Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Jul - Sep 2010 / v.12 / n.3 / 137 - 148

Meeting Embryonic Requirements of Broilers Throughout Incubation: A Review

■ Author(s)

Molenaar R[1]*
Reijrink IAM[1]*
Meijerhof R[2]
Van den Brand H[3]

[1] HatchTech B.V., P.O. Box 256, 3900 AG Veenendaal, the Netherlands.
[2] Poultry Performance Plus, Kleine Enkweg 1, 7383 DB Voorst, the Netherlands.
[3] Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands.

■ Mail Address

R. Molenaar, HatchTech B.V.
P.O. Box 256, 3900
AG Veenendaal, the Netherlands
Tel: +31 (0) 318 512 511
Fax: +31 (0) 318 517 487

E-mail: rmolenaar@hatchtech.nl.

■ Keywords

Broiler embryos, CO_2 concentration, incubation, relative humidity, temperature.

These authors contributed equally to this manuscript.

Arrived: April/2010
Approved: August/2010

ABSTRACT

During incubation of chicken embryos, environmental conditions, such as temperature, relative humidity, and CO_2 concentration, must be controlled to meet embryonic requirements that change during the different phases of embryonic development. In the current review, the effects of embryo temperature, egg weight loss, and CO_2 concentration on hatchability, hatchling quality, and subsequent performance are discussed from an embryonic point of view. In addition, new insights related to the incubation process are described.

Several studies have shown that a constant eggshell temperature (EST) of 37.5 to 38.0°C throughout incubation results in the highest hatchability, hatchling quality, and subsequent performance. Egg weight loss must be between 6.5 and 14.0% of the initial egg weight, to obtain an adequate air cell size before the embryo internally pips. An increased CO_2 concentration during the developmental phase of incubation (first 10 days) can accelerate embryonic development and hatchability, but the physiological mechanisms of this acceleration are not completely understood. Effects of ar increased CO_2 concentration during late incubation also need further investigation.

The preincubation warming profile, thermal manipulation, and *in ovo* feeding are new insights related to the incubation process and show that the optimal situation for the embryo during incubation highly depends on the conditions of the eggs before (storage duration) and during incubation (environmental conditions) and on the conditions of the chickens after hatching (environmental temperature).

INTRODUCTION

During the last century, the poultry industry underwent many changes that also affected the incubation industry. Due to the intensification of poultry production, the brooding hen was first replaced by a small still-air incubator and then by a forced-draught incubator. The forced-draught incubator was used as a multi-stage system in which eggs of different ages were present in the incubator at the same time. Since the early nineties, it has been recognized that multi-stage incubators do not completely fulfill the embryonic requirements and do not optimize hatchling quality (Hill, 2000). Therefore, single-stage incubation was introduced, in which only eggs of one age are incubated in an incubator. In a single-stage incubator, environmental conditions, such as temperature, relative humidity, and CO_2 concentration, can be controlled based on the changing embryonic requirements during the different phases of embryonic development (French, 1997; Hulet, 2007; Bennett, 2010).

Along with the changes in inc.bation technology, genetic selection improved the growth performance of broiler chickens. Consequently,

the production cycle time has decreased in broiler chickens, and the incubation process of 3 weeks has respectively become a larger part of the total chickens lifespan (Wolanski *et al*., 2004; Hulet, 2007), which emphasizes the importance of the incubation process. The challenge of modern incubation is to understand and fulfill the specific embryonic requirements during the different stages of development. In the current review, effects of embryo temperature, egg weight loss, and CO_2 concentration on hatchability and hatchling quality are discussed from an embryonic point of view. In addition, new insights related to the incubation process are described.

