Morphological characteristics of hatching eggs and hatching dynamics in Prelux-Č and Prelux-G laying hybrids and their relationship with chick sex

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Morphological characteristics of hatching eggs and hatching dynamics in Prelux-Č and Prelux-G laying hybrids and their relationship with chick sex

Abstract: This study examined the morphological characteristics of hatching eggs from two Slovenian layer hybrids, Prelux-Č and Prelux-G, and investigated their relationship with hatch window and chick sex. The results showed significant ($p \leq 0.05$) genotype-specific differences in egg morphology, hatching time and chick weight at hatch. Compared to Prelux-G eggs, Prelux-Č eggs were significantly ($p \leq 0.05$) narrower, lighter and had paler shell pigmentation. Chicks from Prelux-Č eggs hatched earlier and had a significantly ($p \leq 0.05$) lower body weight than chicks from Prelux-G eggs. The hatch window of the Prelux-G hybrids was 56 hours, while that of the Prelux-Č hybrids was 64 hours. In the Prelux-Č hybrids, the pullets hatched earlier than the cockerels, while no significant sex-specific differences in hatching time were observed in the Prelux-G hybrids. The duration of egg storage prior to incubation also influenced hatch timing, with chicks from eggs stored for seven days hatching later than chicks from eggs stored for two days; however, this difference was only statistically significant ($p \leq 0.05$) in the Prelux-Č. The statistical analysis showed no significant correlation ($p > 0.05$) between the external egg characteristics and the sex of the chicks, indicating the limited predictive value of egg morphology for sex determination.

Key words: poultry, chicken, genotype, egg morphology, egg storage, hatch window, chick sex

Morfološke značilnosti valilnih jajc in dinamika valjenja pri nesnih hibridih prelux-Č in prelux-G ter njuna povezanost s spolom piščancev

Izvleček: V tej raziskavi so bile proučene morfološke značilnosti valilnih jajc dveh slovenskih nesnih hibridov, prelux-Č in prelux-G, ter njihova povezanost z valilnim oknom in spolom piščancev. Ugotovljene so bile statistično značilne razlike ($p \leq 0,05$) med genotipoma v morfologiji jajc, trajanju valjenja in telesni masi piščancev ob izvalitvi. V primerjavi z jajci prelux-G so bila jajca prelux-Č statistično značilno ($p \leq 0,05$) ožja, lažja in s svetlejšimi lupinami. Piščanci prelux-Č so se izvalili prej in z nižjo telesno maso v primerjavi s piščanci prelux-G. Pri prelux-G je bilo valilno okno dolgo 56 ur, medtem ko je bilo pri prelux-Č daljše, in sicer 64 ur. Pri prelux-Č je bilo ugotovljeno, da so se jarkice izvalile prej kot petelinčki, medtem ko pri prelux-G ni bilo zaznanih statistično značilnih razlik v času izvalitve med spoloma. Na čas izvalitve je vplivala dolžina skladiščenja jajc pred valjenjem; piščanci iz jajc, skladiščenih sedem dni, so se izvalili pozneje kot tisti iz jajc, skladiščenih dva dni. Statistično značilna razlika ($p \leq 0,05$) je bila pri tem parametru opažena le pri hibridu prelux-Č. Statistična analiza ni pokazala značilne korelacije ($p > 0,05$) med zunanjimi lastnostmi jajc in spolom piščancev, kar potrjuje omejeno napovedno vrednost morfologije jajc za določanje spola.

Ključne besede: perutnina, kokoši, genotip, morfologija jajca, skladiščenje jajc, valilno okno, spol piščanca

