Europ.Poult.Sci., **86.** 2022, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2022.360

# Effect of non-ventilation during the first 10 d of incubation on physiology, hatching events and post-hatch performances of two commercial layers strains

## Auswirkung einer nicht belüfteten Brut während der ersten 10 Tage auf die Physiologie, das Schlupfgeschehen und die Leistungen nach dem Schlupf von zwei kommerziellen Legehennenlinien

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Manuscript received 31 January 2022, accepted 13 June 2022

### Abstract

During embryonic development, Lohmann Brown (LB) embryos differ from that of Lohmann White (LW) in terms of embryonic growth. Therefore, these strains might adapt differently under different environmental factors such as exposure to high $CO_2$.

The aim of this study was to investigate the effect of non-ventilation on the physiology and post-hatch performances of LB and LW strains.

A total of 1,200 hatching eggs produced by LB and LW breeders were weighed, individually numbered and randomly assigned to 4 replicates of 75 eggs each/strain. The eggs were then divided equally into 2 incubators that were either ventilated (**V**) for the entire incubation or non-ventilated (**NV**) for the first 10 d of incubation. At d 18, the eggs were candled and fertile eggs were transferred into hatching baskets. Between 468 and 510 h of incubation, hatching eggs were checked individually every 3 h for hatching events. After pull out at 21.5 d, chicks were raised until d 56 to assess post-hatch performances.

The results indicated that $CO_2$ level was higher in the non-ventilated group compared to the ventilated group. In both strains, body weight of the embryos was significantly higher ($p < 0.05$) in the NV group compared to the V group. LW embryos shifted internal pipping ($p = 0.0401$) forward in time compared with LB embryos in both groups. Non-ventilation increased hatchability but this was only obvious in the LB strain ($p = 0.0012$), indicating a significant interaction between strain and non-ventilation ($p = 0.0011$). LB chicks showed higher ($p < 0.05$) thyroid hormone levels compared to LW chicks in both groups. Chicks from LB strains had higher body weight gain ($p = 0.0055$) and feed intake ($p = 0.0002$) compared to LW strains in both treatments.

It can be conclude that non-ventilation during the first 10 d of incubation increased hatchability, but this was genotype-dependant.

### Key words

layer strain; non-ventilation; $CO_2$ level; physiology; hatchability; post-hatch growth; feed intake; thyroid hormones

### Zusammenfassung

Während der Embryonalentwicklung unterscheidet sich das Wachstum der Embryonen der Linie Lohmann Brown (LB) von denen der Linie Lohmann White (LW). Daher könnten sich diese Linien unter verschiedenen Umweltbedingungen, wie z. B. einer hohen $CO_2$-Belastung, unterschiedlich anpassen.

Ziel dieser Studie war es, die Auswirkung der Belüftung während der Brut auf die Physiologie und die Leistungen nach dem Schlupf von LB- und LW-Linien zu untersuchen.

Insgesamt 1.200 Bruteier von LB- und LW-Elterntieren wurden gewogen, einzeln nummeriert und nach dem Zufallsprinzip auf 4 Wiederholungsgruppen mit je 75 Eiern/Linie verteilt. Die Eier wurden dann zu gleichen Teilen auf 2 Inkubatoren verteilt, die entweder während der gesamten Brut belüftet (V) oder während der ersten 10 Tage der Brut nicht belüftet (NV) wurden. Am 18. Bruttag wurden die Eier durchleuchtet, und die befruchteten Eier in Schlupfkörbe übertragen. Zwischen 468 und 510 Stunden der Bebrütung wurden die Bruteier einzeln alle 3 Stunden hinsichtlich des Schlupfvorganges überprüft. Nach 21,5 Tagen wurden die Küken aus dem Brutschrank entnommen und bis zum Tag 56 aufgezogen, um die Leistungen nach dem Schlupf zu bewerten.

Die Ergebnisse zeigten, dass der $CO_2$-Gehalt in der nicht belüfteten Gruppe höher war als in der belüfteten Gruppe. Bei beiden Linien war das Körpergewicht der Embryonen in der NV-Gruppe signifikant höher ($p < 0,05$) als in der V-Gruppe. Bei den LW-Embryonen erfolgte das internal pipping ($p = 0,0401$) im Vergleich zu den LB-Embryonen in beiden Gruppen früher. NV erhöhte die Schlupffähigkeit, aber dies war nur bei der LB-Linie offensichtlich ($p = 0,0012$), was auf eine signifikante Wechselwirkung zwischen Linie und NV hinweist ($p = 0,0011$). LB-Küken wiesen im Vergleich zu LW-Küken in beiden Gruppen einen höheren Gehalt an Schilddrüsenhormonen ($p < 0,05$) auf. Küken der LB-Linie hatten eine höhere Körpergewichtszunahme ($p = 0,0055$) und Futteraufnahme ($p = 0,0002$) im Vergleich zur LW-Linie unter beiden Belüftungsbedingungen.

Daraus lässt sich schließen, dass der Verzicht auf Belüftung während der ersten 10 Tage der Inkubation die Schlupffähigkeit in Abhängigkeit vom Genotyp erhöhen kann.

**Stichworte**

Legehennenlinie; Belüftung; $CO_2$-Gehalt; Physiologie; Schlupffähigkeit; Wachstum nach dem Schlupf; Futteraufnahme; Schilddrüsenhormone

**List of Abbreviations**

| | |
|---|---|
| $CO_2$: | carbon dioxide |
| ED: | embryonic day |
| EP: | external pipping |
| EW: | egg weight |
| HU: | Haugh unit |
| IP: | internal pipping |
| LB: | Lohmann Brown |
| LW: | Lohmann White |
| $O_2$: | oxygen |
| $T_3$: | Tri-iodothyronine |
| $T_4$: | Tétra-iodothyronine or Thyroxine |

## Introduction

The nexus between embryonic development and genotype has been established (TONA et al., 2004). The importance of genetic background and its impact on growth and development of chickens is very well documented. TONA et al. (2010b) reported that there was a different growth trajectory in relation to genotype. The authors stated that Lohmann Brown (LB) and Lohmann White (LW) embryos had different embryonic growth trajectories and these different patterns of growth were attributed to the differences in physiological parameters. However, embryonic development is not only influenced by the genetic makeup but also by the environment in which it develops.

Oxygen ($O_2$) and carbon dioxide ($CO_2$) exchanges are of fundamental importance in embryonic development. High $CO_2$ concentration could play a role in the development of chicken embryo as $CO_2$ increases naturally under the brooding hen (WALSBERG, 1980). EL-HANOUN et al. (2019) showed that the increased $CO_2$ concentration to 1.0%

during the first 10 d of incubation enhanced duck embryo growth, increased corticosterone, $T_3$ and $T_4$ levels, stimulated early hatching and improved hatchability. These results were in agreement with early reports conducted on broilers (De Smit et al., 2006; Willemsen et al., 2008, Tona et al., 2013). Bilalissi et al. (2022) found that the average time of occurrence of internal pipping, external pipping and hatching was shorter in a non-ventilated incubator compared to a ventilated incubator. These studies were conducted on broiler hatching eggs, and the stimulating effect of non-ventilation on incubation time and hatching parameters found in broiler lines could also be beneficial for layers embryos.

In contrast to the extensive knowledge on genetic and environment effects on embryonic development in poultry, there is lack of information on how the interaction (strain – gaseous environment) can affect embryonic growth. In general, it has been proposed that altering the environment of a developing organism, might alter the developmental trajectories of some physiological regulatory systems (De Smit et al., 2006).

As LB and LW strains do not have the same trajectory of development, we hypothesize that these strains might perform differently on different environmental factors such as exposure to different $CO_2$ levels. Therefore, the aim of this study was to compare the embryonic physiological indices, hatching traits and post-hatch performances of LB and LW hatching eggs under ventilation or non-ventilation incubation conditions during the first 10 d of incubation.

## Material and methods

*Experimental design*

The experiment was conducted in accordance with the Institutional Animal Ethics Committee guidelines of the Regional Center of Excellence on Poultry Sciences (CERSA-UL) and consisted of two phases; 1) the incubation phase and 2) the post-hatch phase.

A total of 1,200 layers hatching eggs of two strains were used in this study, 600 each of LB and LW. Eggs were produced by 40 weeks old LB and LW breeders. For each genotype, 30 eggs were used to determine egg quality characteristics.

Prior to setting for incubation, eggs were weighed, individually numbered and randomly assigned into 4 replicates of 75 eggs each/strain. The eggs were then divided equally into 2 incubators with a capacity of 600 each (PasReform, Zeddam, the Netherlands, Smart ProCombi model) that were either ventilated during the entire incubation or non-ventilated during the first 10 days and continued by a ventilated incubation. Only experimental hatching eggs were incubated in these single-stage incubators. Half of the eggs (4 replicates of 75 eggs/strain) (ventilated group) were set for incubation for 18 days in a forced-draft incubator at temperature of 37.8°C, humidity of 60% and were turned once every hour at 90° angle. The other half (4 replicates of 75 eggs/strain) were set for incubation in a forced-draft incubator where the dampers were closed (non-ventilated) during the first 10 days of incubation. In this incubator, the $CO_2$ level was increased gradually from 0.03% at the start of incubation to 0.18% at embryonic day 10 (Figure 1). Then, from the end of day 10 of incubation onward, the incubation was continued in the same way as the ventilated incubation.