INCUBATION - TEMPERATURE

Eggshell temperature

In nature, a clutch of 13 to 15 eggs is incubated by the hen (Romanoff & Romanoff, 1949). Eggs are exposed to the environmental conditions in the nest, which are partly created by the attentiveness of the hen and egg-shifting behavior (Huggins, 1941; Freeman & Vince, 1974). Temperature is one of the most important environmental factors during incubation (Freeman & Vince, 1974; Decuypere & Michels, 1992; Meijerhof, 2009). During the day, temperature in the nest fluctuates due to changes in environmental temperature and attentiveness of the hen (Huggins, 1941; Freeman & Vince, 1974). In addition, a temperature gradient is present within an egg as the bottom of the egg is in contact with the nest material and the top is covered by the brood patch of the hen or surrounded by the air during a recess (Freeman & Vince, 1974; Turner, 1997). After the complete development of the chorio-allantoic membrane around day 12 of incubation (Tullett & Deeming, 1987), the embryo may be able to redistribute heat by its circulation (Turner, 1997) and regulates its temperature within certain limits. This is further indicated by the result that blood flow in the chorioallantoic membrane is found to react to changes in temperature in the last week of incubation (Holland *et al*., 1998; Nichelmann & Tzschentke, 2003; Tzschentke, 2007). In contrast to nature, there is no temperature gradient within the egg during artificial incubation because all the eggs are surrounded by the same air temperature. This means that an embryo is not able to redistribute its blood flow to optimize its temperature, and therefore, incubator temperature is of high importance to maintain embryo temperatures within narrow limits.

Historically, the air temperature of the incubator was controlled and maintained between 36 and 38°C during artificial incubation (Lundy, 1969; Decuypere *et al*., 2001). Heat production increases exponentially after 9 days of incubation in chicken embryos (Lourens *et al*., 2007) due to the increased metabolism of the embryos. If air temperature is maintained between 36 and 38°C, embryo temperature will increase throughout incubation. Because embryo temperature is difficult to measure without killing the embryo, eggshell temperature (EST) is used as an indicator for embryo temperature in practice. Eggshell temperature deviates no more than 0.1 to 0.2°C from the embryo temperature (Meijerhof & Van Beek, 1993; French, 1997). Several studies have shown that a constant eggshell temperature of 37.5 to 38.0°C throughout incubation results in the highest hatchability and hatchling quality (Lourens *et al*., 2005, 2007; Joseph *et al*., 2006; Leksrisompong *et al*., 2007). A constant temperature of the eggshell or embryo is the result of a balance between heat production of the embryo and the heat transfer between the egg and the surroundings (Meijerhof & Van Beek, 1993). Both factors will be described below.

Embryonic heat production

The most important factors that influence embryonic heat production are age of the breeder flock, egg size, and stage of incubation (Tona *et al*., 2004; Lourens *et al*., 2006; Hamidu *et al*., 2007; Meijerhof, 2009). Heat production increases with age of the breeder flock, even when it is corrected for egg sizes (O'Dea *et al*., 2004, Lourens *et al*., 2006), and this may be related to a larger yolk proportion in eggs of older flocks (Hamidu *et al*., 2007). Due to the lower fertility and higher embryonic mortality in old (> 50 weeks) and young flocks (< 35 weeks; Yassin *et al*., 2008), total heat production per incubator can be lower in young and old flocks compared to prime flocks (35-50 weeks). The stage of incubation has the largest influence on heat production (Meijerhof, 2009). As stated earlier, heat production increases exponentially after 9 days of incubation in chicken embryos (Lourens *et al*., 2007). Heat production reaches a plateau phase between day 15 and 18 of incubation and is approximately 140 mW at day 18 of incubation for a 62-g egg (Dietz *et al*., 1998; Lourens *et al*., 2006, 2007). After internal pipping, around day 19 of incubation, embryos switch to lung ventilation, and consequently, heat production is almost increased twofold in broiler embryos (Rahn, 1981; Janke *et al*., 2004).

Heat transfer

In addition to heat production, heat transfer also determines embryo or eggshell temperature. Transfer of heat from the egg to the surroundings or vice versa is influenced by 3 factors, air temperature, air velocity, and relative humidity (Meijerhof & Van Beek, 1993), which will be discussed below.

To maintain an eggshell temperature around the optimum of 37.5 to 38.0°C throughout incubation, air temperature in the incubator must be higher than 37.5 to 38.0°C during the first days of incubation (French, 1997; Lourens *et al*., 2005, 2006; Yahav *et al*., 2009). This is because the heat production by the embryos is lower than the heat loss due to evaporation (Romijn and Lokhorst, 1960). Around day 9 of incubation, embryonic heat production becomes larger than the heat loss due to evaporation and a gradual decrease in air temperature is required to maintain the eggshell temperature at 37.5 to 38.0°C (Romijn & Lokhorst, 1960; Lourens *et al*., 2005, 2006; Yahav *et al*., 2009). The decrease in air temperature depends on factors that influence heat production, such as breed and breeder flock age which affects fertility rates and egg sizes.

