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Timing matters: laying sequence and location effects on egg quality and embryonic growth

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Abstract

In broiler breeders, while floor eggs are associated with a negative impact on hatching performance, the role of laying sequence position remains poorly understood and may contribute a further source of variation to egg quality and embryo development. Two experiments were conducted to evaluate the effects of laying environment and sequence position. In Experiment 1, eggs were classified as first collection floor eggs (FCFE) or second collection floor eggs (SCFE) and incubated to embryonic day 12 (ED12). In Experiment 2, eggs were classified as first collection nest eggs (FCNE), FCFE and SCFE to assess internal and external quality, compositional traits and eggshell mineral composition. In Experiment 1, embryos from FCFE were significantly lighter at ED12 compared with those from SCFE, despite greater initial egg size. In Experiment 2, albumen parameters differed, with first collection eggs, FCNE and FCFE, showing greater albumen weight and proportion than second collection eggs, SCFE. Shell thickness was greater in FCNE and FCFE. Mineral analysis revealed higher sodium concentrations in first collection eggs, lower zinc and molybdenum concentrations in floor eggs, reduced manganese in FCNE and higher arsenic in FCFE. Together, these findings demonstrate that laying sequence and environment alter egg composition, shell quality and mineral deposition, with egg-laying sequence demonstrating potential downstream effects on embryonic development. Improved understanding of the underlying mechanistic contribution of egg sequence to egg quality and embryo development will contribute to our understanding of achieving high and consistent hatch success in broiler breeders.

Introduction

The quality of the egg is a key determinant of success in poultry production. The composition of nutrients within a fertile egg significantly impacts nutrient uptake by the embryo during incubation (Uni *et al.*, 2012). Given the relatively short lifespan of commercial meat strains, the incubation period constitutes approximately one-third of their overall growth period and is therefore a critical phase governing embryonic nutrition, growth and development (Speier *et al.*, 2012).

Eggs laid outside designated nesting areas, known as floor eggs, are particularly problematic in the poultry industry. The environment in which an egg is laid significantly influences its microbial load, with floor eggs presenting a greater risk of contamination due to increased contact with surface dirt, higher levels of bacterial presence on the shell and a greater incidence of shell cracks and reduced hatchability (Berrang *et al.*, 1997; De Reu *et al.*, 2008; Van den Brand *et al.*, 2016; Costa *et al.*, 2022). In line with this, Van den Brand *et al.* (2016) reported that the hatchability of fertile eggs was substantially lower for floor eggs (approximately 74 %) compared to nest eggs (approximately 93 %), highlighting the impact of egg-laying location on hatchability. Additionally, studies have shown that the average body weight of chicks hatching from floor eggs is lower than that of chicks hatching from nest eggs (Van den Brand *et al.*, 2016; Perić *et al.*, 2022).

Beyond the laying location, the sequential pattern in which eggs are laid (referred to as the egg sequence or clutch) also influences egg quality. Eggs are laid in sequences with intervals of approximately 24 to 26 h between ovipositions, resulting in each egg within a sequence being laid progressively later in the day. Once a sequence concludes, a non-laying period, or 'pause', follows before the next sequence commences, which can extend up to 40 h (Robinson *et al.*, 1991). These processes are regulated by the coordinated activity of reproductive hormones and physiological factors such as energy metabolism, with the hypothalamo-pituitary-gonadal axis playing a central role in follicle development and ovulation. As poultry are long day breeders, light plays a crucial role by activating the pineal and pituitary glands, which regulate reproductive hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH)

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and 17β-estradiol, all of which are essential for the growth and number of ovarian follicles (Onagbesan *et al.*, 2006). The pineal gland functions as a circadian oscillator, responding to photic signals to produce melatonin, thereby signalling darkness and influencing circadian regulation (Okano and Fukada, 2003). This circadian mechanism is essential for coordinating the timing of ovulation and subsequent egg-laying. This cycle, spanning approximately 25 to 26 h, includes the progressive development of the yolk, albumen, membranes and shell (Zhang *et al.*, 2022). Consequently, the first egg in a sequence is typically laid early in the morning, approximately 3.5 h after the onset of light during the photoperiod (Gumułka *et al.*, 2010). This timing reflects a tightly regulated physiological response, ensuring synchronization with the internal circadian rhythms of the bird, which are profoundly influenced by light exposure (Lewis *et al.*, 1995). Thus, light emerges as a critical external factor, modulating hormonal activity and the timing of egg-laying through its interaction with the dark phase (Wilson *et al.*, 1985).

Egg sequences vary between the two main types of commercial birds, with laying hens typically having longer sequences, while broiler breeders tend to lay three to four eggs in a sequence with pauses in between (Zuidhof *et al.*, 2007; Gumułka *et al.*, 2010) . The order in which eggs are laid in a sequence affects both the weight and composition of the eggs. Previous research has highlighted the temporal dynamics of egg weights, indicating that eggs laid early in the morning, and thus early in the sequence order, tend to be heavier than those laid later in the day (Robinson *et al.*, 1991; Novo *et al.*, 1997; Zakaria *et al.*, 2005; Tůmová *et al.*, 2008).

The quality of the eggshell is crucial not only for protecting the egg but also for ensuring optimal conditions for embryo development in fertilized eggs (Narushin and Romanov, 2002). The timing of oviposition within a sequence also influences eggshell quality. Eggs laid later in the day tend to have thicker shells and exhibit fewer deformities compared to those laid earlier (Arafa *et al.*, 1982; Yannakopoulos *et al.*, 1994; Tůmová and Ebeid, 2005; Tůmová *et al.*, 2007). The mineralized eggshell provides structural protection and also serves as a major calcium reservoir for embryonic skeletal development (Chien *et al.*, 2009). While mechanistic modelling suggests that the timing of oviposition within the light cycle alters calcium (Ca) absorption, bone mobilization and phosphorus excretion during shell formation (Kebreab *et al.*, 2009), recent studies have shown that Ca and phosphorus (P) metabolism also varies across the oviposition cycle, with shifts in serum concentrations, excretion and transporter expression that reflect the changing demands of eggshell formation (Ren *et al.*, 2019; Yan *et al.*, 2023; Sinclair-Black *et al.*, 2024). These temporal dynamics are mirrored in the composition of the eggshell itself, with Ca levels typically higher in eggs laid earlier in the day, while P and magnesium (Mg) concentrations increase in eggs laid later (Tůmová *et al.*, 2014).

Therefore, in addition to the well-established detrimental effect of floor eggs on hatching performance, the egg sequence may also be having an important impact and could account for some of the variation observed between floor and nest eggs. The concept of egg sequence is key to understanding variations in egg quality and production, which are influenced by a range of physiological and environmental factors. Despite the importance of egg quality, research on egg sequence, particularly in broiler breeders, remains limited. This study aimed to explore how the laying environment and the egg sequence affect egg composition, shell mineralization and embryonic growth. Gaining insights into these dynamics is essential for improving incubation performance and optimizing production outcomes in commercial poultry systems.

