (Julian *et al*., 1986). Image analysis proved to be a valid technique for quantifying the area of broiler hearts (McGovern *et al*., 2001). However, these authors consider that the technique should be standardized to obtain better accuracy. This requires the atria side of the heart cross-section to be placed upward and the heart shape should be maintained as natural as possible. Therefore, differences in results may be attributed to technique variations.

With reference to indicators of heat stress, Al-Murrani *et al*. (1997) mentioned that the heterophil/lymphocyte ratio is widely variable among individuals, but according to Maxwell (1993), this ratio is a more reliable indicator of mild to moderate stress than plasma corticosterone concentration. In the present study, heterophil and lymphocyte counts and the H:L ratio were significantly affected by treatments (Table 03). There was a linear increase in the H:L ratio of broilers submitted to cyclic variation of temperature during the last week of life due to an increase in heterophil counts and decrease in lymphocyte counts as CO_2 incubation levels increased in incubation. This suggests that higher CO_2 levels during incubation did not increase the resistance of broilers to heat stress. During stress, the hormone cortisol is released, increasing heterophil and decreasing lymphocyte counts (Perea *et al*., 1997).

Table 03 – Effect of CO_2 concentrations during incubation and temperature fluctuations between 35 and 42 days of age on heterophil:lymphocyte ratio (H/L), heterophil counts (H) and lymphocytes counts of broilers.

CO_2 levels	H/L	H, %	L, %
4,000 ppm	0.49	30.63	64.88
6,000 ppm	0.75	38.63	54.75
8,000 ppm	0.71	38.13	58.50
10,000 ppm	0.97	45.63	49.63
Mean	0.74	38.45	56.45
Regression	Linear[1]	Linear[2]	Linear[3]
SEM	1.886	1.807	0.061

SEM: Standard Error Mean; [1]$\hat{Y}$ = 0,23250+0,00007094; R^2: 0,84; [2]$\hat{Y}$ = 22,675+0,00223; R^2:0,87 [3]$\hat{Y}$ = 71,6375-0,00210; R^2:0,73

The H/L ratio has become widely accepted as a reliable and accurate physiological indicator of the stress response in chickens (Gross & Siegel, 1983). The differential leukocyte count shows that the normal heterophil: lymphocyte ratio is about 1:2. Nevertheless, this ratio increases when birds are submitted to stress conditions by increasing the number of circulating heterophils. Furthermore, Thaxton & Siegel (1982) and Miller & Qureshi (1991), reported that when broilers are exposed to high temperatures, ranging between 32.2 and 43 ° C for short periods or cycles of constant high temperatures, their immune response is weakened due to the involution of the lymphatic system, including atrophy of the thymus, Bursa of Fabricius, spleen, and pancreas. The release of corticosterone also may cause the involution of lymphoid tissues (thymus, bursa of Fabricius, and spleen) and the suppression of humoral and cell-mediated immunity (Rosales *et al*., 1989). Gross & Siegel (1993) suggested that heterophil to lymphocyte ratio values above 0.8 indicate high stress levels.

CONCLUSION

Although high CO_2 levels during incubation influenced some development parameters of the studied broilers, hypercapnia during incubation did not improve broilers' tolerance to fluctuating temperatures during the last week of rearing, as shown by the heterophil:lymphocyte ratio results. Other effects of hypercapnia during the incubation must be investigated, mainly on the development of the immune system and of the gastrointestinal tract.

REFERENCES

Al-Murrani WK, Kassab A, Al-Sam HZ, Al-Athari AMK. Heterophil/ lymphocyte ratio as a selection criterion for heat resistence in domestic fowls. British Poultry Science 1997;38: 159-63.

Baghbanzadeh A, Decuypere E. Ascites syndrome in broilers: physiological and nutritional perspectives, Avian Pathology 2008;37:117-26.

Bahadoran S, Hassanzadeh M, Zamanimoghaddam AK. Effect of chronic hypoxia during the early stage of incubation on prenatal and postnatal parameters related to ascites syndrome in broiler chickens. Iranian Journal of Veterinary Research 2010;11:64-71.

Bennett B. The advantages of single stage versus multi stage incubation. International Hatching Practice 2010;24:7-9.

Buys NE, Dewil E, Gonzalez E, Decuypere E. Different CO_2 levels during incubation interact withhatching time and ascites susceptibility in two broiler lines selected for different growth rate 1998. Avian Pathology 1998;27:605-12.

