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Green light during incubation: Effects on hatching characteristics in brown and white laying hens

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HIGHLIGHTS

- Light transmission through eggshell is dependent on eggshell color.
- Light during incubation does not impact hatching performance in laying hens.
- Laying hen hybrids and sexes have similar hatching performance scores.
- Future research should focus on effects of lighted incubation on laying hen welfare.

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ABSTRACT

Providing light during incubation is being investigated as a method to improve welfare in later life in poultry. This incubation method would more closely approximate chicken natural environment compared to the current incubation in darkness. Previous studies showed promising results of light during incubation on broiler welfare, but little is known about effects of light during incubation on laying hens. Especially, information about its effects on hatching characteristics (hatch time, hatchability, chick quality, body weight and embryonic age of death) is scarce and requires investigation in both white and brown egg layers. In the current study, Dekalb White (DW) and ISA Brown (ISA) eggs were incubated in complete darkness (dark) or in a light:dark cycle of 12L:12D throughout incubation (light), resulting in four treatment groups: DW-dark, DW-light, ISA-dark, and ISA-light. In the light treatments, green LEDs of 520 nm wavelength were used, at an intensity of 400 lux. First, light transmission through the eggshell was measured through 27 eggs. Then, an analysis of the effects of light during incubation on hatching characteristics was performed on 711 chicks in two consecutive experimental rounds. Light transmission was higher through white eggshells than through brown eggshells ($N = 27$, $p < 0.001$). Light during incubation had no effects on hatching characteristics ($N = 711$, $p \geq 0.1$). Despite the difference of light transmission through eggshell between hybrids, there was no interaction between incubation treatment and hybrid on hatching characteristics ($N = 471$, $p \geq 0.06$). Hatch time was longer and navel quality was better in DW than in ISA, while body weight and embryonic age of death were lower in DW than in ISA (all $p < 0.001$). Males and females had similar chick quality scores except for the beak quality, which was better for males ($N = 486$, $p = 0.003$). To conclude, green light during incubation did not negatively affect hatching characteristics in either DW nor ISA laying hen hybrids. Future research should therefore focus on its potential benefits for laying hen welfare.

1. Introduction

Poultry eggs are typically incubated in complete darkness, while in nature eggs are exposed to some light during the day when the hen leaves the nest. A growing number of investigations are performed on effects of light during incubation in broiler chicks and show negative,

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neutral or positive effects of light during incubation on hatching characteristics (Archer et al., 2009; Archer and Mench, 2014a; Huth and Archer, 2015; Özkan et al., 2012a; Tong et al., 2018; Yu et al., 2018).

Laying hens are a sizable part of the poultry industry, but few studies have investigated effects of light during incubation in laying hens. These studies also showed no negative effects on hatching characteristics, with a more synchronized hatching window and similar hatchability in light-incubated chicks compared to dark-incubated chicks (Özkan et al., 2022; Wang et al., 2020). In addition, effects on laying hen welfare seems positive. Light-incubated layer chicks performed better in a cognitive task (transitive inference) (Daisley et al., 2010) and displayed less severe feather pecking behavior (Özkan et al., 2022) compared to dark-incubated chicks. If light during incubation is promising to improve laying hen welfare, it therefore seems reasonable to investigate further its effects on additional hatching characteristics, such as chick quality, body weight and embryonic death.

This is even more pertinent considering that eggshell color varies between laying hen hybrids. Light is transmitted more readily through a lighter eggshell (white) than through a darker eggshell (brown) (Shafey et al., 2005, 2004, 2002). Chicken pink eggs (Yu et al., 2016) and wild bird eggs (Lahti and Ardia, 2016; Maurer et al., 2015) also showed more light transmission through light-colored eggshells than through dark-colored shells. To our knowledge, these are the only published studies on the effect of eggshell color on light transmission through the eggshell. Light intensity plays a role in the effect of light during incubation on chicken welfare (Tong et al., 2018), thus it may also affect hatching characteristics. Consequently, it seems crucial to broaden our knowledge on the topic. For the present study, we focused on ISA Brown and Dekalb White hybrids. They are the most common Dutch layer hybrids hatching from brown and white eggs, respectively.

Our focus was therefore to investigate interaction effects of light during incubation and hybrid on hatching characteristics. First, we studied whether eggshell color (or the hybrid) influenced green light transmission through the eggshell (experiment 1). Then, we investigated whether a cycle of green 12L:12D throughout incubation affected hatching characteristics of the different hybrids compared to an incubation in complete darkness (experiment 2). Green light was used because of its promising results to improve welfare (Özkan et al., 2022), the long-term goal of using light during incubation.

Based on the results of the forementioned studies (Lahti and Ardia, 2016; Maurer et al., 2015; Shafey et al., 2005, 2004, 2002; Yu et al., 2016), we hypothesized there would be a higher light transmission through white eggshells of Dekalb White hybrids compared to brown eggshells of ISA Brown hybrids. This could potentially lead to stronger light effects on chicks from white than from brown eggs.

In addition, we hypothesized there would be no negative effects of the light during incubation on hatching characteristics (Hannah et al., 2020; Huth and Archer, 2015; Özkan et al., 2012a, 2022; Wang et al., 2020).

Based on the information provided by the breeders and a recent study (Hendrix-Genetics, 2020a, 2020b; Wang et al., 2020), we hypothesized that the only hatching characteristics that would show hybrid differences would be hatch time and body weight.

2. Materials and methods

2.1. Experiment 1: effect of the eggshell color on the light transmission through the eggshell

2.1.1. Ethical statement

The experiment was performed on fertilized eggs of embryonic day 0, which does not require any ethical approvement.

2.1.2. Eggs and preparation

15 eggs from ISA Brown and 12 eggs from Dekalb White hybrids were used for the light transmission experiment, the unbalanced sample size being due to human and technical errors. The fertilized eggs of embryonic day 0 from both hybrids originated from 43-weeks old parental flocks housed in traditional single-tier housing. For the measurements, the eggs were broken open as close as possible to the small pole to remove the egg contents.