Materials and methods

Egg collection and sequence classification

The procedure involving animals was reviewed and approved by the University of Nottingham institutional animal ethics committee (approval no. 244). For both experiments, eggs were collected from Ross-308 broiler breeder flocks and classified based on their approximate position in the laying sequence, following a time-specific collection pattern. This classification was based on the established relationship between oviposition time and sequence progression in broiler breeder hens, whereby the first egg of a sequence is typically laid earlier in the day than subsequent eggs. In both experiments, the first collection of floor eggs (first collection floor eggs, FCFE) was assumed to predominantly represent the first egg in a new egg sequence, laid after a pause. The second collection of floor eggs (second collection floor eggs, SCFE) was assumed to represent eggs laid second in the sequence. In the second experiment, an additional experimental group, the first collection nest eggs (FCNE) was included, was assumed, like FCFE, to represent the first egg in a new sequence after a pause, differing only in laying location.

Experiment 1: incubation

A total of 200 floor eggs were collected from a Ross-308 broiler breeder flock. Eggs were collected at two flock ages: 33 wk (Run 1) and 44 wk (Run 2). For each run, 50 eggs were collected at 07:00 h (FCFE) and 50 eggs were collected at 08:30 h (SCFE).

Eggs were washed for 2 min using a Rota-maid washer and ROTOSAN® solution (15 mL/L). After air drying, eggs were fumigated with Virkon™ TDX solution. The external surface of each egg was evaluated and profiled using the Alltech '20 Common Eggshell Quality Problems' chart. Eggshell quality was assessed, and abnormalities were categorized, translucent and wrinkled eggs were categorized by the affected surface area as minimal (<25 %), moderate (25–75 %) or extensive (>75 %). Each egg was weighed using a digital balance (Ohaus SPU6001), and its length and width were measured using a digital calliper (±0.1 mm). The shape index (SI) was calculated as: SI = (width/length) × 100. Eggs were incubated using a Sanyo MIR-154 (Sanyo, Tokyo, Japan), with temperature and relative humidity maintained according to the breeder's management recommendations. Egg weight was tracked on Embryonic Day 4, 8 and 12 (ED4, ED8 and ED12, respectively) using a digital balance (Ohaus SPU6001). On ED12, eggs were candled and classified as infertile, dead or alive. Embryos were euthanised by storing eggs at −20°C for 1 h. Embryo length was measured using a digital calliper, and embryo weight was recorded a digital balance (Ohaus SPU6001). Eggshell thickness was measured at the blunt end, pointed end and equator using a digital calliper (±0.1 mm).

Experiment 2: egg quality and mineral composition

In Experiment 2, 150 eggs were collected from a 27-week-old Ross-308 breeder flock. Eggs were categorized into three groups as FCFE and FCNE collected at 07:00 h and SCFE collected at 10:00 h ($n = 50$/group). An additional group, FCNE, was included in Experiment 2 to determine whether the differences observed in

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floor eggs were influenced by laying location in addition to collection time.

Each egg was weighed using an Ohaus SPU6001 balance (OHAUS Corporation, Parsippany, New Jersey, USA), and its length and width were measured using a digital calliper. The SI was calculated as: SI = (width/length) × 100. Eggshell quality was assessed, and abnormalities were categorized. The albumen and yolk widths were measured using a digital Vernier calliper. Albumen height was measured with an albumen height gauge equipped with an electronic display (Technical Services and Supplies, York, UK). Yolk colour was assessed using a DSM Yolk Colour Fan (DSM, Switzerland, 2005), with a scale ranging from 1 to 16 units (Ortiz *et al.*, 2021). The Haugh unit was calculated using the formula: $100 \times \log10\ (h - 1.7 \times w^{0.37} + 7.6)$, where h represents the albumen height (mm) and w represents the egg weight (g) (Şekeroglu and Altuntaş, 2009). Albumen and yolk were separated, and individual weights were recorded (Ohaus SPU6001). The albumen and yolk ratios were determined relative to whole-egg weight. The yolk index was calculated as: (yolk height/yolk width) × 100, and the albumen index as: (albumen height/albumen width) × 100. Eggshells were cleaned, air-dried and measured for thickness at the blunt end, pointed end and equator using a digital calliper to the nearest 0.001 mm. Albumen and yolk samples were stored at −80°C, while eggshell samples were stored at −20°C for further analysis. Eggshell mineral composition was assessed on a subset of 15 eggs/group. Eggshell samples were freeze-dried using a Christ Gamma 1–16 LSCplus freeze dryer (Martin Christ, Osterode, Germany). Approximately 0.2 g was digested in nitric acid, Milli-Q water and hydrogen peroxide and analysed via ICP-MS (iCAP-Q, Thermo Fisher Scientific, Bremen, Germany).

For nutrient composition profiling, yolk and albumen samples were dried using a freeze dryer (Christ Gamma 1–16 LSCplus with LyoCube). Yolk fat content was determined by Soxhlet extraction (Gerhardt Analytical Systems, Königswinter, Germany) with petroleum ether (AOAC, 1998). Albumen protein content was quantified by using nitrogen analysis (EA 1112 elemental analyser, Thermo Scientific, UK) using aspartic acid standards (Elemental Lab, Okehampton, UK), applying a conversion factor of 6.25. Three technical replicate measurements were taken per sample.

Statistical analysis

In both experiments, the individual egg was considered the statistical unit. Statistical analyses were performed using SAS University Edition (SAS Institute Inc., Cary, NC, USA). Effects were considered to be significant at $P < 0.05$. For continuous response variables analysed by ANOVA or *t*-test, normality of model residuals was evaluated primarily by inspection of Q–Q plots. Homogeneity of variance was assessed using Levene's test and residual versus fitted plots. For comparisons between two groups, Student's *t*-test was used when the assumption of equal variances was satisfied; otherwise, Welch's *t*-test was applied.

In Experiment 1, initial egg weight, egg length, egg width, SI, ED12 embryo weight, ED12 embryo length and mean eggshell thickness were initially analysed by two-way ANOVA using the PROC GLM procedure. Mean eggshell thickness for each egg was calculated as the average of measurements obtained at the blunt end, equator and pointed end. Collection group (FCFE or SCFE), flock age/run (33 or 44 wk; Runs 1 and 2, respectively) and the collection group × flock age/run interaction were included as fixed effects. As neither flock age/run nor the collection group × flock age/run interaction significantly affected these response variables ($P \geq 0.05$), data from the two runs were pooled, and FCFE and SCFE were subsequently compared using two-tailed *t*-tests. Egg-weight loss at ED4, ED8 and ED12 was analysed using a repeated-measures linear mixed model with the PROC MIXED procedure. Collection group, flock age/run, embryonic day and their interactions were included as fixed effects, with embryonic day specified as the repeated factor and individual egg as the subject. Eggshell abnormality frequencies were compared among treatment groups using Pearson's chi-square test. Fertility and embryonic survival to ED12 were analysed separately as binary outcomes. Fertility was classified as fertile or infertile using all eggs, whereas embryonic survival was classified as alive or dead among fertile eggs only. Binary logistic regression was used to evaluate the effects of collection group, flock age/run and their interaction.