The second factor that has a large influence on heat transfer is air velocity (Meijerhof & Van Beek, 1993). Air velocity is especially important in the first few days when eggs need to be warmed and after day 12 of incubation when embryonic heat production increases exponentially and needs to be removed from the eggs. When air velocity is low, downstream eggs are cooled less efficiently than upstream eggs, which increases the variation in eggshell and embryo temperatures among eggs (Elibol & Brake, 2008). A high air velocity increases the heat transfer capacity of air and reduces variation in eggshell and embryo temperatures.

The last factor that influences heat transfer is relative humidity. Humid air transfers heat better than dry air, and gas sealed incubators use this concept to transfer heat to the eggs and create a more uniform environment at the start of the incubation process. By closing the damper of the incubator, moisture loss of the eggs increases the relative humidity inside the incubator (~80%). Since heat transfer is higher to humid air than to dry air, this decreases variation in temperatures and therefore variation in embryonic development. It needs to be emphasized that increasing relative humidity with the humidifier in the incubator does not have the same effect as closing the damper. Water sprayed in the incubator needs to be evaporated, and this occurs on eggs situated near the humidifier. As a result, eggs close to the humidifier are cooled more than the other eggs, which increases the variation in eggshell and embryo temperatures and embryonic development among eggs (Meijerhof, 2009). Although a high relative humidity level can increase the heat transfer capacity of the air, the method used to increase the relative humidity in the incubator determines the effects on hatchability and hatchling quality.

Single-stage and multi-stage incubation

A constant eggshell temperature throughout incubation can only be achieved by single-stage incubation because incubator settings can be adjusted to compensate for increasing heat production by embryos. During multi-stage incubation, heat produced by the older embryos is used to warm the younger embryos. The advantage of multi-stage incubation is that it is energy efficient, but the disadvantage is that only one climate can be maintained in the incubator because temperature and ventilation rate are fixed throughout incubation. Consequently, eggshell temperatures are maintained below the optimum of 37.5 to 38.0°C for eggs during the first week of incubation and above the optimum in eggs during the last week of incubation (Hulet, 2007). Lourens *et al*. (2005, 2007) showed that any deviation from an EST of 37.5 to 38.0°C could significantly reduce hatchability and hatchling quality.

In both multi- and single-stage incubation, overheating the embryos at the end of incubation occurs because the fixed temperature is too high or because the cooling capacity or air velocity within the incubator is insufficient (French, 1997; Hulet, 2007). Consequently, hatchability can be decreased (Lourens *et al*., 2005, 2007), which can be related to a higher incidence of malpositions such as head between legs and head over wing (French, 2000). Further indications of overheating at the end of incubation are reduced hatchling development indicated by a lower yolk-free body mass, a larger residual yolk, a shorter hatchling length, and a poorer navel condition compared to chickens that were incubated at a normal EST (Lourens *et al*., 2005, 2007; Leksrisompong *et al*., 2007; Piestun *et al*., 2009). The reason for this decrease in hatchling development due to high incubation temperatures may be the reduction in incubation duration, and consequently, the reduction in time for development (Lourens *et al*., 2007). In addition, Molenaar *et al*. (2009) found that the lower development at hatch at high temperatures might be related to the use of egg

protein as an energy source that resulted in lower protein deposition in the embryo from the utilized nutrients from the egg.

Overheating of embryos at the end of incubation decreases development at hatch and can have a negative effect on subsequent performance. Lourens *et al*. (2005) and Joseph *et al*. (2006) found up to 20 g (13%) lower body weight at 1 week of age in chickens incubated at high (38.9-39.5°C) temperatures relative to those incubated at normal (37.8°C) eggshell temperatures in the last week or last 2 days of incubation, respectively. The difference in body weight disappeared at marketing age in the study of Joseph *et al*. (2006), and this may be due to compensatory growth or to the relative short exposure of only 2 days to high temperatures at the end of incubation. Leksrisompong *et al*. (2009) found a negative effect of high temperatures (≥ 39.0°C) from day 17 of incubation until hatching on body weight up to 21 days of age. Body weights of chickens were 54 g (7%) lower at 3 weeks of age after high (≥ 39.0°C) EST compared with normal (± 37.5°C) EST treatment. A decrease in performance due to high EST may be related to the impaired hatchling development related with the earlier hatch time. In addition, chickens spend more time in the hatcher because of the earlier hatch time and are more dehydrated than chickens incubated at a normal temperature (Romanoff, 1936; Wyatt *et al*., 1985). Several studies have shown that a delay in feed and water supply posthatch has a negative effect on subsequent performance (Wyatt *et al*., 1985; Pinchasov & Noy, 1993; Noy & Sklan, 1999; Careghi *et al*., 2005).