2.1.3. Light measurements

An empty eggshell was placed under a green LED strip (Barthelme Y51515213 182,007 LED strip, 520 nm), at the same distance from the LED than it would have in the incubators in Experiment 2. The sensor of a spectrometer AvaSpec-ULS2048 (Avantes, Serial nr: 1601107U1) was placed underneath the eggshell and was connected to a laptop equipped with AvaSoft-Basic software (Avantes). The light of the room was turned off so that only the light from the LEDs was perceived by the sensor, and the measurement was recorded. The data consisted in the number of photons perceived (called "light transmission") (in counts) for 1614 wavelengths from 173.8 to 1100.07 nm.

A control measurement was also made by placing the spectrometer sensor below the light without any eggshell. The highest light transmission corresponded then to a wavelength of 516.32 nm (from now onwards referred to as the control peak). For consistency, the control peak was used to compare the two hybrids in the results (see statistics section).

After the measurements, the eggshells were transported to the lab and their thickness was measured using an electronic micrometer IP 54 (Helios Preisser; precision: 0.001 mm; range: 0 – 25 mm).

2.1.4. Statistical analysis

All the data was analyzed using RStudio 2022.02.3 (Build 492).

Because the eggshells had different colors, we hypothesized they would filter the light differently. We compared the wavelengths corresponding to the highest light transmission through the eggshell of each hybrid, using a Mann-Whitney U test for non-paired data. Non-parametric analyses were used because of problems with distribution and homogeneity of variances.

The eggshell thickness was normally distributed and its variances were homogeneous. A *t*-test was therefore used to determine whether or not the two hybrids had a significantly different eggshell thickness.

Finally, for each hybrid, a Spearman correlation test was performed to investigate the correlation between eggshell thickness and light transmission through the eggshell at the control peak.

All of these analyses were also performed based on the wavelength corresponding to the highest light transmission for each hybrid mentioned earlier; the results were the same to that of the control peak (data not shown).

2.2. Experiment 2: effects of green light during incubation on hatching characteristics of two laying hen hybrids

The experimental design was a 2 × 2 × 2 factorial arrangement (hybrid x incubation treatment x sex) repeated in two consecutive rounds.

2.2.1. Ethical statement

The research project was approved by the Dutch central authority for scientific procedures on animals (the Hague, the Netherlands) under the number AVD1080020198685. The experiment is in accordance with the directive 2010/63/EU on animals used for scientific purposes.

2.2.2. Animals, incubation and hatching procedure

Eggs from the hybrids ISA Brown and Dekalb White were provided by Hendrix Genetics through a commercial hatchery (Het Anker, Ochten, the Netherlands). They originated from parental flocks between 37 and 50 weeks of age housed in traditional single-tier housing.

For practical reasons, the experiment was performed in two consecutive rounds. The first round started in September 2020, the second in September 2021.

In each round, 600 eggs (300 of each hybrid) were incubated at Wageningen University and Research (WUR), Wageningen, the Netherlands. Eggs from each hybrid were equally and randomly distributed over two trays in each of two incubators (each with a maximum setting capacity of 1408 eggs; HatchTech, Veenendaal, the Netherlands) and of two climate respiration chambers (CRC) (each with a maximum setting capacity of 400 eggs) (Verstegen et al., 1987). The rest of the egg positions were left empty. For more information about the HatchTech incubators and CRC (hereafter all called "incubators"), see Güz et al. (Güz et al., 2021).

In one of the HatchTech incubators and one of the CRC, green LED-strips were installed above the eggs (Barthelme Y51515213 182007 LED strip) in such a way that the light intensity was approximately 400 lux at egg level (an average of the light intensity used in other studies (Archer and Mench, 2014a; Özkan et al., 2012a)). The eggs were exposed to a cycle of monochrome green light (520 nm) and dark of 12L:12D throughout the incubation period. This photoperiod was chosen because Archer and colleagues showed on several occasions that was the most promising photoperiod to improve chicken welfare (Archer et al., 2009; Archer and Mench, 2017, 2014a, 2013). The other two incubators were kept in complete darkness.

The first 18 days of incubation, the eggs were turned every hour at an angle of 90°, and the relative humidity and eggshell temperature were set at 57.5% and 37.8 °C, respectively. Eggshell temperature was monitored and regulated with sensors placed on the eggshells of four eggs distributed across each incubator. From embryonic day (ED) 19, the egg-turning stopped, and the relative humidity and incubator temperature were set at 58.5% and 36.3 °C. The sensors were then removed from the eggshells to avoid any disturbance during hatching.

At ED 19, non-fertile eggs and eggs containing dead embryos were identified by candling, and were then removed from the incubators and broken out for investigation (see next section). The remaining eggs were moved to hatching baskets. From then on, and until ED21, hatching and quality checks were performed every six hours (as described in Heijmans et al. (2022)), allowing investigation of the hatch time (in hours after the start of the incubation). The new hatchlings were marked and placed back in the hatching baskets, and quality checks took place six hours later. After the quality checks, the males were culled by cervical dislocation and the females were placed back in the hatching basket for a long-term welfare study (Manet *et al.*, unpublished data). On ED21, all unhatched eggs were broken out for investigation (see next section). To mimic production practice, no feed was provided in the incubators around hatching.

2.2.3. Hatching quality

The chick quality protocol followed the one described by Heijmans et al. (2022). In brief, chick vitality was investigated by placing a chick on its back on a table and measuring after how long it returned on its feet. Less than two seconds led to a score of good quality, and more than two seconds led to a score of poor quality. In addition, body weight was measured, and the absence or presence of red dots on the beak and hocks was recorded as additional measures of good and poor quality, respectively. Finally, the quality of the navel was also evaluated, grading them from good, to moderate, to poor quality (Table 1).

2.2.4. Break outs

Eggs removed from the incubators at ED19 and unhatched eggs at ED21 were broken out and fertility or age of embryonic death were determined by visual inspection (Reijrink et al., 2009).