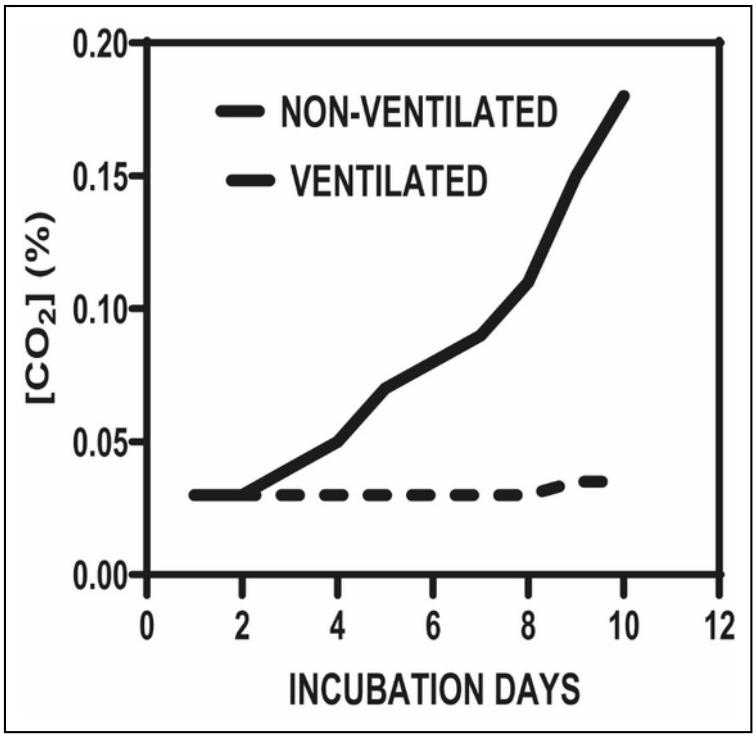



**Figure 1. Changes of $CO_2$ concentration (%) in the ventilated and non-ventilated incubator during the first 10 d of incubation.**

**Veränderungen der $CO_2$-Konzentration (%) im belüfteten und nicht belüfteten Brutschrank während der ersten 10 Tage der Inkubation.**

At day 18 of incubation, the eggs were weighed, candled and those with evidence of living embryos were transferred from the turning trays to hatching baskets. Between 468 and 510 h, hatching eggs were checked individually every 3 h for hatching events and the hatched chicks were recorded according to the treatments.

During rearing phase, the two strains of layers were reared separately under the same condition up to 56 days of age where body weights and feed intake were recorded. These data were used to calculate the body weight gain and the feed conversion ratio to determine post-hatch performances.

*Egg quality measurement*

Prior to setting for incubation, 30 eggs of each genotype were weighed and broken out to measure albumen and yolk weights, eggshell weight and albumen Haugh units (HU). For each HU measurement, the Egg Quality Measurement System (Futura, Lohne, Germany) was used to record egg weight and thick albumen height, by connecting a transmission box and a balance to a personal computer. After calibration of the albumen height gauge, for each weighed and broken egg, the height of albumen was measured (±0.25 mm) with a vertically mounted micrometer with an electronic path. Between eggs, the micrometer was cleaned with distilled water and then dried with absorbent paper. Using the standard software program version 2A, the HU was calculated based on egg weight and albumen height.

*Egg and embryo weighing*

At embryonic days 10, 12, 14, 16, 18 (ED10, ED12, ED14, ED16 and ED18) and at internal pipping, 15 eggs per treatments were weight and broken to determine the weights of the developing embryos. After weighing, each egg was broken; its embryo was removed carefully and separated from all attachments such as yolk sac and chorioallantoic membrane. The embryo was then wiped with absorbent paper before weighing. The relative embryonic body weight (REBW) was defined as the ratio of the embryonic body weight to the egg weight.

The egg weights at ED18 were used to determine egg weight loss using the formula:

$$\text{Weight Loss}(\%) = \frac{EW0-EW18}{EW0} x100$$

Where, EW0 = egg weight at setting and EW18 = egg weight at d18 of incubation.

*Pipping, Hatching Events, hatchability and chick quality*

Between 468 and 510 h of incubation, eggs in the hatching baskets were checked individually every 3 h for hatching events. Eggs in which the beak of embryo penetrates the inner shell membrane (internal pipping, **IP**) where transferred to a new basket and checked individually every 3 h for eggs in which the shell over the air cell is then cracked (external pipping, **EP**) (BILALISSI et al., 2022). The external pipped eggs were put in separate baskets to determine individual hatching time. The hatched chicks were left in the incubator until the machine was stopped. All individual times of IP, EP and hatching were recorded to determine average time and duration of IP, EP and hatch. At IP, EP, or hatching stages, incubation duration was defined as the time between setting and the occurrences of these events for each egg. Then, the timing of the occurrence of hatching events was used to calculate their durations as follows:

EP duration = duration between EP and hatching; and

Hatching duration = duration between IP and hatching.

At the end of incubation process, the numbers of the hatched chicks were recorded according to ventilation treatment and the strain to determine hatchability. Eggs that failed to hatch were recorded, opened, and visually evaluated to determine embryonic mortality. Chick quality assessment was done using the Tona scoring method (TONA et al., 2003). According to this method, physical parameters including reflex, down and appearance, eyes, conformation of legs, navel area, yolk sac and remaining membranes and yolk were scored. The quality score for a chicken was defined as the sum of the scores assigned to each quality parameter.

*Serum Tri-iodothyronine ($T_3$) and thyroxin ($T_4$) determination*

Blood samples were collected from 15 chicks according to treatments, at hatch and at 8-wk post-hatch for $T_3$ and $T_4$ determination. The blood samples were centrifuged at 3,000 rpm for 15 min and the obtained serum was stored at –20°C. Afterwards, a volume of 100 µl of serum was used for determination of $T_3$ and $T_4$ concentrations using the automated VIDAS systems, which is an enzyme-linked fluorescent assay (ELFA) technique. Anti-$T_3$ and Anti-$T_4$ antibody of sheep, provided by VIDAS were used, respectively for determination of $T_3$ and $T_4$ concentrations.

*Rearing*

A total of 112 layer chicks/strain/treatment (4 replicates of 28 chicks/strain/treatment) were reared. The two strains were housed separately in floor pens. Chicks were reared under similar environmental and management conditions. They had *ad libitum* access to commercial available feed [Metabolizable Energy (ME) = 11.51 MJ* and Crude Protein (CP) = 18.21%] and water throughout the rearing period.

Weekly, body weight and feed intake of each replication were recorded to determine the body weight gain and feed conversion ratio of each treatment.

* Metabolisable energy (ME) is calculated according to the method provided by BOURDILLON et al. (1990)

## Statistical analysis

The data were processed with a commercial scientific 2D graphics and statistics software GraphPad Prism 8.0.1 (GraphPad software Inc., California, USA). T-test was used to analyse the effect of genotype on egg quality parameters (HU, albumen, yolk, eggshell weights). The Generalised Linear Model Procedure was used to analyse embryo weights, $T_3$, $T_4$ concentrations, incubation duration, body weight and feed intake. Hatchability was considered as binomial in distribution. Data were analysed as a completely randomised design with a 2 × 2 factorial arrangement of treatments. When the treatment effects of the general model were statistically significant the means were further compared using Tukey's post-hoc-test. For all analyses, p Value of 0.05 was retained as the degree of significance. The model was:

$Yijk = \mu + \alpha i + \tau j + (\alpha\tau)ij + eijk,$

where $Yijk$ = incubation duration, embryonic weight, $T_3$, $T_4$ concentrations, body weight, feed intake of chicken $k$ from incubator ventilation $i$ and line $j$ according to incubation stage; $\mu$= the overall mean; $\alpha i$ = the main effect of incubator ventilation; $\tau j$ = the main effect of strain; $(\alpha\tau)ij$ = the interaction between incubator ventilation and strain; and $eijk$ = the random error term.

## Results

*Effect of strain on hatching eggs characteristics*

Table 1 indicates the egg quality characteristics according to strain of hens. LW eggs weight ($p < 0.0001$) and relative yolk weight ($p = 0.0428$) were significantly higher compared to those of LB strain. However, no difference was observed between the relative albumen weight ($p = 0.8741$), eggshell weight ($p = 0.4154$) and HU ($p = 0.9578$) of both genotypes.

**Table 1. Effect of strain on hatching egg weight, relative albumen, yolk, eggshell weight and Haugh unit.**

**Einfluss der Linie auf das Gewicht der Bruteier, das relative Gewicht von Eiweiß, Dotter, Eischale und Haugh-Einheit.**

| Items | Strains | | p Value |
|---|---|---|---|
| | LB | LW | |
| Egg weight (g) | 56.7 ± 0.14[b] | 57.7 ± 0.14[a] | < 0.0001 |
| Albumen weight (%) | 56.5 ± 1.12 | 56.7 ± 0.72 | 0.8741 |
| Yolk weight (%) | 27.8 ± 0.58[b] | 28.7 ± 0.68[a] | 0.0428 |
| Eggshell weight (%) | 13.3 ± 0.13 | 12.8 ± 0.23 | 0.4154 |
| Haugh unit | 55.1 ± 3.458 | 54.9 ± 1.702 | 0.9578 |

[a, b] Means with a common superscript are not significantly different for $p < 0.05$. LB: Lohmann Brown, LW: Lohmann White.

*Effect of strain and ventilation treatment on embryonic development*

The effect of strain and incubation ventilation treatment on LB and LW embryonic development is shown on Table 2.

