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Kuluçka Döneminde Embriyo Gelişimini Etkileyen Çevresel Faktörler: Isı, Gaz Kompozisyonu, Oksidatif Yanıtlar ve Uygulamalı Yöntemler

Environmental Factors Affecting Embryo Development During Incubation: Temperature, Gas Composition, Oxidative Responses and Applied Methods

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ABSTRACT

The embryonic development process is highly sensitive to environmental conditions. The developing organism strives to survive by developing physiological and biochemical adaptation mechanisms in response to environmental demands. External variables encountered during this process can have various effects on the embryo's biological systems. These effects include many physical and chemical parameters such as temperature, humidity, gas composition (O_2 and CO_2), oxidative balance, and application methods. Historically, as defined by Hans Selye, organisms develop various adaptive responses to environmental changes. These adaptive responses vary among living taxa, but fundamentally, they manifest physiologically at different levels depending on the organism's capacity to adapt to the environment and create a certain level of load. Current experimental studies have revealed various environmental models that affect embryonic development. These models include parameters such as temperature fluctuations, humidity levels, oxygen-carbon dioxide ratio (O_2/CO_2), disruptions in oxidative balance, and the frequency of physical interventions. Temperature changes, in particular, have a significant effect on the physiological systems of birds. Variations in temperature can impact respiration, cardiovascular regulation, muscle development, and even exert effects at the epigenetic level. These environmental conditions may adversely affect the development of the embryo, reducing survival rates. Therefore, optimizing environmental parameters during incubation is of critical importance for both embryonic development and chick quality. In this review study, the effects of factors such as temperature, gas composition, oxidative responses, humidity level, and applied incubation methods on embryonic development have been discussed with literature support, particularly in birds, which are an important tax on both commercially and ecologically.

Keywords: Embryonic development, ıncubation, egg, oxidative stress, thermal stress

ÖZET

Embriyonik gelişim süreci, çevresel koşullara son derece duyarlıdır. Gelişen organizma, çevresel taleplere karşı fizyolojik ve biyokimyasal uyum mekanizmaları geliştirerek yaşamını sürdürmeye çalışır. Bu süreçte karşılaşılan dışsal değişkenler, embriyonun biyolojik sistemleri üzerinde çeşitli etkiler yaratabilir. Tarihsel olarak Hans Selye'nin tanımladığı şekliyle, organizmalar çevresel değişkenlere karşı çeşitli adaptif yanıtlar geliştirir. Bu adaptif yanıtlar canlı taksonları arasında farklılık göstermektedir ama temelde organizmanın çevreye uyum sağlama kapasitesine bağlı olarak farklı düzeylerde fizyolojik olarak etkisini göstermektedir. Günümüzde yapılan deneysel çalışmalar, embriyonik gelişimi etkileyen çeşitli çevresel modeller olduğunu ortaya koymuştur. Bu modeller arasında sıcaklık dalgalanmaları, nem seviyeleri, oksijen-karbondioksit oranı (O_2/CO_2), oksidatif denge bozulmaları ve fiziksel müdahale sıklıkları gibi birçok parametre bulunmaktadır. Özellikle ısı değişimleri, kuşların fizyolojik sistemleri üzerinde belirleyici bir etkiye sahiptir. Sıcaklık farklılıkları, solunum, kardiyovasküler düzenleme, kas gelişimi ve hatta epigenetik düzeyde bile etkiler yaratabilmektedir. Bu çevresel koşullar, embriyonun gelişimini olumsuz etkileyerek hayatta kalma oranını düşürebilir. Dolayısıyla, kuluçka sürecinde çevresel parametrelerin optimize edilmesi, hem embriyonik gelişim hem de yavru kalitesi

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açısından kritik öneme sahiptir. Dolayısıyla hem ticari hem de ekolojik olarak önemli bir takson olan kuşların bu derleme kapsamında; ısı, gaz bileşimi, oksidatif yanıtlar, nem seviyesi ve uygulamalı kuluçka yöntemleri gibi faktörlerin embriyo gelişimi üzerindeki etkileri literatür destekli olarak ele alınmıştır.
Anahtar kelimeler: Embriyonik gelişim, kuluçka, yumurta, oksidatif stres, termal stres