In contrast to the other studies, Hulet *et al*. (2007) found a positive result from an EST of 38.6°C during late incubation on body weights at slaughter age. Chickens incubated at an EST of 38.6°C from day 16 of incubation onward had a higher body weight at day 44 compared with incubation at an EST of 37.5°C or 39.7°C. This positive effect of the high EST may be influenced by the environmental temperatures that the chickens experienced during the grow out period. High ESTs during incubation can improve the thermotolerance of broiler chickens (Yahav *et al*., 2004a,b; Piestun *et al*., 2008a,b; Yalçin *et al*., 2008, 2010), and this may have improved their ability to cope with relatively high temperatures during the grow out period and maintain growth (Yalçin *et al*., 2010). The highest EST of 39.7°C in the study of Hulet *et al*. (2007) did not improve subsequent performance, but this temperature may have greatly impaired development.

In conclusion, eggshell temperatures can be maintained between 37.5 and 38.0°C throughout incubation in a single-stage incubator, which appears to result in the highest hatchability, hatchling quality, and subsequent performance. In a multi-stage incubator, it is not possible to maintain a constant eggshell temperature, and embryos are too cold in the first week and too hot in the last week of incubation. In both single- and multi-stage incubation, there is a risk of overheating the embryos at the end of incubation, which can negatively affect hatchability, hatchling quality, and subsequent performance.

INCUBATION - EGG WEIGHT LOSS

Water and gases are exchanged through the eggshell during both artificial and natural incubation. The amount of water and gases exchanged through the eggshell is a result of eggshell characteristics and a pressure difference between the egg and the surrounding (Walsberg, 1980; Vleck, 1991). The total amount of water inside the egg during embryonic development is a function of two processes (Davis *et al*., 1988). Firstly, an egg loses water by diffusion (Paganelli, 1980). Secondly, the oxidation of yolk lipids produces metabolic water that is added to the total volume of the egg (Ar & Rahn, 1980). One reason for egg weight loss is to create an adequate air cell size inside the egg. This air cell must be large enough at internal pipping for lung ventilation to begin (Ar & Rahn, 1980). Within a batch of eggs, individual egg weight losses are variable due to variations in egg sizes (Marshall & Cruickshank, 1938) and eggshell conductance (Bryant & Sharp, 1934; Fromm, 1959). Visschedijk *et al*. (1985) showed that the coefficient of variation of eggshell conductivity within a batch of eggs was 22%, a much higher variation than observed for egg weight.

Hays & Spear (1951) showed that chickens were able to hatch when egg weight loss was between 6.5 and 12% before external pipping occurred. Hulet *et al*. (1987) showed that hatchability and poult livability was optimal when turkey eggs lost between 9.5 to 11.5% of their egg weight. Meir & Ar (1987) concluded that hatchability was optimal when turkey egg weight loss was between 10 to 14%. In addition, Ar & Rahn (1980) stated that the average egg weight loss should be between 12 to 14% to obtain the highest hatchability of chicken eggs. When eggs lose less than 6.5% of their egg weight before internal pipping occurs, the size of the air cell is not adequate for lung ventilation to begin. On the other hand, when the

average egg weight loss increases above 14%, the risk for dehydration increases. As a result, embryos may die, or hatched chickens are small and dehydrated (Tullett & Burton, 1982). Because the variation in eggshell conductivity is high, there cannot be one optimal percentage of egg weight loss, but there is an optimal range of egg weight loss. As long as the egg weight loss of the majority of eggs is between the optimal range, hatchability and hatchling quality will be maximized. It appears that egg weight loss must be between 6.5 and 14.0% of the initial egg weight to obtain an adequate air cell size before the embryo internally pips.

Because the main reason for egg weight loss is to reach an adequate size of the air cell for lung ventilation to begin, it is probably not important at which moment during incubation the egg loses its weight as long as the air cell reaches an adequate size before the embryo internally pips. Relative humidity within an incubator can be used to change egg weight loss. A high relative humidity towards hatching time will be necessary if eggs evaporated a sufficient amount of water previously but will be detrimental if the relative humidity was high previously during incubation and no substantial amount of water was lost (Robertson, 1961).

