

Effects of ventilation programme and eggshell thickness on hatchability rate and hatching time of broiler eggs

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Abstract

The aim of the research was to determine whether enrichment of the atmosphere in an incubator with carbon dioxide (CO₂) and oxygen (O₂) and eggshell thickness (EST) affected embryonic death (ED), hatchability of fertile eggs (HFE) and hatching time (HT). A total of 320 Ross 308 eggs were used and the experiment was repeated twice. Eggshell thickness was classified as thin (<31 µm), medium (31 - 32 µm) and thick (>32 µm). The incubators were operated with their internal atmosphere enriched with CO₂ (1.57% CO₂; 20.23% O₂) or O₂ (0.50% CO₂; 22.44% O₂). Embryonic death, HFE and HT data were monitored at three periods, namely early (<486 hours), middle (486 - 492 hours), and late (492 - 510 hours). Early ED, late ED and hatchability of fertile eggs were not affected by EST or by the incubator's internal atmosphere ($P > 0.05$). Thus, O₂ supplementation to the incubator was deemed unnecessary at 822 m altitude. There was a highly significant interaction between EST and HT. Eggs with shells 31 to 32 µm thick hatched at an appreciably greater rate between 486 and 492 hours of incubation (17%) than eggs with thicker (0.6%) or thinner (0.4%) shells in both the CO₂ and O₂ enriched atmospheres. The hatching rate was significantly higher in the eggs with an intermediate EST than in thick-shelled eggs. A greater proportion of eggs hatched at the late HT as opposed to earlier, regardless of EST.

Keywords: broiler, eggshell thickness, hatching time, incubator ventilation programme

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Introduction

Successful incubation of broiler eggs depends on management of the incubator (French, 1997; Yildirim & Yetisir, 2004) and certain characteristics of hatching eggs such as genetics, age, maintenance and feeding conditions of breeders, egg weight (EW), and EST (Kirk *et al.*, 1980; Narushin & Romanov, 2002; Sarica *et al.*, 2012; Elibol & Turkoglu, 2014; Hammershoj & Steinfeldt, 2015; Yamak *et al.*, 2015; Popova *et al.*, 2019). The levels of CO₂ and O₂ in the incubator reportedly determine the rate of embryonic development and may affect incubation and post-hatching performance (Metcalf *et al.*, 1981; Lourens, 2007; Molenaar *et al.*, 2010; Tona *et al.*, 2013).

An atmosphere that is low in O₂, such as at high altitudes, can affect incubation efficiency and chick quality negatively (Ahmed *et al.*, 2013). The content of O₂ in the atmosphere is about 21% at sea level (Stock & Metcalfe, 1984). Low (<17%) and high (25%) O₂ levels have both been reported to affect embryonic survival and hatchability negatively (Stock & Metcalfe, 1984; Lourens *et al.*, 2007; Molenaar *et al.*, 2010). In high altitude (3500 - 4000 m) areas, such as India and South America, very low hatchability rates have been reported such as 20% (Ahmed *et al.*, 2013; Tullet 2013). To compensate for reduced levels of O₂, an oxygen concentrator can be used to increase the oxygen level of the atmosphere inside the incubator (Cobb, 2013; Tullet, 2013).

Researchers reported favourable effects from an increase in the level of CO₂ in the incubator. A gradual increase of up to 1.5% in CO₂ levels, which should normally be around 1% in the first 10 days of incubation, improved embryo development, promoted early hatching, and increased overall hatchability (Buys *et al.*, 1998; Onagbesan *et al.*, 2007; Tong *et al.*, 2015).

Eggshell thickness has an important role as an embryonic respiratory component (Hunton, 2005). It can be measured with non-destructive equipment that is produced by a few innovative companies (e.g. Orka Tech Ltd., Israel). Eggshell thickness varies between 33 and 40 µm in broiler eggs (Yamak *et al.*, 2015). In

addition, EST in the range of 35 - 39 μm (Huwaida *et al.*, 2015) apparently does not affect embryo mortality or hatchability.