Introduction

Oviparous species have long been used by developmental biology to examine embryo maturation ex utero to obtain embryos at early developmental stages. The domestic chick embryo is one of the models that has yielded significant insights into organogenesis and development[1]. It has been demonstrated that the embryo's developmental process starts in the oviduct and ends when the embryo reaches the blastodermal and/or gastrulation stages of development at oviposition. Environmental factors throughout the pre- and incubation phases, as well as "maternal effects" can have an impact on bird embryos[2]. "Maternal effects" refer to non-genetic influences of the mother such as her nutritional status, age, hormonal profile, and the physiological conditions in the oviduct on the early developmental state of the embryo. These effects determine the initial developmental quality of the egg even before incubation begins, thereby shaping how the embryo will respond to incubation factors like temperature, gas exchange, humidity, and turning frequency[3,4].

Several key environmental factors play critical roles in determining embryonic development and chick quality during incubation. These factors including temperature fluctuations, controlled thermal manipulations, nutritional interventions, oxygen and carbon dioxide levels, oxidative stress, humidity, and egg turning interact to shape physiological and developmental outcomes. Embryos respond to these variables through adaptive mechanisms that regulate growth, organ development, metabolic activity, and post-hatch performance. On the other hand controlled optimization of these parameters not only enhances hatchability and chick quality but also supports physiological resilience and long-term growth trajectories.

Table 1 provides a comprehensive overview of the main environmental factors and their effects during incubation. The subsequent sections will examine each of these parameters in detail, with particular emphasis on temperature, oxygen and carbon dioxide balance, oxidative stress, humidity, and turning frequency.

Heat/ Temperature Level

While animal physiology is affected by the environment and conditions, the global warming problem, which is becoming increasingly dangerous today, brings heat and heat stress. In addition, one of the most important environmental factors that embryos may encounter during the incubation process is temperature changes in the incubator. There are studies in the literature showing that exposure to this condition, especially during the incubation process, influences vital activities[5,6]. Incubation temperature is an important parameter for both the development and vital activities of the embryo and is also important for its post-hatching performance.

Every living organism in nature responds differently depending on the severity and duration of stress. The embryo is directly affected by temperature fluctuations that include low and high temperatures. The temperature changes experienced during the transition from the incubator to the hatcher can affect the adaptive responses of physiological systems, especially respiration and thermoregulation, and may result in an altered thermotolerance that does not necessarily improve heat resistance, potentially making embryos more vulnerable to heat stress[6-8]. However, incubation temperature is one of the critical elements in determining the effects of other environmental factors[9].One of the periods when heat-temperature mechanisms are most intense is the summer months and is a period that causes negative effects on embryo development and egg production It is reported that heat stress causes effects such as lower than normal body weight, low chick quality, growth retardation, weakening of the immune system, unclosed umbilical cords, increased mortality rates and deterioration in heart functions in offspring[10-12]. In recent years, it has been reported that epigenetic approaches are increasingly used to understand the negative effects of thermal stress in poultry farming. In addition, there are studies that reveal the physiological effects of thermal stress[12-14].

Many studies reveal that thermal stress has serious negative effects on skeletal muscle development by triggering oxidative stress through the generation of ROS and lipid peroxidation in muscles[15,16]. In the study conducted by Li S. et al. (2024), the effects of thermal manipulation during embryonic development on mitochondrial function and muscle growth in broiler chickens were investigated. In this study, eggs were subjected to elevated incubation temperatures in designated windows, and subsequent analyses revealed alterations in muscle fiber cross-sectional area, satellite cell activity, and indicators of oxidative damage in skeletal muscle Although body temperature measurements were included, the key findings focused on the impairment of muscle development and mitochondrial performance under thermal challenge. These results imply that thermal stress during embryogenesis may disrupt skeletal muscle development and thus have lasting impacts on post-hatch performance[17].

Building on these findings, thermal stress has also been shown to exert differential effects based on the sex of the embryo. In the study conducted by Patrone L.G.A. et al., chicks were exposed to control temperature (37.5 °C) or high temperature (39 °C) during the first five days of incubation. The results demonstrated that female chicks exhibited higher respiratory rates and elevated body temperatures under hypercapnia and hypoxia conditions, indicating stronger adaptive responses compared to male chicks. Male chicks, in contrast, showed lower respiratory rates, hypoventilation, and less pronounced adaptation to heat stress. These findings suggest that female embryos may be more sensitive to thermal manipulation and mount more pronounced physiological responses to cope with heat stress[18].

