

Understanding the effects of humidity/ air composition on embryo and post-hatch chick development

E. David Peebles, Mississippi State University, USA



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1 Introduction

Incubation conditions influence embryo growth and viability, which subsequently affect egg hatchability. Along with temperature, environmental factors in the incubator that are of primary importance for optimum hatchability and chick quality include humidity and vital gas [carbon dioxide (CO_2) and oxygen (O_2)] levels. Because the movements of gases across the eggshell occur by diffusion, avian embryos are dependent on the composition of the surrounding air, with their development and hatching success being dependent on the ambient partial pressures of O_2 , CO_2 , and water vapor. The influences of the incubational environment can extend beyond embryonic development into the post-hatch period with ensuing effects on chick survival, quality, and performance. Various studies have been conducted to establish humidity and vital gas levels during incubation that will optimize the embryonic and post-hatch livability and development of poultry. The prospects for the pragmatic commercial use of these incubational regimens, as well as the physiological bases for their observed effects, are explored in this chapter.

2 Physical function of the eggshell as a respiratory organ for the developing avian embryo

2.1 Eggshell porosity

Different integrated physical or structural properties of the avian eggshell determine its porosity, and the transport of water and the vital gases (O_2 and CO_2) across the eggshell occurs by their diffusion through the pores of the eggshell when in their gaseous phases (Paganelli, et al., 1975). These integrated eggshell properties subsequently predispose the proficiencies by which water vapor and the vital gases diffuse and are exchanged between the exterior and interior of the embryonated egg. The pores of the eggshell extend through the shell proper, which is composed of calcite crystals, and terminate on two underlying membrane layers. The membranes overlay and make immediate contact with the vascularized chorioallantois during the first 5–6 days of incubation (Fig. 1). In conjunction with the continual loss of water, the exchange of O_2 and CO_2 occurs across the chorioallantois during the prenatal period (Rahn et al., 1979). The conductance of the eggshell to gases, as governed by its porosity, and differences in the partial pressure of

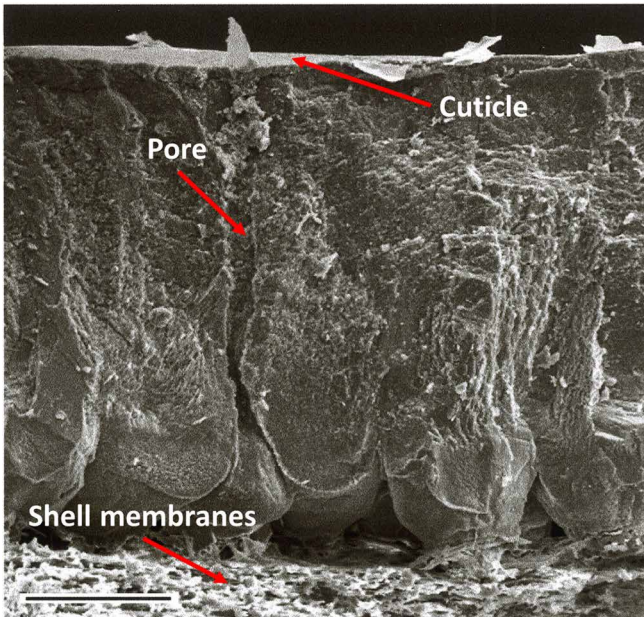


Figure 1 Scanning electron microscope micrograph depicting a longitudinal section of an avian eggshell. The cuticle, pore, and shell membranes are indicated (scale bar = 100 μm , image magnification 200 \times). The chorioallantois will form underneath the shell membranes.

gases between the outside and inside of the egg contribute to the control of gas exchange across the eggshell. It has been summarized that the flux of gas across the eggshell is determined by the product of its effective conductance and the partial pressure gradient of the gases between the inner surface of the eggshell and the ambient air surrounding the eggshell (Visschedijk, 1980). The passage of water vapor and the vital gases through the pores of the eggshell proper and underlying membranes allows for their exchange in the circulation and tissues of the developing avian embryo (Paganelli, 1991). Consequently, embryonic and post-hatch chick development are significantly influenced by the transfer of heat and the exchange of O_2 , CO_2 , and water between the internal and external environments of the egg (Boleli et al., 2016). Meir and Ar (1990) have noted that an increase in the rate of egg mass-specific O_2 consumption by ostrich embryos 1 day prior to external pipping is associated with their active movement inside the egg in accommodation for a change in their means of O_2 uptake during the shift from chorioallantoic to pulmonary respiration. Furthermore, regional gas tensions in the air spaces within the egg are also a consequence of the ratio of diffusive conductance to the perfusion of blood in the chorioallantois and can change with location on the eggshell (Paganelli et al., 1988; Paganelli, 1991).

2.2 Water vapor conductance and incubational egg weight loss

Water vapor, O_2 , and CO_2 share common pathways through the pores that traverse the eggshell (Paganelli et al., 1978), and the shell proper itself has the primary influence on the partial-pressure differences that exist for these gases between the internal and external environments of the egg (Rahn et al., 1979). Moreover, variations in water vapor conductance reflect proportional variations in the conductance values of O_2 and CO_2 (Paganelli et al., 1975). The determination of water vapor conductance is the easiest to measure because it can be calculated from the rate of water loss from an egg, which equates to its weight loss, and egg weight is reciprocally and equally affected by the uptake of O_2 and the loss of CO_2 . Therefore, water vapor conductance is commonly used to also represent the functional conductance of an eggshell to O_2 and CO_2 . In brief, the physiological function of the eggshell as an embryonic respiratory component is more specifically best described in terms of its water vapor conductance since water vapor conductance incorporates the interactions of these gases with the eggshell's physical properties (Peebles and McDaniel, 2004). The water vapor partial pressure gradient that exists between the inside and outside of the egg and which is determined by the difference in the levels of humidity between the egg's internal and external environments is affected by the temperature of the egg and the local barometric pressure.