In Experiment 2, data were obtained from a single 27 wk old flock. Egg weight, egg length, egg width, SI, albumen and yolk measurements, Haugh unit, albumen and yolk weights and ratios, albumen and yolk indices, mean eggshell thickness, nutrient composition and individual eggshell mineral concentrations were analysed by one-way ANOVA using the PROC GLM procedure, with experimental group (FCNE, FCFE or SCFE) included as the fixed effect. Statistical differences among treatments were assessed using Tukey's test. Two a priori contrasts were specified: FCNE versus FCFE, and FCFE versus SCFE. For nutrient composition analyses conducted in technical triplicate, replicate measurements from each sample were averaged to obtain a single egg-level value before statistical analysis.

Results

Experiment 1

The effect of grouping eggs based on collection time into FCFE and SCFE are presented in Figure 1. Throughout the experiment, significant differences were observed between eggs from FCFE and SCFE in several parameters. Initial egg weight at embryonic day 0 (ED0) was significantly higher in FCFE compared to SCFE ($P = 0.034$), and the final egg weight after 12 day of incubation (ED12) also remained greater in FCFE ($P = 0.006$). Additionally, a significant difference in absolute egg weight loss was observed between groups ($P = 0.021$), as was percentage weight loss ($P = 0.008$) between ED0 to ED12. Egg length was significantly longer in FCFE ($P = 0.017$), but there were no differences in egg width or SI between collections.

Embryo weight was significantly lower in FCFE compared to SCFE on ED12 of incubation ($P < 0.001$) as was embryo weight as a percentage of ED12 egg weight ($P < 0.001$). Finally, embryo length was significantly shorter in FCFE compared to SCFE ($P = 0.001$). Figure 2 presents the distribution of shell abnormalities. SCFE exhibited a significantly higher proportion of calcium-coated eggs ($P = 0.044$) as well as a greater incidence of minimal wrinkled eggs ($P = 0.042$). FCFE had a significantly higher proportion of soiled eggs ($P < 0.001$). However, there were no overall significant differences observed in the total number of shell abnormalities or shell characteristics between collections ($P \geq 0.05$). There were no significant differences between collections in the percentages of eggs classified as alive, infertile, early dead, mid dead or rotten after 12 day of incubation. These results highlight that while weight-related parameters and specific shell abnormalities differed by

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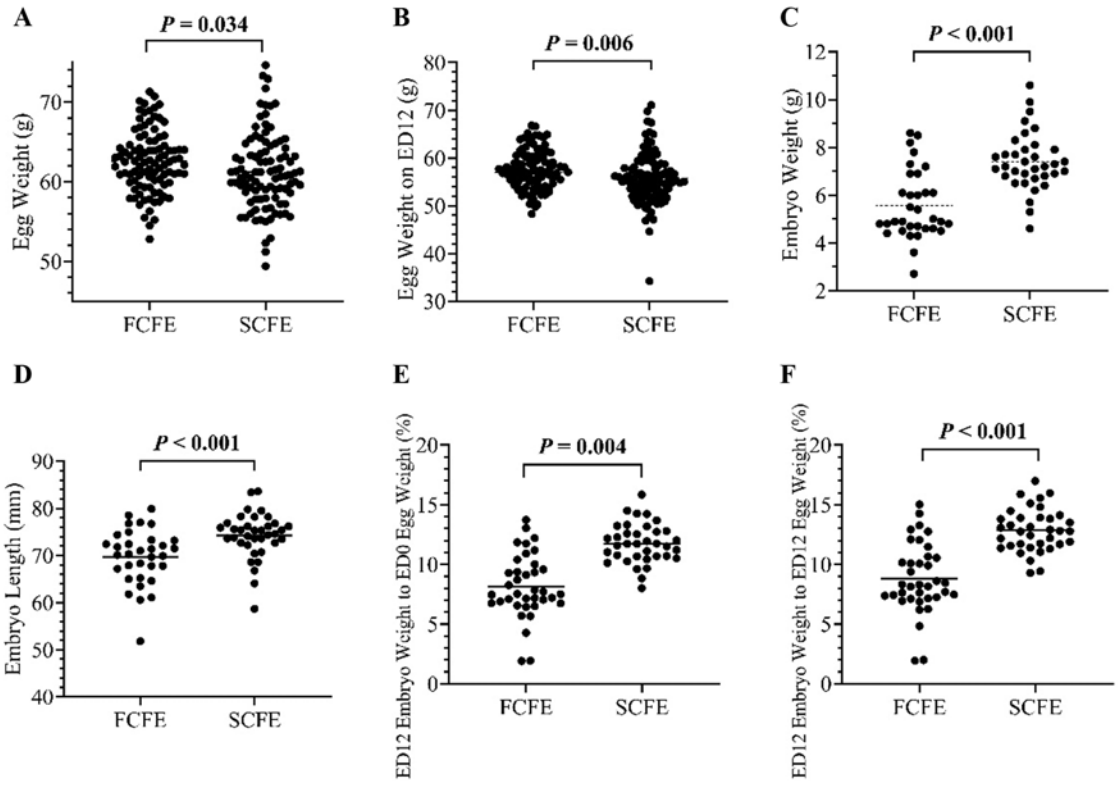


Figure 1. Experiment 1: egg and embryo traits for first and second collection floor eggs. (a) Egg weight on embryonic day 0 (ED0) for first collection floor eggs (FCFE) and second collection floor eggs (SCFE). (b) Egg weight on embryonic day 12 (ED12). (c) Embryo weight on ED12. (d) Embryo length on ED12. (e) Egg weight loss between ED0 and ED12 expressed as a percentage of initial egg weight. (f) ED12 embryo weight expressed as a proportion of ED12 egg weight. Each point represents a single egg or embryo, and horizontal lines indicate group means. Exact *P* values for differences between FCFE and SCFE are shown on the panels. FCFE, first collection floor eggs; SCFE, second collection floor eggs; ED, embryonic day.

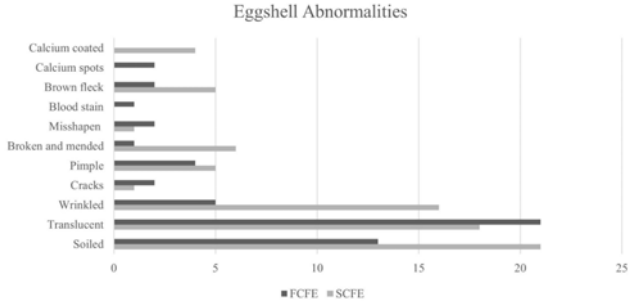


Figure 2. Experiment 1: eggshell abnormalities in first- and second collection floor eggs. The proportion of eggs exhibiting each abnormality type (translucent, wrinkled, soiled, cracked, pimple, broken and mended, misshapen, brown fleck, calcium spot and calcium coated) in first collection floor eggs (FCFE) and second collection floor eggs (SCFE). FCFE, first collection floor eggs; SCFE, second collection floor eggs.

collection time, other factors such as egg width and SI were unaffected.