Some authors found an effect of relative humidity on early embryonic mortality and hatching time, which suggests that egg weight loss does not only affect size of the air cell but also affects embryonic development before the size of the air cell plays a crucial role. Robertson (1961) showed that high relative humidity (75-80%) increased mortality during the first 10 days of incubation. The reason for this is unknown, but a high relative humidity during the early period of incubation may disrupt or retard embryonic growth and development due to the disturbance of the organizing centers or some other unknown deep-set physiological mechanisms (Robertson, 1961) or due to reduced gas exchange (Peebles *et al*., 1987).

Reinhart & Hurnik (1984) decreased hatching time by lowering incubator relative humidity from 57% to 45% between days 3 and 18 of incubation. Peebles *et al*. (1987) suggested that lowering relative humidity might have shorteneds hatching time by promoting the loss of extra metabolic water associated with an increased metabolic rate. However, because air with a low relative humidity has less heat transfer capacity, embryo temperatures may have increased due to the low relative humidity, and this may have caused the shorter hatching time as well.

The difficulty in interpreting results of experiments in which relative humidity is varied during incubation is that embryo temperature is often not kept at the same level in the different treatments. Changes in relative humidity not only affect egg weight loss but also affect the heat transfer capacity of the air and consequently embryo temperatures. The effect on embryo temperatures can cause changes in embryonic development, which is not a direct result of the change in relative humidity. Kosin (1964) stated that air velocity, ventilation rate, machine temperature, and the pattern of air distribution within the incubator depend on the incubator design, and all of these factors affect the percentage of relative humidity in the incubator. Consequently, the effect of a particular level of relative humidity on hatchability depends on incubator design, and therefore, results of different experiments are difficult to compare.

The effect of egg weight loss on hatchling quality has not been investigated extensively. Bruzual *et al*. (2000a) showed that hatchling weight increased when the percentage of relative humidity during incubation was increased (39.4 g, 40.2 g, and 41.2 g when relative humidity was 43%, 53%, and 63%, respectively). Hatching time was not affected by the percentage of relative humidity during incubation in this study. Hamdy *et al*. (1991) showed that the body weight of chickens was 0.7 g higher when eggs were incubated at a relative humidity of 55% compared to 45%. Excess water is probably incorporated into the tissue of the chicken (Davis *et al*., 1988), but in studies by Hamdy *et al*. (1991), Swann & Brake (1990b), and Bruzual *et al*. (2000a), this extra water was rapidly lost after hatching. As a result, no difference in body weight was observed at pull time (Bruzual *et al*., 2000a), and this suggests that the period and conditions between hatch and arrival at the farm affect hatchling quality more than the relative humidity during incubation. Reinhart & Hurnik (1984), Burton & Tullett (1985a), and Swann & Brake (1990a) showed that extra water is not only left in the tissue of the chickens but also in the eggshell or eggshell membranes, and it is possible that this had a negative effect on gas exchange through the eggshell during the last part of incubation.

The relative humidity during incubation may have an effect on later performance, but this also has not been extensively investigated. Bruzual *et al*. (2000b) suggested that chickens of a young breeder flock incubated at a relative humidity level of 43% were more sensitive to sub-optimal brooding conditions than chickens of a young breeder flock incubated at relative humidities of 53 and 63%, but the reason for this

difference was not given. Hamdy *et al*. (1991) showed that chickens that hatched from eggs incubated at 45% relative humidity could better cope with high temperatures during transport than chickens that hatched from eggs incubated at 55% relative humidity. The reason for this difference is unclear, but the chickens in the 45% relative humidity treatment may have produced less heat than the other chickens (Hamdy *et al*., 1991).

It appears that egg weight loss has no detrimental effect on hatchability as long as the majority of eggs lose between 6.5 and 14.0% of their initial egg weight before the embryo internally pips. The time and conditions between hatch and arrival at the farm probably have a more pronounced effect on hatchling quality and on later performance than the relative humidity during the embryonic phase of incubation.