The determination of water vapor conductance is a function of the division of the rate of water loss from the egg by the water vapor gradient across the shell at a particular temperature and barometric pressure (Ar et al., 1974; Tullett, 1984; Peebles and McDaniel, 2004; Pulikanti et al., 2011b). However, Rokitka and Rahn (1987) have reported that there may be regional differences in the rates water vapor conductance over the entire eggshell surface. For example, eggshell water vapor conductance over the air cell may be higher by a factor of 1.6 in comparison to the other regions of the eggshell (Rokitka and Rahn, 1987). It should be noted that embryonic metabolism and hatchability are impaired if eggs that are laid at a low altitude are subsequently incubated at a high altitude (Visschedijk, 1980). A reduction in the atmospheric barometric pressure, associated decrease in O₂ tension, and an increase in eggshell conductance or functional porosity, leading to an excessive loss of CO₂ and water are the major causes of this impairment (Visschedijk, 1991).

The loss of egg weight in association with the loss of water during incubation is known to influence embryogenesis and the post-hatch nutrient utilization, metabolism, growth, and development characteristics of broiler chicks (Peebles et al., 2005). A negative correlation has been observed between the average daily percentage egg weight loss of broiler hatching eggs from 10.5 days to 18.5 days of incubation and the body weight of chicks relative to set egg weight through 12 h post-hatch (Pulikanti et al., 2012b). Pulikanti et al. (2012b) have more specifically suggested that a higher eggshell water vapor conductance adjusted for egg weight results in increased metabolism of the broiler embryo presumably due to increased O₂ uptake, which then increases the successive growth and rate of yolk sac absorption of the chicks through 3 days post-hatch. Influences of the water vapor conductances of eggshells on various physiological attributes of broilers can extend into the middle and late post-hatch grow-out periods (Pulikanti et al., 2013). Pulikanti et al. (2013) further observed that adjusted eggshell water vapor conductance values were positively correlated with breast muscle weight on day 48 post-hatch. Because the incubational weight loss of broiler hatching eggs through the first 10.5 days of incubation has been shown to be negatively correlated with hatchling body weight and to influence day 49 processing yield (Peebles et al., 2014), it should be closely monitored, particularly during the first half of incubation.

2.3 Influences of incubational temperature and airflow

Changes in egg temperature can be affected by changes in the rate of water loss from an egg during incubation. Because the physical change of water from a liquid to vapor requires heat, an increase in the loss of water would result in an increase in the loss of heat from an egg due to evaporative cooling (French, 2009b). An increase in evaporation rate promoted by an increase in

the rate of water vapor conductance can help reduce the effects of increased embryonic metabolic heat production on egg temperature (Meir and Ar, 1990). Nevertheless, other studies have specifically focused on incubation temperature and airflow rate as factors that contribute to the control of water vapor and vital gas exchange across the eggshell (Robertson, 1961b; French, 1997, 2009a). Wilson (1991) indicated that small incremental deviations from 37.0°C to 38.0°C dry bulb incubational temperature can significantly impact embryo development and subsequent hatchability. Nevertheless, it has been emphasized that incubator air temperature and the internal temperature of the egg can be distinctly different, as the temperature that the developing embryo experiences is not only dependent on incubator temperature but also influenced by the transmission of heat between the external and internal environments of the egg and the production of metabolic heat by the metabolizing embryo (French, 1997, 2009a). French (2009a) has precisely indicated that the temperature that the developing embryo inside the egg experiences should be considered as the real or true incubation temperature. The temperature of the external environment of an egg influences heat transfer to or from the egg, and the rise in temperature of the air passing over the egg is inversely proportional to the rate of airflow or airflow volume (French, 2009a). Nevertheless, airflow rate itself can be effectively used to regulate the external temperature of an egg without directly affecting its moisture content, because the rate of airflow over an egg has a negligible effect on its rate of water loss (Kaltofen, 1969; Spotila et al., 1981). When maintaining an optimum air temperature around eggs, a lower air speed is required when the spacing between eggs is increased (French, 1997).

2.4 Measurement of internal egg temperature

Pulikanti et al. (2011a) reported that temperature transponders could be inserted into the air cells of broiler hatching eggs between 12 days and 14 days of incubation without negatively influencing eggshell porosity or causing physiological stress to the growing embryo. Peebles et al. (2012) have suggested that the use of transponders in the air cells of embryonated eggs circumvents the confounding effects that the thermal properties of the eggshell, as well as the flow of air across the shell, may impose on the temperature that the embryo actually experiences. Moreover, Pulikanti et al. (2012a) observed that transponders in the air cells of embryonated eggs detected minute internal temperature fluctuations and recorded mean temperatures that were consistently higher than those of non-embryonated eggs as well as the external microenvironments surrounding the eggs between 13 days and 18 days of incubation. Using transponders, Peebles et al. (2012) have likewise concluded that in comparison to eggshell surface temperatures, air cell temperatures were

higher and closer to those of actual broiler embryo temperatures previously reported in other studies. At a 75% survival rate, it has also been concluded that temperature transponders could be successfully implanted in the air cells of broiler hatching eggs at 10.5 days of incubation to determine internal egg temperature for the subsequent accurate calculation of eggshell water vapor conductance unadjusted or adjusted for set weight (Pulikanti et al., 2011b, 2012a,b), as well as their water vapor conductance constants (Pulikanti et al., 2012a,b).