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1 INTRODUCTION

In chickens, the sex ratio at hatching is usually close to 1:1. In the past, this natural balance did not pose a challenge as both sexes were important in poultry production — hens as layers and cockerels as meat suppliers. However, with the development of highly specialised genetic lines for egg or meat production over the last seven decades, male chicks from layer lines have become a by-product with little or no economic value. These male chicks grow slowly, have poor feed conversion and do not develop enough muscle mass, making them unsuitable for commercial meat production (Popova et al., 2022). Therefore, they are often killed shortly after hatching and processed into protein-rich products or used as feed for carnivorous animals (Kaleta & Redmann, 2008). This routine killing practice has come under increasing ethical criticism and has raised public concern and calls for more humane alternatives (Bruijnis et al., 2015; Hammershøj et al., 2021). One possible solution is the selective incubation of female embryos, which would improve the efficiency of hatcheries as fewer resources would need to be spent on unwanted male chicks and would address major animal welfare concerns (Kaleta & Redmann, 2008). To achieve this, numerous *in ovo* sexing techniques have been developed, including hormone analysis, cytogenetic testing, *in ovo* ultrasound, heart rate monitoring and temperature-dependent sex differentiation (Kaleta & Redmann, 2008; Jia et al., 2023). However, many of these methods are either invasive, technically challenging or unsuitable for commercial application. Recent research has focused on the development of non-invasive and scalable technologies. Hyperspectral imaging, for example, has shown up to 93% accuracy in sex determination, offering a promising tool for early sex determination with minimal impact on the embryo (Jia et al., 2023). Bioimpedance measurement has also been shown to be a novel, non-invasive approach that enables sex determination during incubation (Ching et al., 2023). Despite these advances, all current methods for *in ovo* sex determination involve the destruction of embryos identified as male. This is ethically problematic, not only because of concerns about possible pain sensation in developing embryos, but also because of broader moral and philosophical issues such as the status of the embryo, the instrumentalisation of life, and conflicts with certain cultural or religious values (Wedzerai, 2021; Priesemeister, 2024). Alternatively, researchers have investigated the potential of using external egg characteristics — in particular the shape index (SI), which is calculated as the ratio between the short and long diameter of an egg as a non-invasive indicator of the sex of the chick. Some studies have found statistically significant correlations suggesting that eggs with a lower SI are more likely to produce male chicks, while eggs with a higher SI are more likely to produce female chicks (Kayadan & Uzun, 2023). However, the results in this area are contradictory, as other studies have found no meaningful relationship between egg morphology and chick sex (Burnham et al., 2003). These discrepancies emphasise the need for further research into the extent to which egg characteristics and the hen's genotype can influence the sex of the offspring. In addition to sex determination, the timing of hatching — particularly the duration and synchrony of the hatch window — is another critical factor in poultry production. Efficient and consistent hatching is crucial for optimizing early chick management, ensuring consistent access to feed and water, and improving post-hatch performance (Mesquita et al., 2021). Previous research (e.g. Biesiada-Drzazga, 2020) has shown that genotype, external egg traits and storage conditions can influence hatching dynamics, but the interaction of these factors remains poorly understood, especially in the context of dual-purpose or layer hybrids. For this reason, the main objective of the study was to investigate the influence of genotype, external egg characteristics and egg storage duration on hatching dynamics (especially hatching duration and hatch window), chick sex and chick weight in two Slovenian layer hybrids — Prelux-Č and Prelux-G. The study also aimed to evaluate the predictive value of external egg traits for early sexing as a possible alternative to ethically controversial *in ovo* sexing methods.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE AND ORIGIN OF THE EGGS

The experiment was conducted in the hatchery of the Department of Animal Science of the Biotechnical Faculty of the University of Ljubljana. The hatching eggs used in the study were obtained from the Department's Poultry Educational and Research Center. According to Directive 2010/63/EU on the protection of animals used for scientific purposes (European Parliament and Council of the European Union, 2010), research conducted within a standard production cycle is classified as non-experimental agricultural practice (Chapter I, Article 1, point 5) and therefore does not require ethics committee approval.