2.2.5. Statistical analyses

The models used and their distribution are shown in Table S1. Briefly, the navel quality was analyzed using a Bayesian generalized linear multivariate multilevel model, while all other parameters were analyzed using generalized linear mixed models.

Table 1
Summary of the different chick quality assessments measured at hatching. Each parameter was scored on a binary scale with 0 = good and 1 = poor quality, except for the navel, which had three scores: 0 = good, 1 = moderate and 2 = poor quality.

Measurement	Good quality	Moderate quality	Poor quality
Chick vitality	$\leq$ 2 s		> 2 s
Hock	No red dots		Red dots present
Navel	Flat and closed, nothing protruding	Dried membrane, black button < 2 mm, swollen, red, and/or presence of a string	Black button > 2mm
Beak	No red dots		Red dots present

The models were all first built with the hybrid, the incubation treatment, the sex, the two-way interactions between those three factors, and the round as fixed factors. When relevant, the experimenter was also added as a fixed factor (for the vitality test, beak quality, navel quality and hock quality). Interactions with round or with experimenters were not included as they were not biologically relevant.

Finally, though the experimental unit was the individual chicken, the incubator was corrected for as a random effect. For the embryonic age of death, the tray in which the egg was incubated (up or down) was also corrected for as a random effect. The latter data was only available for the first breakout day, as the eggs were moved after candling to hatching baskets.

When the interactions were not significant, they were stepwise removed from the models until only main factors and significant interactions were left.

For the main effects, only the outcomes from the final models are hereafter reported. For the interactions, the outcome from the last model including them are reported. The estimate (abbreviated as **est**), the 95% confidence interval (hereafter referred to as **95% CI**) and the p-values are reported. An effect was considered significant if the 95% CI did not include 0 and if $p < 0.05$. In the case of the Bayesian model, the statistics software did not provide p-values; these were therefore calculated using Wald's statistic: 2*(1-pnorm(abs(w1)), with w1 = coefficient / standard error.

For practical reasons, the chick quality and body weight of the males were only measured in round 1. To avoid any bias due to the difference in sample size, two analyses were therefore performed for each parameter scored: (1) on the data from the females, to allow round comparison; (2) on the data from round 1, to allow sex comparison. Since only female laying hens are used in practice, the data from the females are presented in details, while only a summary of the results from round 1 is mentioned.

3. Results

The datasets of the two experiments are available at the following address: https://doi.org/10.5281/zenodo.8004765.

3.1. Experiment 1: effect of the eggshell color on the light transmission through the eggshell

The wavelength of the highest light transmission was different between hybrids ($p = 0.0018$). The light transmitted at the control peak was 5.5 times higher for white eggshells compared to brown eggshells ($p < 0.001$). Brown eggshells were thicker than white eggshells ($p = 0.009$). There was no significant correlation between the eggshell thickness and the light transmitted for either hybrid ($p \geq 0.45$) (Fig. 1). More detailed results are available in Table S2.

Table 2.

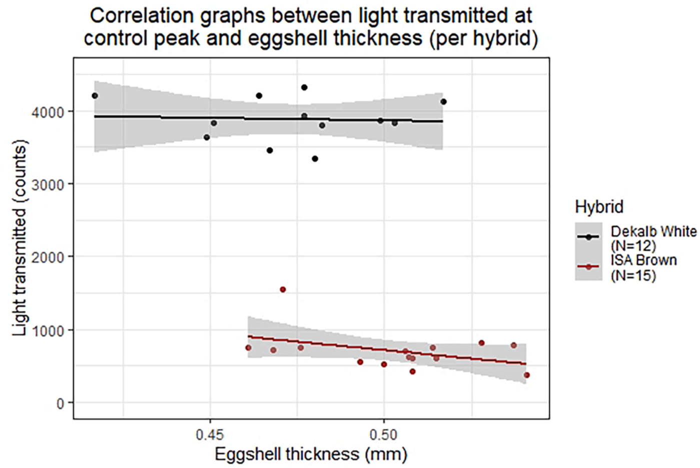

Fig. 1. Correlation between the eggshell thickness light transmitted at control peak for Dekalb White and ISA Brown.

Table 2
Summary of the results of experiment 2. The means for each hybrid and each incubation treatment are given in the relevant columns for each parameter. The estimate, 95% CI and p-value are given (in this order) in the remaining columns. Estimates indicate how the data from white, light-dark cycle, white:light-dark cycle and round 2 changed compared to their respective counterpart. Significant results are in bold, trends are in italics.