INCUBATION - CO_2 CONCENTRATION

In natural incubation, the CO_2 concentration is reported to increase from 0.05 to 0.90% within the nest during incubation due to the increase in embryonic development (Burke, 1925). Consequently, the O_2 concentration declines from 20.9 to 20.3% (Walsberg, 1980). However, gas concentrations are not fixed during incubation because the nest is also ventilated (Chattock, 1925; Rahn *et al*., 1977). In artificial incubation, the CO_2 concentration in a multi-stage incubator is around 0.30% throughout incubation (Gildersleeve & Boeschen, 1983). The CO_2 concentration in a single-stage incubator is around 0.05% at the onset of incubation and gradually increases during incubation due to the CO_2 production of the embryos (Gildersleeve & Boeschen, 1983). Maximal CO_2 concentration in the incubator depends on the number of fertile eggs and ventilation rate but does not normally exceed 0.50% (Onagbesan *et al*., 2007).

Sensitivity of the embryo to CO_2 concentrations during early incubation

Sensitivity of embryos to CO_2 appears to change with embryonic age. During the first 4 days of incubation, the CO_2 concentration can increase up to 1% without affecting hatchability (Taylor *et al*., 1956). Between days 5 and 8 of incubation, embryos can survive CO_2 concentrations up to 3% (Taylor & Kreutziger, 1965). The increase in tolerance of the embryo for high CO_2 concentrations after day 4 of incubation may be caused by the establishment of the respiratory system around 96 hours of incubation (Taylor & Kreutziger, 1965). Between days 9 and 12 of incubation, which is the stage of development in which the greatest rate of growth occurs in the extra-embryonic membranes, embryos can survive CO_2 concentrations up to 5% (Taylor & Kreutziger, 1965). Although the CO_2 concentrations up to 5% do not negatively affect hatchability, it is questionable how it affects embryonic development.

Consequences of high CO_2 concentrations during early incubation

Under standard artificial incubation conditions, the CO_2 concentration will not increase above the indicated tolerance levels, but several studies showed that a gradual increase in the CO_2 concentration to a concentration of 0.7% or 1.5% during the first 10 days of incubation in an air-tight incubator accelerated embryonic development and improved hatchability (De Smit *et al*., 2006, 2008). Bruggeman *et al*. (2007) gradually increased CO_2 concentration to 1.5% during the first 10 days of incubation and also observed a positive effect on early embryonic development but did not find an effect on hatchability. Reasons why an increase in CO_2 concentration does not always improve hatchability are unknown, but effects on hatchability may depend on differences in genetics (De Smit *et al*., 2008) or breeder flock age (Witters, 2009), which both affect the metabolic rate of the embryo.

Another method to increase CO_2 concentration in the incubator is by CO_2 injection. Sadler *et al*. (1954) showed that a CO_2 concentration of 2% to 4% during the first 48 hours of incubation decreased albumen pH and increased early embryonic development in terms of body length, somite counts, and extra-embryonic membrane development. However, Taylor *et al*. (1956) found a reduction in hatchability when eggs were incubated at these CO_2 concentrations during the first 48 hours of incubation. Gildersleeve & Boeschen (1983) maintained different CO_2 concentrations by CO_2 injection into the incubator for different periods when turkey eggs were incubated. A CO_2 concentration of 0.30% during the first 10 days increased hatchability by 5% compared to a CO_2 concentration of 0.10%. Hatchability increased due to a decrease in embryonic mortality during the first 4 days of incubation and after day 21 of incubation. Reijrink *et al*. (unpublished) investigated the effects of high CO_2 concentrations (between 0.7% and 0.8% during the first 5 days of incubation by CO_2 injection into the incubator from the onset of incubation) when

eggs were incubated after 15 days of storage. Hatchability of fertile eggs decreased by 1.3%, and embryonic development was retarded after 66 hours of incubation.

It can be concluded that effects of a gradual increase of the CO_2 concentration or CO_2 injection on embryonic development and hatchability are variable. CO_2 concentrations probably affect albumen pH (Bruggeman *et al*., 2007), the breakdown of the chalaziferous membrane (Sadler *et al*. 1954), and the formation of sub-embryonic fluid (Latter & Baggott, 2002). Genetics, breeder flock age, and storage duration probably affect albumen pH, breakdown of the chalaziferous membrane, and the formation of sub-embryonic fluid. Therefore, the effects of CO_2 concentration on embryonic development and hatchability vary due to differences in genetics, breeder flock age, and storage duration. Because increased CO_2 concentrations do not structurally improve hatchability, high CO_2 concentrations during the first part of incubation have not been extensively used in practice.