2.2 INCUBATION PROCESS AND PROCEDURE AFTER HATCHING OF THE CHICKS

A total of 1,260 hatching eggs from two genotypes of hybrid laying hens, Prelux-G (n = 630 eggs) and Prelux-Č (n = 630 eggs), were used. The eggs were collected from 42-week-old parent flocks and divided into two storage groups (2-day and 7-day storage). The experiment was designed as a 2 × 2 factorial model with two genotypes and two storage durations within each genotype. A total of 315 eggs per genotype and storage period were incubated under identical conditions. Prior to incubation, the eggs were stored in a cold room at 15 °C and 75% relative humidity and turned once a day. Before incubation, each egg was labelled with an identification number and the storage period. The weight, width, height and shell colour of the eggs were recorded, and the shape index was calculated from the width and height using a digital calliper. Shell colour was determined using a reflectometer calibrated with black and white reference tiles (TSS, York, England). Before incubation, the eggs were disinfected with formaldehyde gas. Incubation was carried out in Petersime incubators (Petersime, Zulte, Belgium). During the first 18 days, the eggs were incubated in a setter (S168) at 37.8 °C and 60% humidity, with automatic rotation by 45° every hour. On the 18th day, the eggs were transferred to a hatcher (H168) with a temperature of 37.2 °C and a humidity of 70%, and the turning of the eggs was stopped. At the time of transfer to the hatcher, the eggs were placed in plastic trays with individual wire compartments to ensure separate hatching of the chicks. The start of incubation, or time zero from which the incubation hours were counted, was set as the time at which the temperature and relative humidity in the incubator reached the specified values. The hatching of the chicks was monitored between 469 hours (19 days, 13 hours) and 541 hours (22 days, 13 hours), with a hatch window of 72 hours. Hatching was monitored every 8 hours (at 05:00, 13:00 and 21:00) and the newly hatched chicks were weighed and assigned to their sex. Each chick was individually removed from its compartment and the incubator was immediately closed to minimise temperature loss. The chicks were considered to have hatched when they were completely detached from the egg shell and their feathers were sufficiently dry for examination. The sex of each chick was always determined by two trained persons based on down colour (Prelux-Č) and feathering rate (Prelux-G).

2.3 STATISTICAL ANALYSES

Data analysis was performed using the SAS STAT software (SAS Institute, 2016). The effects of genotype and egg storage duration on hatching duration and chick weight were analysed using a nested ANOVA, with egg storage duration before incubation nested within genotype. Group comparisons were performed using the Tukey-Kramer test. Spearman's rank correlation was used to assess the relationships between egg traits (width, height, shape index, weight and shell colour) and hatching duration, and between chick weight and hatching duration. Ordinal-discrete (hatching duration) and categorical-discrete (sex) variables were analyzed for independence using the chi-square test. Due to the limited number of chicks (< 5 per genotype and sex) hatched at 469, 477, 485, 533 and 541 hours, these time points were excluded from the chi-square analysis. Logistic regression (PROC LOGISTIC) was used to assess the relationship between chick sex as the dependent binary variable and a number of external egg characteristics (egg width, egg height, shape index, egg weight, shell colour) as independent variables. In logistic regression models, odds ratios (OR) are used rather than probabilities. The model coefficients represent changes in the logarithm of the odds, which are often difficult to interpret directly. Therefore, the results were converted into odds ratios by exponentiating the beta estimates of the model.

3 RESULTS AND DISCUSSION

3.1 INFLUENCE OF GENOTYPE ON EGG CHARACTERISTICS, HATCHING TIME AND CHICK WEIGHT

A comparative analysis of the Prelux-Č and Prelux-G genotypes revealed significant differences ($p \leq 0.05$) in the external egg characteristics, the weight of the chicks and the hatching time. Compared to the Prelux-G, the Prelux-Č eggs were significantly ($p \leq 0.05$) narrower, shorter and lighter in mass, with lighter coloured shells. In addition, the chicks hatched from Prelux-Č eggs had a lower body weight and hatched earlier ($p \leq 0.05$) than their Prelux-G counterparts (Table 1).

