doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Veterinaria México OA
Publicación Digital de la Facultad de Medicina Veterinaria y Zootecnia
https://veterinariamexico.fmvz.unam.mx/

Non-Ventilation Conditions During Incubation at High Altitude Modify Embryonic Development and Hatchability of the Broiler Breeder's Eggs

Marco A. Juarez-Estrada[1*]
0000-0003-4376-0651
Erick I. López-Ruiz[1]
0009-0006-0276-4727
Sonia López-Córdova[2]
0009-0008-3609-3188
Guillermo Tellez-Isaias[3]
0000-0002-2416-2747
Omar F. Prado Rebolledo[4]
0000-0001-8802-0177

[1] Universidad Nacional Autónoma de México. Facultad de Medicina Veterinaria y Zootecnia. Departamento de Medicina y Zootecnia de Aves. Ciudad de México, México.

[2] Centro Nacional de Servicios de Diagnóstico en Salud Animal. Dirección General de Salud Animal-Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria. Estado de México, México.

[3] University of Arkansas. Division of Agriculture. Department of Poultry Science. Arkansas, EEUU.

[4] Universidad de Colima. Facultad de Medicina Veterinaria y Zootecnia. Tecomán, Colima, México.

*Corresponding author
Email address:
britoco@unam.mx

Abstract

The current research explores the impact of gradually increasing high carbon dioxide (CO_2) levels during the first 10 days of incubation at high altitude. Two ventilation conditions were compared. In the first, CO_2 concentration gradually accumulated by the embryo's own metabolism in a non-ventilated (NV) incubator during the first 10 days of embryonic development (ED10). In the second condition, the incubator was normally ventilated (V). Both treatments received V conditions for the remainder of the incubation period. The CO_2 concentration in the V incubator remained at 0.13 % during the first ED10 days, whereas in the NV, the concentration gradually increased from 0.14 % to 0.9 %. Throughout the incubation, NV exhibited significantly lower ($P < 0.05$) embryonic mortality compared to V. Remarkably, the hatchability of fertile eggs (HFE) was 10 % significantly higher ($P < 0.05$) in NV conditions than V group. NV conditions at high altitude (2 230 m) produced embryos with heavier yolk-free body mass and a progressive trend of lighter yolk-sac weights from ED10 day until hatch. The weight of hatchlings in the NV treatment was 43.4 g, with a length of 17.5 cm, both traits were were significantly higher ($P < 0.05$) than hatched chicks from the V group (41.5 g and 17.2 cm respectively). The NV condition at high altitude positively influenced the quality of hatchling chicks. We conclude that the NV condition, with a gradual increase of CO_2 concentration during the first 10 days of incubation at high altitude, is preferable to the V conditions.

Keywords: Embryonic mortality; Egg mass loss; Hatchability of fertile eggs; Hypercapnia; Hatchling chick quality.

Submitted: 2024-08-30
Accepted: 2025-03-03
Published: 2025-03-31

Additional information and declarations can be found on page 20

Cite this as:

Juarez-Estrada MA, López-Ruiz EI, López-Córdova S, Tellez-Isaias G, Prado Rebolledo OF. Non-ventilation conditions during incubation at high altitude modify embryonic development and hatchability of the broiler breeder's eggs. Veterinaria Mexico OA. 2025;12. doi: 10.22201/fmvz.24486760e.2025.1399.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Study contribution

The current study highlights the critical role of oxygen and carbon dioxide exchange during the first half of the domestic fowl embryonic development performed at high altitudes. The impact of non-ventilation conditions in the first 10 days of incubation on broiler breeders' fertile eggs was addressed. Restricting ventilation into the incubator allows CO_2 from the embryo´s metabolism to build up. The results show a significant increase in hatchability due to lower embryo mortality, and better chick 1-day-old quality in the non-ventilated group compared to standard ventilation. These findings suggest that controlled CO_2 increase during the early incubation stage can optimize hatchery outcomes. The current methodology is especially valuable for hatchery managers operating at high altitudes, where environmental factors such as oxygen levels and CO_2 concentrations directly influence embryonic development. Understanding these effects can lead to more effective incubation practices, ultimately improving hatchability and the overall health of newborn chicks.

Introduction

Embryonic development (ED) in poultry is influenced by a combination of biotic and abiotic factors, such as genetic background and environmental conditions.[1] The genetic background plays a crucial role in shaping the growth and development of poultry, as evidenced by the distinct characteristics observed in broilers and layers.[2–4] Selective breeding over the years has resulted in significant differences between these two types of chickens, impacting their ED during incubation.[3, 5, 6] The abiotic factors that influence ED requirements for successful incubation include temperature, relative humidity (RH), air movement, air velocity, and the gaseous composition of the environment.[6, 7]

The gaseous environment is particularly important since it can influence the developmental trajectories of physiological regulatory systems in poultry.[6, 8–10] It has been proposed that altering the environment of a developing organism might change the developmental trajectories of some physiological regulatory systems. This phenomenon is known as heterokairy, a specific type of plasticity that describes environmentally driven, altered timing of development within a species.[11, 12] Plasticity in the timing of the onset of developmental events occurs at the individual level during their development.[12, 13]

To achieve optimal embryo development, adequate oxygen levels and the removal of sufficient carbon dioxide are needed.[14] Chronic hypoxia during critical phases of chick embryo development can have varying effects.[15] Chronic hypoxia during critical phases of chick ED can impact embryo survival, body weight, and cause developmental abnormalities.[13, 16–18] It is noteworthy that hypoxia has been observed to accelerate the developmental process of chick embryos, leading to earlier hatching attributed to the earlier maturation of the surfactant system.[13, 17, 19, 20] Hypoxia delays the onset of all cardiovascular responses during the development of certain vertebrate species,[21] and shifts the timing relative to the developmental program. This event illustrates what some researchers describe as the third form of heterokairy: where the onset of a functional regulatory system is moved by altering both the onset of regulation and the length of the developmental program differentially.[8, 11–13]

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

In nature, during early avian embryo development, chronic hypercapnia, with concentrations up to 1 % CO_2, is more prevalent than hypoxia.[9] The atmospheric air contains 0.03 to 0.04 % CO_2 and approximately 21 % O_2. However, CO_2 concentrations can exceed 1 % under breeders during natural incubation, whereas the CO_2 concentration in ambient air remains at only 0.03 %. Although the supply of both O_2 and CO_2 during incubation is crucial for the development of chick embryos and their hatching process, it is also recognized that O_2 consumption and CO_2 production increase as the embryo develops.[22, 23] Research indicates that the role of CO_2 in embryonic development is complex, with studies focusing on factors such as O_2 exchange and pH levels rather than solely on elevated CO_2 levels at the beginning of the ED period.[6, 10]

The atmospheric pressure decreases at high altitude which affects, the partial pressure (Pa or mmHg) of individual gases such as O_2 and CO_2.[24, 25] However, the percentage composition of these gases remains relatively constant, while blood parameters may not change significantly; the development of respiratory organs such as the heart and lungs could be influenced by the O_2 concentration in the incubator, especially regarding the altitude setting during incubation.[16, 26–29], Oxygen and, consequently, CO_2 concentrations are known to influence embryo mortality and hatchability during incubation at high altitudes.[16, 23, 25, 26] In order to compensate for insufficient O_2, hypertrophy is observed, leading to the development of abnormal and overgrown organs.[15, 16, 18, 26] Therefore, it is necessary to compensate for the oxygen content in the air (23–25 %) or increase the atmospheric pressure in the setting at high altitude.[28, 30] However, both measures show technological challenges and dangerous risks. In addition, in high-altitude regions such as India, Pakistan, Iran, China, Turkey, South America, and Mexico (2 200–4 000 m), very poor hatchability rates have been reported.[22, 23, 25]

Traditionally, chicken hatching eggs are incubated in an environment containing 21 % oxygen and 0.5 % carbon dioxide.[22, 31] However, several reports suggest that higher CO_2 levels than those currently used (0.1–0.5 %) for artificial incubation of poultry eggs may be beneficial for embryo development and hatchability, depending on the timing of application during incubation.[8, 22, 23, 27, 32]

The presence of higher CO_2 concentrations during incubation can influence hatchability, embryo weight, and the physiological development of chicken embryos, highlighting the significance of gas exchange conditions for optimal embryonic growth and development.[8–10, 32] Carbon dioxide plays a more significant role in the incubation process of chicken eggs than previously believed.[8, 10, 26] While it was traditionally thought that CO_2 is harmful to the developing embryo, this belief may have been due to the assumption that the embryo requires fresh air and plenty of oxigen for optimal chick development and hatchability.[9, 28, 30] Indeed, with atmospheric CO_2 levels typically around 0.03–0.04 %, and the average measured CO_2 under the hen at around 0.4 %, it is evident that hens incubate their eggs to maintain a certain level of CO_2 above what is found in the ambient environment. Higher CO_2 levels during the incubation process have been shown to stimulate ED during the beginning of the incubation period.[8, 23, 26, 32, 33]

Modifying the ventilation conditions to allow natural increase of CO_2 during the first 10 d of the ED in the incubator has been shown to result in higher absolute

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 2025

and relative body weight (to the egg weight) from ED10 to ED18.[8, 23, 34] This suggests that higher CO_2 levels may accelerate growth and elevate levels of corticosterone and plasma thyroxine.[8, 10, 32–35] El-Hanoun et al.[35] found that duck breeder eggs incubated in a closed incubator with a carbon dioxide concentration of 1 % at the end of incubation exhibited higher HFE and duckling's weights, indicating that the NV condition with circulation of CO_2 for the first 10 d of incubation in ducks is preferable to the V condition.