Furthermore, Amjadian T. et al. reported that daily thermal manipulation at 39.5 °C for three hours did not significantly affect embryonic mortality or hatchability, but led to increased corticosterone and T4 levels, a higher female-to-male ratio, and reduced chick length. Collectively, these studies indicate that thermal stress during embryogenesis not only affects skeletal muscle development and mitochondrial function but also influences physiological adaptation, hormonal regulation, and post-hatch growth patterns, with some effects being sex-dependent. This study shows that heat stress is effective not only during the incubation period but also in later periods. However, there are also studies examining the effect of thermal stress on gender. The effects of thermal stress on gender were examined in the study conducted by Patrone LGA, et al. chicks were exposed to control temperature (CI, 37.5°C) and high temperature (HI, 39°C) during the first 5 days of incubation. The data obtained showed that female chicks showed higher respiratory rate (VE) at high temperature, their body temperature increased more easily, and their respiratory responses were stronger under hypercapnia and hypoxia conditions. In male chicks, lower respiratory rate, hypoventilation, and less pronounced adaptation to heat were observed compared to female chicks. The data obtained show that female chicks show more pronounced adaptive responses to heat stress and are more sensitive to thermal manipulation[18]. In the study conducted by Amjadian T, et al., it was observed that thermal manipulation application for 3 hours per day at 39.5°C did not affect embryonic mortality and hatchability. However, it has been reported that corticosterone level, T4 level and female chick ratio increase, and chick length shortens[19].

Chick quality and hatchability are two important dynamics that express the success of a hatchery[20]. In terms of industrial and livestock sectors, there are many studies aimed at improving and developing these two factors. Having an optimum temperature range during incubation and determining the exposure time are very important. Incubators with optimized temperature conditions can produce chicks that are more resilient, healthy and suitable for commercial production and continuity. In addition, it is stated that thanks to thermal manipulation, chicks have better thermal tolerance, lower mortality rates, grow faster and their immune systems are strengthened[21,22]. In addition, there are different studies showing that chick quality and hatchability can be increased through temperature manipulation during incubation, which improves the physiological and biological functions of the embryo[23,24]. Han G. et al. showed that thermal manipulation improves the metabolism and thermoregulation of hatched chicks and reduces the negative effects of high temperatures[25]. Literature studies show that as the metabolic rate of the embryo increases in the later stages of incubation, the amount of heat it produces also increases[26].

Exposure to high temperatures during incubation can negatively affect chicken embryos, potentially causing morbidity and mortality. In the literature, brief high-temperature exposure is typically described as acute heat stress, whereas prolonged or continuous exposure is considered chronic heat stress[27]. The ideal incubation temperature for chicken embryos is 37.8 °C, and temperatures above this value can negatively affect chick quality, hatchability, and post-hatching growth[28-30]. Thermal manipulations during the embryogenesis process, including growth and developmental physiology, produce positive results in breeder chicks, support healthy chick production, increase productivity, and improve hatching performance [31]. The data obtained show that controlled thermal manipulations can be a safe method for animal welfare. In addition, the above heat and temperature control studies show how important it is to apply heat stress in a controlled manner throughout the incubation process and the significant effects of optimized temperature. The application of nutrient or chemical agents to reduce the harmful effects of extreme temperatures on embryo development stands out as an important research area and includes different techniques. The spraying method is one of them and is applied to alleviate the negative effects of thermal stress on embryo development. In the study conducted by Abuoghaba AAK, Japanese quail eggs were exposed to short-term extreme heat stress during early embryonic development and then treated with a spray method containing betaine. The applied betaine spray supported the health and development of the embryo, reduced the performance loss caused by heat stress, and significant improvements were achieved in chick hatching weight, slaughter performance, hormonal balance and general health indicators[32]. Another study determined that eggs exposed to high temperature stress during incubation and sprayed with curcumin had positive effects on chick performance and T3 hormone levels[27].