The measurement of internal egg temperature is the most accurate and, therefore, the most ideal means to assess embryo temperature; however, it is not practical in commercial operations (French, 2009a). Sotherland et al. (1987) have suggested that the measurement of eggshell temperature is more pragmatic and has been shown to be closely related to internal egg temperature. Using eggshell temperature to measure embryonic temperature (Lourens et al., 2005; Joseph et al., 2006), it has been reported that deviating from a constant eggshell temperature of 37.8°C can result in 10% differences in the yolk-free body mass and hatchability of broiler hatchlings (Lourens et al., 2005). Therefore, because eggshell temperature is affected by embryonic metabolic heat production, and because embryonic heat production can be affected by the amount and efficiency of energy utilization by the embryo, changes in eggshell temperature can subsequently impact embryogenesis, hatchability, and chick quality (Meijerhof, 2002; Lourens et al., 2011).

The topics discussed in this section have described various aspects that are the bases for understanding how humidity and the O₂ and CO₂ concentrations of the air within incubators affect embryo development, hatchability, and post-hatch chick development. The techniques by which the concentrations and exchange or flux of water, O₂, and CO₂ between the external and interior environments of the embryonated egg are measured and the environmental factors that affect their values have also been considered. The following sections describe in more detail the individual effects of humidity, O₂, and CO₂ on embryo and post-hatch chick development.

3 Humidity

3.1 Egg water content and its production and loss during embryonic development

Water is the dominant constituent in eggs, comprising approximately 75% of the internal contents of an egg. The concentrations of water in the albumen, yolk, and eggshell are 88.8%, 47.5%, and 1.0%, respectively (Romanoff and Romanoff, 1949; Vieira and Moran, 1999). A partial loss of this water must occur throughout incubation for embryonic development to be properly achieved

(Barbosa et al., 2013). As summarized by Davis et al. (1988), the total internal water content of an egg during embryonic development is affected by the loss of water through the pores of the eggshell (Paganelli, 1980) and the production of metabolic water by the oxidation of yolk lipids (Ar and Rahn, 1980). Considering the smaller portion of water that is lost to the external environment by its diffusion across the eggshell, the water content of the embryo is increased by its absorption from the extra-embryonic tissues including the amnion, allantois, and yolk sac (Davis et al., 1988; Ar, 1991b). Furthermore, the accretion of metabolic water as a byproduct of the metabolism of lipids by the embryo, which increases with development (Ar, 1991a), is responsible for approximately 8-13% of the water content of the embryo (Ar, 1991b). Not only does the loss of water during incubation have an important association with the loss of heat that is produced by the metabolically active embryo, but it is necessary for the formation of the egg air cell or chamber (Rahn and Ar, 1980; Visschedijk et al., 1980), which will possess a volume of gas that is positively related to the volume of water that is lost from the egg (Durojaye et al., 2018). A substantial air cell with an adequate volume and a well-proportioned supply of vital gases is critical for the subsequent hatching success of the embryo (Rahn et al., 1979; Ar and Rahn, 1980; Ar, 1991b; Barbosa et al., 2013; Uçar et al., 2021). Most importantly, it is essential that the air cell volume is large enough to support the embryo's initial breathing activities and to allow for the adequate filling of its respiratory system, including its lungs and air sacs. The volume of the air cell space needed to accommodate the pipping and subsequent hatching processes is approximately 14% of the total egg volume (Rahn et al., 1976).

3.2 Relative humidity

As described by Cormick (2021), absolute humidity is the amount of water contained in air (g/m^3), regardless of temperature, whereas relative humidity (RH) is the volume of moisture that air can hold at a given temperature and is expressed as a percentage. At a given RH, air at a cold temperature holds less moisture, whereas at a higher temperature it holds more moisture (Cormick, 2021). Relative rather than absolute humidity has been commonly used as the mode of measurement in hatcheries. Because the absolute humidity of the air surrounding the egg modulates the rate of water loss through the pores of the eggshell (Ar and Sidis, 2002), the RH of the incubational environment is a primary means by which the internal water content of the egg can be influenced and altered (Davis et al., 1988). The rate of water loss from an egg increases as the RH of the air surrounding it decreases. Therefore, to augment the proper development of the embryo, the RH of the air that surrounds a fertile hatching egg should be monitored and controlled (Decuyper et al., 2001).

3.3 Egg water loss requirement

Interspecific and intraspecific egg water loss during incubation can vary substantially (Walsberg, 1980). However, it has been well established that prior to pipping, eggs laid by poultry species must lose between 12% and 14% of their fresh weight at set by the loss of water through the pores of their eggshells to hatch successfully (Ar, 1991a). Lundy (1969) has further stated that peak hatching success can be achieved in domestic fowl when total egg water loss equals 10-12% of initial egg weight. In the review article by Barbosa et al. (2013), it was summarized that the RH of an incubator containing broiler hatching eggs should be set between 56% and 60% to allow for a 12% loss of set egg weight through 18 days of incubation and that maintaining a weight loss range within 11-13% during the first 18 days of incubation led to a better rate of hatch than did weight losses that were lower. When considering the effects of altitude changes on the water vapor conductance of eggs, Visschedijk (1991) has noted that in order to compensate for an increase in the water vapor conductance rate of eggs at higher altitudes, the water vapor gradient across the eggshell must be reduced to allow water loss to remain similar to that at lower altitudes (sea level).

3.4 Effects of relative humidity on hatchability and hatchling quality

A 5% change in humidity results in a 1% difference in moisture loss by 18 days of incubation (Cormick, 2021). Although the incubation of broiler breeder eggs of similar weight at either a 33% or 50% RH did not affect the absolute yolk sac weights of hatchlings, Tullett and Burton (1982) observed that, in conjunction with a lower incubational egg weight loss, their body weights were higher when they were incubated at the higher RH. If the RH during incubation is too low, embryonic moisture loss will be excessive, which will affect hatchability and will cause hatchlings to be dehydrated and to have lower body weight. Conversely, if the RH during incubation is too high, an early hatch of mushy chicks may result (Barbosa et al., 2013). The effects of these improper RH settings can further extend into the post-hatch growing period resulting in sub-optimal performance and decreased processing yield (Bruzual et al., 2000b; Pulikanti et al., 2012b; Peebles et al., 2014). Adjustment of an RH in the hatcher that is higher than that in the setter has been used to facilitate the hatching process of chicks. Upon examining the effects of setter and hatcher humidities on the hatchability of Bobwhite quail eggs, Wilson and Dugan (1992) concluded that previous recommendations for setter and hatcher RH levels of 55.4% and 75.0%, respectively, were satisfactory for optimum hatchability. Nevertheless, after conducting studies on the effects of incubational RH during the last 5 days of incubation, Bruzual et al. (2000b)

summarized that despite the lack of an effect on hatchling body weight, optimal performance was achieved overall by incubating eggs from young broiler breeder hens at an RH of 53% rather than at 43% or 63%.