Experiment 2

The internal and external egg quality characteristics for the three groups FCNE, FCFE, SCFE are summarized in Table 1. The egg weight did not differ significantly among the groups ($P = 0.080$); however, pairwise comparison revealed that FCFE (55.4 g) was significantly heavier than SCFE (53.8 g; $P = 0.026$). There were no significant differences in egg height or width across the groups. Internal egg quality varied across groups. Albumen width was significantly greater in FCFE (69.4 mm) and FCNE (68.3 mm) compared to SCFE (65.9 mm) ($P = 0.004$). Albumen weight and percentage were significantly higher in FCNE and FCFE compared to SCFE ($P = 0.020$ and $P = 0.046$, respectively). However, no significant differences were found in albumen height or Haugh units. Yolk characteristics showed that yolk width was significantly larger in FCNE (41.1 mm) compared to FCFE (40 mm) and SCFE (40 mm) ($P = 0.002$), while yolk weight and percentage did not differ significantly between groups. Yolk colour differed significantly among the groups ($P < 0.001$), SCFE had significantly higher yolk colour than both FCFE and FCNE. There were no significant differences in yolk fat content or albumen protein content across the samples. Significant differences were observed in shell characteristics, whereby FCNE and FCFE had thicker shells compared to SCFE ($P < 0.001$).

The eggshell mineral concentrations are summarized in Table 2. Sodium (Na) concentration was significantly lower in SCFE (0.79 mg/g) compared to FCNE (0.85 mg/g) and FCFE (0.83 mg/g) ($P = 0.007$). Magnesium (Mg) concentration also differed among groups, with FCNE having a higher concentration than SCFE, while FCFE was intermediate ($P = 0.026$). For trace minerals, molybdenum (Mo) and zinc (Zn) were significantly higher in FCNE than in FCFE ($P = 0.002$, $P = 0.040$). For manganese (Mn) and arsenic (As), FCFE eggs had significantly higher concentrations than FCNE eggs ($P = 0.007$ and $P = 0.040$, respectively). No significant differences were found in the concentrations of calcium (Ca), phosphorus (P), potassium (K), sulphur (S) or other trace elements ($P \geq 0.05$).

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Table 1. Experiment 2; internal and external egg quality characteristics of eggs designated as first or second collections and from different egg-laying locations

	FCNE	FCFE	SCFE	SEM	*P* value
Egg weight (g)	54.8^{ab}	55.4^{a}	53.8^{b}	0.48	0.075
Egg height (mm)	53.4	53.8	53.9	0.26	0.327
Egg width (mm)	42.3	42.3	42	0.14	0.149
Shape index (%)	79.3^{a}	78.7^{ab}	77.9^{b}	0.44	0.095
Albumen height(mm)	8.8	8.5	8.9	0.18	0.305
Albumen width (mm)	68.3^{a}	69.4^{a}	65.9^{b}	0.74	0.004
Albumen weight (g)	35.52^{a}	35.83^{a}	34.30^{b}	0.40	0.020
Albumen weight (%)	64.89^{a}	64.67^{a}	63.67^{b}	0.36	0.046
Haugh unit	94.7	96.5	95.5	1.28	0.610
Yolk height (mm)	13.5	13.6	14.1	0.20	0.605
Yolk width (mm)	41.1^{b}	40^{a}	40^{a}	0.25	0.108
Yolk weight (g)	15.4	14.6	14.9	0.37	0.002
Yolk weight (%)	28.1	26.5	27.6	0.69	0.373
Yolk colour (score)	7.78^{a}	7.91^{ab}	8.18^{b}	0.073	0.258
Shell weight (g)	4.85^{a}	4.81^{ab}	4.66^{b}	0.06	0.078
Shell weight (%)	8.84	8.80	8.68	0.09	0.404
Shell thickness (mm)	0.32^{a}	0.32^{a}	0.31^{b}	0.001	<0.001
Albumen dry matter (%)	12.94	13.02	13.24	0.19	0.521
Albumen crude protein (FE %)	11.32	11.43	11.45	0.18	0.853
Yolk dry matter (%)	51	50.95	51.02	0.35	0.910
Yolk crude fat (FE %)	24.41	25.18	25.01	0.49	0.520

SEM: standard error of the mean; FCNE: first collection nest eggs; FCFE: first collection. floor eggs; SCFE: second collection floor eggs (FCNE $n = 50$, FCFE $n = 50$, SCFE $n = 50$. means ± SEM); FE %: fresh egg percentage; ^{a,b} means with different superscripts differed significantly according to Tukey's test ($p < 0.05$).

Table 2. Experiment 2; mineral composition of eggshells as eggs designated as from first and second collection times and different egg-laying locations

Mineral concentration	FCNE	FCFE	SCFE	SEM	*P* value
Macro minerals (mg/g)					
Ca	396	395	401	2.40	0.145
P	1.17	1.16	1.15	0.03	0.835
Na	0.85^{a}	0.83^{a}	0.79^{b}	0.01	0.007
K	0.47	0.46	0.47	0.01	0.769
S	1.03	1.17	1.25	0.06	0.193
Mg	3.32^{a}	3.16^{ab}	3.04^{b}	0.07	0.026
Trace minerals (mg/kg)					
Mo	0.023^{b}	0.008^{a}	0.011^{a}	0.003	0.002
Zn	0.42^{b}	0.22^{a}	0.22^{a}	0.06	0.039
Fe	0.72	0.77	0.68	0.08	0.706
Cu	0.65	0.67	0.59	0.04	0.383
Mn	0.07^{a}	0.31^{b}	0.17^{ab}	0.05	0.007
Pb	0.008	0.005	0.004	0.0018	0.295
Co	0.007	0.008	0.007	0.0002	0.357
Ni	0.046	0.044	0.047	0.003	0.662
Se	0.078	0.078	0.077	0.004	0.338
Cd	0.001	0.001	0.001	0.0002	0.123
As	0.002^{a}	0.004^{b}	0.003^{ab}	0.0005	0.044

SEM: standard error of the mean; FCNE: first collection nest egg; FCFE: first collection. floor eggs; SCFE: second collection floor eggs; ^{a,b} means with different superscripts differed significantly according to Tukey's test ($P < 0.05$). Macro minerals are expressed as mg/g and trace minerals as mg/kg.

