The Effect of Supplemental Carbon Dioxide in Chicken Incubation with Eggs from Heavy Breeder Parents

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Abstract
The study followed the results of 0.85% CO_2 influence on chick embryonic development. Biological material was composed of chicken eggs obtained from COBB500 hybrid broiler breeder parents. After weight determination of chick embryos in different stages of development, egg components and embryos annexes, pH measurements of albumen and yolk sac were made. All of this analysis was made in order to determine the positive influence of 0.85% CO_2 level on multistage chick incubation.

Keywords: chick embryo, CO_2, CO_2 multistage incubation

1. Introduction

In chicken, as for whole species, the development of the embryo is a very dynamic and a complex process, influenced mainly by the genetic background of the organism but also by the environment in which this process takes place. Different level of CO_2 plays a important role in the development of the chicken embryos [1]. Despite early studies [2-4] showed that different concentrations of CO_2 applied in different stages of embryo development depress hatchability, other studies [5-9] reported that a concentration higher than 1% increased gradually in the first 10 days of incubation enhanced embryo growth, and improved hatchability. However, when applied after the 10^{th} day of incubation it had no effect on hatchability [5]. Using high levels of CO_2 for the single-stage incubation system in the first 10 days of incubation become a commune practice since it is assumed that CO_2 in high concentrations enhances chick quality and broiler performance. This approach is based on empirical findings and it is not yet documented with many scientific results. In literature the effect of CO_2 levels on chick hatchability is fairly described and more than that, the effects of physiological parameters of a developing chick embryo have not been reported since now. The aim of this study was to investigate the effects of incubated eggs from heavy breeder parents in multistage incubation system using a closed incubator designed for CO_2 injection. The concentration of CO_2 was raised to a level of 0.85% during incubation, weights of egg components, egg weight loss, embryo, embryo annexes, pH of yolk sac and albumen was measured, incubation indices were recorded.

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2. Materials and methods

Six hundred Cobb500 eggs from 30 and 32 weeks old broiler breeders provided by S.C. Transavia S.A. Brasov, were weighted, numbered and randomly divided to form 5 incubation series. Later on, the series were set for incubation at a 3,5 days interval in a air-tight forced-draft incubator modified for automatic CO_2 injection. Dry bulb temperature and wet bulb temperature was monitored and regulated every step of incubation time and they were set for 37.77ºC and 28.88ºC

(50%) respectively. Egg turning was made automatically every hour at an angle of 45º from horizontal.

In the experimental group the incubation conditions were identical with the incubation conditions from the control group except the CO_2 level. For the experimental group the level of CO_2 was set to rise and remain at a level of 0.85% from the beginning of incubation of the first series until the end of the 5-th series. Raising the level of relative humidity was possible by placing inside the incubator a tray with distilled water and decreasing it was possible by opening the air dampers together with using a thermoelectric element closed in a plastic box. Over the cold surface of this thermoelectric element air from incubator was directed so the suspension water from the incubator air was condensed and collected in a plastic container.

For the experimental group an extra humidification of the air inside of the incubator it was no necessary. Eggs evaporated enough water to maintain the relative humidity to a level of 50%. Additionally the dampers from experimental incubator were opened in situations like humidity rising over 50% or when CO_2 level passing over the 0.85% setting value. Temperature, wet bulb humidity and CO_2 level was monitored and regulated with a PC through a data acquisition board (National Instruments NI DAQ-6008). The software for controlling the data acquisition board was written in "real-time" mode and recorded data was possible in a interval of 10 minutes for every incubation environmental factor (temperature, humidity and CO_2 level). Dry bulb thermometer and wet bulb thermometer were precision Centigrade thermometer sensors (LM35).For measuring the CO_2 level inside the incubator, a high precision sensor was used (AS45-aSENSE CO2 sensor). Each series of eggs in the experimental group was subjected to the same environmental conditions for all days of incubation. After an egg candling control clear eggs and eggs with dead embryos were removed and the remaining eggs were transferred to the hatchery. In the hatchery, environmental factors like temperature and humidity were monitored, regulated and registered by the same hardware and software as for the incubator. The control group also contained six hundred Cobb500 eggs from 32 and 36-weeks old broiler breeders provided by S.C. Transavia S.A. Brasov, were weighted, numbered and randomly divided to form 5 incubation series. The incubation conditions were the same for the control group as they were for the experimental group, except the level of CO_2. For the control group the level of CO_2 was the same as it was in the ambient air.