In a study, incubating Ross 308 young breeder´s eggs at high altitude in an air-tight incubator allowing the CO_2 level to rise to 1.2 % at ED10 followed by a normal incubation procedure, there was 7 % improvement in HFE compared to the control.[22] Recently, Fares et al.[32] discovered that high carbon dioxide levels (0.9 %) during early ED (0–9 d) contributed to earlier and narrower spread of hatch, higher HFE and hatched chick weight compared to the control V group. While the effects of CO_2 levels on egg hatchability have been fairly documented in the literature, the assumption in the incubation industry that increased levels of CO_2 at the beginning of incubation at high altitude enhance hatchability and chick quality is not yet supported by enough scientific results.[22, 23, 26, 36]

The research goal was to investigate whether the manipulation of ventilation had any impact on the natural evolution of CO_2 levels in the incubator, embryo growth, viability, hatching events, and the quality of one-day-old broiler chicks. The current study also evaluated whether allowing CO_2 concentration to naturally increase without ventilation during the first 10 days, followed by normal ventilated incubation until hatch, had clear beneficial effects on hatchability and embryo survival at an altitude of 2 230 meters.

Materials and methods

Ethical statement

The experimental procedures reported in this experiment were carried out according to the Internal Committee for the Care and Use of Animals (Comité Interno para el Cuidado y Uso de Animales, CICUA) of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Nacional Autónoma de México.

Broiler breeder eggs

The current study was performed using fertile hatching eggs from a flock of 37-week-old Ross 308 breeders. The breeder farm was located at 1 350 m of altitude in Jiutepec, Morelos, Mexico. A total of 420 hatching eggs were collected and stored with the pointed end down in a cool room (18 °C and 75 % RH) for 3–7 days before setting in the incubators. All egg trays were transferred to experimental incubation setting located in Mexico City at 2 230 m of altitude. Upon arrival, all eggs were randomly assigned to two groups of equal size, identified, weighed, and placed in ten forced-draft commercial incubators (Hova-Bator® Mod. #1583 GQF Inc. Savannah, Georgia, USA).

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Experimental design

The first group of hatching chick eggs was incubated in a normally ventilated incubator (V) throughout the incubation period. The second group of hatching eggs was incubated in a non-ventilated incubator (NV) during the first ten days of incubation. The air-tight condition was achieved by closing 8/12 bottom vent dampers and two top outlets of the commercial incubator with Polypropylene adhesive Tuk®. Both treatments were kept under the same V condition from the tenth day of embryonic development (ED10) to ED18. A total of 42 eggs were incubated in each treatment, with five replicates per treatment. For the first two days all eggs were incubated at 100.0 °F dry bulb, from 3 to 7 days at 99.9 °F, from 8 to 10 days at 99.7 °F, from 11 to 13 days at 99.5 °F, from 14 to 15 days at 99.3 °F, 16 and 17 at 99.0 °F, and from 18 to 21.5 days at 98.4 °F. In V condition from ED1 to ED18 days, and in the NV conditions from ED10 to ED18 days wet bulb was kept at 84.5 °F.

Eggs were turned every hour from 1 to 18 days of incubation. At 444 h of incubation, the eggs were weighed and candled. Those with living embryos were transferred to fixed-hatching baskets. The eggs were hatched in the same incubators converted into hatchers. The wet bulb of hatcher from ED18 to hatch was kept at 90.0 °F. The incubators were monitored six times daily to ensure proper operation. To accurately track hatch time, the time when the eggs were set in every incubator was recorded as hour zero. Concentrations of CO_2 and O_2 within each machine were measured three times a day (morning, afternoon, and night) using an infrared (NDIR) sensor and a galvanic cell sensor, respectively (Analox®, Analox Inst. Ltd. The Vale, London W3 7QE, UK). Prior to every measurement, the gas analyzers were calibrated in the same manner using atmospheric air and precision calibration gases.

Hatching egg and embryonic mass weight

Eggs were individually weighed at 0, 10 and 18 days of incubation. The percentages of egg weight loss (EWL) during different incubation intervals (0—10, 10—18 and 0—18 days) were calculated for each ventilation condition. Fifteen eggs from each incubator were randomly picked to calculate EWL using the following equation:

$$EWL = \left(\frac{EW\ at\ every\ sample\ d\ ED\text{-}EW\ of\ same\ egg\ at\ ED0}{EW\ of\ same\ egg\ at\ ED0} \right) \times 100$$

The ratio of yolk-free embryo body weight and yolk-sac weight to egg weight was determined in 5 eggs randomly sampled per incubator at 10, 12, 14, 16, and 18 days of incubation, and 9 chicks per hatching basket. The wet and dry weights of the yolk-free embryo, yolk-sac, heart, and liver of every sampled egg and newly hatched chick were measured according to the methodology previously described by Willemsen et al.[37] Briefly, after careful separation of the embryo and yolk-sac, both were weighed (Figure 1 at left). Embryos were weighed after excessive fluid was dried off using absorbent paper (Versi-Dry® Thermo Scientific). For the purpose of drying the embryo, internal organs, skin, and extra-embryonic membranes were excluded, while the complete yolk-sac was dried as one piece. The yolk-free embryo, yolk-sac, heart, and liver were dried through desiccation by heating them in a dry oven set at 140 °F for approximately 72 h, until reaching a stable final weight (Figure 1 at right).

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Figure 1. Yolk-free embryo mass and yolk-sac were separated at 10, 12, 14, 16, and 18 days of incubation and in newly hatched chicks (left). Desiccation was performed by heating them in a dry oven (right).

Hatching events

Following transfer to the hatching baskets on day 18, eggs were individually monitored at 2-h intervals, starting at 468 h and continuing for 48 h. During this period, the internal pipping, external pipping, and hatching were recorded for each egg. Chick hatchlings that had fully emerged from their eggs, exhibiting healed navels and dryness around the head and neck, were removed. For each egg, the incubation duration was defined as the period between setting and hatching. At the end of incubation, the hatchability of fertile eggs (HFE) was calculated according to the following equation:

$$HFE = \left(\frac{Total\ of\ hatchlings\ chicks}{Total\ fertile\ eggs} \right) \times 100$$

The hatchability of total eggs set (HES) was also determined using the following equation:

$$HES = \left(\frac{Total\ of\ hatchlings\ chicks}{Total\ eggs\ set} \right) \times 100$$

After each collection of hatched chicks, they were weighed to the nearest 0.1 g, measured for length, and received a quality score under random double-blind conditions. The hatch window was recorded as the time elapsed between the first and last chick hatched within each treatment group.

Embryonic mortality

Eggs that failed to hatch after 21.5 days of incubation were opened and examined macroscopically to estimate the infertility rate and assess the developmental stage reached before the embryo died. The time of embryonic death was estimated in days to the extent feasible. The embryonic mortality percentage, expressed as a

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 2025

percentage of fertile eggs set, was recorded and classified into different periods of the ED. Early embryonic mortality occurs during stage I (days 1 to 7 of ED), mid embryonic mortality during stage II (day 8 to 17 of ED), late embryonic mortality during stage III (days 18 to 21 of ED) and cases with pipped but unhatched eggs before 516 hours were classified as stage IV.

Quality score grading newly hatched chicks

All one-day-old chicks were individually pulled out and numbered after the entire batch of chicks had hatched. Every chick was examined macroscopically to identify traits associated with excellent, good, average, poor, and unacceptable quality. This methodology assessed chick quality based on field observations of various physical conditions crucial for successful chick development. The scoring of newly hatched chick quality was based on thirteen comprehensive characteristics (Table 1). These traits included physical conditions, such as body dryness and cleanness, activity level, appearance of eyes, retracted yolk-sac, remaining yolk-sac, conformation of legs, tarsometatarsus and toes integrity, appearance of the navel, presence of remaining membranes and debris, appearance of the vent, hydration condition, body weight and chick length (Table 1).

The quantitative traits, body weight and chick length, were recorded for each hatched chick. Chicks were individually weighed in grams. To measure chick length, each chick was positioned face down on a flat surface, ensuring that the neck and right leg were fully extended to their maximum length. Chick length was defined as the distance from the tip of the beak to the point where the nail is attached on the third toe (Figure 2).[34, 38–40] All these methods are described in Table 1.