**Table 2. Effect of strain and ventilation treatment on embryonic development (g).**

**Einfluss der Linie und Belüftung auf die Embryonalentwicklung (g).**

| Embryonic Day | Lohmann Brown | | Lohmann White | | p Value | | |
|---|---|---|---|---|---|---|---|
| | NV | V | NV | V | I | S | I × S |
| ED10 | 2.70 ± 0.15 | 2.92 ± 0.22 | 2.74 ± 0.15 | 2.75 ± 0.10 | 0.8902 | 0.9378 | 0.8997 |
| ED12 | 6.11 ± 0.18[a] | 5.52 ± 0.19[b] | 5.48 ± 0.14[b] | 5.07 ± 0.14[b] | 0.0498 | 0.0445 | 0.1764 |
| ED14 | 12.4 ± 0.28[a] | 10.4 ± 0.11[c] | 11.1 ± 0.14[b] | 9.09 ± 0.20[d] | 0.0001 | 0.0012 | 0.9735 |
| ED16 | 18.2 ± 0.25 | 17.9 ± 0.24 | 18.0 ± 0.32 | 17.6 ± 0.29 | 0.0585 | 0.6344 | 0.937 |
| ED18 | 25.2 ± 0.22 | 25.3 ± 0.73 | 26.0 ± 0.25 | 25.5 ± 0.32 | 0.0727 | 0.0535 | 0.0568 |
| IP Stage | 42.4 ± 0.43[a] | 37.3 ± 1.54[c] | 39.3 ± 1.21[b] | 37.1 ± 0.79[c] | < 0.0001 | 0.0004 | 0.0005 |

[a, b, c, d] Means with a common superscript are not significantly different for $p < 0.05$. V: ventilated, NV: non-ventilated; I = incubation ventilation, S = strain, I × S = interaction.

No weight difference was found at ED10, ED16 and ED18 across incubation ventilation treatment ($p > 0.05$). However, embryonic body weights from the LB strain were significantly greater at ED12 ($p = 0.0445$) and ED14 ($p = 0.0012$) compared to embryos from the LW strain in both ventilated and non-ventilated treatments. In both strains, embryos body weight were significantly higher at ED12 ($p = 0.0498$) and ED14 ($p = 0.0001$) in the non-ventilated group compared to the ventilated group and an interaction ($p = 0.0005$) was found between strain and ventilation treatment at the IP stage. In fact, non-ventilation increased body weight of the embryo both in LB and LW strains, but the effect was more pronounced in the LB strain.

*Effect of strain and ventilation treatment on egg weight loss up to ED18*

The effect of strain and incubation ventilation treatments on LB and LW egg weight loss (%) up to ED18 is presented in Figure 2.

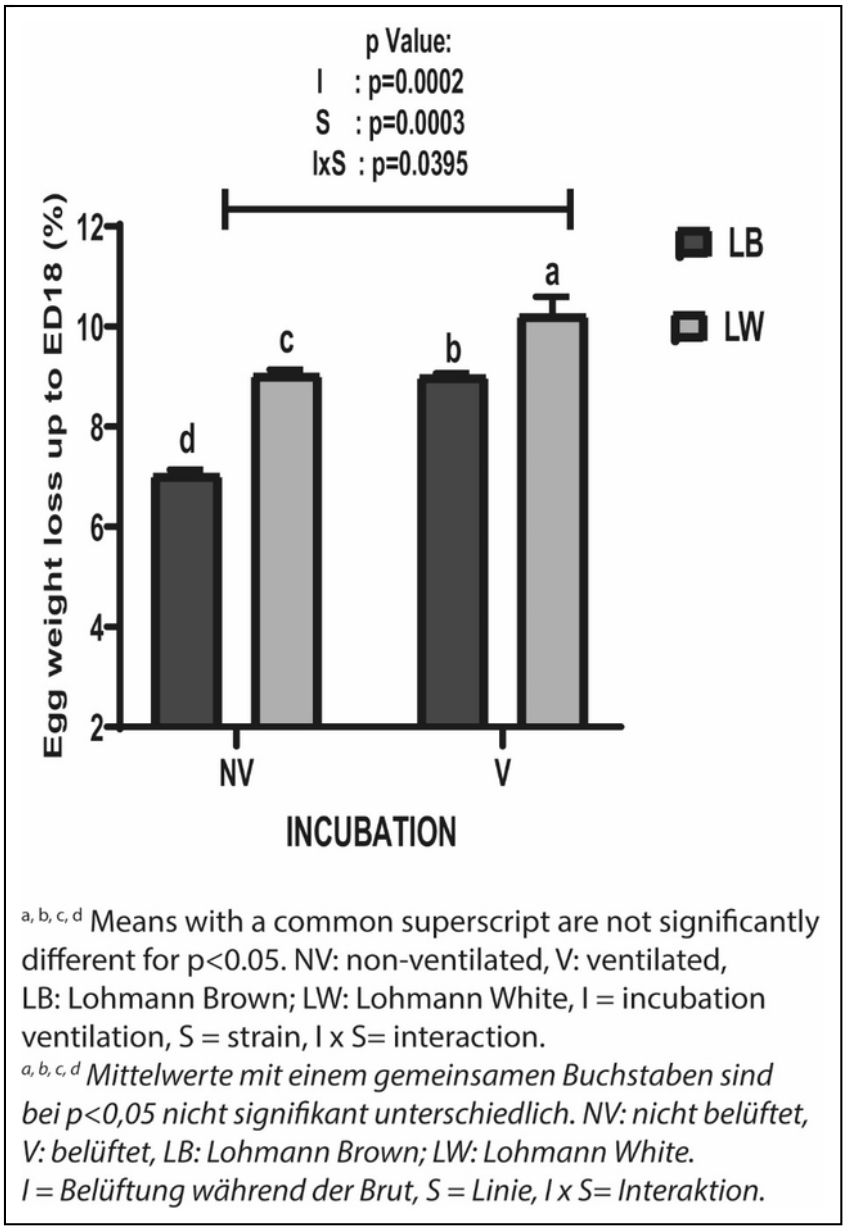



**Figure 2. Egg weight loss up to ED18 according to strain and ventilation treatment.**

**Gewichtsverlust der Bruteier bis zum ED18 je nach Linie und Belüftung.**

In both non-ventilated and ventilated incubators the egg weight loss from the LW strain was significantly higher compared to eggs from the LB strain ($p = 0.0003$). The effect of non-ventilation on egg weight loss was more pronounced in the LW stain compared to the LB strain witch reflect an interaction between ventilation treatment and strain ($p = 0.0395$).

The percentage of egg weight loss up to ED18 was significantly higher in eggs from the ventilated group compare to eggs from non-ventilated group in both LB and LW strains ($p = 0.0002$).

*Effect of strain and ventilation treatment on average time for IP, EP, duration of EP, duration of hatch and average hatching time*

Table 3 shows the average incubation time for occurrence of IP, EP, durations of EP, hatch and hatching time according to ventilation treatment during incubation and chicken strain.

**Table 3. Effect of strain and ventilation treatment on average time for IP, EP, duration of EP, duration of hatch and hatching time.**

**Einfluss der Linie und Belüftung auf die durchschnittliche Zeit für IP, EP, Dauer des EP, Dauer des Schlupfes und Schlupfzeit.**

| Strain and Incubation | Incubation time up to IP | Incubation time up to EP | Duration between IP and EP | Duration between EP and hatch | Average hatching time (h) |
|---|---|---|---|---|---|
| Lohmann Brown | | | | | |
| NV | 462 ± 0.47[b] | 471 ± 0.53[b] | 9.63 ± 0.40[b] | 18.4 ± 0.68[b] | 480 ± 0.42[b] |
| V | 463 ± 0.56[b] | 471 ± 0.69[b] | 10.0 ± 0.27[b] | 18.0 ± 0.63[b] | 481 ± 0.5[b] |
| Lohmann White | | | | | |
| NV | 464 ± 0.58[a] | 475 ± 0.43[a] | 11.0 ± 0.72[a] | 21.7 ± 0.69[a] | 486 ± 0.31[a] |
| V | 465 ± 0.48[a] | 476 ± 0.42[a] | 10.7 ± 0.23[a] | 21.9 ± 0.59[a] | 486 ± 0.42[a] |
| p Value | | | | | |
| Incubation | 0.0084 | 0.0593 | 0.0102 | 0.3128 | 0.3419 |
| Strain | 0.0002 | < 0.0001 | 0.0006 | < 0.0001 | 0.0081 |
| Incubation × Strain | 0.8835 | 0.0121 | 0.1304 | 0.4594 | 0.7031 |

[a, b] Means with a common superscript are not significantly different for $p < 0.05$.