Before the start of incubation, 15 eggs from each series belonging to control and experimental group were weighted. Afterwards they were broken and measures of pH of albumen and yolk sac were done with a electronic pH-meter, egg shell thickness was measured with an electronic caliper. Furthermore, during incubation at different time intervals 15 eggs were removed from each series from both groups to determine the embryonic weight loss during incubation. Relative embryonic weight loss (EWL) was calculated with formula

$$EWL=\left(\frac{EW\ (at\ day\ X)-EW(at\ day0)}{EW(at\ ED0)}\right)x100$$

where $EW(at\ day\ X)$ –egg weight at the day when measurements are taken place; $EW(at\ day0)$- egg weight at setting time.

On day 3, 10 and 18.5 of incubation, 15 eggs from each series of the two groups, were weighed and then sectioned. Eggs removed on day 3 of incubation were sectioned at the blunt end of the egg and the other eggs coming from day 10, 18.5 were sectioned at the sharp end. After removing the shell, embryo was exposed. Afterwards the embryo was removed and weighed. Albumen and yolk sac were poured in Berzelius beakers and weighed separately. PH was also measured. The shell was weighed too and it's thickness was measured with an electronic caliper. At day 10 and day 18.5 weights of embryo chorioalantoic membrane and yolk sac vascular membrane was weighed (Table 2). The purpose of weighing those embryo annexes is to see if using CO2 as incubation environmental factor has any influence on the embryo blood vessel system.

After hatching, every day old chic from every series belonging to both groups, was weighed, hatching and fertility percent were calculated (Table 4).

3. Results and discussion

Egg weight between groups did not differ significantly (control 61.89±0.140g, experimental 61.25±0.180 g). Not significant differences were observed also between eggs collected for analysis from both experimental groups on all incubation time (Table 1).

Table 1. Dynamic weight loss (Mean±SEM)

Specification	n	Setting egg	Egg component parts						Egg weight loss
			Yolk/Yolk sac		Albumen		Shell		
		grams	grams	%	grams	%	grams	%	%
Setting									
Control	75	61.37± 0.504	20.12± 0.188	32.81± 0.210	34.37± 0.340	55.97± 0.210	6.880 ± 0.0805	11.21± 0.101	X
Experimental	75	60.89± 0.547	19.92± 0.257	32.67± 0.225	33.97± 0.316	55.82± 0.226	7.000± 0.0828	11.51± 0.116	X
3 days of incubation									
Control	75	62.09± 0.467	23.52± 0.383	38.77± 0.581	31.08± 0.441	51.17 ± 0.563	6.107± 0.075 **	10.06± 0.104 ***	2.244± 0.109
Experimental	75	61.36± 0.448	23.20± 0.257	38.81± 0.434	30.31± 0.439	50.48 ± 0.487	6.400± 0.071 **	10.71 ± 0.129 ***	2.373 ± 0.104
10 days of incubation									
Control	75	62.59±0 .362	23.69±0 .272 **	40.36± 0.364 **	12.77± 0.455 *	21.81± 0.808 *	5.907± 0.081	10.07± 0.123	6.253± 0.158
Experimental	75	61.29± 0.547	22.05± 0.461 **	38.21± 0.566 **	11.55± 0.214 *	20.05± 0.254 *	5.893± 0.079	10.29± 0.139	6.316± 0.142
18.5 days of incubation									
Control	75#	62.12± 0.350	11.71± 0.209 ***	21.40± 0.315 ***	5.571± 1.645 n=7	10.49± 3.027 n=7	5.627 ± 0.062 *	10.31± 0.094 **	12.09± 0.337
Experimental	75#	61.32± 0.483	9.107± 0.236 ***	16.71± 0.358 ***	n=0	n=0	5.813 ± 0.049 *	10.75± 0.107 **	11.55± 0.135

Means significance is based on P<0.05

75# = the sample size for some egg component parts is specified in the cell;

Since day 3 of incubation significantly differences were observed between embryo weights from control and experimental group, the last ones presented higher weights. In the 10-th day of incubation, as well as in day 3 of incubation, embryos coming from the experimental group presented higher weights than control, differences between two groups being very significantly. More than that, the weight of chorioalantoic membrane together with yolk sac vascular membrane presented no significantly differences between groups at day 10 of incubation. The situation changed at day 18.5 days of incubation when, between groups, differences were extremely significantly, weights being higher for control group (Table 2).