Parameter	Hybrid:Incubation Statistics outcome	Incubation Means D[1]	 L[2]	Statistics outcome	Hybrid Means B[3]	 W[4]	Statistics outcome	Round Statistics outcome
Hatch time (h)	est = 0.5 95%CI = [−1.1 – 2.1] *p* = 0.53	467.2	466.7	est = −0.5 95% CI = [−1.2 – 0.3] *p* = 0.26	**462.7**	**471.3**	**est = 8.6** **95% CI = [7.8 – 9.3]** ***p* < 0.001**	**est = 2.2** **95% CI = [1.4 – 2.9]** ***p* < 0.001**
Fertility (%)	est = −0.2 95% CI = [−2.8 – 2.4] *p* = 0.88	98.96	99.16	est = 0.2 95% CI = [−1 – 1.5] *p* = 0.71	98.82	99.29	est = 0.5 95% CI = [−0.7 – 1.9] *p* = 0.42	est = −1.0 95% CI = [−2.5 – 0.3] *p* = 0.15
Hatchability (%)	est = −0.4 95% CI = [−1 – 0.2] *p* = 0.22	83.38	83.36	est = −0.008 95% CI = [−0.3 – 0.3] *p* = 0.96	82.52	84.22	est = 0.09 95% CI = [−0.2 – 0.4] *p* = 0.56	est = −0.1 95% CI = [−0.4 – 0.2] *p* = 0.70
Vitality	est = 0.1 95% CI = [−0.9 – 1.1] *p* = 0.80	0.236	0.181	est = −0.4 95% CI = [−0.9 – 0.1] *p* = 0.10	0.267	0.151	est = −0.29 95% CI = [−0.8 – 0.3] *p* = 0.30	est = −0.3 95% CI = [−0.9 – 0.4] *p* = 0.42
Beak	est = −0.7 95% CI = [−1.8 – 0.4] *p* = 0.25	0.120	0.152	est = 0.3 95% CI = [−0.3 – 0.8] *p* = 0.30	0.134	0.138	est = 0.2 95% CI = [−0.5 – 0.8] *p* = 0.58	est −0.3 95% CI = [−0.9 – 0.3] *p* = 0.39
Navel	est = 0.04 95% CI = [−0.7 – 0.8] *p* = 0.96	1.845	1.892	est = 0.08 95% CI = [−0.3 – 0.5] *p* = 0.64	**2.082**	**1.655**	**est = 1.07** **95% CI = [−1.5 – −0.7]** ***p* < 0.001**	est = 0.2 95% CI = [−0.2 – 0.7] *p* = 0.30
Hock	est = −0.8 95% CI = [−1.9 – 0.4] *p* = 0.19	0.155	0.182	est = 0.05 95% CI = [−0.5 – 0.6] *p* = 0.88	*0.194*	*0.142*	*est = 0.6* *95% CI = [−0.07 – 1.3]* *p = 0.08*	**est = 1.2** **95% CI = [0.4 – 2.0]** ***p* = 0.0034**
BW[5] (g)	*est = −0.8* *95% CI = [−1.6 – 0.07]* *p* = 0.07	41.21	41.33	est = 0.02 95% CI = [−0.6 – 0.6] *p* = 0.94	**42.46**	**40.07**	**est = −2.43** **95% CI = [−3.0 – −1.8]** ***p* < 0.001**	**est = 0.7** **95% CI = [0.2 – 1.3]** ***p* = 0.01**
EAD[6] (d)	est = −1.3 95% CI = [−3.4 – 1.1] *p* = 0.31	9.18	9.11	est = 0.2 95% CI = [−0.9 – 1.4] *p* = 0.68	**9.34**	**8.92**	**est = −1.3** **95% CI = [−2.4 – −1.3]** ***p* = 0.02**	est = 1.1 95% CI = [−0.1 – 2.3] *p* = 0.21

[1] Dark.
[2] Light-dark cycle.
[3] Brown.
[4] White.
[5] Body Weight.
[6] Embryonic Age of Death.

3.2. Experiment 2: effects of green light during incubation on hatching characteristics of two laying hen hybrids

The results of experiment 2 are summarized in Table 3.

3.2.1. Hatch time

Hatch time was not influenced by the interaction between incubation treatment and hybrid ($p = 0.53$) nor by the incubation treatment alone ($p = 0.26$). Chicks from white eggs hatched on average 8.6 h later than chicks from brown eggs ($p < 0.001$) (Fig. 2). Chicks from round 1 hatched 2.2 h earlier than chicks from round 2 ($p < 0.001$). Hatch time was also not influenced by sex (est = −0.06, 95% CI = [−0.8 – 0.7], $p = 0.89$) or any other interaction ($p > 0.1$; for detailed estimates, 95% CI and p-values, see Table S3).

3.2.2. Fertility and hatchability

Fertility was not influenced by the interaction between incubation treatment and hybrid, incubation treatment, hybrid or round ($p \geq 0.15$).

Table 3
Mean quality scores and body weight per hybrid, incubation treatment and sex of chicks from round 1. Estimate, 95% CI and p-values of the fixed effects are given. The estimates indicate how the data from white, light-dark cycle and males change compared to their respective counterparts. *P*-values calculated with Wald's statistics are indicated with an [a]Significant results in bold, trends in italics.

Parameter	Factor	Mean	Estimate	95% CI	*p*
Vitality	Hybrid	B[1]: 0.244 W[2]: 0.165	0.2	[−0.3 – 0.8]	0.43
	Incubation	D[3]: 0.232 L[4]: 0.180	−0.3	[−0.8 –0.1]	0.14
	Sex	F[5]: 0.201 M[6]: 0.211	0.003	[−0.5 –0.5]	0.99
	Exp[7]		**−1.4**	**[−2 −−0.9]**	**< 0.001**
Beak	Hybrid	B: 0.136 W: 0.131	0.1	[−0.5 –0.8]	0.64
	Incubation	D: 0.112 L: 0.155	0.4	[−0.2 –1.1]	0.12
	Sex	**F: 0.180 M: 0.089**	**−0.9**	**[−1.4 −−0.3]**	**0.003**
	Exp		−0.3	[−0.99 –0.3]	0.29
Navel	**Hybrid**	**B: 2.064 W: 1.602**	**−1.1**	**[−1.6 −−0.7]**	**< 0.001[a]**
	Incubation	L: 1.846 D: 1.833	−0.06	[−1.3 –0.8]	0.92[a]
	Sex	F: 1.854 M: 1.826	−0.1	[−0.5 –0.3]	0.50[a]
	Exp		**−0.99**	**[−1.4 −−0.6]**	**< 0.001[a]**
Hock	Hybrid	B: 0.172 W: 0.068	−0.5	[−1.2 –0.4]	0.13
	Incubation	D: 0.124 L: 0.118	−0.05	[−0.6 –0.5]	0.86
	Sex	F: 0.096 M: 0.146	0.5	[−0.1 –1.1]	0.11
	Exp		**−1.2**	**[−1.9 −−0.5]**	**< 0.001**
Body weight (g)	**Hybrid**	**B: 41.81 W: 40.40**	**−1.4**	**[−2.0 −−0.8]**	**< 0.001**
	Incubation	D: 41.10 L: 41.16	0.09	[−1.7 –1.9]	0.94
	Sex	F: 40.96 M: 41.30	0.3	[−3.3 –0.9]	0.36

[1] Brown.
[2] White.
[3] Dark.
[4] Light-dark cycle.
[5] Female.
[6] Male.
[7] Experimenter.