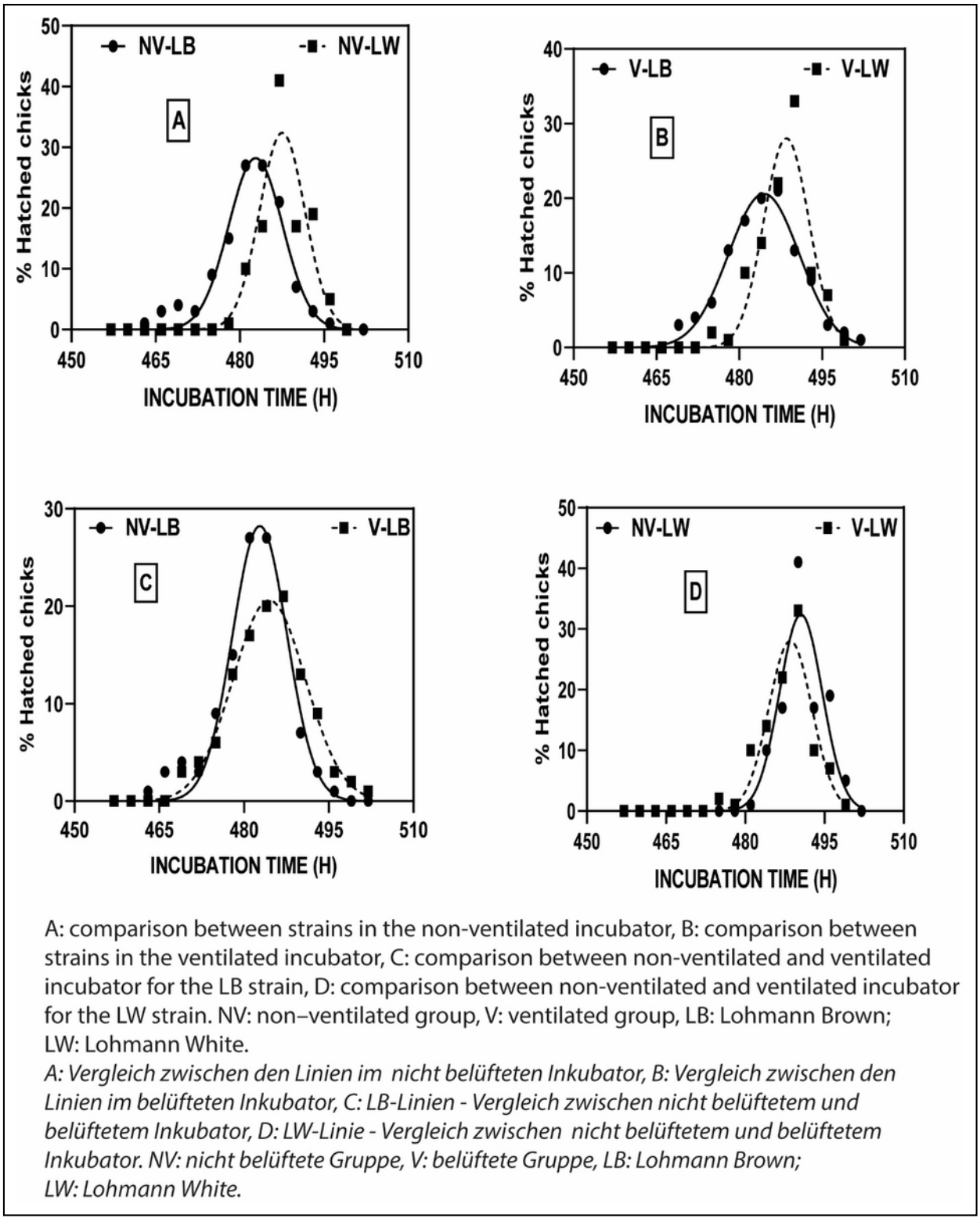
V: ventilated, NV: non-ventilated; IP: internal pipping; EP: external pipping.

Non-ventilation did not impact the average time for IP, EP, durations of EP and hatch which tended to be shorter, but not significantly ($p > 0.05$) in non-ventilated group compare to ventilated group in both LB and LW embryos. However, LW embryos shifted internal pipping ($p = 0.0002$) forward in time compared with LB embryos in both ventilated and non-ventilated group. Non-ventilation reduced the average of EP duration for 1 h compared to the ventilated group in the LW strain (475 ± 0.43 vs 476 ± 0.42) but not in the LB strain, indicating an interaction between strain and ventilation treatment on average EP duration ($p = 0.0121$). Both durations of EP ($p = 0.0006$) and total hatch ($p < 0.0001$) were delayed in LW eggs compared to LB eggs in both ventilated and non-ventilated groups.

LB embryos had a shorter average hatching time ($p = 0.0081$) compared to the LW strain but there was no difference between the non-ventilated and the ventilated groups in both strains ($p = 0.3419$).

*Effect of strain and ventilation treatment on hatching curve*

Figure 3 shows the spread of hatch for the eggs of ventilated and non-ventilated incubation according to the strains. The eggs of the LB strain started hatching earlier than those of the LW strain under both incubation conditions (A and B). The peak of hatch of the LB strain was 3 h earlier in the ventilated group and 6 h in the non-ventilated group. In the LB strain, the spreading around the average hatching time was significantly shorter than those of the LW strain. Overall, the eggs of non-ventilated incubation had a shorter, although not significantly different, spread of hatch compared to ventilated incubation in both strains (C and D).




A: comparison between strains in the non-ventilated incubator, B: comparison between strains in the ventilated incubator, C: comparison between non-ventilated and ventilated incubator for the LB strain, D: comparison between non-ventilated and ventilated incubator for the LW strain. NV: non–ventilated group, V: ventilated group, LB: Lohmann Brown; LW: Lohmann White.

*A: Vergleich zwischen den Linien im nicht belüfteten Inkubator, B: Vergleich zwischen den Linien im belüfteten Inkubator, C: LB-Linien - Vergleich zwischen nicht belüftetem und belüftetem Inkubator, D: LW-Linie - Vergleich zwischen nicht belüftetem und belüftetem Inkubator. NV: nicht belüftete Gruppe, V: belüftete Gruppe, LB: Lohmann Brown; LW: Lohmann White.*

**Figure 3. Spread of hatch for the eggs of ventilated and non-ventilated incubation expressed as percentage of hatched chicks according to the strains.**

**Streuung des Schlupfes der Eier aus belüfteter und nicht belüfteter Brut, ausgedrückt als Prozentsatz der geschlüpften Küken, in Abhängigkeit von der Linie.**

*Effect of strain and ventilation treatment on hatchability, embryo mortality and day old chick quality*

The effects of strain and incubation ventilation treatment on hatchability, embryonic mortality, average hatching time and chick quality are presented in Table 4.

**Table 4. Effect of strain and ventilation treatment on hatchability, embryo mortality and chick quality.**

**Einfluss der Linie und Belüftung auf die Schlupffähigkeit, embryonale Mortalität und Kükenqualität.**

| Strain and Incubation | Hatchability (%) | Embryo mortality (%) | Average chick scores |
|---|---|---|---|
| Lohmann Brown | | | |
| NV | 94.5 ± 0.48$^{c}$ | 5.02 ± 0.02$^{c}$ | 94.4 ± 0.77$^{a}$ |
| V | 90.1 ± 0.09$^{d}$ | 9.41 ± 0.41$^{a}$ | 90.4 ± 0.17$^{b}$ |
| Lohmann White | | | |
| NV | 96.3 ± 0.25$^{b}$ | 3.25 ± 0.25$^{b}$ | 88.0 ± 0.60$^{c}$ |
| V | 98.1 ± 0.11$^{a}$ | 1.39 ± 0.39$^{d}$ | 82.0 ± 0.1$^{d}$ |
| p Value | | | |
| Incubation | 0.0012 | 0.0015 | 0.0006 |
| Strain | 0.0083 | 0.0083 | < 0.0001 |
| Incubation × Strain | 0.0011 | 0.0011 | 0.1161 |

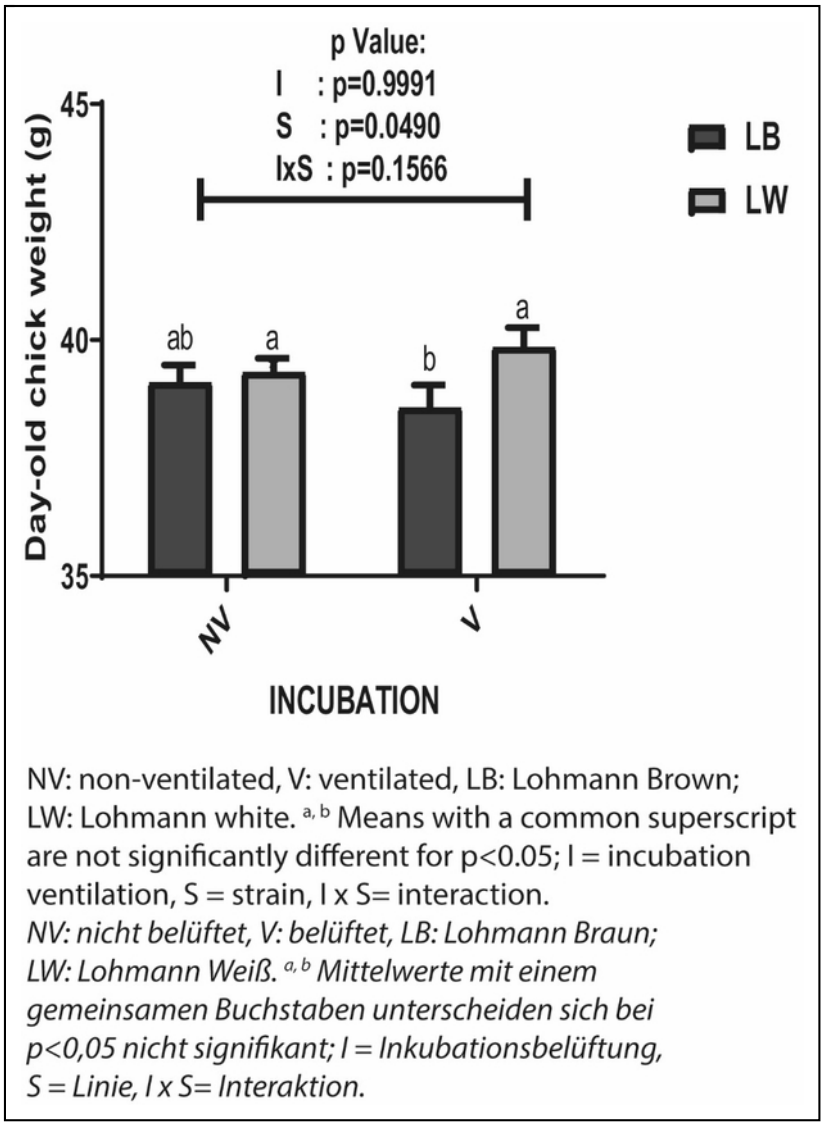
$^{a, b}$ Means with a common superscript are not significantly different for $p < 0.05$.
V: ventilated, NV: non-ventilated.