The grading score assigned to every newly hatched chick was used to create an index of chick hatchling quality. Every parameter was scored based on the subsequent performance of the birds during their rearing at the farm, as described previously by Tona et al.[41] and Willemsen et al.[34, 42] Discrete traits were scored on a scale of 0 to 100 points, as outlined in Table 2.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Table 1. Assessment of different characteristics to index newly hatched chicks quality

Parameter	Assessment
Down and appearance	The 1-day-old broiler chick was examined for dryness and cleanness. It was regarded as normal if it is dry and clean. If it is wet or dirty or both (which can be a source of contamination), then it is not a normal condition.
Activity	It was assessed by laying the chick hatchling on its back to determine how quickly it returned to its feet. A quick spring back onto its feet was regarded as good, but dragging back onto its feet or remaining on its back was assessed as weak or very weak.
Eyes	The newborn broiler chick was put in front of the observer. The condition of eye brightness and the wideness of the gape of the eyelids were determined.
Retracted yolk	The hatchling was put on its back obliquely on the palm until abdominal movement stopped. The height and consistency of its abdomen were estimated through direct touching and gentle pressing. If the height of the abdomen was estimated to be higher and harder to touch than normal, then retracted yolk was regarded as large and consistent.
Remaining yolk	Observation of the navel area allowed estimation of the size of any remaining yolk. The size of any remaining yolk-sac was classified as large (clear detection), small (almost no detectable), or no yolk-sac.
Legs	The broiler chick was put on its feet to determine if it remained upright well. If the chick remained upright with difficulty, articulations of the knees were examined to detect signs of inflammation, redness, or both.
Tarsometatarsus and toes	The broiler chick was put on its feet, and the tarsometatarsus and toes were examined for their integrity. The toes were analyzed for how straight or crooked they were.
Navel	The navel and its surrounding areas were examined for closure and coloration conditions. If the color was different from the skin color, it was regarded as poor; if this had an appearance like a navel button or a leaky navel, it was very bad.
Remaining membrane	Observation of the navel area allowed estimation of the size of any remaining membrane or debris. If present, the size was classified as large, small, or no membrane and debris.
Vent appearance	The broiler chick's cloacal area was examined for cleanness grading. It was regarded as normal if it is clean. If it was wet, with adherence of chalky white material, dirty or vent pasting or both (which can be a source of contamination), then it was not good.
Dehydration	The assessment of the skin and vascular vessels of the neck, wing and leg allowed estimation of dehydration condition. It was regarded as none, mildly dry, or severely dry.
Weight	All one-day-old broiler chicks were individually weighed in grams.
Lenght	The one-day-old broiler chick was laid on its ventral side, with the neck and right leg extended to their maximum length. Chick length was defined as the length from the tip of the beak to the point where the nail is attached on the third toe.

Figure 2. Newly hatched chick length. The chick was positioned face down, ensuring that the neck and right leg were fully extended to their maximum length.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Table 2. Assignment of scores to different quality traits observed in hatchling broiler chicks

Parameters	Characteristics	Scores
Down and appearance	Clean and dry Wet Dirty and wet	8 4 0
Activity	Good Weak Very weak, chick remained lying down	8 4 0
Eyes	Both opened and bright Opened and not bright One or both closed	8 4 0
Retracted yolk	Chick body with a normal swallowed yolk-sac Chick body with a regular swallowed yolk-sac Chick body with a large yolk-sac, and rather than being hard to touch	8 4 0
Remaining yolk	No yolk-sac Small yolk-sac Large yolk-sac	8 4 0
Legs	Normal legs One infected leg or swelling of the hock joint Two infected legs or swelling of both hock joints	8 4 0
Tarsometatarsus and toes	Normal toes Lighter twisted toes Twisted toes	8 4 0
Navel	Completely closed and clean Unhealed (< 1.5 mm) and not discolored Unhealed (> 1.5 mm) and discolored, black navel button or leaky navel	10 4 0
Remaining membrane and debris	No membrane or debris Small membrane Large membrane	8 4 0
Vent appearance	Clean Wet Dirty or vent pasting	8 4 0
Dehydration	None Skin dry and wrinkled Skin severily dry and wrinkled	8 4 0
Length 36–45 week-old Broiler breeders	≥ 18 cm 15–18 cm ≤ 15 cm	10 4 0
Weight 36–45 week-old Broiler breeders	≥ 40 g 37–40 g ≤ 37 g	8 4 0

The quality score rank assignment was as follows: 91–100 points = Excellent, 81–90 = Good; 71–80 = Average; 61–70 = Poor, and < 60 = Unacceptable. The final score allowed grading each percentage rank in each ventilation treatment (V and NV). All measurements were performed in a double-blind manner, meaning that each parameter was measured for all chicks taken in random order from the entire batch before the next parameter was assessed.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 2025

Statistical analysis

The data were collected and analyzed using an ANOVA with a general linear model (GLM) (SAS/STAT 9.2. SAS Institute Inc., Cary, NC, USA). The response traits included egg weights at setting, the weight of the yolk-sac-free embryo body, the weight of the embryo yolk-sac, the weights of the heart and liver, and the weight of the newly hatched chicks in grams, as well as the length of the newly hatched chicks in centimeters. Before the statistical analysis, data from hatching events, chick hatchling quality, and egg weight losses underwent an arcsine square root transformation. All of this data was also analyzed using an ANOVA via the GLM. When significant differences were revealed between treatments, *post hoc* pairwise comparisons were conducted using Tukey's test ($P < 0.05$) to discriminate specific differences among NV and V treatment groups. All values were expressed as mean ±standard deviation (SD). Chi-square test of independence was employed for assessing embryonic mortality stages (I, II, III, and PUH), as well as quality scoring on one-day-old broiler chicks. Statements of statistical significance were based on a threshold of $P < 0.05$.

Results

Incubation environment

Figure 3A shows a lower (0.13 %) but steady CO2 concentration in the V condition throughout the first 10 d of embryonic development, whereas the NV condition showed a gradual increase in CO_2 concentration from the outset of incubation until ED10, reaching a CO_2 concentration of 0.88 % at ED10 (Figure 3). During the first 10 days of embryonic development, the V condition showed a higher O_2 concentration of 19.85 %, which was significantly greater ($P < 0.05$) than the 19.55 % of O_2 recorded in the NV treatment. From ED10 until hatching, the NV condition exhibited higher CO_2 concentration at 13, 14, and 21 d of embryonic development (Figure 3A), whereas both ventilation conditions maintained similar levels of O_2 during this period (Figure 3B). The hatchery room maintained an average temperature of 24.4 °C and an RH of 53.3 % throughout the experimental period. O_2 levels were measured at 19.88 %, with CO_2 concentration at 0.10%.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

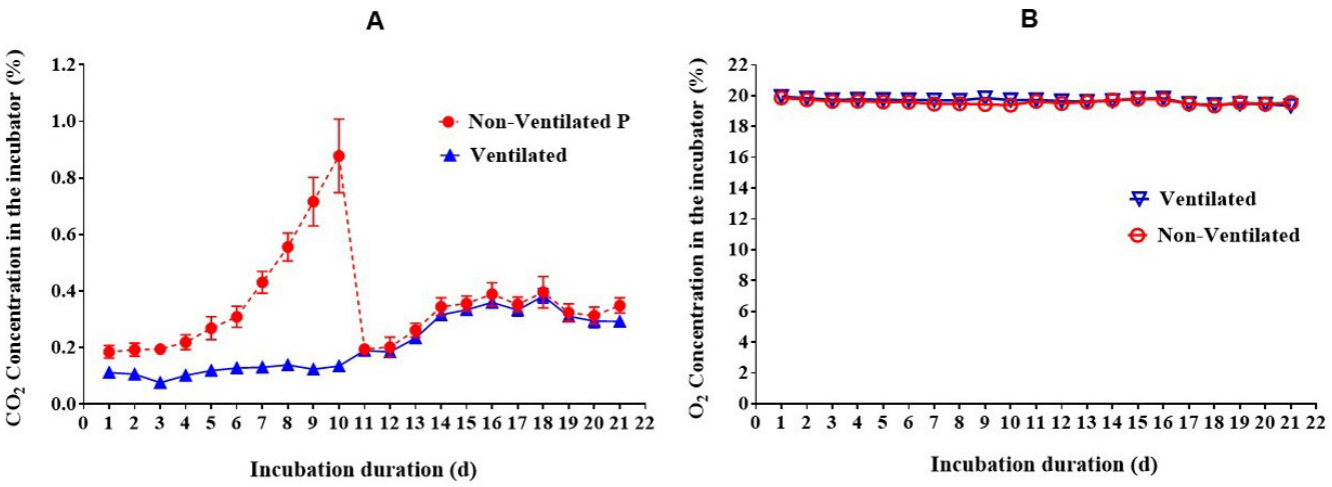

Figure 3. Dynamics of the CO_2 (A) and O_2 concentration (B), into the air-tight and standard incubators (n = 5).

Egg-weight losses

The mean egg weight at the outset of incubation was 61.3 ± 3.9 g in the V group, and 61.9 ± 3.5 g in the NV group, with no significant difference observed between the treatments. In the V condition, the EWL at ED10 was measured at 6.23 ± 1.16 %, significantly higher ($P < 0.05$) than the 5.47 ± 0.89 % recorded in the NV group (Table 3).

Table 3. Egg-weight loss of broiler breeder's hatching eggs

Egg weight variable	Ventilated	Non-ventilated [1]
Egg hatching weight at set (g)	61.34 ± 3.98	61.86 ± 3.50
Egg weight at day ED10 d (g)	58.27 ± 4.00	59.19 ± 3.51
Egg-weight loss at ED10 d (%)	6.23 ± 1.16[a]	5.47 ± 0.89[b]
Egg weight at day ED18 (g)	54.28 ± 3.77[b]	55.41 ± 3.61[a]
Egg-weight loss at ED18 d (%)	11.65 ± 1.71[a]	10.47 ± 1.57[b]
Egg-weight loss ED10-ED18 (%)Ψ	4.92 ± 0.71	5.28 ± 0.75

[1] Non-ventilated air-tight incubator condition achieved through Polypropylene Tuk® tape during the first 10 days of embryonic development.
[a, b]Means (± SD) in the same row with different superscript letter are significantly different ($P < 0.05$).
n = 75 Broiler eggs by group Ψ Ventilated (n = 26) Ψ Non-ventilated (n = 28)

When all eggs were transferred to baskets, the V group exhibited a mean EWL of 11.65 ± 1.71 %, which was significantly higher ($P < 0.05$) than the 10.47 ± 1.57 % observed in the NV group. At the same time, the mean egg weight in the V group was significantly lower ($P < 0.05$) compared to the mean egg weight recorded in the NV group. Egg-weight loss from ED10 to ED18 did not exhibit any difference between treatments (Table 3).