Discussion

In both experiments, eggs were collected and sampled early and late in the morning, to capture, at a population level, eggs broadly representative of early and later positions in the laying sequence respectively, as supported by the consistent egg weight gradient observed between collection groups. In Experiment 1, there was no significant effect of collection time on the proportion of eggs classified as alive, infertile or dead at various stages of incubation. This indicates that both SCFEs and FCFEs contributed similarly to reductions in fertility and hatchability. However, clear differences in embryonic growth emerged between groups. Embryos from FCFE were significantly lighter at ED12, even though these eggs were initially heavier and lost less weight during incubation. The initial egg size and nutrient content determine substrate availability and necessitate corresponding adjustments in metabolic pathways during embryonic development (Hu, 2013). However, these differences were not explained by the egg weight on ED12 alone, suggesting that additional factors – such as water vapour conductance or differences in shell functional properties – may have influenced the trajectory of embryonic growth. Comparable trends have been reported by Zakaria *et al.* (2009) indicating that mid-sequence eggs experienced greater weight loss during storage than those laid earlier or later, a pattern they associated with increased shell porosity and variation in albumen quality, although oviposition time did not affect weight loss during incubation. Such mechanisms may help explain the elevated weight loss observed in SCFEs in the present study. Notably, embryo length mirrored embryo weight, further reinforcing the interpretation of slower developmental progression in FCFEs. This contrasts with findings by Ruiz and Lunam (2002), who reported that chicks from morning-laid eggs were consistently heavier than those from afternoon-laid eggs, supporting the assumption that egg weight is positively associated with chick or embryo weight. To our knowledge, the magnitude of differences observed in the current study have not been previously reported, making it difficult to speculate on the specific mechanisms contributing to these variations. The current results suggest that this relationship may not hold under all conditions. Variation in pre-incubation thermal exposure is a potential contributing factor to the observed differences in growth. Earlier studies (Fasenko *et al.*, 1991; Akil and Zakaria, 2015) proposed that prolonged pre-incubation at ambient temperature can lead to partial embryonic development prior to storage-induced diapause. While this may initially explain the advanced embryonic growth observed in FCFEs, studies have shown that embryos progressing beyond stage XIII of the Eyal-Giladi and Kochav (1976) classification – toward primitive streak formation – may be more vulnerable to developmental arrest during storage due to ongoing cellular migration and differentiation (Bellairs, 1986; Fasenko *et al.*, 2001). In contrast, embryos that remain at stage XIII, a more quiescent stage, are better suited

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to resume development following storage. Taken together, these observations suggest that SCFEs, which likely experienced shorter exposure to ambient conditions prior to collection, may have been developmentally less advanced at the onset of storage and therefore more stable during the early stages of incubation. It is worth noting that any difference in pre-incubation thermal exposure between collection groups is itself downstream of sequence-dependent differences in oviposition timing and therefore cannot be considered entirely independent of laying sequence. However, the underlying mechanisms remain unclear. Findings from Experiment 2 provide some support for this, as differences in egg characteristics across collection times may help to explain the variation in embryonic growth outcomes observed in Experiment 1.

Shell quality also showed variation between collection times in Experiment 1. While the overall incidence of abnormal shells was not affected, the nature and distribution of defects differed between collection times. FCFEs were more likely to exhibit structural flaws such as misshapen, cracked, translucent and calcium-spotted shells, whereas SCFEs were more often affected by shell surface irregularities such as pimples, wrinkles, soiling or signs of mending. The presence of calcium-coated shells exclusively among SCFEs may indicate transient oviposition delays, during which additional calcium is deposited on the shell surface (Reynard and Savory, 1999). The greater frequency of soiled eggs in FCFEs, despite post-collection washing, may reflect extended contact with the floor or residual surface contamination. These traits, commonly used as proxies for shell quality, may reflect underlying variation in shell gland function, calcium metabolism or the timing of shell calcification (Solomon, 1997; Roberts, 2004; Kebreab *et al.*, 2009). Although the precise mechanisms remain unclear, these patterns suggest that the processes underlying shell formation are not static throughout the laying period and may shift over the course of shell formation. These findings indicate temporal variation in shell quality that warrants further investigation.

Experiment 2 extended this by assessing whether time of collection was also associated with variation in other structural and compositional egg traits. It confirmed the pattern already observed in Experiment 1: eggs collected earlier were heavier than eggs collected later. These findings align with prior studies which have consistently demonstrated that oviposition time significantly impacts egg weight, with eggs laid earlier in the day tending to be heavier than those laid later (Robinson *et al.*, 1991; Patterson, 1997; Zakaria *et al.,* 2005; Tůmová *et al.*, 2009). There are two possible underlying biological processes to consider here. Firstly, differences in egg weight may partly reflect variation in maternal investment during egg formation, as resources are deposited maternally into avian eggs and the amount of major resources is generally related to egg size, while maternal diet and nutritional condition may also influence the composition of resources allocated to eggs (Williams, 1994; Nager *et al.*, 2006; Rao *et al.*, 2009; Rubolini *et al.*, 2011). In wild birds, mothers influence embryonic development by allocating nutrients, with the amounts varying across the laying sequence and with maternal condition or social environment (Reed and Vleck, 2001; Reed and Clark, 2011). Maternal mechanisms have been proposed to regulate hatching synchrony (Boonstra *et al.*, 2010). In both synchronously hatching Canada geese and asynchronously hatching American coots, later-laid eggs receive lower parental investment, yet their embryos exhibit developmental strategies that mitigate these disadvantages. While egg components were once thought to unilaterally direct development, growing evidence suggests that embryos are not passive recipients but can actively sense and respond to their environment, even modulating maternally derived factors such as yolk steroids potentially to their own benefit (Reed and Clark, 2011). Secondly, the first egg in a new sequence is often retained in the reproductive tract for an extended period of time, which may account for the greater egg weight observed. This prolonged residence is particularly relevant for the shell gland, where the amount of deposited shell is a linear function of uterine residence time after plumping (Tůmová and Ebeid, 2005; Ketta and Tůmová, 2016). Prolonged transit may also influence albumen deposition in the magnum, as albumen synthesis proceeds continuously in the tubular gland cells while the rate of secretion rises when an egg is present (Muramatsu *et al.*, 1991). Although direct evidence for such a time-dependent effect is more limited.

The timing of egg-laying is known to influence various components of the egg. The yolk weight and percentage did not vary with collection time. This is broadly consistent with previous work by Gumułka *et al.*, (2010), who reported no consistent trend across oviposition times. However, other studies have reported greater yolk mass in eggs laid earlier in the day (Tůmová and Ebeid, 2005; Tůmová and Gous, 2012), suggesting that yolk dynamics may be more context dependent. In the present study, the greater egg weight observed in early-collection eggs was not attributable to yolk content, indicating that albumen allocation is the more responsive component of egg mass under these conditions. Albumen proteins are deposited around the yolk during passage through the magnum, where synthesis proceeds continuously but the rate of secretion rises when an egg is present in the region (Muramatsu *et al.*, 1991). Both types of first collection egg had higher absolute weight, proportional weight and albumen width than SCFEs, a consistent reduction that points to a sequence-linked mechanism.

Eggshell thickness was also influenced by the collection time, with thicker shells observed in first collection eggs. This corresponded to an increase of approximately 3% in thickness compared with SCFEs. This difference may be associated with a longer laying interval, as the duration of eggshell formation and the time an egg spends in the shell gland influence shell deposition and eggshell thickness (Nys, 1986; Tůmová and Ebeid, 2005; Ketta and Tumová, 2016). Longer laying intervals have also been positively correlated with the duration of eggshell formation, deposition rate and quality (Chen *et al.*, 2021). While previous research has often focused on factors such as genotype, temperature, bird age and nutrition (Michalak *et al.*, 2011; Gualhanone *et al.*, 2012; Abdelqader *et al.*, 2013; Tůmová *et al.*, 2014; Kibala *et al.*, 2015), the findings of this study highlight the role of laying sequence disruptions in influencing eggshell characteristics. These structural differences may carry functional consequences: thicker shells reduce water vapour conductance and weight loss (Ar *et al.*, 1974), while also modulating gas exchange critical for embryonic development (Burton and Tullett, 1983). As highlighted in Experiment 1, variations in shell thickness may influence water vapour loss and gas conductance during incubation. While shell conductance was not measured directly, the observed differences in shell characteristics provide a plausible link between laying pattern and embryonic growth, as shell conductance has been proposed as a potential mechanism underlying laying-sequence differences in embryonic metabolism in Canada geese (Boonstra *et al.*, 2010).