CO_2 concentrations during late incubation

Due to the high metabolic rate of the embryo and limited conductance of the eggshell (Burton & Tullett, 1985b), the O_2 concentration in the air cell decreases to approximately 14.2%, and the CO_2 concentration increases to 5.6%, at the end of incubation around the start of the hatching process (Romijn and Roos, 1938; Visschedijk, 1968). This triggers the embryo to pip the air cell and emerge from the egg. Even within species, there is a large variation in eggshell conductance (Burton & Tullett, 1985b; Visschedijk *et al*., 1985), resulting in a large variation in gas exchange, and this creates differences in hatching time. These differences in hatching time can be further increased among batches of eggs by differences in storage time, egg size, breeder flock age, and incubation conditions. The variation in hatching time within a batch of eggs is expressed as the hatch window, the time difference between the first and last chicken hatching. In practice, a short hatch window is preferred to achieve a uniform flock at pull time. To achieve a short hatch window, the CO_2 concentration is sometimes increased to 2% at the onset of pipping to stimulate the chickens to hatch (French, 2010). CO_2 concentrations up to 7% from day 17 of incubation onward do not have a negative effect on hatchability (Taylor *et al*., 1971). However, the effect of high (> 1 %) compared to normal (< 0.3%) CO_2 concentrations during the hatching phase on hatchling quality and subsequent performance is unclear. In general, high CO_2 concentrations stimulate embryos to start the hatching process and may reduce the hatch window of a batch of eggs. However, some embryos may require a longer incubation time to maximize their development during the incubation process, for instance due to a higher initial egg weight. Because of the high CO_2 concentration, they are forced to hatch, and this will probably reduce the overall hatchling quality, indicated by a lower yolk-free body mass and shorter hatchling length. In addition, high CO_2 concentrations at the end of incubation may negatively affect heart and lung maturation (Coleman & Coleman, 1991). Effects of high CO_2 concentration on hatchling quality and subsequent performance are unclear, and more (practical) research is required to evaluate the effects of high CO_2 concentrations.

NEW INSIGHTS RELATED TO THE INCUBATION PROCESS

Although the optimal embryo requirements regarding temperature, relative humidity, and CO_2 concentration to obtain maximum hatchability, hatchling quality, and subsequent performance are still not completely known, new insights related to the incubation process have been gained. In this chapter, a few new insights related to the incubation process are described. Firstly, the effect of the preincubation warming profile on hatchability is described. Secondly, the effects of thermal manipulation during incubation on thermotolerance of the embryos during incubation and the birds later in life are explained. Furthermore, the effect of in ovo feeding during the end of incubation on the nutritional status of the embryo at hatch and subsequent performance is described.

Preincubation warming profile

At the onset of incubation, eggs need to be warmed from the storage temperature to the incubation temperature. The preincubation warming profile is the time and pattern used to increase the internal egg temperature from the storage temperature to the incubation temperature. The preincubation warming profile affects condensation on eggs at the onset of incubation. Condensation creates an optimal environment for bacteria to grow and increases the risk for contaminated eggs. Therefore, condesation should be prevented at all times. On the other hand, the preincubation warming profile may also affect

embryo viability. Some authors have suggested that it is beneficial to warm eggs quickly to the incubation temperature because prolonged exposure to temperatures between 25 and 35°C may increase embryonic mortality or abnormal embryonic development (Wilson, 1991; Renema *et al*., 2006). On the other hand, Hodgetts (1999) suggested that eggs should be warmed slowly to reduce the temperature shock to the embryo. Reijrink *et al*. (2010) showed that hatchability was not affected by the preincubation warming profile (4 or 24 hours) when eggs were stored for 4 days. However, after storage for 13 days, embryonic mortality during the first 2 days of incubation decreased from 17.1 to 12.7% when eggs were warmed over 24 hours instead of 4 hours. Long-stored eggs are probably more sensitive to the preincubation warming profile than short-stored eggs due to the negative effect of prolonged egg storage on embryo viability. Rhyne *et al*. (2009) showed that female broilers of stored eggs (14 days) had significantly higher body weights at 6 weeks of age when they were warmed over 18 hours compared to 2 hours. Body weights of the male broilers were significantly higher when warmed over 2 hours instead of 18 hours. The reason for this difference is unclear.