These results are consistent with previous studies showing the genetic influence on egg quality. Uçar and Kahya (2024) reported significant differences in egg weight and shape between different chicken genotypes, emphasising the role of genetic predisposition in determining these parameters. In addition, the observed delayed hatching in heavier eggs is consistent

with the findings of Iqbal et al. (2014), who associated greater egg mass with a longer incubation period and higher chick weight after hatching. However, the results of Sklan et al. (2000) indicate no significant correlation between egg weight and hatch duration in Cobb 500 broilers, suggesting possible variability between genetic lines and incubation conditions. The relationship between egg morphometrics and hatch duration remains a topic of interest in evolutionary biology and developmental ecology. In general, larger eggs require longer incubation times due to higher yolk content and nutrient availability, which may prolong embryonic development (Nangsuay et al., 2011). Nevertheless, there are species-specific differences, with some studies suggesting that larger eggs within the same breed may hatch earlier due to a better nutrient supply and more favourable thermal characteristics (Lourens et al., 2006;

identical conditions, in the same incubator and without direct sunlight. Consequently, differences in heat absorption due to eggshell pigmentation were not a factor contributing to the observed differences in hatching time. Egg shape, defined by the ratio of width to height, can also influence hatching dynamics. Longer eggs can alter the surface-to-volume ratio, which affects gas exchange and moisture retention, which in turn affects embryo development (Duursma et al., 2018). Conversely, it has been hypothesised that rounder eggs may improve heat retention, which could reduce incubation time under certain conditions. However, in the present study, the shape index of Prelux-Č and Prelux-G eggs did not differ significantly ($p > 0.05$), suggesting that egg shape does not contribute to the observed differences in hatch duration. Recent advances in developmental biology indicate that embryo metabolic rate,

Table 1: The influence of the genotype on the external characteristics of the eggs, the weight of the chicks and the hatch duration

	LSM ± SE	
Trait	Prelux-Č	Prelux-G
Egg width (mm)	43.03[a] ± 0.05	43.87[b] ± 0.05
Egg height (mm)	57.60[a] ± 0.10	58.99[b] ± 0.10
Shape index (%)	74.80[a] ± 0.13	74.46[a] ± 0.13
Egg weight (g)	59.57[a] ± 0.19	63.93[b] ± 0.19
Shell color (%)	46.84[a] ± 0.26	34.29[b] ± 0.26
Chick weight (g)	42.12[a] ± 0.15	44.52[b] ± 0.16
Hatch duration (hours)	506.92[a] ± 0.30	508.32[b] ± 0.31

LSM = least squares mean; SE = standard error; a, b values in the same row of the table marked with different letters are statistically different at $p \leq 0.05$.

Table 2: Spearman correlation coefficients between hatch duration and external egg characteristics and chick weight in two Prelux hybrids

	Hatch duration	
	Prelux-Č	Prelux-G
Egg width	0.121 ($p = 0.004$)	0.088 ($p = 0.044$)
Egg height	0.083 ($p = 0.052$)	0.052 ($p = 0.231$)
Shape index	−0.0003 ($p = 0.993$)	0.014 ($p = 0.735$)
Egg weight	0.138 ($p = 0.001$)	0.093 ($p = 0.034$)
Shell color	0.041 ($p = 0.336$)	0.018 ($p = 0.674$)
Chick weight	0.079 ($p = 0.064$)	0.046 ($p = 0.294$)

Yalçin et al., 2022). In this study, two external morphometric characteristics of the eggs — width and weight — showed a weak correlation with hatching time (Table 2).

The results presented in Table 2 indicate that egg width and weight may play a role in embryonic development, but their predictive power for hatching time is limited, especially when genetic factors are not taken into account. The influence of eggshell colour on hatching time remains a complex phenomenon that is influenced by environmental factors such as light exposure and incubation temperature. It has been hypothesised that darker shells retain more heat, possibly accelerating embryo metabolism; however, empirical evidence is inconclusive (Westmoreland et al., 2007). In the present study, eggs of both genotypes were incubated under

oxygen availability and eggshell porosity contribute significantly to the differences in hatching time (Peebles, 2023; Lourens et al., 2007). These physiological and environmental factors may obscure or attenuate direct correlations between external egg characteristics and hatch duration. In addition, epigenetic mechanisms, such as maternally transmitted hormones, have been shown to influence hatching plasticity (Hukkanen et al., 2023), adding another level of complexity to genotype-dependent variability. Given the limited predictive power of egg width and weight alone, future research should incorporate a broader range of parameters, including eggshell structure, yolk composition and thermal environment, to improve our understanding of the factors that determine hatch duration. In addition, genomic analyses could elucidate the genetic basis for variation in developmental timing and provide a more comprehensive model of embryonic growth dynamics.