As the timing of shell calcification may differ depending on when the egg is laid, we considered whether variation in calcium availability might influence shell quality (Mongin and Sauveur, 1974). Final stages of shell formation occur overnight, when hens

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are not feeding, and calcium must be drawn primarily from the medullary bone (Bain *et al*., 2016). Late-laid eggs may follow a different temporal pattern relative to feed intake as calcium absorption is not tightly synchronized with shell deposition, hens rely on reserves from the crop or medullary bone to support shell formation (Mongin and Sauveur, 1974). This may contribute to variation in shell quality depending on laying time.

The intriguing difference in the eggshell thickness suggests that there may be differences in eggshell mineral composition. It is emphasized that the mineral composition of eggshells can vary based on dietary supplementation and environmental conditions (Drabik *et al*., 2021)., which may change throughout the day. For instance, hens that lay eggs in the morning may have different access to nutrients compared to those that lay later in the day, potentially leading to variations in the mineral content of the eggshells produced during these times. In the present study, the higher eggshell sodium (Na) levels in first collection eggs may reflect temporal changes in Na^+ related ion transport in the shell gland during egg formation (Lavelin *et al*., 2001). In terms of trace minerals, the results showed that zinc (Zn) and molybdenum (Mo) levels were significantly lower in FCFE and SCFE compared to FCNE. The observed differences may reflect underlying hen-level factors, such as differences in mineral metabolism, stress or access to feed associated with floor-laying behaviour. Previous research has shown that eggshell mineral content can be influenced by the hen's physiological status, dietary intake and environmental or management conditions (Ketta and Tůmová, 2016). Thus, the reduced Zn and Mo content in FCFE and SCFE eggshells may indicate that floor-laying hens experience physiological or nutritional challenges that impact mineral deposition in the shell, independent of oviposition time. The higher As content observed in first sequence floor eggshells may partly reflect greater contact with floor or litter material, as arsenic can occur in poultry manure and associated surface soils and environmental metal exposure may vary geographically (Liu *et al*., 2021; Drabik *et al*., 2024). Eggs laid on the floor are in direct contact with dust, litter or bedding material, which can serve as sources of arsenic deposition. Levels of lead (Pb), cobalt (Co), nickel (Ni), copper (Cu) and iron (Fe) did not differ significantly among the egg types. Overall, in this study the findings suggest that the mineral composition of eggshells was more influenced by location than sequence.

Conclusion

Oviposition time influenced both egg composition and embryonic growth. Eggs laid early in the sequence were heavier and had greater albumen weight and width, yet embryos from these eggs were lighter and shorter at mid-incubation compared with those from later-laid eggs. By contrast, smaller eggs laid later in the sequence supported more advanced embryonic growth, suggesting that nutrient utilization might differ across the laying sequence. Shell properties also varied, with early-laid eggs exhibiting greater thickness and higher sodium concentrations, while later-laid eggs produced thinner shells with more irregularities. These differences in albumen allocation and shell structure may affect water balance and gas conductance during incubation. Whether such variation in egg composition translates into measurable effects on hatchability, chick viability or post-hatch performance remains an important question for future research.

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References

Abdelqader A, Al-Fataftah AR and Daş G (2013) Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. *Animal Feed Science and Technology* **179**, 103–111. https://doi.org/10.1016/j.anifeedsci.2012.11.003.

Akil R and Zakaria AH (2015) Egg laying characteristics, egg weight, embryo development, hatching weight and post-hatch growth in relation to oviposition time of broiler breeders. *Animal Reproduction Science* **156**, 103–110. https://doi.org/10.1016/j.anireprosci.2015.03.006.

AOAC International (1998) *Official Methods of Analysis of AOAC International*, 16thEdn, 4th revision. Gaithersburg, MD, USA: AOAC International.

Ar A, Paganelli CV, Reeves RB, Greene DG and Rahn H (1974) The avian egg: Water vapour conductance, shell thickness and functional pore area. *Condor* **76**, 153–158.

Arafa AS, Harms RH, Miles RD, Christmas RB and Choi JH (1982) Quality characteristics of eggs from different strains of hens as related to time of oviposition. *Poultry Science* **61**, 842–847. https://doi.org/10.3382/ps.0610842.

Bain MM, Nys Y and Dunn IC (2016) Increasing persistency in lay and stabilising egg quality in longer laying cycles. *What Are the Challenges?. British Poultry Science* **57**, 330–338. https://doi.org/10.1080/00071668.2016.1161727.

Bellairs R (1986) The primitive streak. *Anatomy and Embryology* **174**, 1–14.

Berrang ME, Frank JF, Buhr RJ, Bailey JS, Cox NA and Mauldin JM (1997) Microbiology of sanitized broiler hatching eggs through the egg production period. *Journal of Applied Poultry Research* **6**, 298–305. https://doi.org/10.1093/japr/6.3.298.

Boonstra TA, Clark ME and Reed WL (2010) Position in the sequence of laying, embryonic metabolic rate, and consequences for hatching synchrony and offspring survival in Canada Geese. *The Condor* **112**, 304–313. https://doi.org/10.1525/cond.2010.090043.

Burton FG and Tullett SG (1983) A comparison of the effects of eggshell porosity on the respiration and growth of domestic fowl, duck and turkey embryos. *Comparative Biochemistry and Physiology Part A: Physiology* **75**, 167–174. https://doi.org/10.1016/0300-9629(83)90065-8.

Chen X, He Z, Li X, Song J, Huang M, Shi X, Li X, Li J, Xu G and Zheng J (2021) Cuticle deposition duration in the uterus is correlated with eggshell

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cuticle quality in White Leghorn laying hens. *Scientific Reports* **11**, 22100. https://doi.org/10.1038/s41598-021-01718-0.

Chien YC, Hincke MT and McKee MD (2009) Ultrastructure of avian eggshell during resorption following egg fertilization. *Journal of Structural Biology* **168**, 527–538. https://doi.org/10.1016/j.jsb.2009.07.005.

Costa BTA, Lopes TSB, Sousa LS, Barbosa HJS, Gonçalves TF, Franco CMA, Lara LJC and Araújo ICS (2022) Floor eggs: Hatchability, microbiological analysis, and effects of post-hatch use of ceftiofur on performance of broiler chicks. *Research in Veterinary Science* **152**, 610–617. https://doi.org/10.1016/j.rvsc.2022.09.020.

De Reu K, Messens W, Heyndrickx M, Rodenburg TB, Uyttendaele M and Herman L (2008) Bacterial contamination of table eggs and the influence of housing systems. *World's Poultry Science Journal* **64**, 5–19. https://doi.org/10.1017/S0043933907001687.

Drabik K, Karwowska M, Wengerska K, Próchniak T, Adamczuk A and Batkowska J (2021) The variability of quality traits of table eggs and eggshell mineral composition depending on hens' breed and eggshell colour. *Animals* **11**, 1204. https://doi.org/10.3390/ani11051204.