Non-ventilation increased hatchability but the magnitude of the effect was higher in the LB strain ($p = 0.0012$), indicating a significant interaction between strain and ventilation treatment ($p = 0.0011$). Consequently, non-ventilation reduced embryonic mortality significantly in LB eggs ($p = 0.0015$).

Chick quality was significantly impacted by the strain ($p < 0.0001$) in both ventilated and non-ventilated groups. The chick quality score was higher for the LB strain compared to the LW strain chicks in both the ventilated and non-ventilated incubator. In both strains, the chick quality score was higher in the non-ventilated group compared to the ventilated group ($p = 0.0006$).

*Effect of strain and ventilation treatment on day old-chick weight*

At hatch, the effect of strain was only obvious in the control group where day old chicks from the LW strain were heavier than chicks from the LB strain ($p = 0.0490$). Ventilation treatment did not affect the day old chick body weight in both LB and LW strains ($p = 0.9991$) (Fig. 4).




NV: non-ventilated, V: ventilated, LB: Lohmann Brown; LW: Lohmann white. [a, b] Means with a common superscript are not significantly different for p<0.05; I = incubation ventilation, S = strain, I x S= interaction.
*NV: nicht belüftet, V: belüftet, LB: Lohmann Braun; LW: Lohmann Weiß.* [a, b] *Mittelwerte mit einem gemeinsamen Buchstaben unterscheiden sich bei p<0,05 nicht signifikant; I = Inkubationsbelüftung, S = Linie, I x S= Interaktion.*

**Figure 4. Effect of strain and ventilation treatment on day old-chick weight.**

**Einfluss der Linie und Belüftung auf das Gewicht der Eintagsküken.**

*Effect of strain and ventilation treatment on post-hatch growth, feed intake, body weight gain and feed conversion ratio*

Figure 5 shows the effect of strain and incubation ventilation treatment on the post-hatch growth of the chicks up to 8 weeks. No significant effects of strains ($p > 0.05$) or ventilation ($p > 0.05$) were found on post-hatch growth up to 6 weeks. At 7 and 8 weeks post-hatch, chicks from the LB strain were significantly heavier than chicks from the LW strain ($p < 0.0001$) in both ventilated and non-ventilated groups. However, in both strains, ventilation treatment did not affect chicks body weight ($p > 0.05$) although chicks body weight from non-ventilated group tended to be higher than chicks from control group.

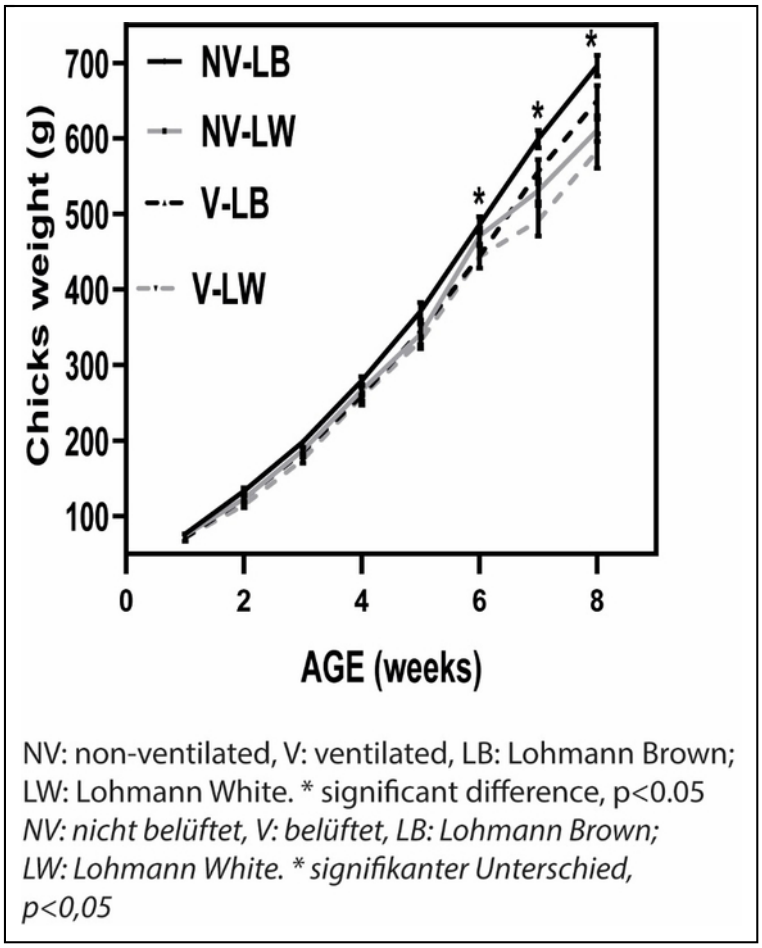



**Figure 5. Effect of strain and ventilation treatment on post-hatch growth.**

**Einfluss der Linie und Belüftung auf das Wachstum nach dem Schlupf.**

Feed intake was significantly higher in the LB strain compared to the LW strain in both non-ventilated and ventilated groups ($p = 0.0002$). However, ventilation treatment did not affect feed intake in both strains ($p = 0.0887$) and no interaction was found between ventilation treatment and the strain ($p = 0.0646$) (Figure 6).

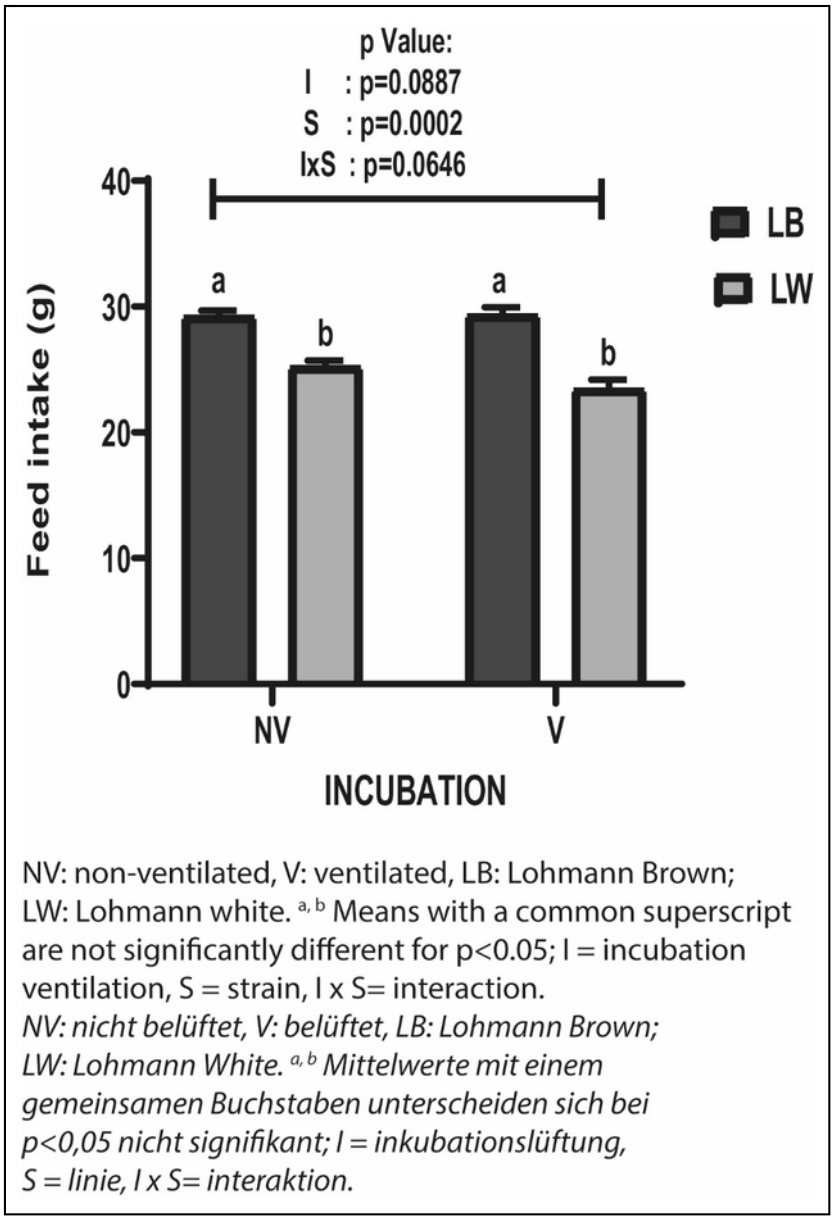
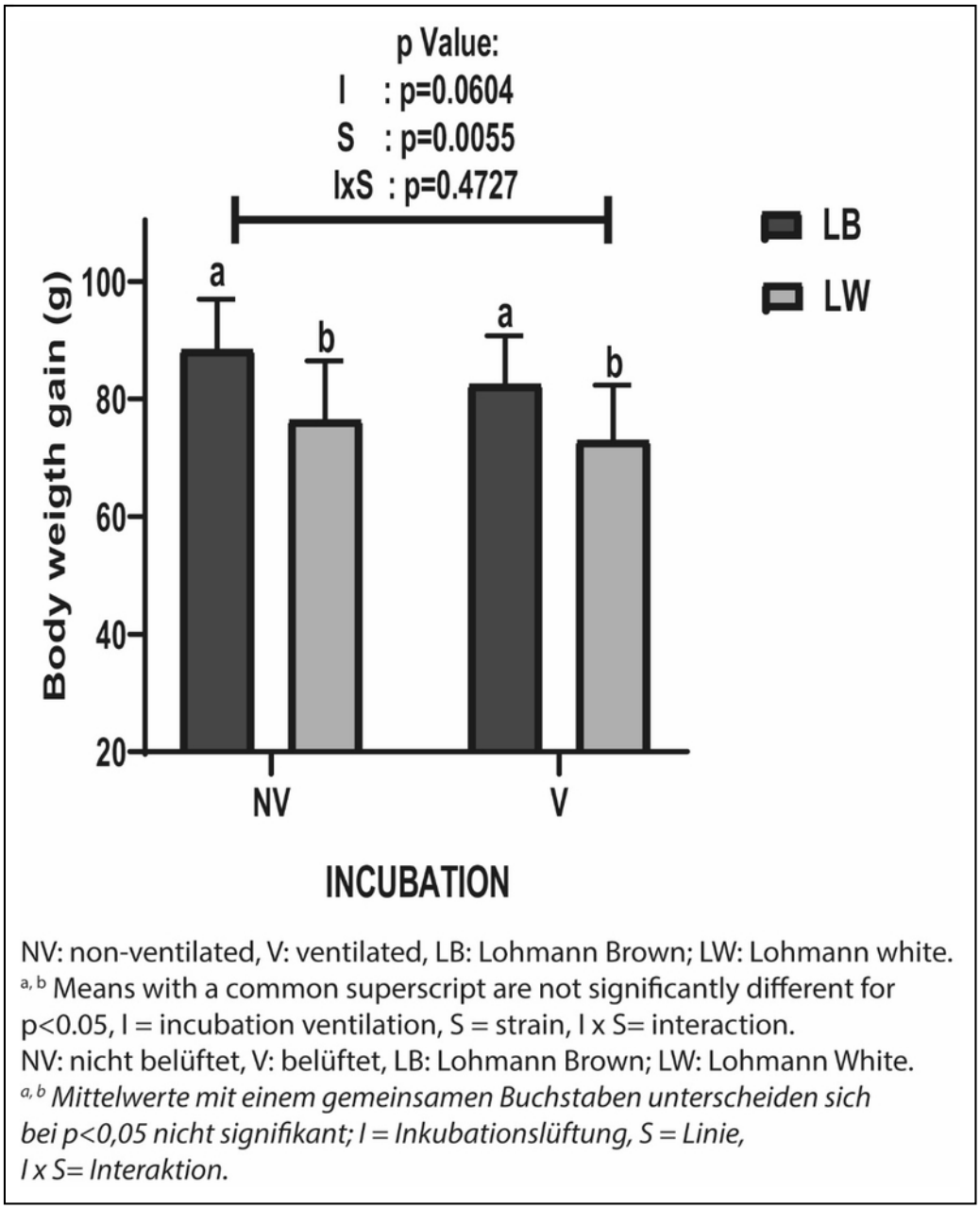