The average fertility was of 99.1% (Fig. 2).

Hatchability of fertile eggs was not influenced by the interaction between incubation treatment and hybrid, incubation treatment, hybrid or round ($p \geq 0.22$). The average hatchability of fertile eggs was of 83.3% (Fig. 2).

3.2.3. *Chick quality*

The chick quality results are summarized in Fig. 2.

Vitality and beak scores were not influenced by the interaction between hybrid and incubation, hybrid, incubation treatment or round ($p \geq 0.10$).

Navel score was not influenced by the interaction between hybrid and incubation, incubation treatment or round ($p \geq 0.30$). The navel score of chicks from white eggs was on average 1.07 unit lower (better) than that of chicks from brown eggs ($p < 0.001$).

Hock score was not influenced by the interaction between hybrid and incubation or by incubation treatment ($p \geq 0.19$). Chicks from white eggs tended to have a 0.6 unit higher (worse) hock score than chicks form brown eggs ($p = 0.08$). The hock score was 1.2 units higher (worse) in round 2 than in round 1 ($p = 0.003$).

There was an experimenter effect on vitality score (est = −1.8, 95% CI = [−2.5 – −1.1], $p < 0.001$), navel score (est = −0.76, 95% CI = [−1.3 – −0.2], $p = 0.01$) and hock score (est = −2.63, 95% CI = [−3.5 – −1.8], $p < 0.001$), but not on beak score (est = 17.6, 95% CI ∋ 0, $p = 0.90$).

3.2.4. *Body weight*

Body weight was not influenced by the interaction between hybrid and incubation or incubation treatment alone ($p \geq 0.07$). Chicks from white eggs weighed 2.4 g less than chicks from brown eggs ($p < 0.001$). Body weight was 0.7 g lower in round 1 than in round 2 ($p = 0.01$) (Fig. 3).

3.2.5. *Embryonic age of death*

Embryonic age of death was not influenced by the interaction between incubation treatment and hybrid, incubation alone or round ($p \geq 0.21$). Non-hatched embryos of white eggs died on average 1.3 days earlier than those of brown eggs ($p = 0.02$).

3.2.6. *Sex comparison*

The analyses of the vitality, beak, navel and hock scores, and body weight with only the data from round 1 are summarized in Table 3. Briefly, the males' beak score was 0.9 unit lower (better) than the females, while none of the other parameters differed between sexes.

The only slight difference regarded the hock score: here, there was no significant difference due to the hybrid, while in the female data, there was a trend for the chicks of white eggs to have a higher (worse) hock score than chicks of brown eggs.

4. Discussion

Light transmission was measured through eggshells of Dekalb White and ISA Brown eggs (white and brown shells, respectively). Considerably more light was transmitted through the white shells compared to the brown shells. That confirmed our hypothesis and was consistent with previous studies by Shafey et al. (2002, 2004, 2005). This difference might be related to the thickness of the eggshells: brown eggshells were thicker than white eggshells. However, in this experiment, eggshell thickness was not correlated to light transmission through the eggshell. That supports the hypothesis that eggshell color, rather than eggshell thickness, was responsible for this difference in light transmission.

We hypothesized that light during incubation might affect chickens differently depending on their eggshell color. If that was the case, we could not conclude whether the differences were only due to the color of the eggshell, or also to other egg characteristics or to the genetic background of the different lines. Future investigation could focus on disentangling effects of the color of the eggshell and of the genetic background. One way would be to filter the light so it is transmitted the same way for both hybrids (*e.g.* using different light intensities, different LED colors, a physical filter, or paint).

Our main argument to use light during incubation was its close similarity to natural incubation. In natural incubation conditions, effects of genetics and eggshell color cannot be disentangled. For that reason, we decided to use the same LED lights (resulting in different light transmission levels) for both hybrids in experiment 2.

The use of light during incubation as an intervention to improve welfare has had promising results in broiler chickens (Archer and Mench, 2017, 2014a, 2014b, 2013). In the second experiment, the incubation treatment did not affect any of the hatching characteristics,