Table 3: The influence of egg storage time before incubation on egg external characteristics, chick weight and hatch duration

Genotype	Storage (days)	Egg width (mm)	Egg height (mm)	Shape index (%)	Egg weight (g)	Shell color (%)	Chick weight (g)	Hatch duration (h)
Prelux-Č	2	42.98 ± 0.07[a]	57.58 ± 0.14[a]	74.73 ± 0.18[a]	59.62 ± 0.26[a]	47.10 ± 0.36[a]	42.18 ± 0.21[a]	505.57 ± 0.43[a]
	7	43.08 ± 0.07[a]	57.62 ± 0.14[a]	74.88 ± 0.18[a]	59.52 ± 0.27[a]	46.58 ± 0.37[a]	42.06 ± 0.22[a]	508.28 ± 0.43[b]
Prelux-G	2	43.82 ± 0.07[b]	58.85 ± 0.14[b]	74.55 ± 0.19[a]	63.93 ± 0.27[b]	34.95 ± 0.38[b]	44.37 ± 0.22[b]	507.55 ± 0.45[b]
	7	43.92 ± 0.07[b]	59.13 ± 0.14[b]	74.37 ± 0.18[a]	63.93 ± 0.27[b]	33.63 ± 0.37[b]	44.68 ± 0.22[b]	509.09 ± 0.44[b]

p-values (effect of storage duration within genotype): Egg width = 0.615; Egg height = 0.601; Shape index = 0.804; Egg weight = 0.996; Shell color = 0.044; Chick weight at hatch = 0.350; Hatch duration = 0.0001.
LSM = least squares mean; SE = standard error. Values in the same column and within genotype marked with different superscripts (a, b) differ significantly at $p \leq 0.05$.

3.2 EFFECTS OF EGG STORAGE TIME BEFORE INCUBATION ON HATCHING TIME

The hatching time of the Prelux-Č chicks was significantly influenced by the duration of egg storage prior to incubation ($p \leq 0.05$). Specifically, chicks hatched earlier from eggs stored for a shorter pre-incubation period (two days) compared to those stored for a longer period (seven days). Prelux-Č chicks emerging from eggs stored for two days hatched after 505.57 hours of incubation, whereas those from eggs stored for seven days required 508.28 hours (Table 3). A similar trend was observed in the Prelux-G hybrids, where chicks from younger eggs hatched earlier (507.55 hours for two-day stored eggs) compared to those from older eggs (509.09 hours for seven-day stored eggs). However, this difference was not statistically significant (p > 0.05) for the Prelux-G hybrids (Table 3).

The results presented in Table 3 are consistent with previous studies showing that prolonged egg storage prior to incubation can delay embryonic development and prolong hatch duration. The mechanisms underlying this phenomenon are related to metabolic and physiological changes that occur in the egg during storage. Research suggests that prolonged storage leads to a number of changes, such as cracks in the cuticle, increase in the pH of the egg contents, water evaporation, enlargement of air cells, thinning of the albumen, weakening of the yolk membrane, protein degradation, loss of vitamins and lysozyme, reduced embryo viability and delayed onset of embryonic development due to impaired gas exchange and moisture loss (Biesiada-Drzazga, 2020; Reijrink et al., 2010). The observed differences between the Prelux-Č and Prelux-G can be attributed to genetic factors that influence the embryo's resistance to storage-related stress. Previous studies have shown that different poultry breeds and hybrid lines are differently sensitive to storage duration, with some lines showing greater adaptability in maintaining embryo viability under prolonged storage conditions

(Nasri et al., 2020). Given these findings, optimising hatchery storage conditions and duration according to genotype should be a priority in order to maximise hatchability and minimise variability in incubation times.