Because of their potential effects on economic criteria, including hatchability of fertile eggs and hatching time, it was hypothesized that differences in the CO_2 and O_2 levels of the atmosphere inside the incubator and their interrelationship with EST will affect embryonic survival, hatchability of fertile eggs and time of hatching of broiler chicks.

Material and Methods

The study was conducted in accordance with the Turkish Animal Welfare Act, and all procedures involving handling of eggs and chicks were approved by the Animal Ethics Committee of Bolu Abant İzzet Baysal University (Decision no: 2015/45 dated 30 December 2015). It took place in Bolu Province where more than 30% of poultry production in Turkey occurs. The average altitude of this province is 822 m.

The entire experiment was done twice under identical conditions. In each experiment, 2880 broiler hatching eggs were obtained from Ross 308 breeder flocks. All eggs were stored for three days before incubation, and the egg storage room was kept at 18 °C and 75% relative humidity during this period. Before the incubation period, the eggs were preheated for six hours in incubators set at 24 °C.

The eggs were weighed and classified as heavy (69.83 ± 0.25 g), medium (65.10 ± 0.10 g), and light (60.97 ± 0.21 g), and placed in trays so that there was an equal number of eggs of similar weight in all treatment groups. The incubators (Cimuka 960SH, Cimuka Ltd. Co., Turkey) were located in the incubation laboratory of Bolu Abant İzzet Baysal University Faculty of Agriculture Department of Poultry Science. Each incubator was equipped with six egg trays with a capacity of 80 eggs, and six chick baskets with the same capacity. The six trays were divided into groups of two, according to their location in the incubator. The eggs that were used in this research were positioned in the middle part of each tray. Therefore, 320 hatching eggs were weighed (± 0.1 mg) individually using an analytical balance (Radwag AS 220.R2, Radwag Balance and Scales, Poland) and numbered. Eggshell thickness was measured with an ultrasonic EST gauge (Orka Tech. Ltd., Israel) with a sensitivity of ± 1 μm and classified as thin (<31 μm), medium (32 - 33 μm) and thick (>33 μm). During the incubation period, both incubators were operated to achieve 37.8 °C eggshell temperature.

To achieve a CO_2 -rich atmosphere in one of the incubators, the ventilation flaps were closed during the first 10 days of incubation and CO_2 was allowed to increase. After this time, the ventilation flaps were opened and the normal ventilation regime was applied. In the second incubator, an O_2 -rich atmosphere was achieved with an oxygen concentrator (Hikoneb Oxybreath 10LPM, Kare Medical Ltd. Co., Turkey), which produced $92 \pm 3\%$ pure O_2 and was introduced at approximately 10% of the total air drawn into the incubator. The CO_2 level was measured with Hatch Eco2-01 sensors (Cimuka Ltd., Turkey), and the O_2 levels were recorded by PAC 7000 data loggers (Dräger Safety AG & Co., KGaA, Germany). Eggshell temperature values were measured with an infrared ear thermometer (Braun Thermoscan, Braun, Germany). In both incubators relative humidity was maintained at 57.0% throughout the incubation period. The trays were turned automatically 24 times a day.

On the 18th day of incubation, the eggs were transferred from the trays to the hatching baskets in the same incubators and maintaining their position. The experimental eggs were held in a specially prepared plastic compartment that was covered to maintain the association of eggs and chicks with the treatment to which they had been subjected. Hatching time was categorized as early, middle, and late. To determine HT, the incubators were opened after 486, 492, and 510 hours of incubation. At these times, the eggs were checked, and hatched chicks were counted, recorded, and removed. Hatching rates for each treatment were calculated as:

$$\text{Hatching rate, \%} = \frac{\text{Number of chicks that hatched in the specified time interval}}{\text{Total number of eggs hatched}} \times 100$$

Once hatching was finished, EDs were determined as early (0–5 days), middle (6–17 days) and late (18–21 days, and as pipped but unhatched eggs). Percentages of early-, middle-, and late-stage EDs were calculated relative to the total number of eggs set. The percentage hatchability of fertile eggs was expressed relative to the total number of fertile eggs set.