Albumen weight in both groups decreased in the first 3 days of incubation. Until 10-th day of incubation a significantly decrease of albumen weight in both groups was observed. Knowing that the albumen consume starts at day 12-13 of incubation and until day 16 all the albumen is consumed [10] so at day 18.5 of incubation, a comparison was not possible because the eggs from experimental group consumed the whole quantity of albumen. Before start of incubation process, pH value of albumen was 9.504±0.011 for the control group and 9.315±0.010 for the experimental group.

Table 2. Embryo and annexes weight dynamics (g)

Specification	Groups			
	n	Control	n	Experimental
		$X \pm s_x$		$X \pm s_x$
		Embryo		
3 day of incubation	73	0.01837± 0.001*	75	0.02112± 0.0002*
10 day of incubation	71	2.778 ± 0.039***	75	3.074 ± 0.057***
18, day of incubation	75	28.24 ± 0.315***	75	32.96 ± 0.266***
		Embryo annexes		
10 day of incubation	71	14.39 ± 0.157	75	14.81 ± 0.258
18, day of incubation	75	8.547 ± 0.239***	75	6.400 ± 0.223***

Means significance is based on P<0.05

At day 3 of incubation, the pH values were lower for both groups than the pH values for the setting time. This descending pattern was observed for all the incubation time. Comparing the pH values between groups, distinct significantly differences were observed. Over the incubation times, those differences became lower so in the 10-th day of incubation the differences between two groups were very significant (Table 3).

Table 3. Albumen pH and Yolk/Yolc sac pH dynamics

Specification	Groups			
	n	Control	n	Experimental
		$X \pm s_x$		$X \pm s_x$
		Albumen pH		
setting	75	9.504 ± 0.011***	75	9.315 ± 0.010***
3 day of incubation	75	9.393 ± 0.018***	75	8.656 ± 0.023***
10 day of incubation	75	7.994 ± 0.076**	75	7.651 ± 0.073**
18, day of incubation	7	7.70±0.064	0	
		Yolk/Yolk sac pH		
setting	75	6.251 ± 0.0138***	75	6.140 ± 0.022***
3 day of incubation	75	6.615 ± 0.032***	75	6.412 ± 0.044***
10 day of incubation	75	7.451 ± 0.078	75	7.397 ± 0.026
18, day of incubation	59	8.021 ± 0.0227***	67	7.754 ± 0.0118***

Means significance is based on P<0.05

Regarding to the yolk sac, its weight had high values both in day 3 of incubation as in day 10 of incubation, comparing to the setting time. From day 10 until 18.5-th day of incubation the weight of yolk sac decreased until 11.71±0.209 grams for control group and 9.107±0.236 grams for the experimental group. Between groups, the weight of yolk sac was very significant in day 10 of incubation and extremely significant in day 18.5 of incubation (Table 1).

Egg wheigt loss in the incubation time presented no significant differences between both groups. As [10] presented the weight loss of the egg was between 12-14 % which represents the normal loss for the multistage of incubation system (Table 1).

pH values for yolk sac were very significant at setting, day 3 and day 18.5 of incubation. Not significant differences of yolk sac were observed only at day 10 of incubation at the moment when the alantoic membrane completes its development (Table 3).

Embryo annexes had not significant weight differences in day 10 of incubation but this changed in the 18.5 day of incubation when the differences had significantly higher values. As for the egg shell the weight of it had a descending pattern from setting until end of incubation. Significant differences were observed in day 3 of incubation and significantly differences were observed in day 18.5 of incubation (Table 2).

As for the hatching results the newly hatched chicks were weighed and fertility percent and hatched percent were calculated. As we can see in table 4, very significantly differences occurred only between the weight of the chicks.

Chick weight at hatching was higher for the control group, as was egg weight at the setting eggs for incubation. Because there is a strong positive correlation between egg weight and chick weight [11], the difference in egg weight may explain the chick weight difference.