3.5 Effects of relative humidity on embryo metabolism and embryonic and post-hatch chick development

Although varying RH outside a 42–70% range was not found by Barott (1937) to significantly affect the time of hatch of chicks, when set at 42% or 70%, it decreased embryonic energy metabolism and thereby compromised their growth. Barott (1937) concluded that the metabolic activity of the embryos occurred at a higher level at an RH of 60%. Lundy (1969) later reported that the optimal RH range is wide and can be between 40% and 70%, but Robertson (1961a) has concluded that RH should be approximately 50% to maximize hatchability. Robertson (1961b) further noted that within a 40–70% range in RH, hatchability and hatchling body weight, which reflect embryonic growth, were not significantly affected. However, it was found that the two endpoints of that RH range inversely altered the rate of egg weight loss, thereby potentially disrupting embryo metabolism at various stages of development, with the subsequent consequence of increasing embryonic mortality. In that report, it was also indicated that egg size may be a confounding factor in determining an optimum RH, with larger eggs requiring a lower RH. Because both incubation RH and temperature not only affect the loss of heat from the embryonated egg but also affect its rate of water loss, eggs that are incubated at a high or low RH may need to be incubated at a different incubation temperature to maintain the same embryo temperature when incubated in a more normal (55–60%) RH range (Molenaar et al., 2010; van der Pol et al., 2013; Boleli et al., 2016). A higher level of hatchability for broiler eggs was obtained by van der Pol et al. (2013) when they were incubated within a 55–60% range of RH and when their eggshell temperature was 37.8°C.

Upon comparing the effects of incubating layer hatching eggs at a 45% or 55% RH, Hamdy et al. (1991) reported that chicks hatched from eggs incubated at 55% RH had higher body weights at the hatch in comparison to those from eggs that had been incubated at the 45% RH. However, this difference ceased to exist by day 2 post-hatch, suggesting that the chicks from eggs incubated at the 45% RH were able to soon adapt physiologically and compensate in growth. Furthermore, although incubational RH treatment did not affect chick feed intake, feed conversion, or rectal temperature through 4 weeks post-hatch, after exposure to 39.0°C for 48 h, fewer chicks in the 45% RH treatment group died in comparison to those in the 55% RH treatment group. Bruzual et al. (2000a) tested the effects of 43%, 53%, and 63% RH incubational settings from the time that broiler hatching eggs were set to the time that the hatch was pulled. With increasing RH, the body weight of

the hatchlings was increased, but the time of hatch was not significantly affected. In that study, it was also observed that the percentage hatchability of fertile eggs was highest at 53% RH, whereas the percentage of late dead embryonic mortalities was highest at 63% RH. Therefore, although increasing RH to 63% produced the heaviest hatchling body weight, it had a detrimental effect on embryonic development. Bruzual et al. (2000b) further tested the effects of 43%, 53%, and 63% RH incubational settings during the last 5 days of incubation. Conversely, although the time of hatch was likewise not significantly affected by these same RH settings, no significant differences in hatchling body weight were noted. Restricting these changes in RH to only the last 5 days of incubation, therefore, does not appear to impact broiler hatchling body weight. The effects of RH on hatchling BW may be associated with the rate of yolk uptake from the yolk sac by embryos. Upon comparing the effects of 43%, 53%, and 63% RH incubational environments on yolk utilization in broiler hatching eggs, Burnham et al. (2001) noted that the rate of embryonic yolk uptake was increased by incubating eggs at the 53% RH. The concentration of palmitic acid in the yolk was also reported to be lower at 17 days of incubation at the 53% RH setting.

3.6 Influences of breeder age on the effects of relative humidity on embryonic development

The results of experiments conducted by Vick et al. (1993) have suggested that an incubational RH that produces the best hatch results for broiler breeder eggs is influenced by the age of the breeder hen. A 50% rather than a 58% RH resulted in a higher level of hatchability and a lower early embryonic mortality in eggs as the age of the breeder hen decreased between 28 weeks and 64 weeks. Conversely, the 58% RH resulted in a higher hatchability and a lower late embryonic mortality in eggs laid by hens that were 60 weeks of age. The 50% RH also led to a lower hatchability of eggs from hens that were 66 weeks of age, whereas young hens exhibited a higher level of hatchability. The differential influences of breeder age on the effects of RH on hatchability can be attributed to changes in the diffusivity of the eggshell to water vapor with hen age (Peebles and Brake, 1987; Vick et al., 1993). In a study exploring the influence of incubational RH on the characteristics of broiler embryo progeny from young breeder hens, Peebles et al. (2001) found that changes in RH between 43% and 63% did not affect embryo moisture content and did not have consistent effects on their crude fat and protein contents. However, a reduction in RH to 43% depressed embryogenesis, which may augment the inferior performance of broiler chicks from young breeder hens. Upon testing the effects of 43%, 53%, and 63% RH settings on broiler hatching egg yolk composition, Burnham et al. (2001) observed that the effects of these RH levels on yolk lipid content were influenced by breeder age. More specifically, the percentage of yolk lipid was higher when eggs from 26-week-old breeders

were incubated at a 63% rather than a 53% RH. However, yolk lipid content was lower in eggs from 30-week-old breeders when they were incubated at an RH of 43% rather than an RH of 53% or 63%.