The required number of observations was calculated with PASS 11 (Hintze, 2011) power analysis. The data were analysed using IBM SPSS Statistics 22 (SPSS Inc., 2013). The Shapiro-Wilk test was used to confirm the normal distribution of the data. One-way analysis of variance and post-hoc Tukey test were used to assess the treatment effects. *P*-values lower than 0.05 were considered significant. The results are reported as means \pm standard error.

Results and Discussion

The data in Table 1 confirm the categorization of EW as heavy, medium and light and EST as thin, medium and thick. Likewise, they demonstrate the similarity of eggs that were allocated to the treatments ($P > 0.05$). Overall, the average EW was 65.17 g with a coefficient of variation (CV) of 6.17 and the average EST was 31.26 μm with a CV of 4.34. In the light of these data, the uniformity of the eggs used in the present research was high. These eggs were slightly heavier than those used by Kirk *et al.* (1980) and had thinner shells compared with other studies (Christensen *et al.*, 1994; Huwaida *et al.*, 2015 and Yamak *et al.*, 2015).

Table 1 Description of experimental materials used to assess effects of the ventilation programme and eggshell thickness on hatchability and hatching time of broiler eggs

	Egg weight, g	Eggshell thickness, μm
Egg weight		
Heavy	69.56 \pm 0.24 ^a	31.38 \pm 0.13
Medium	64.98 \pm 0.10 ^b	31.18 \pm 0.12
Light	60.97 \pm 0.20 ^c	31.20 \pm 0.14
Incubator atmosphere		
Carbon dioxide rich	63.77 \pm 0.74	31.33 \pm 0.11
Oxygen rich	64.05 \pm 0.71	31.19 \pm 0.11
Eggshell thickness		
Thin (<31 μm)	64.11 \pm 1.04	29.63 \pm 0.06 ^c
Medium (32 - 33 μm)	63.69 \pm 0.59	31.49 \pm 0.04 ^b
Thick (>33 μm)	66.56 \pm 0.50	33.27 \pm 0.06 ^a
Egg position in incubator		
Top	63.31 \pm 1.31	31.30 \pm 0.13
Medium	63.90 \pm 0.87	31.29 \pm 0.13
Bottom	64.42 \pm 0.44	30.99 \pm 0.14
<i>P</i> -value		
Egg weight	0.000	0.497
Incubator ventilation programme	0.878	0.344
Eggshell thickness	0.412	0.000
Egg position	0.679	0.162

*Carbon dioxide rich (1.57% CO₂ and 20.23% O₂) and oxygen rich (0.50% CO₂ and 22.44% O₂)

^{a,b,c} Within a set of observations of a trait, means with common superscripts did not differ with probability $P = 0.05$

In the first 10 days of incubation, the atmosphere inside the incubator could be enriched gradually with CO₂ by keeping the ventilation flaps closed (Figure 1). Thus, on the tenth day of incubation, eggs in that incubator were exposed to a CO₂ level of 1.50% and an O₂ level of 20.25%. In contrast, eggs in the incubator in which the atmosphere was enriched with O₂, were exposed to a CO₂ level of 0.90% and an O₂ level of 21.50% at that time.