Table 4. Incubation indices

Specification	Groups			
	n	Control	n	Experimental
		$X \pm s_x$		$X \pm s_x$
Fecundity%	5	94.67 ± 1.434	5	96.00 ± 2.449
Hatching %	5	97.19 ± 1.052	5	99.33 ± 0.666
Chick weight	276	43.57 ± 0.168**	286	42.61 ± 0.238**

Means significance is based on $P<0.05$

The study conducted by Romanoff and Romanoff [12] sugested that the alkalinity of albumen in the incubated eggs reach the highest point at day 2-3 of incubation. The cause of this albumen alkalinty is the physical release of CO_2 from the albumen. After this turn to alkalinity, the albumen moves to acidity during incubation. The increased concentration of carbon dioxide during the first days of incubation seems to start earlier some physiological functions related to the embryonic development. Moreover these effects persist for the whole period of development. Sadler [5] sustained that the beneficial effects of carbon dioxide is represented by the acidity of pH of the albumen which might have slowed down the breakdown of the chalaza and the thick layer of albumen. Other possible explanations are related to the pH-dependent enzyme- carbonic anhydrase which is involved in formation of subembryonic fluid involved in early stages of development [13]. Deeming [13] in his study also suggested that carbonic anhydrase starts it's expression earlie when high concentration of carbon dioxide is applied in incubation environment. The enzyme catalyses the hydration of carbon dioxide and in result protons and bicarbonateions are produced.

It is possible that high concentrations of carbon dioxide during early incubation, to improve the production of subembryonic fluid [14]. Our results show that a higher concentration of carbon dioxide applied in multistage incubation causes a faster rate of albumen pH towards acidity in the first 10 days of incubation, due to reaction of carbon dioxide with water content of the albumen.

Within the decrease of water content of the albumen the turn rate of albumen pH towards acidity slows down.

This was observed in the 10-th day of incubation when the differences between groups turned from extremely significant to very significant ($P<0.05$).

Not much data exists on how a higher concentration of carbon dioxide during incubation is affecting the yolk-sac .We observed in our study that during incubation a part of water from albumen moves toward the yolk forming the yolk sac. This grows in weight in the first 10 days but the rate of consume of it it's small. Nevertheless, absolute value of embryo weight was statistically significant for the experimental group. Also after a visual analyze, the vascularization of the yolk sac was better formed in the experimental group than the control group. This confirmed the result of [16] regarding to hypercapnia that might influence the cardiovascular system, by inducing vasodilatation and increasing the blood flow. This could mean that the carbonic anhydrase did express itself earlier and the albumen liquefaction taken place earlier in the experimental group and the embryo started earlier to consume the yolk sac content comparing to the control. The differences in yolk sac pH between experimental group and control were statistical different from setting time until day 10 of incubation. Both groups presented a movement toward alakalinity of yolk sac. As the chorioalantoic membrane starts to express itself at full capacity from day 10 onwards it is highly possible that until this incubation day the carbon dioxide to have no direct effect over the yolk sac pH. Nevertheless in the 18.5 day of incubation the yolk sac pH presented a significantly higher rate of alkalinzation in the control group. Knowing that the albumen consume starts at day 12-13 of incubation and until day 16 all the albumen is consumed [10], it is not know how the yolk sac pH is affected by the high level of carbon dioxide

after, or if the embryo has a role in alkalinization of the yolk sac during incubation.

As we showed the embryo weight at 3 days of incubation was significantly higher for the experimental group. The embryos continue their development but at day 10 of incubation and afterwards at day 18.5 of incubation the embryo weight was higher for the experimental group, differences between groups being extremely significant. The results in our study are sustained by the results obtained by other authors [5-9] when they shown that carbon dioxide higher levels than 1% during periods up to 10 days of incubation enhanced embryo growth. As a result of incubation, differences between incubation indices were not statistical significant. As for the embryo weight, this presented very significant differences between groups.

4. Conclusions

In conclusion, this study shows that the carbon dioxide concentration in the incubator during multistage incubation it may play an important role but its effect in multistage incubation needs future investigations.

A striking and consistent result of the higher CO_2 levels in the incubator was the explicit lowering of albumen pH, which illustrates that the albumen is, besides its known role in protein and water source and antimicrobial protection, a player in coping with high environmental CO_2. Concomitantly, with recent studies showing that a high level of CO_2 during the first half of incubation results in accelerated embryonic growth our study shows that the same effect is achieved when a high level of CO_2 is applied for the multistage incubation system.

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