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Hatchings events

In the V condition, the HFE was 56.71 %, which was significantly different ($P < 0.05$) to the 66.93 % of HFE observed in the NV group (Table 4). The hatchability of all eggs set in the V condition was 53.18 %, which was significantly lower ($P < 0.05$) compared to the 57.95 % recorded in the NV group (Table 4).

Table 4. Incubation variables and embryo mortality

Incubation variables	Ventilated	Non-ventilated [1]
Fertility (%)$^{\Psi}$	92.26 ± 4.91	92.26 ± 7.87
Hatchability from fertile eggs (%)$^{\Psi}$	56.71 ± 10.19^{b}	66.93 ± 12.96^{a}
Hatchability of all eggs set (%)$^{\Psi}$	53.18 ± 10.01^{b}	57.95 ± 9.33^{a}
Early embryo mortality (%)$^{\Phi}$	17.62^{a}	16.32^{b}
Middle embryo mortality (%)$^{\Phi}$	17.09^{a}	9.33^{b}
Late embryo mortality (%)$^{\Phi}$	17.35^{a}	7.43^{b}
Pipped but unhateched (%)$^{\Phi}$	1.20^{a}	0.0^{b}
Total embryo mortality (%)	53.26	33.08

[1]Non-ventilated air-tight incubator condition achieved through Polypropylene Tuk® tape during the first 10 days of embryonic development.
a, b $^{\Psi}$Means (± SD) in the same row with different superscript letters are significantly different ($P < 0.05$).
a, b $^{\Phi}$Means in the same row with different superscript letters are significantly different ($P < 0.05$).
n = 210 Broiler hatching eggs by group.

The embryos in the NV group started hatching 11.5 h earlier ($P < 0.05$) than those in the V group. The hatch window was 49 h for chick hatchlings in the NV treatment and 41 h for the V treatment, with no significant difference observed between groups.

Embryonic mortality

During the first ED stage, no significant difference in embryo mortality was observed between the NV and V conditions. Results in Table 4 revealed differences in embryo mortality between NV and V treatments from the middle stage onward. The NV treatment showed a middle embryo mortality rate of 9.33 %, which was significantly lower ($P < 0.05$) compared to the 17.09 % observed in the V group (Table 4). Chick embryos that died in the late stage (18–21 days of ED) were predominantly observed in the V condition (17.35 %), and this was significantly higher ($P < 0.05$) compared to the 7.43 % of embryo mortality observed in the NV group (Table 4). In the NV group, no eggs were recorded as pipped but unhatched (0 %), a value significantly lower than the 1.20 % observed in the V group. The higher total embryonic mortality was observed in group V (53.26 %), which was significantly higher than the 33.08 % observed in the NV group (Table 4).

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Embryonic development and newly hatched chicks

The one-day-old broiler chick weight from the NV group, at 43.41 ± 1.84 g, was significantly heavier ($P < 0.05$) than the 41.51 ± 1.58 g recorded in those chicks from the control V group. However, there was no significant difference in the length of hatched broiler chicks between the two ventilation conditions (Table 5).

Table 5. Quality grade scoring of one-day-old broiler chicks from ventilated and non-ventilated conditions during the first half of incubation at high altitude

Chick quality feature	Ventilated	Non-ventilated[1]
High quality (%)	0 ± 0	0 ± 0
Good quality (%)	24.72 ± 8.80[b]	44.44 ± 11.11[a]
Middle quality (%)	33.89 ± 10.46	31.11 ± 4.97
Poor quality (%)	25.28 ± 10.41	24.44 ± 9.29
Unclassified (%)	16.11 ± 6.92[a]	0 ± 0[b]
Chick hatchling mass (g)	41.51 ± 1.58[b]	43.41 ± 1.84[a]
Chick hatchling lenght (cm)	17.20 ± 0.60	17.48 ± 0.96

[1]Non-ventilated air-tight incubator condition achieved through Polypropylene Tuk® tape during the first 10 days of embryonic development.
[a, b]Means (± SD) in the same row with different superscript letter are significantly different ($P < 0.05$).
n = 45 Chick hatchlings/group

Yolk-free body mass from the NV treatment, whether fresh or dry, was consistently heavier compared to the control V group from ED10 to ED18, and this trend was maintained in the hatched broiler chicks (Figures 4–7).

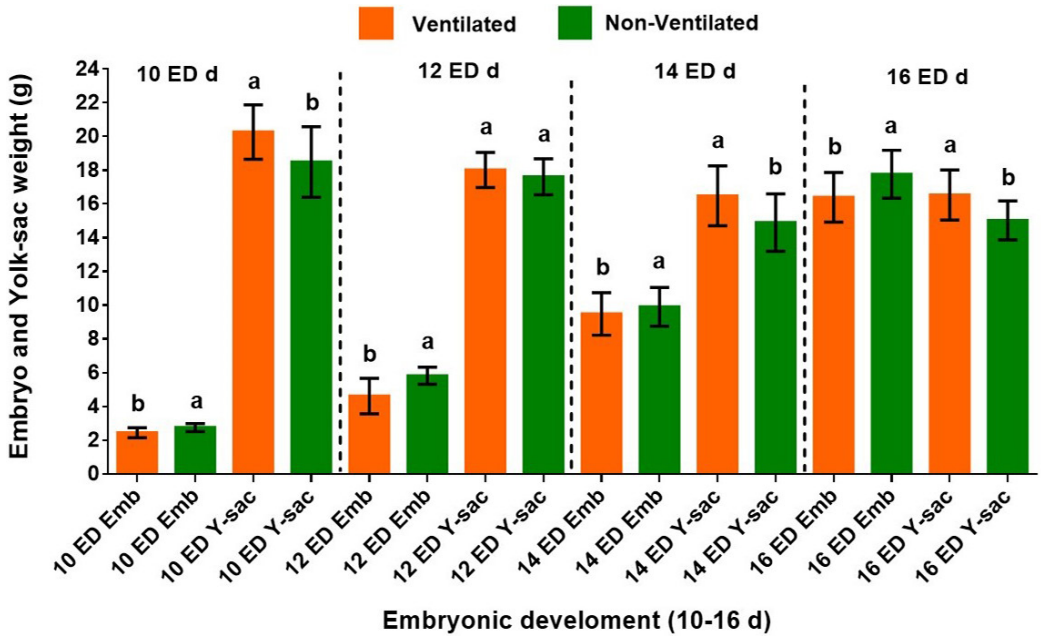

Figure 4. Effect of non-ventilation during the first ten days of incubation at high altitude on wet yolk-free body mass and wet yolk-sac mass at 10, 12, 14 and 16 days of embryonic development (n = 25).

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 2025

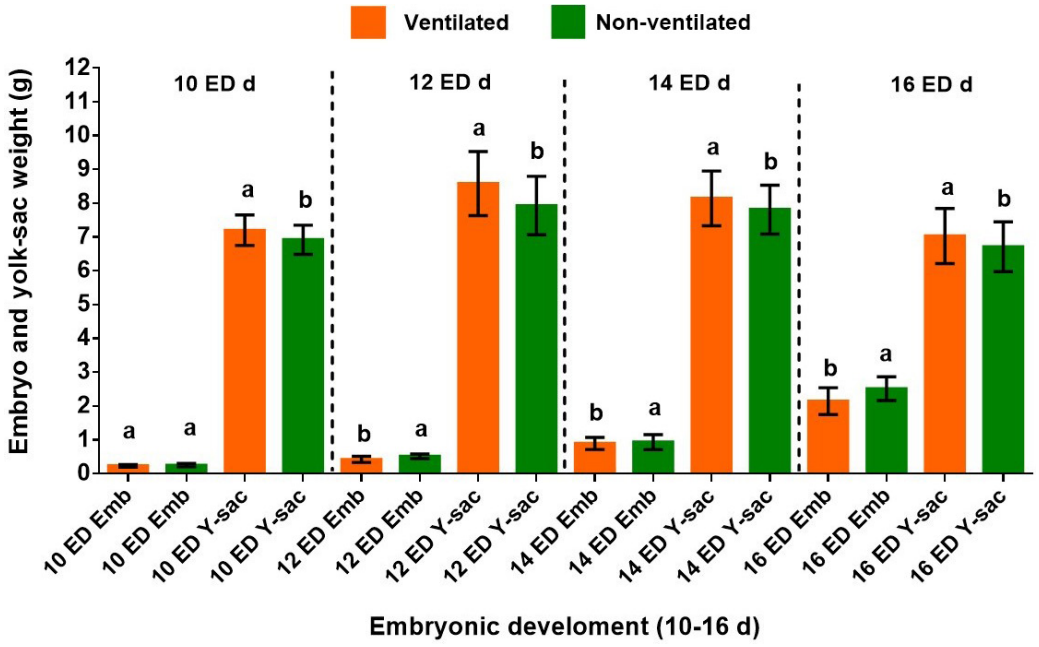

Figure 5. Effect of non-ventilation during the first ten days of incubation at high altitude on dry yolk-free body mass and dry yolk-sac mass at 10, 12, 14 and 16 days of embryonic development (n = 25).