Oxygen and Carbon Dioxide Levels

Another parameter that plays an important role in chick quality and embryonic development during the incubation process is oxygen and carbon dioxide levels[33]. Under normal incubation conditions, the CO2 concentration in the environment surrounding the eggs is initially approximately 0.05%, but this rate increases to 0.9% as embryonic development progresses. During the same period, oxygen O2 concentration is reported to decrease from 20.9% to 20.3%[34,35]. These metabolic changes are critical for proper embryonic development, as they directly affect the oxygen uptake and carbon dioxide excretion of the embryo. Commercial incubators generally aim to provide high ventilation rates and low CO2 levels in the environment, but this may not fully reflect the CO2 increase and O2 decrease seen in natural conditions. As a result, these machines may have negative effects on embryo development and chick quality. In this sense, appropriate CO2 regulation strategies should be investigated to support embryonic development and improve chick quality. Increasing CO2 levels in a controlled manner may help regulate metabolic processes necessary for normal embryo development. Therefore, optimizing CO2 levels in incubators may contribute to both healthier chicks and reduced embryonic mortality rates. Research on this subject will allow the development of new strategies that will make incubation processes more efficient.. Research on this subject will allow the development of new strategies that will make incubation processes more efficient. There are studies in the literature reporting that high CO2 concentrations in early or late incubation periods support embryo development, shorten the hatching time and cause rapid embryo development. It is stated that the embryo can tolerate high CO2 concentrations for a short time and this tolerance increases with the growth of the embryo During the first 4 days of incubation, approximately 3% CO2reduced embryo viability by 50%, while the unchanged concentration did not affect the hatching rate in the last stages of hatching[35]. During the first 4 days of incubation, approximately 3% CO2 reduced embryo viability by 50%, while the unchanged concentration did not affect the hatching rate in the last stages of hatching[36]. However, in the study conducted by De Smit L, and colleagues, not ventilating the incubator until day embryonic day 10 (E10) resulted in a higher carbon dioxide partial pressure in the air cell of the chick egg. Especially, in the non-ventilated groups, CO2 levels increased between embryonic day 11 (E11) and embryonic day 14 (E14), while O2 levels decreased. It has been reported that this increased CO2 level accelerates embryonic development and leads to positive outcomes such as higher embryonic body weight, earlier hatching and narrower hatching spread[37]. Furthermore, in the study conducted by Bruggeman V, et al., the effects of increasing CO2 to 1.5% between 25 and 96 h of incubation and maintaining it at this level for up to 240 h were investigated on embryonic growth, blood gas parameters and hatching parameters. High CO2 levels led to faster acidification of albumin *(egg white)* and a decrease in pH. Blood CO2 levels increased on embryo days 10 and 11, but pH did not change. The increase in plasma H_2CO_3 acted as a buffer to balance pH.

Embryonic growth accelerated, but chick weight did not differ between treatment groups. Namely, it has been reported that high CO2 levels accelerate embryonic growth but do not cause a difference in postnatal chick weight[38]. On the contrary, there are also studies reporting that exposure to high CO2 and low O2 concentrations has a serious effect on both embryo morphology and physiological development. In the study conducted by Everaert N, et al., acid sensitivity, genetic predisposition, incubation conditions and hatching performance were investigated. In the study, resistant (E) and acid sensitive (A) chick lines were incubated with typical or extreme CO2 conditions from embryonic day 10. The chicks were exposed to cold weather conditions from day 15. The results showed that line A had higher blood pCO2 levels, post-hatching body weight, hematocrit and plasma corticosterone concentrations, regardless of the incubation conditions. Incubation under high CO2 conditions reduced body weight, affected blood gases, increased hematocrit and decreased thyroxine and triiodothyronine levels. These effects were observed in both lines, and it was reported that CO2 incubation affected the metabolic programming of the hens and changed the offspring performance[39]. In the study conducted by Fernandes JIM, et al., the effects of different CO2 concentrations during incubation on live performance, heart morphology and blood cells were investigated. Eggs incubated at different CO2 levels for the first 10 days were then incubated with 4000 ppm CO2. In the evaluations made on the 42nd day, it was found that high CO2 levels did not affect the resistance of the chickens to variable temperature conditions, but the heterophil:lymphocyte (H:L) ratio increased with the increase in CO2[40]. In the study conducted by Okur N, et al., the effects of CO2 and O2 levels in incubators on egg weight, embryo mortality rate (EM) and hatchability rate of fertilized eggs (HFE) were investigated. A total of 1920 eggs placed in four incubators with different ventilation programs were incubated at various CO2 and O2 levels. The results showed that high O2 levels increased early embryo mortality and decreased hatchability, and egg weight made this effect more pronounced. These findings indicate that incubation O2 levels are as important as CO2[33]. De Smit L, et al. investigated the effects of not ventilating until day E10 of incubation on the physiological development of the embryo and postnatal developmental stages. Two different incubation conditions, adequate ventilation (V) and no ventilation (NV) incubators, were compared. CO2 levels in the NV incubator remained at high levels for the first 10 days. This led to an acceleration of embryo growth. NV embryos showed higher body weight, plasma corticosterone and T3 levels. Chicks incubated in NV condition hatched earlier and had higher body weight in the postnatal period. The results report that high CO2 levels produce epigenetic effects during hatching and early postnatal period[41].