Drabik K, Skałecki P, Spasowska H, Melnyk OP, Kutrzuba M and Batkowska J (2024) Organic eggs as an indirect indicator of agricultural environmental pollution – Preliminary research. *Journal of Food Composition and Analysis* **131**, 106214. https://doi.org/10.1016/j.jfca.2024.106214.

Eyal-Giladi H and Kochav S (1976) From cleavage to primitive streak formation: A complementary normal table and a new look at first stages of the development of the chick. *I. General Morphology. Developmental Biology* **49**, 321–337.

Fasenko GM, Robinson FE, Armstrong JG, Church JS, Hardin RT and Petitte JN (1991) Variability in pre-incubation embryo development in domestic fowl. I. Effects of nest-holding time and method of egg storage. *Poultry Science* **70**, 1876–1881. https://doi.org/10.3382/ps.0701876.

Fasenko GM, Robinson FE, Whelan AI, Kremeniuk KM and Walker JA (2001) Prestorage incubation of long-term stored broiler breeder eggs. *I. Effects on Hatchability. Poultry Science* **80**, 1406–1411. https://doi.org/10.1093/ps/80.10.1406.

Gualhanone A, Furlan RL, Fernandez-Alarcon MF and Macari M (2012) Effect of breeder age on eggshell thickness, surface temperature, hatchability and chick weight. *Brazilian Journal of Poultry Science* **14**, 9–14. https://doi.org/10.1590/S1516-635X2012000100002.

Gumułka M, Kapkowska E and Maj D (2010) Laying pattern parameters in broiler breeder hens and intrasequence changes in egg composition. *Czech Journal of Animal Science* **55**, 428–437.

Hu Q (2013) *Energy sensing in developing chicken embryos and post-hatch chicks from different-size eggs. PhD thesis*, College Park, MD, USA: University of Maryland.

Kebreab E, France J, Kwakkel RP, Leeson S, Kuhi HD and Dijkstra J (2009) Development and evaluation of a dynamic model of calcium and phosphorus flows in layers. *Poultry Science* **88**, 680–689. https://doi.org/10.3382/ps.2008-00157.

Ketta M and Tumová E (2016) Eggshell structure, measurements, and quality-affecting factors in laying hens: A review. *Czech Journal of Animal Science* **61**, 299–309. https://doi.org/10.17221/46/2015-CJAS.

Kibala L, Rozempolska-Rucińska I, Kasperek K and Zięba G (2015) Ultrasonic eggshell thickness measurement for selection of layers. *Poultry Science* **94**, 2360–2363. https://doi.org/10.3382/ps/pev254.

Lavelin I, Meiri N, Genina O, Alexiev R and Pines M (2001) Na(+)-K(+)-ATPase gene expression in the avian eggshell gland: Distinct regulation in different cell types. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **281**, R1169–R1176. https://doi.org/10.1152/ajpregu.2001.281.4.R1169.

Lewis PD, Perry GC and Morris TR (1995) Effect of photoperiod on the mean oviposition time of two breeds of laying hen. *British Poultry Science* **36**, 33–37. https://doi.org/10.1080/00071669508417750.

Liu Y, Tian X, Cao S, Li Y, Dong H and Li Y (2021) Pollution characteristics and health risk assessment of arsenic transformed from feed additive organoarsenicals around chicken farms on the North China Plain. *Chemosphere* **278**, 130438. https://doi.org/10.1016/j.chemosphere.2021.130438.

Michalak I, Chojnacka K, Dobrzański Z, Górecki H, Zielińska A, Korczyński M and Opaliński S (2011) Effect of macroalgae enriched with microelements on egg quality parameters and mineral content of eggs, eggshell, blood, feathers and droppings. *Journal of Animal Physiology and Animal Nutrition* **95**, 374–387. https://doi.org/10.1111/j.1439-0396.2010.01065.x.

Mongin P and Sauveur B (1974) Voluntary food and calcium intake by the laying hen. *British Poultry Science* **15**, 349–359. https://doi.org/10.1080/00071667408416118.

Muramatsu T, Hiramoto K and Okumura J-I (1991) Changes in ovalbumin and protein synthesis in vivo in the magnum of laying hens during the egg formation cycle. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **99**, 141–146.

Nager RG, Monaghan P, Houston DC, Arnold KE, Blount JD and Verboven N (2006) Maternal effects through the avian egg. *Acta Zoologica Sinica* **52**, 658–661.

Narushin VG and Romanov MN (2002) Egg physical characteristics and hatchability. *World's Poultry Science Journal* **58**, 297–303. https://doi.org/10.1079/WPS20020023.

Novo RP, Gama LT and Chaveiro Soares M (1997) Effects of oviposition time, hen age and extra dietary calcium on egg characteristics and hatchability. *Journal of Applied Poultry Research* **6**, 335–343. https://doi.org/10.1093/japr/6.3.335.

Nys Y (1986) Relationships between age, shell quality and individual rate and duration of shell formation in domestic hens. *British Poultry Science* **27**, 253–259. https://doi.org/10.1080/00071668608416878.

Okano T and Fukada Y (2003) Chicktacking pineal clock. *Journal of Biochemistry* **134**, 791–797. https://doi.org/10.1093/jb/mvg221.

Onagbesan OM, Metayer S, Tona K, Williams J, Decuypere E and Bruggeman V (2006) Effects of genotype and feed allowance on plasma luteinizing hormone, follicle-stimulating hormone, progesterone, estradiol levels, follicle differentiation and egg production rates of broiler breeder hens. *Poultry Science* **85**, 1245–1258. https://doi.org/10.1093/ps/85.7.1245.

Ortiz D, Lawson T, Jarrett R, Ring A, Scoles KL, Hoverman L, Rocheford E, Karcher DM and Rocheford T (2021) Biofortified orange corn increases xanthophyll density and yolk pigmentation in egg yolks from laying hens. *Poultry Science*, **100**(7), 101117. https://doi.org/10.1016/j.psj.2021.101117.

Patterson PH (1997) The relationship of oviposition time and egg characteristics to the daily light: Dark cycle. *Journal of Applied Poultry Research* **6**, 381–390. https://doi.org/10.1093/japr/6.4.381.

Perić L, Mitraković M, Tomić B, Orehovački I and Meijerhof R (2022) Effects of flock age, place of oviposition and cleaning treatments of hatching eggs on hatchability in broiler breeders. *Journal of Applied Poultry Research* **31**, 100279. https://doi.org/10.1016/j.japr.2022.100279.

Rao K, Xie J, Yang X, Chen L, Grossmann R and Zhao R (2009) Maternal low-protein diet programmes offspring growth in association with alterations in yolk leptin deposition and gene expression in yolk-sac membrane, hypothalamus and muscle of developing Langshan chicken embryos. *British Journal of Nutrition* **102**, 848–857. https://doi.org/10.1017/S0007114509276434.

Reed WL and Clark ME (2011) Beyond maternal effects in birds: Responses of the embryo to the environment. *Integrative and Comparative Biology*, **51**, 73–80. https://doi.org/10.1093/icb/icr032.