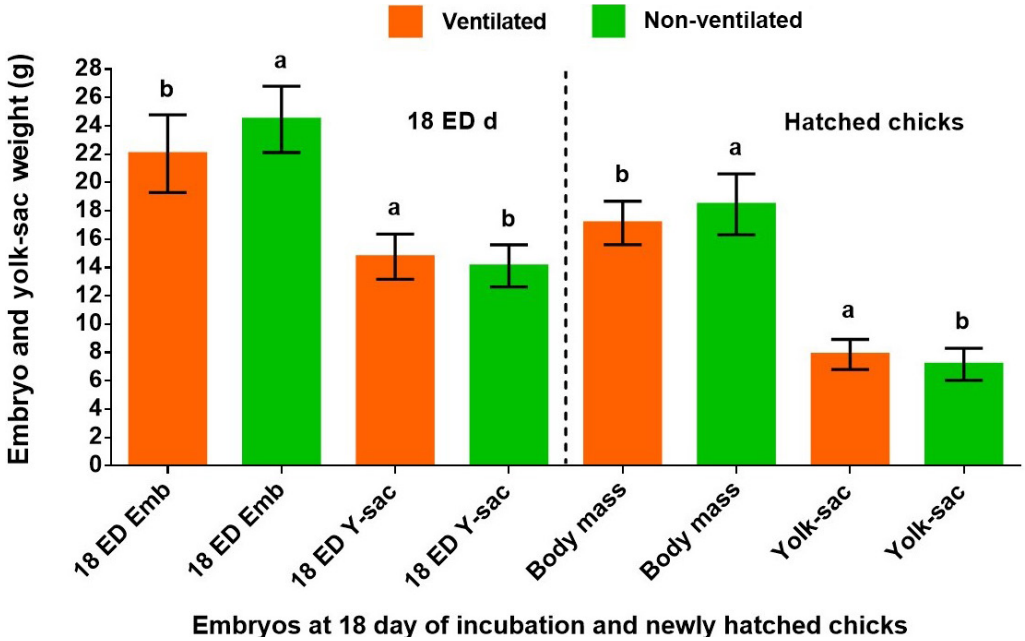

Figure 6. Effect of non-ventilation during the first ten days of incubation at high altitude on wet yolk-free body mass and wet yolk-sac mass at 18 days of embryo development and hatching time (ED18 n = 25; one-day-old chicks n = 45).

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

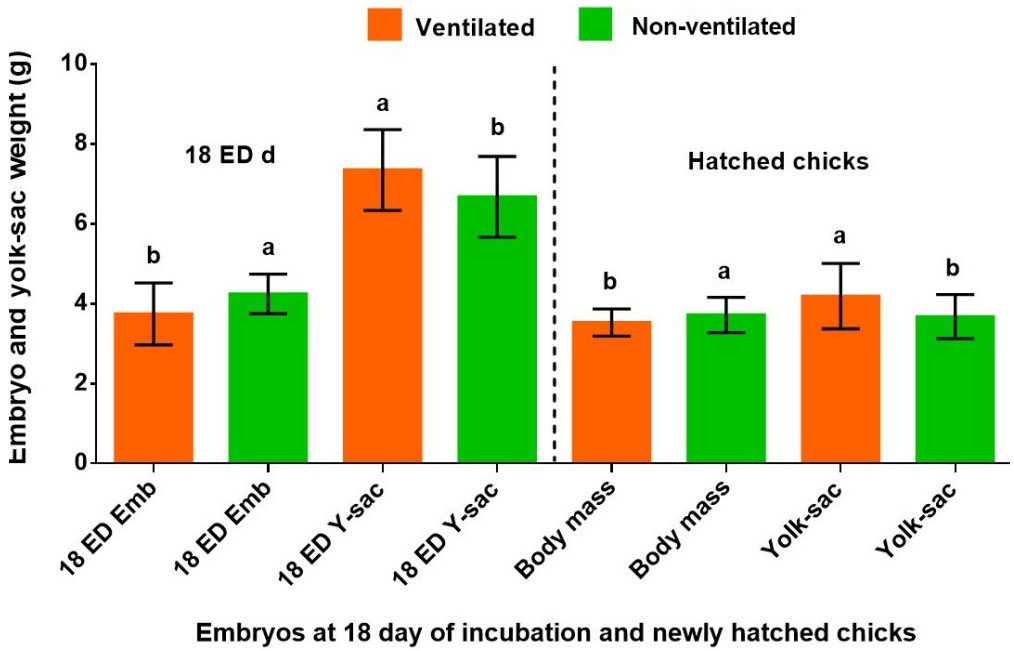

Figure 7. Effect of non-ventilation during the first ten days of incubation at high altitude on dry yolk-free body mass and dry yolk-sac mass at 18 days of embryo development and hatching time (ED18 n = 25; one-day-old chicks n = 45).

Otherwise, fresh and dry yolk-sac mass from the control V group was heavier ($P < 0.05$) compared to that from NV group at 10, 14, 16, and 18 ED days, as well as in hatched broiler chickens (Figures 4–7). The fresh yolk-sac mass from the control V group did not show any difference at ED12 compared to the fresh yolk-sac mass from the NV group. Dry yolk-sac mass did not show any difference between groups, only at ED10 d (Figure 5). Hearts from embryos from ED12 d to ED16 d were significantly heavier ($P < 0.05$) in the NV group compared to those from the control V group, as well as in the newly hatched chicks (Table 6). At ED18 d, there were no differences in heart weight between groups. However, the liver was significantly heavier ($P < 0.05$) in the NV group compared to that from the control V group from ED12 d to newly hatched chicks (Table 6).

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Table 6. Heart and liver weight (g) of embryos and newly hatched chicks during the first half of incubation at high altitude

Quality features	Ventilated	Non-ventilated [1]
Heart weight on ED12 dΨ	0.054 ± 0.01[b]	0.067 ± 0.01[a]
Liver weight on ED12 dΨ	0.089 ± 0.03[b]	0.107 ± 0.05[a]
Heart weight on ED14 dΨ	0.091 ± 0.02[b]	0.125 ± 0.02[a]
Liver weight on ED14 dΨ	0.194 ± 0.02[b]	0.220 ± 0.04[a]
Heart weight on ED16 dΨ	0.120 ± 0.02[b]	0.140 ± 0.02[a]
Liver weight on ED16 dΨ	0.300 ± 0.06[b]	0.320 ± 0.05[a]
Heart weight on ED18 dΨ	0.240 ± 0.02	0.240 ± 0.03
Liver weight on ED18 dΨ	0.420 ± 0.10[b]	0.530 ± 0.11[a]
Heart weight on hatchlingsΦ	0.320 ± 0.04[b]	0.350 ± 0.04[a]
Liver weight on hatchlingsΦ	0.900 ± 0.11[b]	0.950 ± 0.09[a]

[1]Non-ventilated air-tight incubator condition achieved through Polypropylene Tuk® tape during the first ten days of the embryo development.
[a, b]Means (± SD) in the same row with different superscript letters are significantly different ($P < 0.05$).
Ψ n = 25 Embryos/group Φ n = 45 Newly hatched chicks/group

Hatchling chick quality

None of the chickens received a high-quality grading score, and while there was a slight trend toward better quality in the NV-hatched chicks, ultimately, there was no significant difference between the groups (Table 5). Forty-four percent of newly hatched chicks from the NV condition received a good-quality score, a percentage significantly higher ($P < 0.05$) than the 24.7 % of hatched chicks from the control V group receiving the same ranking score (Table 5). Otherwise, the control V group showed significantly higher proportion ($P < 0.05$) of newly hatched chicks in the unclassified ranking score (16.1 %), compared to the NV group.

Discussion

The lower hatchability observed in the standard V group, incubated at higher altitude, could be attributable to elevated rates of middle and late embryonic mortality, as well as pipped but unhatched chicks. In contrast, the NV group exhibited significantly reduced embryo mortality during the last two stages of embryonic development. The NV group displayed higher embryo weights from the 10th day of ED until hatch, suggesting a more robust ED than those observed in the control V group.

According to Willemsen et al.[34] embryos surviving the early NV condition exhibit enhanced morphophysiological conditions that enable them to accelerate their ED compared to embryos from the V condition. Several research groups have indicated that a gradual increase in CO_2 from 0.8 % to 1.5 % during the first half of chicken incubation accelerates ED, enhances embryo morphophysiological conditions, and contributes to increased hatchability and quality of hatched chicks.[8, 22, 23, 26, 32, 43] Bruggeman et al.[43] assessed hypercapnia conditions (NV) in the first half of incubation and found accelerated embryonic growth, increased embryo weight, faster hatching, and enhanced quality of the hatched chicks. These findings are similar to the results obtained in our NV group. However, Bruggeman

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 2025

et al.[43] reported no significant improvement in hatchability or a decrease in the total embryonic mortality rate.