Oxidative Stress

Oxidative stress is an imbalance resulting from increased levels of ROS generated during cellular metabolism - such as hydroxyl radicals, superoxide radicals, and hydrogen peroxide - and insufficient antioxidants to neutralize these substances. The shift in the balance between oxidant production and antioxidant defense in favor of oxidants disrupts the oxidative balance at the cellular level, which can lead to damage to cellular functions. It is known that developing embryos are extremely sensitive to oxidative stress[42]. Embryos grown in commercial incubators under different environmental conditions can be affected by parametric changes within the machine, and this can be associated with oxidative stress mechanisms. In the studies by Stock MK, et al., which examined the effects of oxygen access and oxidative stress on the embryo, chicken embryos were exposed to 15% and 60% O_2 for 72 hours. The effects of hyperoxia and hypoxia on oxidative stress parameters were assessed by measuring malondialdehyde (MDA) levels, a marker of lipid peroxidation. The data obtained showed that hyperoxia did not cause a significant increase in lipid peroxidation; however, it induced adaptive responses, increasing the embryo's capacity to cope with oxidative stress. It suggests that this situation strengthens the antioxidant defenses of the embryo and protects it from oxidative damage[43].

In order to understand and reduce the effects of oxidative stress that may develop during the incubation period, different substances have been investigated in the literature. Arsenic, an environmental toxin with serious effects, can affect health through drinking water and industrial pollution. Han and colleagues investigated the role of arsenic exposure in their study on neural tube defects (NTD), a developmental disorder. Their data revealed that arsenic-related NTD were associated with oxidative stress and epigenetic changes. Arsenic exposure decreased manganese superoxide dismutase activity and downregulated DNA methyltransferase (DNMT) 1 and 3A expression. In addition, decreased S-adenosylmethionine (SAM) levels and increased S-adenosylhomocysteine (SAH) levels were observed, and these were reported to contribute to DNA hypomethylation by causing a decrease in the SAM/SAH ratio[44]. Xiao X et al. investigated the protective effects of sodium selenite (SS) or selenomethionine (SM) in chick embryos exposed to oxidative stress. The obtained data reported that lower levels of oxidative stress markers and higher antioxidant enzyme activities were observed in chick embryos. In addition, SM supplementation was reported to be more effective than SS supplementation and more efficiently reduced oxidative stress[45] Elsayed M et al. investigated the effects and consequences of ochratoxin A (OTA) injection administered in ovo to the embryo on oxidative stress. OTA in-ovo application primarily decreased glutathione (GSH) levels in the liver and other organs and increased the expression of thiobarbituric acid reactive substances (TBARS), a marker of oxidative stress. Other examination parameters included increased enzyme activities in the liver, impaired liver function, decreased cholesterol levels, and degenerative changes in brain and liver tissues. This study reported that OTA in-ovo application had toxic and teratogenic effects on the embryo[46]. Thompson J, et al. investigated the effects of cadmium (Cd) in-ovo application on oxidative stress and teratogenesis. While it was reported that MDA levels associated with oxidative stress increased after in-ovo applications, it was reported that only N-acetyl cysteine among antioxidants decreased MDA levels to control values. In this context, it has been stated that Cd-induced teratogenesis and oxidative stress are not directly related[47]. Hilscherova K et al., in their studies examining the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and oxidative stress on teratogenicity and toxicity, reported that TCDD altered oxidative stress markers in the liver of chicks but was not effective in the brain. While lipid peroxidation and DNA damage increased in the liver, glutathione levels decreased. It has also been reported that antioxidants provide incomplete protection against oxidative stress caused by TCDD[48].