To summarize, an approximate 12% loss of the internal water content of an embryonated egg prior to pipping, in conjunction with the formation of an air cell with well-proportioned concentrations of O_2 and CO_2 , must occur for the embryo to metabolize nutrients, develop, and hatch successfully. The rate of water loss during incubation is primarily a consequence of the functional porosity of the eggshell and the RH of the air surrounding the egg at a given temperature. In addition, hen species and age, egg size, stage of incubation, and the elevation at which incubation occurs are influential factors that can further modify the rate of water loss. Because RH affects post-hatch chick performance as well as embryogenesis, hatchery managers must carefully monitor incubator RH while considering the various influential factors which can modify the effects of RH.

4 Vital gases

4.1 Oxygen

4.1.1 *Eggshell porosity, air cell oxygen tension and the transition from chorioallantois vasculature to pulmonary respiration*

Burton and Tullett (1983) predicted the O_2 consumption of embryos based on the diffusion as well as air cell gas composition measurements of O_2 . In the report, it was shown that eggshell porosity affects O_2 availability and subsequent embryonic metabolism. Throughout incubation up until external pipping, all embryonated eggs consume a total amount of O_2 equivalent to 90 cm³ per gram of fresh egg weight (Ar and Rahn, 1978; Tullett, 1984). The events of internal and external pipping are associated with changes in the partial pressure of O_2 in the air cell and various physiological responses of the late-stage embryo. It is known that the O_2 tension in the gas space of the air cell is associated with the metabolic rate of the embryo and that an increase in embryonic metabolism lowers O_2 tension within the air cell (Rahn et al., 1974; Tullett and Deeming, 1982). The tension of O_2 in the air cells of eggs reaches approximately 14% (100 mmHg) immediately prior to internal pipping (Ar and Rahn, 1978; Tullett, 1984). Subsequently, endocrine-induced external pipping and successive hatching occur in response to decreased O_2 concentrations in the air cell (Decuyper et al., 2006; Decuyper and Bruggeman, 2007; Mortola, 2009). When O_2 availability for the embryo becomes limited, its physiological demands increase considerably (Tazawa et al., 1983). The loss of access to O_2 requires a transition to pulmonary ventilation (Vince, 1976; Menna and Mortola, 2002). There is a transition from gas exchange that is facilitated by the

chorioallantoic vasculature to pulmonary respiration after internal pipping. The increased availability of O_2 after this transition allows for the predominant use of aerobic fatty acid oxidation to produce energy by the embryo (Moran, 2007).

4.1.2 Interactive effects of eggshell porosity, embryo metabolism and temperature on embryonic oxygen consumption and hatchling quality

As previously discussed, the water vapor conductance of an eggshell is known to be controlled by the temperature of the egg. However, as eggshell water vapor conductance increases with an increase in egg content surface temperature, the O_2 diffusive conductance of the eggshell decreases (Meir and Ar, 1990). Upon examining the interactive effects of incubational O_2 concentration and RH on the hatchability of alligator eggs, Reigh and Williams (2020) concluded that when RH is adequate, a good level of hatching success can be achieved when eggs are incubated under normal ambient O_2 concentrations. Moreover, Deeming and Thompson (1991) have further indicated that eggs can experience higher conductance rates to respiratory gases, such as O_2 and CO_2 , when incubated in high humidity environments. Nevertheless, Burton and Tullett (1983) have more specifically noted that high porosity eggshells are able to adequately provide O_2 to late-stage embryos, whereas low porosity eggshells may lead to a reduction in embryonic metabolism. In accordance with an increase in the metabolic demands of the embryo that are associated with its various successive developmental stages, there is an increased demand for gas exchange through the pores of the eggshell (Boleli et al., 2016). French (2009b) has likewise suggested that an increase in eggshell conductance facilitates an increase in embryonic O_2 consumption in association with an increase in their rate of metabolism. Even though the diffusivity of gases through the eggshell is known to be controlled by egg temperature, Lourens et al. (2007) showed that the O_2 concentration of the surrounding air in an incubator during the third week of incubation had a greater effect in determining embryo development than did the eggshell temperature. More specifically, in comparing high (38.9°C) to normal (37.8°C) eggshell temperatures and high (25%) to low (17%) O_2 concentrations, it was found that embryonic heat production was highest when both eggshell temperature and O_2 concentration were highest and that heat production was lowest when eggshell temperature was high but when O_2 concentration was low. Facilitation of the high metabolic demands for increased O_2 availability by the late-stage embryo must, therefore, include a sufficiently high partial pressure of O_2 in an egg's external environment regardless of eggshell temperature and porosity. It was subsequently observed by Lourens et al. (2007) that an increased O_2 concentration increased the yolk-free body weight and length of hatchlings, while residual yolk sac weight was decreased.

4.1.3 Physiological responses of the embryo and hatchling to environmental oxygen concentrations

Diverse physiological responses also occur to increase the gas exchange demands of the late embryo and hatchling that include increases in circulating hematocrit, hemoglobin, and red blood cell count values (Morita et al., 2009; Tazawa et al., 2011, 2012). Özge et al. (2006) tested the hypothesis that O₂ supplementation could improve the survival rates of broiler breeder embryos during the late stage of incubation. Although the time of hatch and circulating red blood cell, packed cell volume, and hemoglobin values were not affected by subjecting the eggs to 23% O₂ concentrations between 18 days and 21 days of incubation, an improvement in embryonic survival rate, in association with a decrease in late embryonic mortality, were observed. The hatchlings were also heavier and had higher blood glucose concentrations in response to the O₂ supplementation. The use of supplemental O₂ as high as 23% may, therefore, be a useful means by which to meet the higher O₂ demands of late-stage embryos. Christensen et al. (1997) tested the hypothesis that providing an O₂-enriched environment to embryonated turkey eggs between 25 days and 28 days of incubation would increase the survival rate of the embryos from genetic lines selected for growth or egg production when compared to their respective random-bred controls. They observed significant interactions between O₂ treatment and genetic line for embryonic survival, heart growth, and hepatic glycogen concentration. It was concluded that the two types of genetic selection resulted in a diminishment in the response of the embryos to O₂ enrichment, which may be attributed to decreases in their eggshell conductances and their altered metabolisms. In a later related study by Christensen et al. (1999), in which the metabolism of carbohydrates and lipids was examined, it was confirmed that embryos selected for growth or egg production are unable to adequately respond to elevated environmental O₂ partial pressures by normally modifying the metabolism of their energy sources, thereby leading to an increase in embryonic mortality during the plateau stage of O₂ consumption that exists during the latter stage of incubation.