**Figure 6. Effect of strain and ventilation treatment on feed intake.**

**Einfluss der Linie und Belüftung auf die Futteraufnahme.**

The body weight gain was significantly higher in the LB strain compare to the LW strain in both non-ventilated and ventilated groups at 8 weeks (Figure 7) ($p = 0.0055$). However, ventilation treatment did not affect body weight gain in both strains ($p = 0.0604$) and no interaction was found between ventilation treatment and the strain ($p = 0.4727$).




NV: non-ventilated, V: ventilated, LB: Lohmann Brown; LW: Lohmann white.
[a, b] Means with a common superscript are not significantly different for p<0.05, I = incubation ventilation, S = strain, I x S= interaction.
NV: nicht belüftet, V: belüftet, LB: Lohmann Brown; LW: Lohmann White.
*[a, b] Mittelwerte mit einem gemeinsamen Buchstaben unterscheiden sich bei p<0,05 nicht signifikant; I = Inkubationslüftung, S = Linie, I x S= Interaktion.*

**Figure 7. Effect of strain and ventilation treatment on weekly body weight gain.**

**Einfluss der Linie und Belüftung auf die wöchentliche Körpermassezunahme.**

Figure 8 shows the effect of ventilation treatment on feed conversion ratio according to the genotype of the chickens up to 8 weeks.

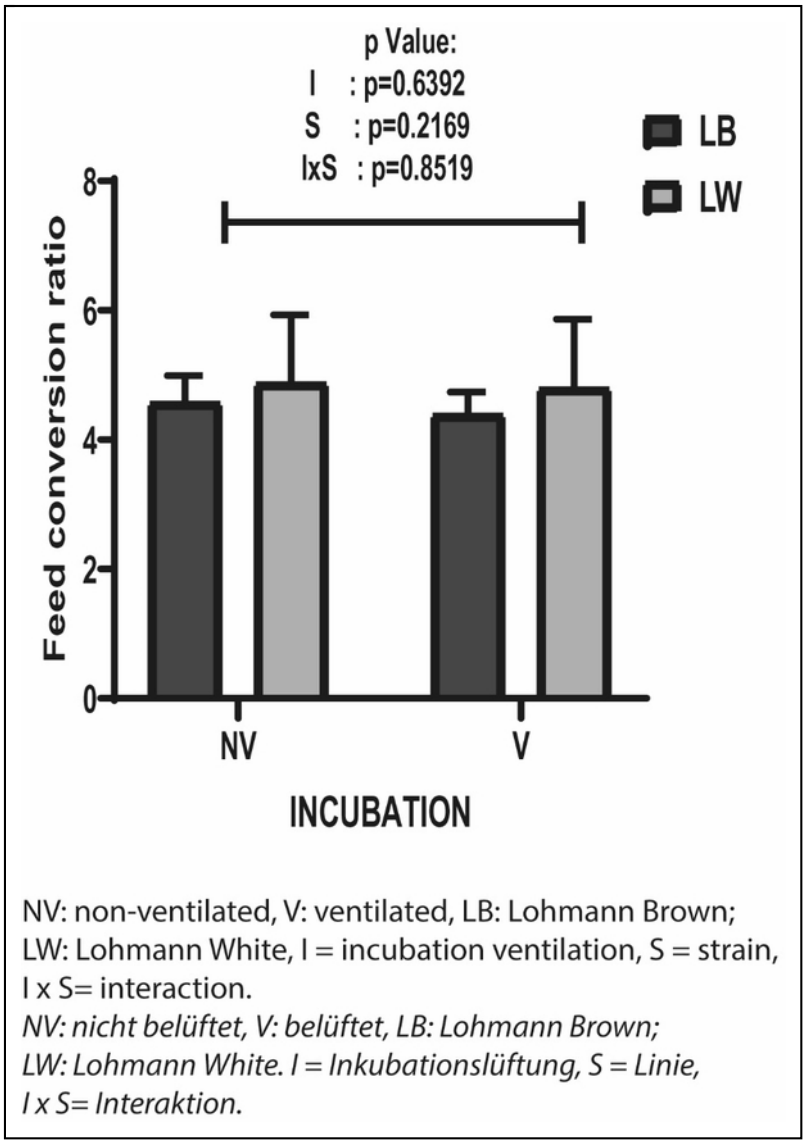



**Figure 8. Effect of strain and ventilation treatment on feed conversion ratio.**

**Einfluss der Linie und Belüftung auf die Futterverwertung.**

No significant effects of strain (p = 0.2169) or ventilation treatment (p = 0.6392) were found on feed conversion ratio up to 8 weeks.

*Effect of strain and ventilation treatment on $T_3$ and $T_4$ levels, and $T_3/T_4$ ratio*

There was a significant effect of strain on $T_3$ concentration at hatch (p = 0.0003). LB chicks showed higher $T_3$ concentration compared to LW chicks in both ventilated and non-ventilated groups. The effect of ventilation treatment was only significant (p = 0.0492) at 8 weeks post-hatch and this was obvious in LB chicks where chicks from non-ventilated group showed higher $T_3$ concentration compared to chicks from ventilated group, indicating a significant interaction between strain and ventilation treatment (p = 0.0263) (Figure 9).