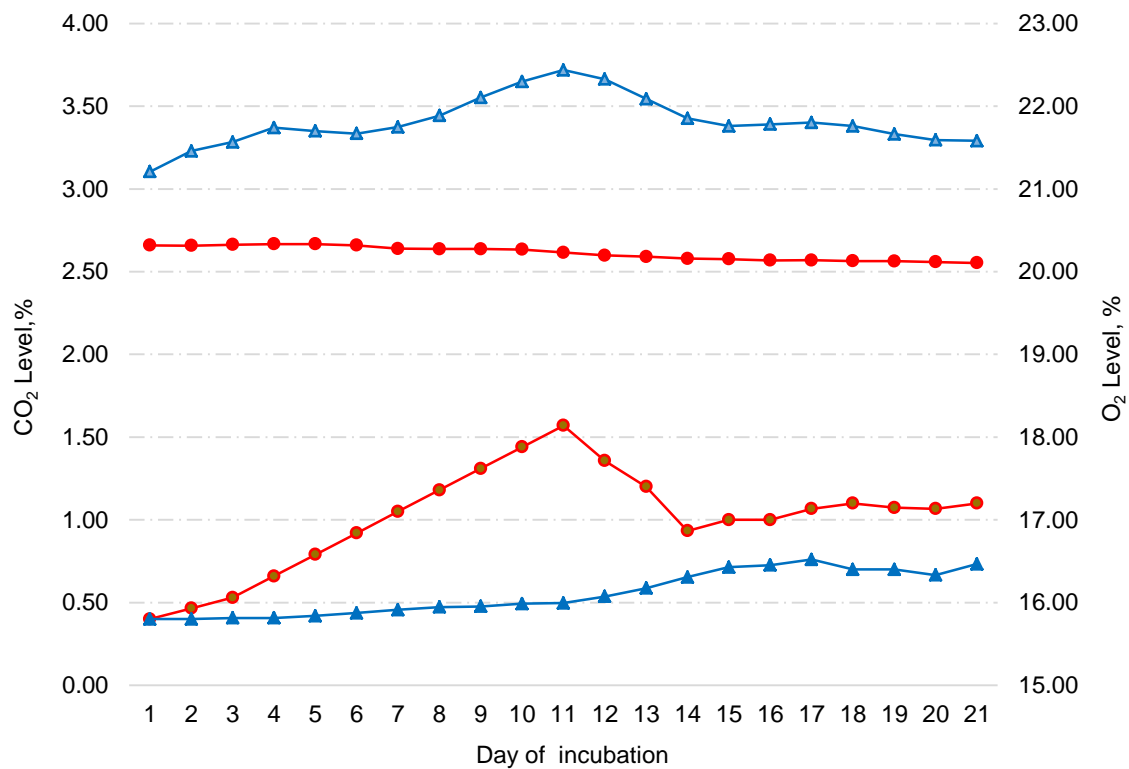


Figure 1 Carbon dioxide and oxygen levels in incubators with enriched atmospheres

Red line: Carbon dioxide (CO₂) enriched atmosphere; blue line: oxygen (O₂) enriched atmosphere; top panel and right axis: O₂ levels; lower panel and left axis: CO₂ levels

Enriching the atmosphere inside the incubator with either CO₂ or O₂ through day 10 of incubation did not affect ($P > 0.05$) the levels or timing of ED or HFE (Table 2). These results differ from the observations of Tona *et al.* (2013), in which a gradual increase in level of CO₂ up to 1.50% improved embryo development, promoted early hatching, and increased the hatchability of chicken eggs. However, the broiler breeding companies indicated that O₂ enrichment of the atmosphere inside an incubator should not be expected to alter embryo development or hatchability in places with an altitude less than 1000 m above sea level. The location in which the current study was conducted is 822 m above sea level (Cobb, 2013; Tullet, 2013). Accordingly, it can be concluded that in areas with similar altitude, the use of an oxygen concentrator to maintain the O₂ level in the incubator is not necessary. Eggshell thickness did not affect the timing and level of ED or HFE ($P < 0.05$). This result was consistent with the findings of Huwaida *et al.* (2015), who reported that EST did not affect ED and hatchability. The interaction of EST and atmospheric conditions in the incubator did not affect these traits ($P > 0.05$), either.

Table 2 Embryonic death and hatchability of fertile eggs as related to differences in eggshell thickness and atmospheric conditions inside the incubator

Shell thickness	Atmosphere	Early embryonic death	Later embryonic deaths	Hatchability of fertile eggs
<31 µm	CO ₂ enriched	1.39 ± 1.39	5.28 ± 3.11	93.33 ± 3.20
31 - 32 µm	CO ₂ enriched	2.70 ± 1.55	6.25 ± 4.49	91.05 ± 4.41
>32 µm	CO ₂ enriched	3.79 ± 2.60	14.70 ± 9.01	81.52 ± 9.39
<31 µm	O ₂ enriched	6.13 ± 4.20	7.87 ± 4.76	86.00 ± 5.78
31 - 32 µm	O ₂ enriched	2.96 ± 1.77	1.29 ± 0.87	95.75 ± 2.00
>32 µm	O ₂ enriched	5.00 ± 3.56	4.17 ± 2.85	90.83 ± 4.72
<i>P</i> -values				
Incubator atmosphere		0.333	0.295	0.644
Eggshell thickness		0.846	0.481	0.389
Interaction		0.838	0.502	0.462