An adequate influx of O₂ is vital to the metabolic function of embryos, as the partial pressure of CO₂ increases in the blood and extraembryonic fluids during the period of incubation (Boutilier et al., 1977). In enclosed environments, higher rates of embryonic respiration during the later stages of incubation can increase deviations from normal atmospheric O₂ tensions (Deeming and Thompson, 1991). However, higher porosity eggshells that allow for increased O₂ influx through the eggshell to the embryo counteract the natural accumulation of CO₂ in the blood and extraembryonic fluids as incubation progresses. This effect is manifest in the report by Burton and Tullett (1983) showing that a decreased availability of O₂ to a developing embryo,

due to the low porosity of an eggshell, is a major factor that influences embryo weight late in incubation. The atmosphere consists of approximately 21% O₂ at sea level (Stock and Metcalfe, 1984; Weekley and Bland, 2021). Walsberg (1980) has noted that the fractional O₂ concentration in the typical nests of birds is like that in the general atmosphere but can decline during incubation from 20.9% to about 20.0–20.3%. Changes in the O₂ levels in an incubator can affect hatching results (Okur, 2019). It has been further observed that hatchability and subsequent performance can be adversely affected by O₂ levels that are either too low (<17%) or too high (25%) (Stock and Metcalfe, 1984; Lourens et al., 2007; Molenaar et al., 2010). During the early stages of incubation, chick embryos are very sensitive to O₂ deprivation; however, embryos display an improved tolerance to acute hypoxia as their age increases. The embryo also becomes more tolerant to hyperoxia as its age increases between the middle and late periods of incubation (Taylor et al., 1956; Taylor and Kreuziger, 1965, 1966; Onagbesan et al., 2007). Nevertheless, upon testing the effects of progressively increasing atmospheric O₂ concentrations between 15% and 50% on chick hatchability, Barott (1937) observed that a 21% level of O₂ produced the best hatch results.

4.1.4 Influences of altitude on the effects of atmospheric oxygen concentrations on the embryo and hatchling

Although the O₂ concentration in air at any altitude is 21%, there is a near-linear decrease in the atmospheric partial pressure of O₂ as altitude increases (Weekley and Bland, 2021). This is a result of a reduction in barometric pressure as altitude increases (Visschedijk, 1991). A hypoxic environment, due to a reduction in the partial pressure of O₂ that occurs at high altitudes (approximately 600 m above sea level), can cause increased early embryonic mortality (Şahan et al., 2011), reduced organ growth (Bagley and Christensen, 1989), and reductions in hatchability (Francis et al., 1967; Şahan et al., 2011) and hatchling weight (Şahan et al., 2011; Boleli et al., 2016). Boleli et al. (2016) more specifically summarized that the adverse effects of hypoxia on embryonic development during the first half of incubation are related to increased chorioallantoic development and vascularization, whereas its effects during the second half of incubation are related to the compensatory responses of the organs. After assessing the effects of incubational O₂ concentrations at high altitudes on the hatchability of chicken and turkey eggs in the studies conducted by Ells and Morris (1947) and Meshew (1949), Davis (1955) summarized that the hatchability of chicken eggs incubated at a high altitude (2195 m) could be increased with the use of supplemental O₂ concentrations as high as 25%, which equated to a 19% O₂ concentration at sea level. However, it was also suggested that more rapid genetic

improvements in hatchability may be made possible by natural selection in an un-supplemented environment at high altitudes and that a higher level of hatchability in an environment without supplemental O₂ may be a partial result of a reduction in the variation of O₂ concentrations that is experienced between trials in which supplemental O₂ is used.

Broiler embryos in eggs incubated at high altitudes, in which hypoxic conditions existed, have been observed by Şahan et al. (2011) to show increased plasma triiodothyronine (T₃) and thyroxine (T₄) concentrations and higher T₃:T₄ ratios. The effect of high altitude carried over into the newly hatched chicks, which also exhibited higher circulating T₃:T₄ ratios. Şahan et al. (2011) noted that newly hatched chicks that had been incubated at a high altitude exhibited higher hemoglobin and plasma hematocrit values. Chan and Burggren (2005) examined the effects of continuous hypoxia (15% O₂) exposure on the development of various organs, including the chorioallantoic membrane, at several successive critical intervals of development in chicken embryos. The differential effects that hypoxia had on the various organs were dependent on the developmental stage of the embryo. More specifically, although chorioallantoic membrane mass was not affected by hypoxia during the early and middle developmental stages, a remarkable compensatory increase in its mass was observed by day 18 of development to mitigate the physiological effects of the hypoxic condition. Furthermore, eye mass and beak length were reduced in middle development, while the masses of the liver, brain, heart, kidneys, stomach, intestines, and skeletal long bones were not affected at any developmental stage.

In summary, eggshell porosity and the availability of O₂ in the external environment, in conjunction with changes in air cell O₂ partial pressure, affect the metabolism, O₂ consumption, induction of pipping, and transition from chorioallantois vasculature to pulmonary respiration in embryos. Moreover, the impacts of O₂ availability on embryo development and its physiological responses depend on the stage of embryogenesis and the altitude at which incubation occurs.