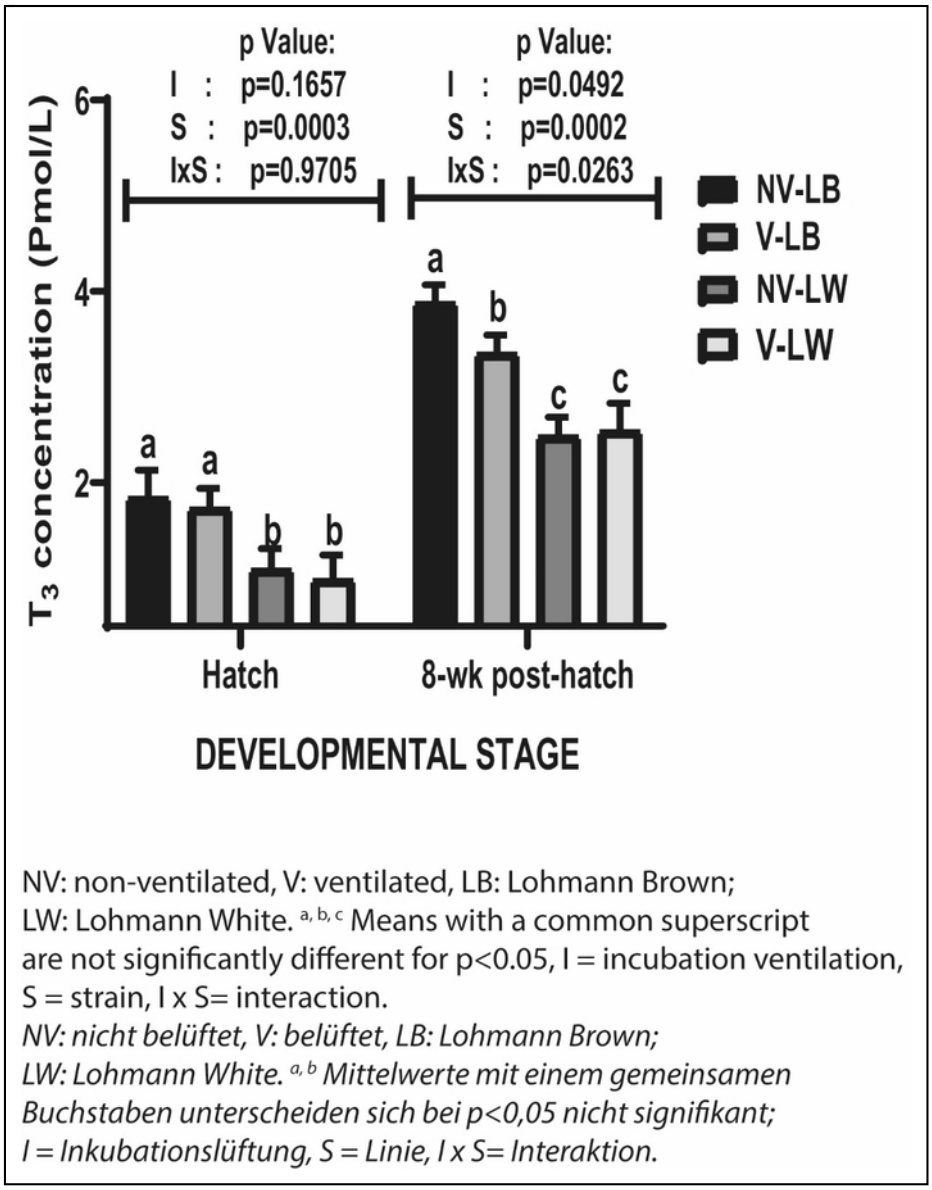



**Figure 9. Tri-iodothyronine ($T_3$) levels according to strain and ventilation treatment.**

**Einfluss der Linie und Belüftung auf den Tri-iodothyronine ($T_3$)-Gehalt.**

$T_4$ concentration was not affected by ventilation treatment both at hatch (p = 0.3772) and at 8 weeks post-hatch (p = 0.1219). But, the effect of strain was significant at hatch (p = 0.0216) and at 8 weeks post-hatch (p = 0.0014). LB chicks showed higher $T_4$ concentration compared to LW chicks in both non-ventilated and ventilated groups (Figure 10).

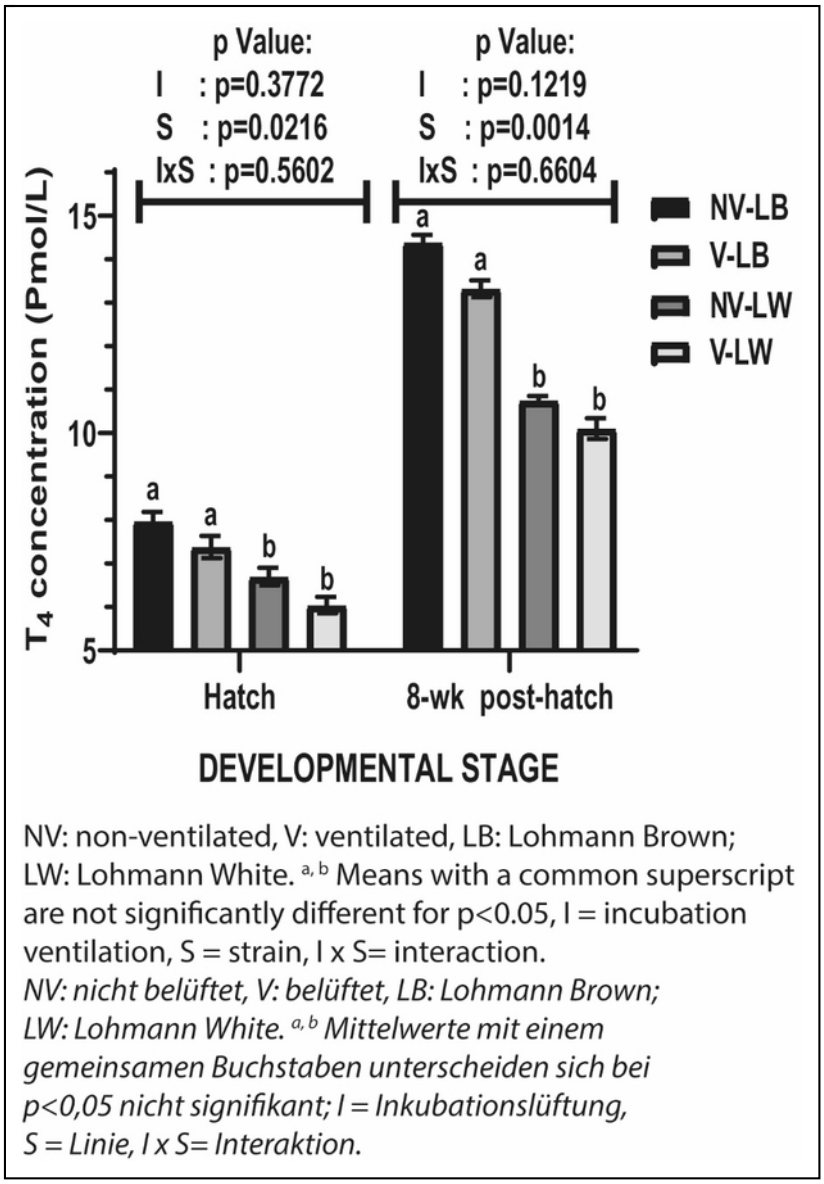



**Figure 10. Thyroxin ($T_4$) levels according to strain and ventilation treatment.**

**Einfluss der Linie und Belüftung auf den Thyroxin ($T_4$)-Gehalt.**

At hatch, LB chicks showed higher ($p = 0.0003$) $T_3/T_4$ ratio compared to LW chicks in both groups but there was not significant effect ($p = 0.1047$) of ventilation treatment in both strains. At 8 weeks post-hatch, $T_3/T_4$ ratio was higher in non-ventilated group compare to ventilated group for LB chicks but the result was the reverse for LW chicks indicating a significant interaction ($p < 0.0001$) between strain and ventilation treatment (Figure 11).

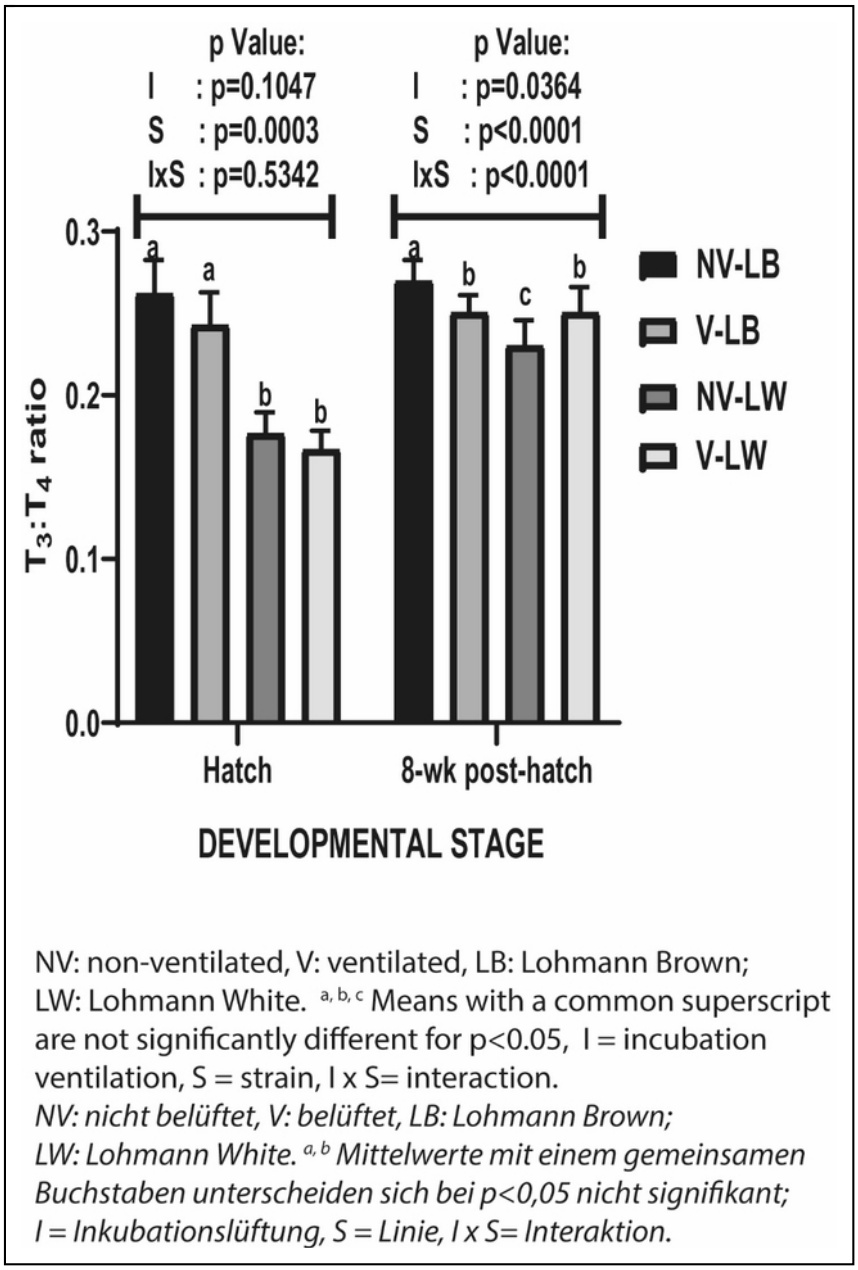



**Figure 11. $T_3/T_4$ ratio according to strain and ventilation treatment.**

**Einfluss der Linie und Belüftung auf das $T_3/T_4$-Verhältnis.**

## Discussion

During incubation, eggs release $H_2O$ and $CO_2$. The removal of $CO_2$ during incubation is therefore considered one of the most important tasks of ventilation. Several reports have suggested that during early incubation, the chicken embryo can tolerate high level of $CO_2$ to a great extent. Previous studies increased carbon dioxide level up to 1% and 1.5% by non-ventilation (BRUGGEMAN et al., 2007, TONA et al., 2013, EL-HANOUN et al., 2019) into the incubator at the beginning of incubation and showed a positive effect on broilers or duck embryonic development and hatchability.