The precise reasons for the inconsistent improvement in hatchability parameters observed throughout all studied cases with increased of CO_2 during early incubation are not yet fully understood.[8, 22, 23, 27, 32, 35, 36, 43] Some studies have highlighted serious problems in the incubation of commercial broiler breeders, especially at high altitudes.[16, 22, 25, 27, 28, 30] For this reason, hatcheries for commercial breeder genotypes are generally established at low altitudes. Since local genotypes are adapted to high altitudes, there are not many problems with their incubation.[25, 44] However, this situation can be problematic in geographical areas with high altitudes and the need for commercial broiler chicks.[16, 22, 25, 27]

Leghorn chicks can oxygenate their hemoglobin fully at 2 500 to 3 000 m of altitude. However, broiler chicks showed low arterial blood oxygen even at low altitudes. Recently, Juárez-Estrada et al.[23] showed that high CO_2 during the first half of ED in Leghorn breeders´ eggs produced positive results on major hatchability traits, but these were not as high as the outcomes observed here with Broiler breeders´ eggs. Higher CO_2 during the first half of incubation could have more positive outcomes for high-altitude broiler breeder hatcheries than for other genotypes.

The beneficial effects of an early NV condition on hatchability seem to be attributed to several factors including genotype, breeding stock age, and even the altitude of the incubation setter, rather than solely the CO_2 concentration achieved during the first half of the incubation period.[4, 8, 22, 23, 26, 35] The 0.9 % CO_2 concentration reached in our NV group during the first half of incubation at high altitude when compared to that in the control V group, resulted in a gradual increase of benefits, including accelerated ED, improved hatchability, and increased weight and quality of newly hatched chickens. This is aligned with the observations made by De Smit et al.,[8] who applied NV incubation on embryos from 60-week-old broiler breeders and achieved 1.0 % CO_2 concentration remarkably similar to CO_2 levels recorded in our NV incubators.

De Smit et al.[8] observed higher hatchability and increased embryo weights from ED10 to ED17 when compared to their control V group. In the NV condition, the weight of yolk-free body mass consistently exceeded that of embryos in the V group throughout the incubation period. Similarly, the weight of dry yolk-free body mass followed an identical pattern for almost the whole experiment. NV incubation resulted in accelerated embryonic growth and utilization of the primary energy reserves, particularly from the yolk-sac. From the 10^{th} d of ED onward, as the embryo's weight increased, the volume and weight of the yolk-sac decreased. Yolk-free body mass serves as a more reliable indicator of post-hatch broiler development than embryo body weight, because it includes the weight of the residual, unmetabolized yolk.[40, 45]

According to Wolanski et al.,[45] there is an important correlation between wet and dry mass weight for the yolk sac compared to the embryo, a pattern that closely aligns with our findings. The NV group's accelerated embryo development suggests the embryo's ability to grow faster in conditions of hypercapnia and relative hypoxia throughout the first half of ED. The environmental conditions applied in the NV group could contribute to maximizing the growth and functionality of the chorioallantoic membrane (CAM).[15, 17, 29, 43] After 10 days of incubation, early stimulation for enhanced CAM development and functionality might result in improved oxygen

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

uptake during the exponential phase of ED (second half of ED). This could explain the beneficial effects of early hypercapnia and relative hypoxia in the later stages of ED observed in our NV group, perhaps enhancing the rate of energy supply from the yolk-sac through biochemical mechanisms such as fatty acid beta-oxidation and gluconeogenesis.[17, 29, 45–47]

As ED progresses during natural incubation, the CO_2 concentration increases from 0.04 % to 0.5 %, while the O_2 levels decrease from 20.9 % to 20.3 %, as noted by Walsberg.[48] Similarly, our study found a similar trend of O_2 declining over the first ten days of ED. The NV group experienced a decrease from 19.8 % to 19.5 %. Conversely, the V group did not show any decrease in O_2 levels. Rahn et al.[44] discovered that gas levels in the nest do not remain constant throughout natural incubation and often change as ED progresses. Hens play a crucial role in maintaining the nest temperature by constantly monitoring the egg's temperature in nature. Once the embryos reach a stage when they can generate their heat autonomously, hens prefer to leave the nest more frequently to drink and feed, especially after the embryos' endothermic period (1—10 days of setting).[49] Hens improve ventilation by leaving the nest more frequently, which increases CO_2 removal and oxygen supply surrounding the nest eggs. This process continues progressively until hatching.[44, 48, 49]

The embryos in the NV group consistently weighed more than those in the V group. Willemsen et al.[34] observed that the weight of chicks at hatch had the highest predictive value for post-hatch performance, surpassing even the length measurement of the chick when it was implemented as the sole predictive metric measurement. In the current study, the NV group showed the highest weights for one-day-old chicks. However, the chick length was found not to differ between the groups. Wolanski et al.[40, 50] found a correlation ($r = 0.56$) between chick length and yolk-free body mass at hatch. However, several other authors have expressed doubts regarding the usefulness of chick length as a meaningful indicator of chick quality.[34, 51]

The NV treatment enabled the attainment of heavier yolk-free embryos along with a progressively lighter yolk-sac weight, a phenomenon previously reported by several research groups.[8, 22, 23, 40, 43] This may be attributed to the embryo's elevated early metabolic activity, particularly in tissue formation, and the fulfillment of energy requirements for its own development.[45, 46, 52, 53] The weight and volume of the remaining yolk-sac serve as measures of both the energy invested in the egg and the energy used by the embryo during its development.[45, 53–55] According to Moran[56] starting from the 12^{th} d of ED, the gain in yolk-free body mass exhibits a negative correlation with yolk-sac weight. The embryos in the NV group exhibited a relatively smaller proportion of yolk-sac compared to the yolk-free body mass, suggesting that the chick hatchlings in this group may have matured earlier. In addition to increasing yolk-free body mass, surviving NV embryos exhibited higher heart and liver weights than the control V group. The observed increase in liver and muscle in NV embryos at 18 ED days suggests elevated storage of glycogen, indicating a more robust ED, as previously suggested by Druyan.[57] The glycogen storage in the liver and glycolytic muscles is needed to fuel the "pipping" and hatching processes and serve as the main energy source until the newly hatched chick can access external food.[45, 46, 54, 55, 58]

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Early incubation under NV not only generates optimal CO_2 and O_2 profiles but also promotes uniformity in eggshell temperature (EST). These combined effects promote the achievement of optimal ED and post-hatch growth.[37, 52, 53, 59] Post-hatch growth and organ function can be impaired if growth rates during ED deviate from the optimal EST.[6, 52, 59, 60–62] NV incubation helps to mitigate temperature oscillations caused by the forced exchange of fresh air from outside, which is commonly observed during conventional V incubation.[7, 22, 25, 60, 62] NV incubation ensures a balanced thermal environment over the first half of the incubation period.[22, 61] This ventilation approach is specifically applied during the most critical growth phase of the ED (1—10 d of ED), where keeping a warm and uniform EST is critical.[6, 7, 49, 52, 60, 61] Indeed, wet air transfers heat more efficiently than dry air.[7, 60] As a result, restricting ventilation during the first 10 d of ED promotes a uniform, humid and warm environment throughout the incubation period. This is particularly important at high altitudes, where the air is dry and cold.[7, 22, 23, 25]

A single exposure to a certain environment during early embryonic growth can have long-lasting consequences for the life of birds.[7, 11, 13, 33, 62] In the current study, the NV condition contributed to optimizing the subsequent development stages of embryos exposed to hypercapnia and relative hypoxia at an early stage.[10, 61] Some research groups have proposed that the high CO_2 content during the early stages of ED may exert a specific influence on the pH of the albumin.[36, 43, 47, 63, 64] Albumin pH is affected by an early upregulation in the expression of pH-dependent enzymes, such as carbonic anhydrase, which plays a critical role in the early stages of ED.[29, 47] Early hypercapnia enhances the rupture of the chalaziferous membranes, causing a rapid loss of hardness in both the dense and aqueous phases of the albumen.[65] Furthermore, early hypercapnia has been shown to improve the formation of sub-embryonic fluid during the early stages of ED.[66] This phenomenon could potentially contribute to improved early ED and subsequently enhance hatchability outcomes.[8, 22]

Moreover, genotype, breeder flock age, and egg storage time have been shown to influence albumin pH, chalaziferous membrane breakdown, and the optimal production of sub-embryonic fluid.[65, 66] These interactions may explain the disparities in the effects of early hypercapnia observed throughout different studies, perhaps leading to contradictory conclusions.[10, 23, 26, 32, 33, 36] Further research will hopefully reveal more precisely the exact mechanisms by which CO_2 modifies early ED. Meanwhile, the development of novel incubation programs that consider optimal concentrations of O_2 and CO_2 during specific stages of ED for poultry breeding of different ages and genotypes is essential.

Conclusions

The gaseous environment, specifically elevated carbon dioxide concentrations during critical incubation periods at high altitude, improves embryonic development, optimizes quality, and increases body weight in one-day-old broiler chicks. Incubating fertile eggs with increased CO_2 levels at high altitudes for the first ten days resulted in 10 % higher hatchability than the standard ventilated group. This method offers an effective strategy for increasing hatchability and the quality of newly hatched broiler chicks, particularly in high-altitude commercial breeder hatcheries.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

Data availability

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Acknowledgments

The authors are grateful to Professor Dr. Bilgehan Yilmaz-Dikmen, Bursa Uludağ University, Görükle Bursa Turkey, for her valuable discussion and helpful suggestions on the preparation of the current manuscript.