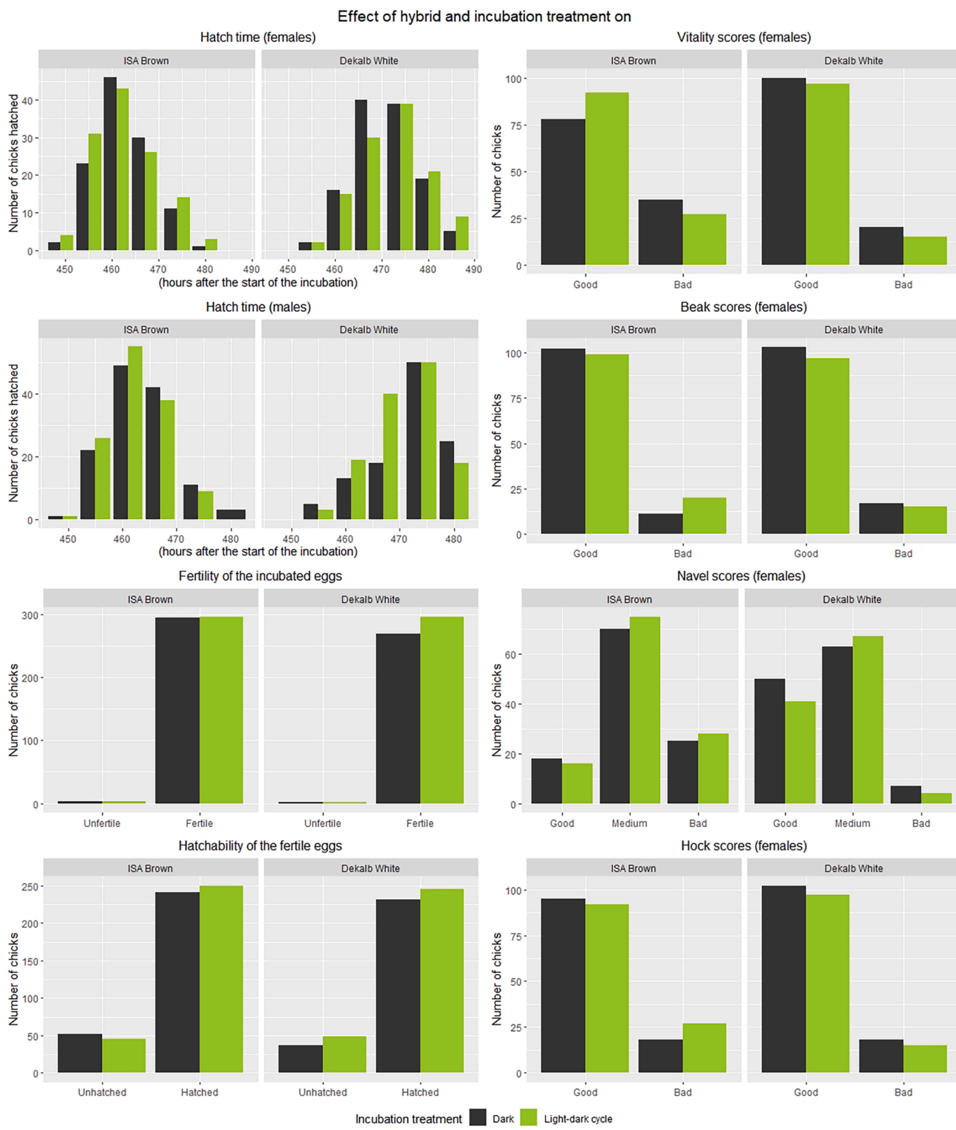

Fig. 2. Effect of hybrid and incubation treatment on hatch time, fertility, hatchability and chick quality.

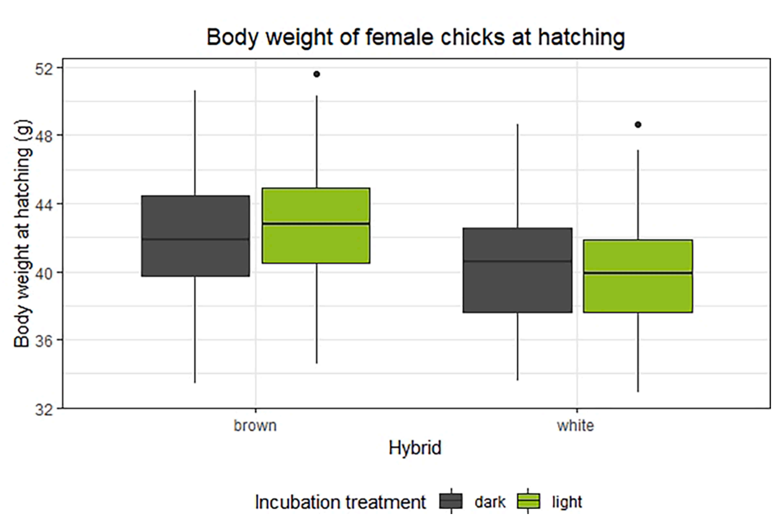

Fig. 3. Female chicks body weight at hatching depending on the hybrid and the incubation treatment. Sample sizes: brown, Dark: 113. Brown, Light-dark cycle: 121. White, Dark: 121. White, Light-dark cycle: 116.

regardless of the hybrid. The two hybrids differed for a few parameters. The Dekalb White chicks were sometimes of better, sometimes of lesser quality than the ISA Brown chicks. The only difference between sexes was found for the beak quality.

Consistently with literature, the incubation treatment had no effect on hatchability, body weight or age of embryonic death (Huth and Archer, 2015; Özkan et al., 2012b, 2022; Wang et al., 2020). However, light incubated chicks were expected to hatch sooner and to be of better quality (Hannah et al., 2020; Özkan et al., 2022; Wang et al., 2020), which we did not observe. The differences with the aforementioned studies could be due to the light intensity, color, photoperiod or schedule.

The light intensity in these studies was either 200 or 250 lux, while ours was 400 lux. It seems unlikely that a higher intensity led to fewer differences between treatments. However, our photoperiod and light schedule (12L:12D; 21 days) were also different (12L:12D or 16L:8D; 4, 13, 18 or 21 days). (Hannah et al., 2020) showed that the light schedule does not influence hatch time, but the combination with the light intensity and photoperiod may have played a role in our results. In addition, light color has shown to greatly influence the effects of light during incubation on behavior and physiology (Archer, 2017; Özkan et al., 2022). Our study was the first to use green light in a cycle of 12L:12D during 21 days at an intensity of 400 lux, and knowledge on the interaction between these factors (color, photoperiod, schedule and intensity) is lacking to compare our results to others.

Other possible explanations for the difference with other studies are the types of incubators and the hybrids used, both in terms of genetics and of eggshell pigmentation variation (Huth and Archer, 2015).

In addition, no significant interactions between incubation treatment and hybrid were found, despite the differences in light transmission through the eggshell. A first explanation might be the absence of an incubation treatment effect. There was a trend for an interaction between incubation treatment and hybrid on body weight. However, the difference only represented 2% of the average body weight of each hybrid, which does not seem biologically significant. Indeed, the weight difference was smaller than the margin allowed by the Management Guides (Hendrix-Genetics, 2020a, 2020b). A second hypothesis to explain the non-significant interaction is that Dekalb White may be biologically adapted to a higher light exposure during incubation and ISA Brown to a lower one, comparatively. That is, however, still an open question which requires research on light reception by Dekalb White and ISA Brown embryos.

This research showed several hybrid differences. Most of these are consistent with literature: embryonic age of death, hatchability, hatch time, beak score and hock score (Wang et al., 2020). Several explanations are possible, such as hybrid differences or non-hybrid flock characteristics (egg size, egg composition, storage duration and conditions, flock health, etc.) (Bouba et al., 2021), which makes it difficult to draw any conclusions.