In this study, although the absolute concentration reached at ED10 was lower than obtained in previous experiments, the $CO_2$ concentration curve followed the same pattern. The differences in $CO_2$ concentration can be attributed to the method used. In previous studies, the increased $CO_2$ level was done by $CO_2$ injection while in the present study, the $CO_2$ concentration was increased only by closing incubator dampers. In this condition, the lower $CO_2$ level could be attributed to egg fertility. Moreover, this increase in carbon dioxide concentration seems to influence some hatching processes related to embryonic development, which might differ between strains. The

present experiment demonstrated with the use of non-ventilation in the first 10 days of incubation that the increase of $CO_2$ levels in the incubator affected the physiology and the hatching events of the LB and LW strains differently.

The effect of non-ventilation up to ED10 on embryo growth was even evident at ED12 and ED14 where embryo weights from non-ventilated group were higher than that of the ventilated group in both strains. Similar result was published by BILALISSI et al. (2022). DE SMIT et al. (2006) reported that this beneficial effect on embryonic growth was the result of the expression of pH-dependent enzymes like carbonic anhydrase, which is involved in the formation of sub-embryonic fluid (SEF), which is a pivotal event in early development. The higher embryonic weight in non-ventilation treatment in the LB compared to the LW strain suggests that the effect of ventilation treatment on embryonic growth may be genotype-dependant. These differences in LB and LW embryos growth both in non-ventilated and ventilated groups observed at ED12 indicate different developmental trajectories in both genotypes as reported by TONA et al. (2010a). Although non-ventilation is known to be a stimulus for a higher concentration of plasma corticosterone and $T_3$, leading to the initiation of the hatching process in chickens (DECUYPERE et al., 1991), the average time of IP, EP and the average hatching time did not differ between non-ventilated and ventilated groups in both strains. This result can be attributed to thyroid hormones levels which did not differ between ventilated and non-ventilated groups. In the study of TONA et al. (2013), the average time was shorter in the non-ventilated group compared with the ventilated group when the $CO_2$ level reached 1.5% at ED10. The observed differences between the study of TONA et al. (2013) and the present study could be explained by the level of $CO_2$ reached at ED10 of incubation for early hatching.

A previous report of TONA et al. (2010a) demonstrated that the LB strain hatched earlier than the LW strain. This result was confirmed in this study, where hatching of the LB strain started 6 h earlier compared to the LW strain, and the time between IP and hatching (duration of hatch) was shorter in the LB strain than in the LW strain. This resulted in the shorter hatching window and a higher chick quality score for the LB compared to the LW strain. This phenomenon is related to increased levels of corticosterone, $T_3$ and $T_4$ as a result of increased $CO_2$ in the air cell at internal pipping under the non-ventilation condition (EL-HANOUN et al., 2019). According to ARAÙJO et al. (2016), the shorter the hatch window is, the better the physical quality of broiler chicks. The shorter hatching time observed for LB embryos can be linked to their higher embryonic thyroid activity. It is well known that the interval between IP (the start of pulmonary respiration) and hatching is thyroxin-dependent (DECUYPERE et al., 1991). Thus, the shorter interval between IP and hatch for the chicks of the LB strain compared with the LW strain might be explained by their higher thyroxin concentrations until the moment of hatch. Our result is in accordance with DE SMIT et al. (2008) who demonstrated that the shorter interval between IP and hatch for the chicks of Cobb compared with SAS strains was linked to their plasma tetra-iodothyronine ($T_4$) levels. Indeed, the main secretory product of the thyroid gland is thyroxin ($T_4$), which is converted to tri-iodothyronine ($T_3$) by 5'-monodeiodination. It is accepted that the conversion of $T_4$ to $T_3$ takes place mainly in the liver. This conversion is determined by the amount of nutrient that reaches the liver. The higher $T_3/T_4$ ratio observed in the LB strain at hatch and at 8 weeks post hatch indicated a rapid conversion of $T_4$ into $T_3$ and this may be related to egg nutrient consumption or feed intake which was higher for the LB strain; thus providing the liver with a high availability of nutrients to be metabolized. Thus, the higher $T_3$ concentration observed for LB chicks compared to LW chicks both at hatch and post-hatch point to a higher metabolic rate culminating in a higher body weight up to 8 weeks for LB chicks as it is generally assumed that $T_3$ is the calorigenically active form of thyroid hormones (TONA et al., 2013).

Eggs incubated during the first 10 days under non-ventilation conditions were under higher humidity condition which is reflected in the lower egg weight loss compared to the ventilated group. This result is in line with studies by ÖZLÜ et al. (2018), EL-HANOUN et al. (2019) and BILALISSI et al. (2022) who showed lower egg weight loss for eggs incubated at non-ventilation condition. However, egg weight loss was higher in LW eggs compare to LB eggs. Thus, the higher hatchability in the LW strain, which corresponded with the lower embryonic mortality compared to the LB strain might be related to their egg weight loss. In fact, the lower egg weight loss observed in LB eggs would have reduced the gas exchange through the egg membrane and promoting a decreased hatchability. Moreover, non-ventilation increased hatchability in the LB strain but not in the LW strain. Thus, the effect of non-ventilation on hatchability might be genotype-dependant. This finding was in agreement with studies by EL-HANOUN et al. (2019) who reported that the hatchability of duck fertile eggs was significantly higher in non-ventilated than in ventilated groups.

Chick weight at hatch mainly depends on the initial egg weight. The higher chick weights of the LW strain compared to the LB strain in ventilated groups was due to their egg weight, which was heavier than those of the LB strain eggs.

This may be due to the higher amount of yolk in LW eggs compared to LB eggs. The higher chick weight from the LW strain could be linked to the amount of yolk present in the eggs that the chicks would have utilized before they had access to feed and water. Moreover, ABIOLA (1999) have shown that the positive correlation between egg weight and day old chick weight at hatching is related to the amount of yolk. In fact the correlation between egg weight and chick weight has been reported to be $r^2 = 0.99$ (MBAJIORGU, 2011). Non-ventilation did not affect the day old chick weight in both LB and LW strains. This result is in agreement with DE SMIT et al. (2006) who did not find any difference between the 1-day old chicks of the ventilated and the non-ventilated groups.

In this study, non-ventilation did not affect chick's body weight up to 8-wk post-hatch. But, DE SMIT et al. (2006) showed that chick's weight from the non-ventilated group was higher compared to chicks from the ventilated group at day 7 post-hatch when the $CO_2$ level was increased up to 1.5% at ED 10 of incubation which was higher compared to the $CO_2$ level reached in this study. In this reflection, one has to bear in mind the importance of $CO_2$ level during the first 10 days of incubation for possible effect on post-hatch growth. Moreover, chicks from the LB strain had higher body weights and higher body weight gain than those of the LW strains as a result of higher feed intake observed in the LB strain compare to the LW strain. These differences can be attributed to higher thyroid hormones which point to higher metabolic rate for LB chicks compare to LW chicks. It can also be suggested that the observed differences are linked to the genetic make-up of the two strains. However, no difference was observed on feed conversion ratio. These results are in agreement with the report of AL-NASSER et al. (2002) who showed that the body weight, body weight gain and feed intake of brown pullets were higher than that of white pullets.

## Conclusion

From the results of this study, it can be concluded that non-ventilation during the first 10 d of incubation increased the levels of $CO_2$ in the incubator, which affect differently the physiological indices of the two layers strains. The average time and the time intervals of the hatching process were different between the two strains and can be related to the changes in thyroid hormones. LB and LW strains do not have the same developmental trajectory and the effect of non-ventilation during the first 10 days of incubation on some hatching and post-hatch parameters may depend on the level of carbon dioxide content in the incubator.

## Acknowledgment

The study was supported by CERSA (Regional Excellence Center on Poultry Sciences) of University of Lome (Togo). Authors express a warm gratitude to World Bank IDA 5424 who is the main sponsors of CERSA.

## Author's contributions

**A.B** conceived, designed, performed the study and wrote the paper. **H.T.M** participated in the practical work and critical revision of the manuscript. **O.E.O, H.L** and **O.O** conducted constructive criticism and English language assistance during the preparation of the manuscript. **K.T** supervised and approved the experimental design of the study, clever advisor for statistical analysis, critical revision of the manuscript and final approval for paper submission.

## Conflict of interest

None.

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