Humidity Level

Humidity is a concept that expresses the amount of water vapor in the air and is usually measured as a percentage (%). Especially during the incubation process, the humidity of the environment has a critical function in the growth of the embryo. Humidity level is an important factor that regulates the development of the embryo, the passage of respiratory gases and important steps for embryogenesis such as energy metabolism by affecting the evaporation rate of water inside the eggs. All embryonic life is completed in water as a whole and insufficient water in the environment stops life. As embryo development progresses during incubation, the egg loses water through evaporation. The total water cost incurred affects both hatching and the formation of cells that will provide embryonic lung ventilation. However, it has been reported that embryonic mortality increases when water decreases below 9.1% or above 18.5%[49].

Ideal humidity levels change during incubation. Barott HG et al. reported that a relative humidity (RH) of 54% during the first period of incubation is considered ideal for the continuation of the embryonic process[50]. Furthermore, the optimum low RH range is between 40% and 70% RH depending on the incubation period. The maximum hatching rate achieved has been stated as occurring at 50% RH[51]. Maintaining this level is of vital importance, especially for the healthy development and hatching of the embryo. In one study, it was reported that keeping the RH at 50–60% until embryonic day 19 (E19) of incubation and at approximately 70% from E19 until hatching increased hatching success[52]. Accordingly, there are studies in the literature using different humidity parameters to increase both chick quality and hatching rate. Peebles ED, et al. investigated the consequences of incubation humidity on embryo growth and chick weight from young breeders. Data from 26-week-old breeders indicate that chicks with lighter weight and lower RH have higher egg water loss. While embryogenesis is accelerated in young breeders, development is impaired, and chick performance is adversely affected at 43% RH[53]. In a study conducted by Bruzaul JJ, et al., the consequences of RH on eggs from young broiler breeders were investigated through incubation. Eggs collected from breeders aged 26, 28 and 30 weeks were incubated at 43%, 53% and 63% RH. Although hatching weight increased as RH increased, this difference in body weight decreased when the chicks were removed from the incubator after the incubation period was completed. Late embryo mortality was highest at 63% RH and the hatching rate from fertilized eggs was highest at 53% RH. Although high RH increased hatching weight, it adversely affected embryonic development[54]. Van der Pol et al. studied the effects of RH on embryo mortality rate, chick quality and egg weight loss through incubation. In this study, Egg Shell Temperature

(EST) was kept constant at 37.8°C from embryonic day 0 (E0) to embryonic day 18 (E18) and high (55–60%) or low (30–35%) RH was applied from embryonic day 2 (E2) onwards. The results showed that low RH reduced hatchability but had no major effect on chick quality and post-hatching performance[49]. In the study conducted by Bassareh M et al., it was reported that incubating eggs at 82.5% RH provided the highest hatchability compared to other RH levels[55].

Number of Cycles and Turning Frequency of the Incubator

Incubators are the most important tools for ensuring the initiation and continuation of embryonic development. These machines must be of efficient standards and used correctly to improve the formation and survival of healthy chicks, hatching rate, chick quality and the development of productive individuals in later periods and economic indicators. There are studies in the literature that correlate the turning frequency with embryo health. Deeming examined the results of egg turning on embryo growth in his study. In his study, he emphasized the role of egg turning, especially on the immune system, embryo growth and mortality rates. Deeming stated that turning before the 12th day is necessary for embryo and albumin *(egg white)* development but stopping the turning process after the 15th day can lead to negative effects, which can prevent the normal development of the embryo and increase mortality rates. Corticosterone levels were examined to test embryo stress and the highest corticosterone levels were reported to be in the turning stopped on day 15 (T15) group[56].

Turning can affect the position and developmental process of the embryo and therefore a certain turning frequency is critical for optimum development. In the study of Moraes TGV, et al., the effects of different positions and turning on egg weight loss, chick weight, hatchability and embryonic mortality rates through unnatural incubation of quail eggs were investigated. It was reported that eggs in the horizontal position during incubation had the highest hatching rate (77%). Data obtained from eggs incubated in positions other than this reported higher late embryo mortality rates. It was reported that egg position and turning influenced weight loss and water loss and that groups with better hatchability showed lower weight loss and higher chick weight [57]. Yoshizaki N. et al. reported that turning eggs thinned the limiting membrane, accelerated water permeability and that albumin *(egg white)* prevented the passage of water through the membrane[58]. It is stated that the number of turns plays an important role in embryonic growth as it prevents the embryo from adhering to the inner shell membrane and supports the absorption and metabolism of albumin and nutrients in the egg in the embryo[59-61]. It is clear that the number of egg turns in the machine during incubation is a physical factor that can affect the success and quality of hatching. Accordingly, it is noteworthy that there is no standard protocol on the frequency of turning among incubator manufacturers and researchers Freeman stated that the frequency of egg turning in commercial incubators is 24 times per day until embryonic day 18 (E18) of incubation[62]. On the contrary, recent findings show that eggs are typically turned 12 times per day throughout the incubation procedure[63,64].