The only inconsistency with literature resides in the navel score. (Wang et al., 2020) found no difference in navel score between White Leghorn and Rhode Island Red (the pure lines Dekalb White and ISA Brown respectively descend from). On the contrary, the Dekalb White chicks in the present study had better navel scores than ISA Brown chicks. The main driver for navel quality is temperature (Lourens et al., 2006). Since ISA Brown chicks were heavier than Dekalb White ones, it is likely that the brown eggs were heavier too (Pinchasov, 1991). Yet, heavier eggs are at larger risk to be overheated (Lourens et al., 2006), and white and brown eggs were mixed in the incubators. It is therefore possible that brown eggs experienced a higher temperature than white eggs, resulting in a worse navel quality.

In the literature, studies on laying hens focus on females for obvious reasons. In addition, studies on broilers usually do not look into sex differences as both sexes are used during rearing. However, it is known that, in chickens, females usually hatch earlier than males (Burke, 1992). In addition, a recent study compared vitality, beak, navel and hock quality between sexes in broilers. They found that females had worse vitality and navel scores than males, but better hock scores, and equal beak scores (Souza Da Silva et al., 2021). Our results are in contradiction with these findings. A sex difference was found in beak score, but not in hatch time, vitality, navel or hock scores. This may be due to the differences between broilers (used in those two studies) and layers (used in the present study) (Huth and Archer, 2015).

This research took place in two different rounds, and differences were found between rounds in terms of hatch window, hock quality and body weight. Performing experiments in batches brings in uncontrollable factors, such as seasonal differences or characteristics of the parent flocks. Overall, round effects found in the present research reflect the difficulty to repeat experiments. This in turn might even explain the inconsistencies found in literature.

To conclude, green light during incubation appears to have minor effects on hatching characteristics. Future investigations should be performed in an effort to disentangle the effect of light from the genetic effect of the hybrid used. Testing the effect of light during incubation on more white and brown hybrids is also required. Without that, it is impossible to know whether this intervention would suit all laying hens or should be hybrid-adapted. Finally, the incubation treatment had no effect on any hatching characteristics in these two hybrids. Research can therefore be pursued to assess the effects of green light during incubation on welfare of white and brown pullets without compromising productivity.

CRediT authorship contribution statement

Maëva W.E. Manet: Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Saskia Kliphuis:** Investigation, Methodology, Project administration, Writing – review & editing. **Henry van den Brand:** Investigation, Methodology, Writing – review & editing. **Rebecca E. Nordquist:** Supervision, Writing – review & editing. **Vivian C. Goerlich:** Supervision, Writing – review & editing. **T. Bas Rodenburg:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: T. Bas Rodenburg reports financial support was provided by MSCA.

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Supplementary materials

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References

Archer, G.S., 2017. Exposing broiler eggs to green, red and white light during incubation. Animal 11, 1203–1209. https://doi.org/10.1017/S1751731117000143.

Archer, G.S., Mench, J.A., 2017. Exposing avian embryos to light affects post-hatch anti-predator fear responses. Appl. Anim. Behav. Sci. 186, 80–84. https://doi.org/10.1016/j.applanim.2016.10.014.

Archer, G.S., Mench, J.A., 2014a. The effects of the duration and onset of light stimulation during incubation on the behavior, plasma melatonin levels, and productivity of broiler chickens. J. Anim. Sci. 92, 1753–1758. https://doi.org/10.2527/jas.2013-7129.

Archer, G.S., Mench, J.A., 2014b. Natural incubation patterns and the effects of exposing eggs to light at various times during incubation on post-hatch fear and stress responses in broiler (meat) chickens. Appl. Anim. Behav. Sci. 152, 44–51. https://doi.org/10.1016/j.applanim.2013.12.010.

Archer, G.S., Mench, J.A., 2013. The effects of light stimulation during incubation on indicators of stress susceptibility in broilers. Poult. Sci. 92, 3103–3108. https://doi.org/10.3382/ps.2013-03434.

Archer, G.S., Shivaprasad, H.L., Mench, J.A., 2009. Effect of providing light during incubation on the health, productivity, and behavior of broiler chickens. Poult. Sci.

Bouba, I., Visser, B., Kemp, B., Rodenburg, T.B., Van Den Brand, H., 2021. Predicting hatchability of layer breeders and identifying effects of animal related and environmental factors. Poult. Sci. 100, 101394 https://doi.org/10.1016/j.psj.2021.101394.

Burke, W.H., 1992. Sex differences in incubation length and hatching weights of broiler chicks. Poult. Sci. 71, 1933–1938. https://doi.org/10.3382/PS.0711933.

Daisley, J.N., Vallortigara, G., Regolin, L., 2010. Logic in an asymmetrical (social) brain: transitive inference in the young domestic chick. Soc. Neurosci. 5, 309–319. https://doi.org/10.1080/17470910903529795.

Güz, B.C., Molenaar, R., de Jong, I.C., Kemp, B., van Krimpen, M., van den Brand, H., 2021. Effects of green light emitting diode light during incubation and dietary organic macro and trace minerals during rearing on tibia characteristics of broiler chickens at slaughter age. Poult. Sci. 100, 707–720. https://doi.org/10.1016/j.psj.2020.11.042.

Hannah, W.A., Astatkie, T., Rathgeber, B.M., 2020. Hatch rate of laying hen strains provided a photoperiod during incubation. Animal 14, 353–359. https://doi.org/10.1017/S1751731119002039.

Heijmans, J., Duijster, M., Gerrits, W.J.J., Kemp, B., Kwakkel, R.P., van den Brand, H., 2022. Impact of growth curve and dietary energy-to-protein ratio of broiler breeders on offspring quality and performance. Poult. Sci. 101, 102071 https://doi.org/10.1016/J.PSJ.2022.102071.

Hendrix-Genetics, 2020a. ISA Brown Product Guide.

Hendrix-Genetics, 2020b. Dekalb White Product Guide.