Funding statement

The research was supported by the National Autonomous University of Mexico (www.unam.mx), grant number PAPIIT IN220909-3 "The increased CO_2 during early incubation modifies poultry embryonic-development", and was granted to MA Juarez-Estrada. The funder had no involvement in the study's design, data collection and analysis, publication decisions, or preparation of the manuscript.

Conflicts of interest

The authors have no conflict of interest to declare concerning this publication

Author contributions

Conceptualization: MA Juarez-Estrada.
Data curation: EI Lopez-Ruiz, MA Juarez-Estrada.
Formal analysis: EI Lopez-Ruiz, MA Juarez-Estrada.
Funding acquisition: MA Juarez-Estrada.
Investigation: EI Lopez-Ruiz, S Lopez-Córdova, MA Juarez-Estrada.
Methodology: EI Lopez-Ruiz, MA Juarez-Estrada.
Project administration: MA Juarez-Estrada.
Resources: MA Juarez-Estrada.
Supervision: MA Juarez-Estrada, G Tellez-Isaias.
Validation: MA Juarez-Estrada, G Tellez-Isaias, S Lopez-Córdova.
Visualization: MA Juarez-Estrada, OF Prado-Rebolledo.
Writing-original draft: MA Juarez-Estrada, EI Lopez-Ruiz.
Writing-review and editing: MA Juarez-Estrada, G Tellez-Isaias, OF Prado-Rebolledo.

References

1. Mortola JP, Al Awam K. Growth of the chicken embryo: implications of egg size. Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology. 2010;156(4):373–379. doi: 10.1016/j.cbpa.2010.03.011.
2. Cooper CB, Voss MA, Ardia DR, Austin SH, Robinson WD. Light increases the rate of embryonic development: implications for latitudinal trends in incubation period. Functional Ecology. 2011;25(4):769–776. doi: 10.1111/j.1365-2435.2011.01847.x.
3. Ho DH, Reed WL, Burggren WW. Egg yolk environment differentially influences physiological and morphological development of broiler and layer chicken embryos. Journal of Experimental Biology. 2011;214(4):619–628. doi: 10.1242/jeb.046714.
4. Bilalissi A, Meteyake HT, Kouame YAE, Oke OE, Lin H, Onagbesan O, *et al.* Effects of pre-incubation storage duration and nonventilation incubation pro-

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

cedure on embryonic physiology and post-hatch chick performance. Poultry Science. 2022:101(5):101810. doi: 10.1016/j.psj.2022.101810.

5. Bednarczyk M, Dunislawska A, Stadnicka K, Grochowska E. Chicken embryo as a model in epigenetic research. Poultry Science. 2021;100(7):101164. doi: 10.1016/j.psj.2021.101164.
6. Molenaar R, Reijrink IAM, Meijerhof R. Van den Brand H. Meeting embryonic requirements of broilers throughout incubation: a review. Brazilian Journal of Poultry Science. 2010;12:(3):137—148. doi: 10.1590/S1516-635X2010000300001.
7. Boleli IC, Morita VS, Matos Jr JB, Thimotheo M, Almeida VR. Poultry egg incubation: integrating and optimizing production efficiency. Brazilian Journal of Poultry Science. 2016;8(spe 2):1—16. doi: 10.1590/1806-9061-2016-0292.
8. De Smit L, Bruggeman V, Tona JK, Debonne M, Onagbesan O, Arckens l, *et al.* Embryonic developmental plasticity of the chick: increased CO_2 during early stages of incubation changes the developmental trajectories during prenatal growth. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology. 2006;14582):166—175. doi: 10.1016/j.cbpa.2006.06.046.
9. Everaert N, Kamers B, Witters A, de Smit L, Debonne M, Decuypere E, Bruggeman V. Effect of four percent carbon dioxide during the second half of incubation on embryonic development, hatching parameters, and posthatch growth. Poultry Science. 2007;86(7):1372—1379. doi: 10.1093/ps/86.7.1372.
10. Tona K, Voemesse K, N'nanlé O, Oke OE, Kouame YAE, Bilalissi A, *et al.* Chicken incubation conditions: role in embryo development, physiology, and adaptation to the post-hatch environment. Frontiers in Physiology. 2022;13:895854. doi: 10.3389/fphys.2022.895854.
11. Spicer JI, Burggren WW. Development of physiological regulatory systems: altering the timing of crucial events. Zoology. 2003;106(2):91—99. doi: 10.1078/0944-2006-00103.
12. Rundle SD, Spicer JI. Heterokairy: a significant form of developmental plasticity? Biology Letters. 2016;12(9):20160509. doi: 10.1098/rsbl.2016.0509.
13. Blacker HA, Orgeig S, Daniels CB. Hypoxic control of the development of the surfactant system in the chicken: evidence for physiological heterokairy. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2004;287(2):R403—R410. doi: 10.1152/ajpregu.00399.2003.
14. Ar A, Deeming DC. Roles of water and gas exchange in determining hatchability success. Avian Biology Research. 2009;2(1—2):61—66. doi: 10.3184/175815509x431830.
15. Mortola JP. Gas exchange in avian embryos and hatchlings. Comparative Biochemistry and Physiology, A: Molecular and Integrative Physiology. 2009;153(4):359—377. doi: 10.1016/j.cbpa.2009.02.041.
16. Hassanzadeh M, Fard MHB, Buyse J, Bruggeman V, Decuypere E. Effect of chronic hypoxia during embryonic development on physiological functioning and on hatching and post-hatching parameters related to ascites syndrome in broiler chickens. Avian Pathology. 2004;33;(6):558—564. doi: 10.1080/03079450400013188.
17. Chan T, Burggren W. Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (*Gallus gallus*). Respiratory Physiology & Neurobiology. 2005;145:(2—3):251—263. doi: 10.1016/j.resp.2004.09.005.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

18. Tintu A, Rouwet E, Verlohren S, Brinkmann J, Ahmad S, Crispi F, *et al.* Hypoxia induces dilated cardiomyopathy in the chick embryo: mechanism, intervention, and long-term consequences. PloS ONE. 2009;4(4):e5155. doi: 10.1371/journal.pone.0005155.
19. Sharma SK, Lucitti JL, Nordman C, Tinney JP, Tobita K, Keller BB. Impact of hypoxia on early chick embryo growth and cardiovascular function. Pediatric Research. 2006;59(1):116–120. doi: 10.1203/01.pdr.0000191579.63339.90.
20. Haron A, Ruzal M, Shinder D, Druyan S. Hypoxia during incubation and its effects on broiler's embryonic development. Poultry Science. 2021;100(3):100951. doi: 10.1016/j.psj.2020.12.048.
21. Galli GLJ, Lock MC, Smith KLM, Giussani DA, Crossley DA. Effects of developmental hypoxia on the vertebrate cardiovascular system. Physiology (Bethesda). 2023;38(2):53–62. doi: 10.1152/physiol.00022.2022.
22. García HJ, Juárez EMA, López CS. Gradual increase of CO_2 during first stages of incubation with late change of O_2 partial pressure, modifies the hatch trajectory of broiler chicks. Veterinaria México. 2013;44(1):1–16.
23. Juárez-Estrada MA, López-Ruiz EI, López-Cordova S, Tellez-Isaias G, Prado-Rebolledo OF. Increased CO_2 levels during the first half of incubation at high altitude modifies embryonic development of fertile Leghorn breeder eggs. Food and Nutrition Sciences. 2024;15(7):524–547. doi: 10.4236/fns.2024.157035.
24. Visschedijk AHJ. Physics and physiology of incubation. British Poultry Science. 1991;32(1):3–20. doi: 10.1080/00071669108417323.
25. Ahmed M, Biswas A, Roy BG, Srivastava RB. Frequently encountered problems during hatching in cold arid high altitude regions such as Ladakh in India: causes and remedies. World's Poultry Science Journal. 2013;69(4):897–901. doi: 10.1017/S0043933913000895.
26. Okur N, Eratalar SA, Yigit AA, Kutlu T, Kabakçi R, Özsoy SY. Effects of incubator oxygen and carbon dioxide concentrations on hatchability of fertile eggs, some blood parameters, and histopathological changes of broilers with different parental stock ages in high altitude. Poultry Science. 2022;101(2):101609. doi: 10.1016/j.psj.2021.101609.
27. Okur N. Effects of incubator carbon dioxide and oxygen levels, and egg weight on broilers´ hatchability of fertile eggs. Brazilian Journal of Poultry Science. 2019;21(3):1–7. doi: 10.1590/1806-9061-2019-1038.
28. Yilmaz-Dikmen B, Sahan Ü, Ipek A, Aydin C, Kederli E. Effect of oxygen supplementation in a hatchery at high altitude and growth performance of broiler reared at low altitude. South African Journal of Animal Science. 2014;44:(4)350–359. doi: 10520/EJC163898.
29. Fernandes JIM, Bortoluzzi C, Schmidt JM, Scapini LB, Santos TC, Murakami AE. Single stage incubators and hypercapnia during incubation affect the vascularization of the chorioallantoic membrane in broiler embryos. Poultry Science. 2017:96(1):220–225. doi: 10.3382/ps/pew274.
30. Şahan Ü, İpek A, Yilmaz-Dikmen B, Aydin C. Kederli E. Effect of oxygen supplementation in the hatcher at high altitude on the incubation results of broiler eggs laid at low altitude. British Poultry Science. 2011;52(3):388–394, doi: 10.1080/00071668.2011.578122.
31. Cobb Genetics.com. Cobb Hatchery Management Guide [L-015-01-20 EN]. Arkansas, US: Cobb-Vantress; 2021. https://www.cobbgenetics.com