Coleman MA, Coleman GE. Ascites control through proper hatchery management. World Poultry Science 1991;7:33-5.

Chan T, Burggren W. Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (Gallus gallus). Respiratory Physiology and Neurobiology 2005;145:251-63.

De Smit L, Bruggeman V, Debonne M, Tona JK, Kamers B, Everaert N, Witters A,Onagbesan O, Arckens L, De Baerdemaeker J, Decuypere E. 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;87:551-60.

De Smit L, Bruggeman V, Tone JK, Debonne M, Onagbesan O, Arckens L, De Baerdemaeker J, Decuypere E. 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 2006 Part A;145:166–75.

Decuypere E, Buyse J, Buys N. Ascites in broiler chickens: Exogenous and endogenous structural and functional causal factors. World's Poultry Science 2000;56:367–77.

Druyan S, Levi E,Shinder D, Stern T. Reduced O_2 concentration during CAM development-Its effect on physiological parameters of broiler embryos. Poultry Science 2012;91:987–97.

Dzialowski E, Von Plettenberg D, Elmonoufy NA, Burggren WW. Cronic hypoxia alters the physiological and morphological trajectories of developing chicken embryos. Comparative Biochemistry and Physiology A molecular and Integrative Physiology 2002;131:713-24.

Everaert N, De Smit L, Debonne M, Witters A, Kamers B, Decuypere B, Bruggeman B. Changes in Acid-Base Balance and Related Physiological Responses as a Result of External Hypercapnia During the Second Half of Incubation in the Chicken Embryo. Poultry Science 2008;87:362–67.

Everaert N, Kamers B, Witters A, De Smit L, Debonne M, Decuypere E, Bruggeman V. Effect of four percent carbon dioxide during the second half of incubation on embryonic development, hatching parameters, and post hatch growth. Poultry Science 2007;86:1372–9.

Everaert N, Willemsen H, Debonne M, Witters A, Kamers B, Darras VM, De Baerdermaeker J, Decuypere E, Bruggeman V. Interaction betweeb ascites susceptibility and CO_2 during the second half of incubation of two broiler lines: the effect on post-hatch development and ascites motality. British Poultry Science 2012;53:262-9.

Gross WB, Siegel HS. Evaluation of the heterophil/ lymphocyte ratio as a measure of stress in chickens. Avian Disease 1983; 27: 972-9.

Hassanzadeh M, Buyse J, Decuypere E. Further evidence for the involvement of cardiac β-adrenergic receptors in right ventricle hypertrophy and ascites in broiler chickens. Avian Pathology 2002;31:177-81.

Hulet RM. Managing the embryo for performance, managing incubation: where are we and why? Poultry Science 2007;86:1017–9.

Julian RJ, Friars GW, French H, Quinton M. The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. Avian Disease 1986;31:130-5.

Maxwell MH. Avian blood leucocycte responses to stress. World's Poultry Science Journal 1993;49:34-43.

McGovern RH, Feddes JJR, Zuidhof MJ, Hanson JA, Robinson FE. Growth performance, heart characteristics and the incidence of ascites in broilers in response to carbon dioxide and oxygen concentrations. Canadian Biology Engineer 2001;43:4.1-4.6.

Miller L, Queshi MA. Introduction of heat shock proteins and phagocytic function of chicken macrophage following in vitro heat exposure. Veterinary ImmunolyImmunopatholy 1991;37:34-42.

Nichelmann, M, Tzschentke, B. Ontogeny of thermo-regulation in precocial birds. Comparative Biochemistry and Physiology, Part A 2002;131:751-63

Perea AT, Isaías GT, Maldonado FG.Techniques for measuring stress in poultry. Veterinary Mexican 1997;28:345-51.

Rosales AG, Villegas P, Lukert PD, Fletcher OJ, Mohamed MA, Brown J. Isolation, identification and pathogenicity of two field strains of infectious Bursal virus. Avian Disease 1989;33:35-41.

Thaxton JP, Siegel HS. Immmunodepression in young chickens by high environmental temperature. Poultry Science 1982;42:202-5.

Tona K, Onagbesan O, Bruggeman V, De Smit L, Figueiredo D, Decuypere E. 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;33:32–46.

Walsberg GE. The gaseous microclimate of the avian nest during incubation. American Zoologist 1980;20:363–372.