4.2 Carbon dioxide

4.2.1 Effects of changes in carbon dioxide production by the developing embryo on incubator carbon dioxide concentrations and the influences of temperature

Increased levels of CO₂ early in incubation are known to cause a more rapid acidification and liquefaction of the albumen, and development of the sub-embryonic fluid (Benton and Brake, 1996; Bruggeman et al., 2007). Tona et al. (2013) have further noted that the albumen pH of layer hatching

eggs decreases more rapidly than that in broiler hatching eggs. The normal production of CO₂ by the embryo is 0.7 times the uptake of O₂ (Visschedijk, 1991). In association with the output of CO₂, a byproduct of embryonic metabolism under standard incubation conditions, the CO₂ concentration in a single-stage incubator can gradually increase from 0.05% at the beginning of incubation to approximately 0.3-0.5% at the end of incubation (Özlü et al., 2019). Because the CO₂ concentration in the air surrounding eggs in a nest is linearly related to the production of CO₂ by the embryo (Romanoff and Romanoff, 1967; Walsberg, 1980), the CO₂ concentration in nests peaks near the end of incubation, but it is when the embryo is less sensitive to elevated levels (Lundy, 1969). Deeming and Thompson (1991) have further stated that increased rates of metabolic CO₂ production, in conjunction with the higher respiratory rates of late-stage embryos, can cause a more intense divergence from normal atmospheric CO₂ partial pressures in the air within an incubator. However, Tona et al. (2013) have also hypothesized that reductions in elevated incubator temperature and CO₂ concentrations resulting from the increased production of heat and CO₂ by late-stage embryos can be achieved when the eggs are incubated at standard ventilation rates. Lourens et al. (2006) found that when standard incubator temperature was decreased by 0.30°C for 1 h, embryonic CO₂ production was initially increased by 0.5%, but then decreased thereafter. Conversely, when machine temperature was increased, CO₂ production initially decreased by 0.4%, but then increased thereafter. The embryos, therefore, displayed an initial inverse response followed by a more long-term direct response to the short-term change in incubational temperature. It was surmised that the changes in CO₂ production were not due solely to the level of embryonic heat production, but that alterations in the flow of blood in the chorioallantois that followed changes in incubation temperature affected heat transfer and the rate of CO₂ diffusion. Lourens et al. (2006) further concluded that the ability of the embryo to liberate CO₂ may limit its development under higher temperatures.

4.2.2 Effects of changes in incubator carbon dioxide concentrations on embryogenesis and hatchability

Carbon dioxide levels ranging between 0.1% and 0.5% are customarily used in the incubation of poultry eggs (Onagbesan et al., 2007). Hatching results are affected by changes in CO₂ concentration in an incubator (Okur, 2019). Despite the possible confounding influences of various environmental factors during experimentation, it has been suggested that incubational CO₂ levels above 1.0% can adversely affect hatching success in domestic fowl (Lundy, 1969). In a series of studies (Taylor et al., 1956; Taylor and Kreutziger, 1965, 1966), it was

shown that the effects of hypercapnia on the hatchability of chicks were related to the period of incubation in which they occurred in a setter unit. For instance, hatchability was reduced when CO₂ levels were equal to or above 1.0% between days 1 and 4 (Taylor et al., 1956), 3.0% between days 5 and 8 (Taylor and Kreuziger, 1965), and greater than 6.0% between days 9 and 12 (Taylor and Kreuziger, 1966). The increased tolerance of embryos to hypercapnia as they age during incubation can be partially attributed to the different buffering systems that they possess (Onagbesan et al., 2007). Nevertheless, embryos in low conductance eggshells not only can experience low water loss and O₂ uptake levels, but an accumulation of CO₂ in the egg can lead to a decrease in their blood pH levels (French and Tullett, 1991). The processes of internal and external pipping are influenced by CO₂ partial pressure changes in the air cell. A CO₂ tension of approximately 6% (40 mmHg) is reached in the air cell just before the initiation of internal pipping (Ar and Rahn, 1978; Tullett, 1984). Increased CO₂ concentrations in the air cell are associated with hormonally stimulated external pipping and the complete emergence of hatchlings from the eggshell (Decuypere et al., 2006; Decuypere and Bruggeman, 2007; Mortola, 2009). Nevertheless, it has been suggested in other studies that not only may high CO₂ concentrations not be detrimental to embryonic development and subsequent hatchability, but that higher levels of CO₂ can be beneficial to embryonic development and to further improve hatchability and to stimulate an early hatch response (Bruggeman et al., 2007; Tona et al., 2007). The results that were observed were alleged to be related to the conservation of energy in association with a greater uptake of O₂ in response to physiological adaptations including increases in circulating erythrocyte numbers and a general improvement in cardiovascular development (Tazawa et al., 2002; Decuypere et al., 2006; Verhoelst et al., 2011).

4.2.3 Potential benefits of elevated atmospheric carbon dioxide concentrations during incubation on embryogenesis, hatchability and post-hatch performance