Huth, J.C., Archer, G.S., 2015. Effects of LED lighting during incubation on layer and broiler hatchability, chick quality, stress susceptibility and post-hatch growth. Poult. Sci. 94, 3052–3058. https://doi.org/10.3382/ps/pev298.

Lahti, D.C., Ardia, D.R., 2016. Shedding light on bird egg color: pigment as parasol and the dark car effect. Am. Nat. 187, 547–563. https://doi.org/10.1086/685780.

Lourens, A., Molenaar, R., Van Den Brand, H., Heetkamp, M.J.W., Meijerhof, R., Kemp, B., 2006. Effect of Egg Size on Heat Production and the Transition of Energy From Egg to Hatchling. Poult. Sci. 85, 770–776. https://doi.org/10.1093/PS/85.4.770.

Maurer, G., Portugal, S.J., Hauber, M.E., Mikšík, I., Russell, D.G.D., Cassey, P., 2015. First light for avian embryos: eggshell thickness and pigmentation mediate variation in development and UV exposure in wild bird eggs. Funct. Ecol. 29, 209–218. https://doi.org/10.1111/1365-2435.12314.

Özkan, S., Yalçin, S., Babacanoğlu, E., Kozanoğlu, H., Karadaş, F., Uysal, S., 2012a. Photoperiodic lighting (16 h of light: 8 h of dark) programs during incubation: 1. Effects on growth and circadian physiological traits of embryos and early stress response of broiler chickens. academic.oup.com.

Özkan, S., Yalçin, S., Babacanoğlu, E., Uysal, S., Karadaş, F., Kozanoğlu, H., 2012b. Photoperiodic lighting (16 h of light:8 h of dark) programs during incubation: 2. Effects on early posthatching growth, blood physiology, and production performance in broiler chickens in relation to posthatching lighting programs. Poult. Sci. 91, 2922–2930. https://doi.org/10.3382/ps.2012-02427.

Özkan, S., Yalçın, S., Bayraktar, Ö.H., Bilgen, G., Dayıoğlu, M., Bolhuis, J.E., Rodenburg, T.B., 2022. Effects of incubation lighting with green or white light on brown layers: hatching performance, feather pecking and hypothalamic expressions of genes related with photoreception, serotonin and stress systems. Poult. Sci. 102114 https://doi.org/10.1016/J.PSJ.2022.102114.

Pinchasov, Y., 1991. Relationship between the weight of hatching eggs and subsequent early performance of broiler chicks. Br. Poult. Sci. 32, 109–115. https://doi.org/10.1080/00071669108417332.

Reijrink, I.A.M., Meijerhof, R., Kemp, B., Graat, E.A.M., Van Den Brand, H., 2009. Influence of prestorage incubation on embryonic development, hatchability, and chick quality. Poult. Sci. 88, 2649–2660. https://doi.org/10.3382/ps.2008-00523.

Shafey, T.M., AL-Batshan, H.A., Ghannam, M.M., Al-Ayed, M.S., 2005. Effect of intensity of eggshell pigment and illuminated incubation on hatchability of brown eggs. Br. Poult. Sci. 46 (2), 190–198. https://doi.org/10.1080/00071660500065789.

Shafey, T.M., Al-Mohsen, T.H., Al-Sobayel, A.A., Al-Hassan, M.J., Ghnnam, M.M., 2002. Effects of eggshell pigmentation and egg size on the spectral properties and characteristics of eggshell of meat and layer breeder eggs. Asian-Australas. J. Anim. Sci. 15, 297–302. https://doi.org/10.5713/ajas.2002.297.

Shafey, T.M., Ghannam, M.M., Al-Batshan, H.A., Al-Ayed, M.S., 2004. Effect of pigment intensity and region of eggshell on the spectral transmission of light that passes the eggshell of chickens. Int. J. Poult. Sci. 3, 228–233. https://doi.org/10.3923/ijps.2004.228.233.

Souza Da Silva, C., Molenaar, R., Giersberg, M.F., Rodenburg, T.B., Van Riel, J.W., De Baere, K., Dosselaer, I.Van, Kemp, B., Van Den Brand, H., De Jong, I.C., 2021. Day-old chicken quality and performance of broiler chickens from 3 different hatching systems. https://doi.org/10.1016/j.psj.2020.12.050.

Tong, Q., McGonnell, I.M., Demmers, T.G.M., Roulston, N., Bergoug, H., Romanini, C.E., Verhelst, R., Guinebretière, M., Eterradossi, N., Berckmans, D., Exadaktylos, V., 2018. Effect of a photoperiodic green light programme during incubation on embryo development and hatch process. Animal 12, 765–773. https://doi.org/10.1017/S1751731117002117.

Verstegen, M.W.A., Van Der Hel, W., Brandsma, H.A., Henken, A.M., Bransen, A.M., 1987. The Wageningen respiration unit for animal production research: a description of the equipment and its possibilities. Energy Metab. farm Anim. Eff. housing, Stress Dis. 21–48.

Wang, P., Sun, Y., Fan, J., Zong, Y., Li, Y., Shi, L., Isa, A.M., Wang, Y., Ni, A., Ge, P., Jiang, L., Bian, S., Ma, H., Jiang, R., Liu, X., Chen, J., 2020. Effects of monochromatic green light stimulation during embryogenesis on hatching and posthatch performance of four strains of layer breeder. Poult. Sci. 99, 5501–5508. https://doi.org/10.1016/j.psj.2020.06.074.

Yu, Y., Li, Z., Pan, J., 2016. Changes in pigment, spectral transmission and element content of pink chicken eggshells with different pigment intensity during incubation. PeerJ 2016, 1–13. https://doi.org/10.7717/peerj.1825.

Yu, Y., Li, Z., Zhong, Z., Jin, S., Pan, J., Rao, X., Yu, Y., 2018. Effect of monochromatic green LED light stimuli during incubation on embryo growth, hatching performance, and hormone levels. Trans. ASABE 61, 661–669. https://doi.org/10.13031/trans.12485.