Reed WL and Vleck CM (2001) Functional significance of variation in egg-yolk androgens in the American coot. *Oecologia* **128**, 164–171. https://doi.org/10.1007/s004420100642.

Ren Z, Sun W, Liu Y, Li Z, Han D, Cheng X, Yan J and Yang X (2019) Dynamics of serum phosphorus, calcium and hormones during egg-laying cycle in Hy-Line Brown laying hens. *Poultry Science* **98**, 2193–2200. https://doi.org/10.3382/ps/pey572.

Reynard M and Savory CJ (1999) Stress-induced oviposition delays in laying hens: duration and consequences for eggshell quality. *British Poultry Science* **40**, 585–591. https://doi.org/10.1080/00071669986945.

Roberts JR (2004) Factors affecting egg internal quality and eggshell quality in laying hens. *Journal of Poultry Science* **41**, 161–177. https://doi.org/10.2141/jpsa.41.161.

Robinson FE, Hardin RT, Robinson NA and Williams BJ (1991) The influence of egg sequence position on fertility, embryo viability and embryo

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weight in broiler breeders. *Poultry Science* **70**, 760–765. https://doi.org/10.3382/ps.0700760.

Rubolini D, Romano M, Navara KJ, Karadas F, Ambrosini R, Caprioli M and Saino N (2011) Maternal effects mediated by egg quality in the Yellow-legged Gull Larus michahellis in relation to laying order and embryo sex. *Frontiers in Zoology* **8**, 24. https://doi.org/10.1186/1742-9994-8-24.

Ruiz J and Lunam CA (2002) Effect of pre-incubation storage conditions on hatchability, chick weight at hatch and hatching time in broiler breeders. *British Poultry Science* **43**, 374–383. https://doi.org/10.1080/00071660120103648.

Şekeroğlu A and Altuntaş E (2009) Effects of egg weight on egg quality characteristics. *Journal of the Science of Food and Agriculture* **89**, 379–383. https://doi.org/10.1002/jsfa.3454.

Sinclair-Black M, Garcia-Mejia RA, Blair LR, Angel R, Arbe X, Cavero D and Ellestad LE (2024) Circadian regulation of calcium and phosphorus homeostasis during the oviposition cycle in laying hens. *Poultry Science* **103**, 103209. https://doi.org/10.1016/j.psj.2023.103209.

Solomon SE (1997) *Egg and Eggshell Quality*, London, UK: Manson Publishing Ltd.

Speier JS, Yadgary L, Uni Z and Wong EA (2012) Gene expression of nutrient transporters and digestive enzymes in the yolk sac membrane and small intestine of the developing embryonic chick. *Poultry Science* **91**, 1941–1949. https://doi.org/10.3382/ps.2011-02092.

Tůmová E and Ebeid T (2005) Effect of time of oviposition on egg quality characteristics in cages and in a litter housing system. *Czech Journal of Animal Science* **50**, 129–134. https://doi.org/10.17221/4006-CJAS.

Tůmová E, Englmaierová M and Zita L (2009) The effect of genotype, housing system and egg collection time on egg quality in egg-type hens. *Czech Journal of Animal Science* **54**, 17–23. https://doi.org/10.17221/1736-CJAS.

Tůmová E and Gous RM (2012) Interaction of hen production type, age, and temperature on laying pattern and egg quality. *Poultry Science* **91**, 1269–1275. https://doi.org/10.3382/ps.2011-01951.

Tůmová E, Gous RM and Tyler N (2014) Effect of hen age, environmental temperature and oviposition time on eggshell quality and eggshell and serum mineral contents in laying and broiler breeder hens. *Czech Journal of Animal Science* **59**, 435–443. https://doi.org/10.17221/7655-CJAS.

Tůmová E, Zita L, Hubený M, Skřivan M and Ledvinka Z (2007) The effect of oviposition time and genotype on egg quality characteristics in egg-type hens. *Czech Journal of Animal Science* **52**, 26–30. https://doi.org/10.17221/2326-CJAS.

Tůmová E, Zita L, Hubený M, Skřivan M and Ledvinka Z (2008) Effect of time of oviposition on egg quality in egg and meat type hens. *Scientia Agriculturae Bohemica* **39**, 269–272.

Uni Z, Yadgary L and Yair R (2012) Nutritional limitations during poultry embryonic development. *Journal of Applied Poultry Research* **21**, 175–184. https://doi.org/10.3382/japr.2011-00486.

Van den Brand H, Sosef MP, Lourens A and Van Harn J (2016) Effects of floor eggs on hatchability and later life performance in broiler chickens. *Poultry Science* **95**, 1025–1032. https://doi.org/10.3382/ps/pew008.

Williams TD (1994) Intraspecific variation in egg size and egg composition in birds: Effects on offspring fitness. *Biological Reviews of the Cambridge Philosophical Society* **69**, 35–59. https://doi.org/10.1111/j.1469-185x.1994.tb01485.x.

Wilson SC, Jennings RC and Cunningham FJ (1985) Effects of an advance of darkness on the ovulatory cycle of the hen. *British Poultry Science* **26**, 83–96. https://doi.org/10.1080/00071668508416790.

Yan J, Wang J, Chen J, Shi H, Liao X, Pan C, Liu Y, Yang X and Ren Z (2023) Adjusting phosphate feeding regimen according to daily rhythm increases eggshell quality via enhancing medullary bone remodelling in laying hens. *Journal of Animal Science and Biotechnology* **14**, 17. https://doi.org/10.1186/s40104-023-00829-0.

Yannakopoulos AL, Tserveni-Gousi AS and Nikokyris P (1994) Egg composition as influenced by time of oviposition, egg weight and age of hens. *European Poultry Science* **58**, 206–213. https://doi.org/10.1016/S0003-9098(25)01421-3.

Zakaria AH, Plumstead P, Romero-Sanchez H, Leksrisompong N, Osborne J and Brake J (2005) Oviposition pattern, egg weight, fertility and hatchability of young and old broiler breeders. *Poultry Science* **84**, 1505–1509. https://doi.org/10.1093/ps/84.9.1505.

Zakaria AH, Plumstead PW, Romero-Sanchez H, Leksrisompong N and Brake J (2009) The effects of oviposition time on egg weight loss during storage and incubation, fertility and hatchability of broiler hatching eggs. *Poultry Science* **88**, 2712–2717. https://doi.org/10.3382/ps.2009-00069.

Zhang Z, Du X, Lai S, Shu G, Zhu Q, Tian Y, Li D, Wang Y, Yang J, Zhang Y and Zhao X (2022) A transcriptome analysis for 24-hour continuous sampled uterus reveals circadian regulation of the key pathways involved in eggshell formation of chicken. *Poultry Science* **101**, 101531. https://doi.org/10.1016/j.psj.2021.101531.

Zuidhof MJ, Renema RA and Robinson FE (2007) Reproductive efficiency and metabolism of female broiler breeders as affected by genotype, feed allocation and age at photostimulation. *III. Reproductive Efficiency. Poultry Science* **86**, 2278–2286. https://doi.org/10.1093/ps/86.10.2278.

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