It has been shown that upon exposing broiler hatching eggs to hypercapnic conditions, through gradual increases in CO₂ levels from 0.7% to 1.5% during the first 10 days of incubation, that embryogenesis was accelerated, and hatchability was improved (De Smit et al., 2008). Accelerated embryo growth was likewise observed by Carlea et al. (2012) when they exposed Cobb 500 broiler hatching eggs to 0.85% CO₂ concentrations during the first half of incubation. Willemsen et al. (2008) concluded that a gradual increase in incubator air CO₂ concentration up to 1.0% during the first 10 days of incubation decreased embryonic mortality and subsequently increased fertile egg hatchability by lowering embryo

malposition incidence. However, although higher CO₂ levels increased broiler body weight through 2 weeks post-hatch, it had no consistent effects on body weight at slaughter age. Conversely, Fernandes et al. (2014) showed in a subsequent study that when broiler hatching eggs were exposed to graded levels of CO₂ between 0.4% and 1.0% during the first 10 days of incubation, that although the circulating heterophil:lymphocyte ratios of the birds were increased at 42 days of post-hatch age, it did not affect their post-hatch livability, performance, heart and liver weights, or heart characteristics. These same results were observed even when they were subjected to fluctuating temperatures between 35 days and 42 days of age. Gildersleeve and Boesch (1983) have indicated that CO₂ levels between 0.3% and 1.5% early in incubation improve hatch, and De Smit et al. (2006) have also indicated that levels in that same range early in incubation can also stimulate embryonic growth. In comparison to low (0.2%) CO₂ concentrations, when high (1.0%) CO₂ concentrations were applied during only the hatching phase (beginning on day 19) of incubation, higher relative heart and lung weights were observed at 12 h after hatch, thereby indicating that when applied during the hatching phase, high CO₂ levels in an incubator may only exert a temporary physiological effect during the early post-hatch period (Maatjens et al., 2014a). In a subsequent study by Maatjens et al. (2014b), in which the same experimental design was employed, it was reported that the high CO₂ concentration resulted in a lower blood pH and hepatic glycogen concentration in embryos that had been internally piped.

4.2.4 Physiological responses of embryos and hatchlings to elevated atmospheric carbon dioxide concentrations and the influences of genotype

Increased plasma T₃ and T₄ concentrations and higher T₃:T₄ ratios have been observed in broiler embryos in eggs incubated under hypercapnic conditions at high altitudes. Higher circulating T₃:T₄ ratios in response to the high altitude also subsequently occurred in hatchlings (Şahan et al., 2011). Tona et al. (2013) conducted experiments to determine the effects of incubator CO₂ concentration on the physiological variables of chicken eggs from broiler and layer genotypes exhibiting different growth trajectories. It was reported that increasing incubator CO₂ concentration during the first 10 days of incubation differentially affected the growth trajectories and the physiological variables of late-stage embryos. It was also noted that the higher CO₂ level during incubation led to higher plasma T₃ and corticosterone concentrations in both genetic lines at the time of internal pipping. However, the higher CO₂ concentration resulted in a shorter length of incubation in only the slower-growing genetic line. Buys et al. (1998) further explored the effects of different incubational CO₂ levels (0.2% and 0.4%) on the hatch results and physiological characteristics of embryo and post-hatch

chicks from ascites-sensitive and ascites-resistant broiler lines. At the 0.2% but not the 0.4% level, the ascites-resistant line hatched earlier than the ascites-sensitive line. Embryos from both lines had higher plasma T_3 concentrations when incubated under a 0.4% rather than a 0.2% CO_2 concentration. In the post-hatch period, lower ascites mortalities occurred in chicks that hatched from eggs that were incubated at the 0.4% level in comparison to the 0.2% level, and heart right ventricular to total ventricular ratios were higher in the 0.2% CO_2 treatment group in comparison to the 0.4% CO_2 treatment group.

To summarize, incubational temperature and the increased production of CO_2 by developing embryos can affect atmospheric CO_2 concentrations in incubators. However, although increased incubational CO_2 concentrations can affect hatching success, late-term embryos become more tolerant to hypercapnia. Nevertheless, elevated CO_2 concentrations during the first half of incubation may be used to stimulate embryo growth and improve hatch. These effects are mediated by various physiological responses and are influenced by altitude and bird genotype.

5 Conclusion

To achieve a successful hatch, an approximate 12% loss of the internal water content of an embryonated egg must occur prior to pipping in conjunction with a well-formed air cell at the large end of the egg containing a correct proportion of O_2 and CO_2 . The rate of water loss is primarily a product of an eggshell's functional porosity and the RH, at a given temperature, of the air immediately surrounding an egg. While carefully monitoring the incubational environment with these factors in mind, it is also necessary to consider other confounding factors previously described in this chapter that can further modify the rate of water loss from the embryonated egg.

Knowing the need for the adequate elimination of CO_2 and the supply of O_2 for the developing embryo, it can be summarized that maintenance of the vital gas concentrations across the eggshell by proper adjustments of their concentrations in the internal incubator environment during the setter and hatcher phases of incubation is necessary to meet the embryo's physiological requirements. The physiological needs and adjustments of the embryo in response to O_2 availability include its metabolism, pipping activity, and transition from vascular to pulmonary respiration. Like that of RH, other modifying factors that can affect the impact of O_2 availability on embryogenesis include the stage of development of the embryo and the altitude at which incubation occurs. In contrast to the required uptake of O_2 , the elimination of CO_2 as a metabolic byproduct of the developing embryo is also required. A subsequent accumulation of CO_2 in an incubator can occur with an increased growth of the embryo. However, after taking into consideration the modifying effects

of altitude and bird genotype, the practice of elevating CO₂ concentrations during the first half of incubation may be used to aid in stimulating embryonic growth and improving hatchability.

To understand the subsequent effects of air humidity and vital gas composition in an incubator on embryo and post-hatch chick development, it is necessary to have a comprehensive knowledge of the interactive effects of the physiological function of the eggshell and the modifying influences of the various physical factors in the incubational environment. With this knowledge, hatchery managers will be better equipped to optimize productivity in their hatcheries.

6 Where to look for further information

It is recommended that researchers consult current company guidelines for the incubation of their specific hatching eggs. This would include following their recommended procedures for monitoring incubational egg weight loss for the proper adjustment of incubational conditions necessary to achieve an optimal weight loss prior to pipping. Further information can also be obtained from updated extension articles published by a university Poultry Science department. Future research should focus on determining fine incubational regimen adjustments, including those for temperature and humidity, that could be made at various periods during incubation to better accommodate the changing growth and metabolic rates of embryos of modern strain hatching eggs.

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