In conclusion, hatchability and hatchling quality appear to be affected by preincubation warming profile, but the effect depends on storage duration and may depend on gender.

Thermal manipulations

Periods of high temperatures during the incubation period may alter the thermotolerance of broiler chickens in a process called thermal manipulation (Piestun *et al*., 2008a; Yahav *et al*., 2009). Thermal manipulation is applied during the period that the thermoregulatory center in the brain develops and matures (e.gs., days 6 to 16 of incubation) to alter the 'setpoint' of the systems controlling thermoregulation (Piestun *et al*., 2008b). Temperatures used for thermal manipulation during incubation are around 39.5°C and are applied for 6 to 12 hours per day (Piestun *et al*., 2008a; 2009; Yahav *et al*., 2009; Yalçin *et al*., 2010). The result of thermal manipulation during incubation is that chickens are better able to cope with high temperatures during the grow out period (Piestun *et al*., 2008b). Therefore, benefits on performance from thermal manipulation appear to be particularly found when broiler chickens experience high temperatures during the grow out period (Yalçin *et al*., 2010). When chickens are raised under normal temperatures, subsequent performance can be negatively affected in chickens subjected to frequent short exposures to a high incubation temperature (39.6°C for 6 hours/day from days 10 to 18 of incubation) compared to chickens exposed to a constant incubation temperature of 37.8°C (Yalçin *et al*., 2010).

Other studies (Yahav *et al*., 2004a,b; Collin *et al*., 2005, 2007; Tona *et al*., 2008; Tzschentke & Halle, 2009) applied short periods of high incubation temperatures from day 16 to 18 of incubation, when the axis in the brain related to thermoregulation is activated (Wise & Frye, 1973; Yahav *et al*., 2004a). Most studies (Yahav *et al*., 2004a,b; Collin *et al*., 2005, 2007; Tona *et al*., 2008) found no negative effects on hatchability or hatchling weight in broiler chickens and a positive effect on short-term thermotolerance (e.gs., up to 1 week of age). However, long-term effects on thermotolerance (e.gs., up to 6 weeks of age) do not appear to be influenced by thermal manipulation between day 16 and 18 of incubation (Collin *et al*., 2007; Tona *et al*., 2008). It can be concluded that in the future, thermal manipulation during incubation may be used in hot climates to maintain performance (Yalçin *et al*., 2010). However, when normal temperatures can be maintained during the grow out period, thermal manipulation can have a negative effect on subsequent performance (Yalçin *et al*., 2010).

***In ovo* feeding**

During in ovo feeding, an isotonic solution is injected in the amnion of the embryo around day 18 of incubation to improve the nutritional status of the embryo and chicken. The injected solution contains carbohydrates, proteins or a mixture of both. The in ovo feed is consumed by the embryo before pipping the air cell (Uni & Ferket, 2004). In practice, in ovo feeding is applied when chicken embryos are transferred from the setter to the hatcher. Hatchling weight can increase by 5 to 6% due to in ovo feeding (Uni *et al*., 2005; Foye *et al*., 2006). Furthermore, immune system development, health status, muscle development, and breast meat yield can improve due to in ovo feeding (Uni & Ferket, 2004; Uni *et al*., 2005). The effect of in ovo feeding is influenced by genetics, age of the breeder flock, egg size, and incubation conditions (Ferket, 2009). When incubation conditions fulfill the embryonic requirements, effects of in ovo feeding may be limited.

CONCLUSIONS

Incubation temperature, egg weight loss, and CO_2 concentrations need to be controlled to fulfill the specific embryonic requirements throughout incubation. Embryo and eggshell temperature need to be maintained between 37.5 and 38.0°C throughout incubation to optimize hatchability, hatchling quality, and subsequent performance. In a single-stage incubator, incubation conditions can be adjusted to the embryo requirements that change during development. Egg weight loss must be between 6.5 and 14.0% of the initial egg weight to obtain an adequate air cell size before the embryo internally pips. Effects of CO_2 concentrations during early and late incubation on hatchability, hatchling quality, and subsequent performance are ambiguous and need further practical and scientific investigation.

New insights related to the incubation process of broiler chickens are the preincubation warming profile, thermal manipulation, and *in ovo* feeding. These new insights show that the optimal situation for the embryo during incubation highly depends on the conditions of the eggs before (storage duration) and during incubation (environmental conditions) and the conditions of the chickens after hatching (environmental temperature).

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