Several studies have investigated the effects of egg turning frequency on hatchability and embryonic development. Although the optimum turning frequency was generally found to be 96 times per day, turning 24 times per day is often considered more practical under commercial conditions due to the relatively small differences in hatchability between the two frequencies [65–68.] Robertson IS et al. showed that no turning or very low turning frequency resulted in poor hatching, whereas very high frequencies (up to 480 times per day) caused only slight decreases in hatchability. Wilson HR reported that turning 96 times per day provided the maximum yield, but 24 times was sufficient for commercial purposes, with the most beneficial period for turning being between days 3 and 7[66]. Similarly, Elibol O and colleagues confirmed that 96 times per day was optimal for hatchability, but 24 times per day was more practical under market conditions[65]. Oliveira et al. also found that reducing turning frequency decreased hatching rates and increased early and late embryonic mortality, while 24 times per day provided the highest hatching rate[68].

Table 1. Environmental and Applied Effects of Incubation Conditions on Embryo Development

Factor	Treatment / Condition	Effect / Outcome	Sex / Notes
Heat / Thermal Stress	39–39.5°C first 5 days	↑ ROS, skeletal muscle & mitochondrial damage, growth retardation	Females higher adaptation
Heat / Thermal Manipulation	39.5°C, 3 h/day	↑ Corticosterone & T4, ↓ chick length	Females > Males response
Heat + Nutritional	Betaine spray	↑ Hatch weight, ↑ performance, improved hormonal balance	–
Heat + Nutritional	Curcumin spray	↑ T3 hormone, ↑ performance	–
CO_2 Increase (Early)	3% CO2 first 4 days	50% embryo reduction; later hatching unaffected	Short-term tolerated
CO_2 Increase (No Ventilation)	NV until E10; CO2 rise E11–E14	↑ Embryonic weight, earlier hatching	O2 decreased; metabolism ↑
CO_2 1.5%	25–96 h, maintained to 240 h	Faster albumin acidification, ↓ pH, accelerated growth	Plasma H_2CO_3 buffered
Extreme CO2 + low O2	Exposure from E10	↓ Body weight, altered blood gases & hormones	Metabolic programming affected
High O2	High O2 incubation	↑ Early embryo mortality, ↓ hatchability	Heavier eggs more affected
Oxidative Stress	Arsenic	↑ MDA, ↓ SOD & DNMT	Neural tube defects
Oxidative Stress	Sodium selenite / Selenomethionine	↓ Oxidative stress, ↑ antioxidant enzymes	SM more effective
Oxidative Stress	OTA / Cd / TCDD	↑ Lipid peroxidation, ↓ GSH	Toxic / teratogenic
Humidity	50–60% RH → 70% until hatching	↑ Hatching success	Stage dependent
Humidity	43%, 53%, 63% RH	↑ Hatching weight at higher RH, late embryo mortality highest at 63%	–
Turning / Cycles	24–96 turns/day	Adequate hatchability, prevents adhesion to shell	24/day sufficient for industry

Cd: Cadmium; DNMT: DNA methyltransferase; ED: Embryonic day; H_2CO_3: Carbonic acid; MDA: Malondialdehyde; NV: No Ventilation; OTA: Ochratoxin A; RH: Relative Humidity; ROS: Reactive Oxygen Species; SM: Selenomethionine (organic selenium compound); SOD: Superoxide dismutase; T3: Triiodothyronine hormone; T4: Thyroxine hormone; TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin

Conclusion

Environmental factors such as temperature, gas composition, oxidative stress, humidity, and turning frequency play a critical role in embryonic development, chick quality, and hatchability. Controlled applications, including thermal manipulation, nutritional supplementation, and optimized incubation practices, can improve physiological adaptation, growth, and survival of embryos. Moreover, these interventions may have broader implications for human health and food quality by enhancing the nutritional value of poultry products and reducing stress-related residues. Future research integrating environmental management and biotechnological approaches may provide more sustainable, efficient, and safer poultry production systems.

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