The highly significant interaction effect resulted primarily from eggs with shells 31 to 32 µm thick, which had an appreciably greater rate of hatching between 486 and 492 hours of incubation than eggs with thicker or thinner shells in the CO₂- and O₂-enriched atmospheres (Table 3). In addition, eggs with this intermediate shell thickness hatched at appreciably higher rates than thin- or thick-shelled eggs. A greater proportion of eggs hatched at the late HT than at the earlier time, regardless of EST. For the late hatching, the hatching rate was significantly higher in eggs with an intermediate EST than in thick-shelled eggs.

Table 3 Mean hatching rates (%) for the highly significant interaction of eggshell thickness and hatching time in incubators with carbon dioxide- and oxygen-enriched atmospheres

Eggshell thickness	Hatching time	Carbon dioxide	Oxygen	Average
<31 µm	<486 h	1.52 ± 0.85 ^c	4.17 ± 4.17 ^b	2.90 ± 2.18 ^{cd}
<31 µm	486 - 492 h	0.38 ± 0.38 ^c	0.35 ± 0.35 ^b	0.36 ± 0.25 ^d
<31 µm	492 - 510 h	14.39 ± 4.40 ^{bc}	16.67 ± 4.62 ^{bc}	15.58 ± 3.13 ^{bc}
31–32 µm	<486 h	0.38 ± 0.38 ^c	13.89 ± 8.84 ^{bc}	7.43 ± 4.74 ^{bcd}
31–32 µm	486 - 492 h	17.42 ± 9.68 ^{bc}	16.67 ± 6.71 ^{bc}	17.03 ± 5.67 ^{bc}
31–32 µm	492 - 510 h	43.94 ± 5.28 ^a	29.51 ± 5.72 ^a	36.41 ± 4.12 ^a
>32 µm	<486 h	0.38 ± 0.38 ^c	0.35 ± 0.35 ^b	0.36 ± 0.25 ^d
>32 µm	486 - 492 h	0.76 ± 0.51 ^c	0.35 ± 0.35 ^b	0.54 ± 0.30 ^d
>32 µm	492 - 510 h	20.83 ± 4.67 ^b	18.06 ± 3.71 ^b	19.38 ± 2.90 ^b

Carbon dioxide (1.57% CO₂ and 20.23% O₂) and oxygen (0.50% CO₂ and 22.44% O₂) enriched atmospheres during the first 10 days of incubation

^{a,b,c,d} Means with a common superscript were not different with probability *P* = 0.05

Prolonged holding of chicks without food and water in an incubator can lead to their becoming dehydrated and losing weight. The results for the effect of EST show that it is an important parameter of egg quality (Ketta & Tumova, 2016; Dahloum *et al.*, 2018; Ketta & Tumova, 2018) and of HT. It is known that EST decreases as the egg yield increases (Christensen *et al.*, 1994; Yamak *et al.*, 2015). Modern technology allows non-destructive measurement of EST (Khaliduzzaman *et al.*, 2020; Kibala *et al.*, 2015) and could be used in managing the incubation process. An alternative way of reducing holding time would be to empty the incubators twice instead of once.

Conclusion

Although they are beneficial at higher altitudes, changes in the internal atmosphere of the incubators were deemed unnecessary at 822 m altitude. However, HT was affected by EST, and the incubation process might be made more efficient and the welfare of hatched chicks could be improved by sorting the eggs according to EST when placing them in an incubator.

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Authors' Contributions

NO designed the study and analysed data. NO and SAE collected data and prepared the manuscript.

Conflict of Interest Declaration

The authors declare that they have no affiliations with any organization or entity with any financial or non-financial interest that could bias the subject matter and outcomes discussed in this manuscript.

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