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

32. Fares WA, Ahmed MRM, Rizk RE, Shahein EHA, Boutrous NG, El-Sabrout K. Influence of non-ventilating intervals during early incubation stage on egg hatching process. Veterinary World. 2023;16:(7):1534—1540. doi: 10.14202/vetworld.2023.1534-1540.
33. De Smit L, Bruggeman V, Debonne M, Tona JK, Kamers B, Everaert N, *et al.* The effect of nonventilation during early incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. Poultry Science. 2008;87(3):551—560. doi: 10.3382/ps.2007-00322.
34. Willemsen H, Everaert N, Witters A, De Smit L, Debonne M, Verschuere F, *et al.* Critical assessment of chick quality measurements as an indicator of posthatch performance. Poultry Science. 2008;87(11):2358—2366. doi: 10.3382/ps.2008-00095.
35. El-Hanoun A, El-Sabrout K, Abdella M, Eid M. Effect of carbon dioxide during the early stage of duck egg incubation on hatching characteristics and duckling performance. Physiology and Behavior. 2019;208:112582. doi: 10.1016/j.physbeh.2019.112582.
36. Özlü S, Uçar A, Banwell R, Elibol O. The effect of increased concentration of carbon dioxide during the first 3 days of incubation on albumen characteristics, embryonic mortality, and hatchability of broiler hatching eggs. Poultry Science. 2019; 98(2):771—776. doi: 10.3382/ps/pey464.
37. Willemsen H, Li Y, Willems E, Franssens L, Wang Y, Decuypere E, *et al.* Intermittent thermal manipulations of broiler embryos during late incubation and their immediate effect on the embryonic development and hatching process. Poultry Science. 2011;90(6):1302—1312. doi: 10.3382/ps.2011-01390.
38. Tona K, Bamelis F, De Ketelaere B, Bruggeman V, Moraes VMB, Buyse J, *et al.* Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poultry Science. 2003;82(5):736—741. doi: 10.1093/ps/82.5.736.
39. Tona K, Onagbesan OM, Jego Y, Kamers B, Decuypere E, Bruggeman V. Comparison of embryo physiological parameters during incubation, chick quality, and growth performance of three lines of broiler breeders differing in genetic composition and growth rate. Poultry Science. 2004;83(3):507—513. doi: 10.1093/ps/83.3.507.
40. Wolanski NJ, Renema RA, Robinson FE, Carney VL, Fancher BI. Relationship between chick conformation and quality measures with early growth traits in males of eight selected pure or commercial broiler breeder strains. Poultry Science. 2006;85(8):1490—1497. doi: 10.1093/ps/85.8.1490.
41. Tona K, Bruggeman V, Onagbesan O, Bamelis F, Gbeassor M, Mertens K, *et al.* Day-old chick quality: relationship to hatching egg quality, adequate incubation practice, and prediction of broiler performance. Avian and Poultry Biology Reviews. 2005;16(2):109—119. doi: 10.3184/147020605783438787.
42. Willemsen H, Debonne M, Swennen Q, Everaert N, Careghi C, Han H, *et al.* Delay in feed access and spread of hatch: importance of early nutrition. World's Poultry Science Journal. 2010;66(2):177—188. doi: 10.1017/S0043933910000243.
43. Everaert N, Kamers B, Witters A, De Smit L, Debonne M, Decuypere E, *et al.* Effect of four percent carbon dioxide during the second half of incubation on embryonic development, hatching parameters, and posthatch growth. Poultry Science. 2007;86(7):1372—1379. doi: 10.1093/ps/86.7.1372.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

44. Rahn H, Carey C, Balmas K, Bhatia B, Paganelli C. Reduction of pore area of the avian eggshell as an adaptation to altitude. Proceedings of the National Academy of Sciences. 1977;74(7):3095—3098. doi: 10.1073/pnas.74.7.3095.
45. Uni Z, Ferket PR, Tako E, Kedar O. *In ovo* feeding improves energy (*sic.*) status of late-term chicken embryos. Poultry Science. 2005;84(5):764—770. doi: 10.1093/ps/84.5.764.
46. Uni Z, Ferket RP. Methods for early nutrition and their potential. World's Poultry Science Journal. 2004;60(1):101—111. doi: 10.1079/WPS20038.
47. Everaert N, Willemsen H, Willems E, Franssens L, Decuypere E. Acid–base regulation during embryonic development in amniotes, with particular reference to birds. Respiratory Physiology and Neurobiology. 2011;178(1):118—128. doi: 10.1016/j.resp.2011.04.023.
48. Walsberg GE. The gaseous microclimate of the avian nest during incubation. American Zoologist. 1980;20(2):363—372. doi: 10.1093/icb/20.2.363.
49. Scanes CG, Dridi S. Sturkie's Avian Physiology. 7th. ed. San Diego, US: Academic Press; 2021. 1462 pp.
50. Wolanski NJ, Renema RA, Robinson FE, Carney VL, Fancher BI. Relationships among egg characteristics, chick measurements, and early growth traits in ten broiler breeder strains. Poultry Science. 2007;86(8):1784—1792. doi: 10.1093/ps/86.8.1784.
51. Deeming DC. Yolk sac, body dimensions, and hatchling quality of ducklings, chicks and poults. British Poultry Science. 2005;46(5):560—564. doi: 10.1080/00071660500255042.
52. Lourens A, van den Brand H, Meijerhof R, Kemp B. Effect of eggshell temperature during incubation on embryo development, hatchability, and posthatch development. Poultry Science. 2005;84(6):914—920. doi: 10.1093/ps/84.6.914.
53. Sato M, Tachibana T, Furuse M. Heat production and lipid metabolism in broiler and layer chickens during embryonic development. Comparative biochemistry and physiology. Part A, Molecular and Integrative Physiology. 2006;143(3):382—388. doi: 10.1016/j.cbpa.2005.12.019.
54. Van der Wagt I, de Jong IC, Mitchell MA, Molenaar R, van den Brand H. A review on yolk sac utilization in poultry. Poultry Science. 2020;99(4):2162—2175. doi: 10.1016/j.psj.2019.11.041.
55. Şahan Ü, Ipek A, Sözcü A. Yolk sac fatty acid composition, yolk absorption, embryo development, and chick quality during incubation in eggs from young and old broiler breeders. Poultry Science. 2014;93(8):2069—2077. doi: 10.3382/ps.2013-03850.
56. Moran Jr ET. Nutrition of the developing embryo and hatchling. Poultry Science. 2007;86(5):1043—1049. doi: 10.1093/ps/86.5.1043.
57. Druyan S. The effects of genetic line (broilers vs. layers) on embryo development. Poultry Science. 2010;89(7):1457—1467. doi: 10.3382/ps.2009-00304.
58. Christensen VL, Wineland MJ, Fasenko GM, Donaldson WE. Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. Poultry Science. 2001;80(12):1729—1735. doi: 10.1093/ps/80.12.1729.

doi: 10.22201/fmvz.24486760e.2025.1399
Vol. 12 | 2025

59. Ipek A, Sahan Ü, Baycan SC, Sözcü A. The effects of different eggshell temperatures on embryonic development, hatchability, chick quality, and first-week broiler performance. Poultry Science. 2014;93(2):464—472. doi: 10.3382/ps.2013-03336.
60. French NA. Modeling incubation temperature: the effects of incubator design, embryonic development, and egg size. Poultry Science. 1997;76(1):124—133. doi: 10.1093/ps/76.1.124.
61. Maatjens CM, Reijrink IAM, Molenaar R, van der Pol CW, Kemp B, van den Brand H. Temperature and CO_2 during the hatching phase. I. Effects on chick quality and organ development. Poultry Science. 2014;93:(3):645—654. doi: 10.3382/ps.2013-03490.
62. Sözcü A, İpek A, van den Brand H. Eggshell temperature during early and late incubation affects embryo and hatchling development in broiler chicks. Poultry Science. 2022;101(10):102054. doi: 10.1016/j.psj.2022.102054.
63. Reijrink IAM, Meijerhof R, Kemp B, van den Brand H. The chicken embryo and its micro environment during egg storage and early incubation. World's Poultry Science Journal. 2008;64(4):581—598. doi: 10.1017/S0043933908000214.
64. Burggren WW, Andrewartha SJ, Tazawa H. Interactions of acid–base balance and hematocrit regulation during environmental respiratory gas challenges in developing chicken embryos (*Gallus gallus*). Respiratory Physiology and Neurobiology. 2012;183(2):135—148. doi: 10.1016/j.resp.2012.06.011.
65. Sadler WW, Wilgus HS, Buss EG. Incubation factors affecting hatchability of poultry eggs: 2. Some effects of carbon dioxide upon morphogenesis. Poultry Science. 1954;33(6):1108—1115. doi: 10.3382/ps.0331108.
66. Latter GV, Baggott GK. Role of carbon dioxide and ion transport in the formation of subembryonic fluid by the blastoderm of the Japanese quail. British Poultry Science. 2002;43(1):104—116. doi: 10.1080/00071660120109944.