3.3 GENOTYPE-SPECIFIC VARIABILITY OF HATCHING TIME

The incubation period in hens is about 21 days (504 hours), which is the average time from egg deposition in the incubator to chick hatching (Hedlund and Jensen, 2021). Since chick hatching does not occur simultaneously, there is a period of time between the first and the last hatched chick called the hatch window. The main aim of commercial hatcheries is to maximise the yield of high-quality day-old chicks. Typically, chicks are collected as soon as all viable eggs have hatched, usually after 21.5 days of incubation. This schedule favours late hatching chicks to ensure the highest possible yield. However, in cases where the hatch window is more than 48 hours, conditions for early hatched chicks may be suboptimal (Cobb-Vantress, 2020). In the present study, the hatch window for Prelux-G hybrids was 56 hours (485 to 541 hours), while Prelux-Č hybrids had a longer window of 64 hours (469 to 533 hours) (Figure 1). Initially, more pullets hatched than cockerels; however, this trend reversed after 509 hours. These observations are consistent with the results of Burke (1992), who found shorter incubation times for pullets in different breeds of hens. Burke (1992) recorded an average hatching time of 499.8 hours for pullets and 502.8 hours for cockerels, with pullets also having a larger hatch window (41 hours compared to 33 hours for cockerels). Our results indicate significant genotype-dependent differences in hatching dynamics. In the Prelux-G hybrids, the cockerels hatched in a shorter period (48 hours) than the pullets (56 hours), whereas in the Prelux-Č hybrids, the cockerels had a longer
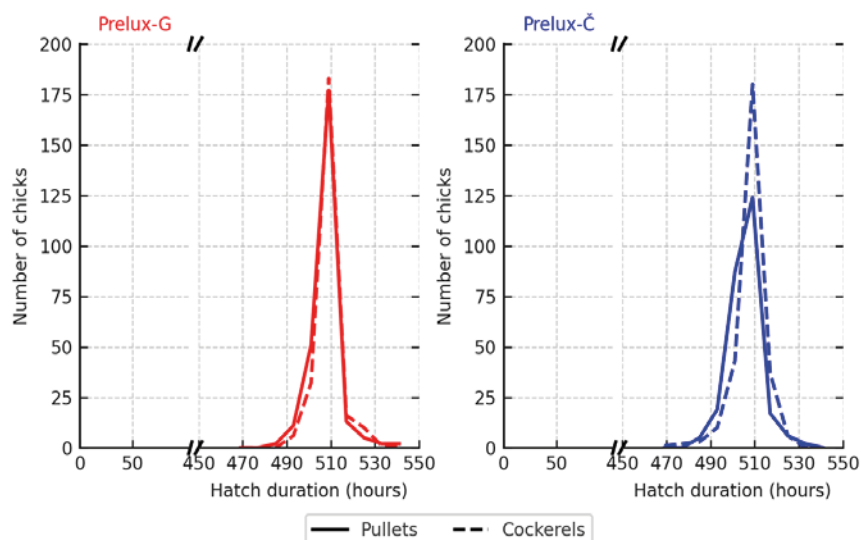
Figure 1: Graphical representation of the hatch window in the Prelux-G and Prelux-Č hybrid layers

hatch window (56 hours) than the pullets (48 hours). These results indicate that the duration of the hatch window is influenced by the genotype of the chick.

3.4 RELATIONSHIPS BETWEEN CHICK SEX AND HATCH DURATION

In both Prelux-Č and Prelux-G hybrids, the relationship between chick sex and hatch duration showed genotype-specific differences. In the Prelux-Č hybrids, a significantly ($\chi^2 = 34.47$; $p = 0.0001$) higher number of pullets than cockerels hatched between 493 and 501 hours after the start of incubation, whereas in Prelux-G hybrids no statistically significant sex-specific differences in hatching time were observed ($\chi^2 = 7.70$; $p = 0.1031$) (Figure 2). By 493 hours, a numerically higher proportion of pullets than cockerels hatched in both Prelux-G and Prelux-Č. However, binomial tests performed separately for each genotype at this time point showed that the observed sex ratios did not deviate from the expected 1:1 ratio ($p > 0.05$) (Figure 2).
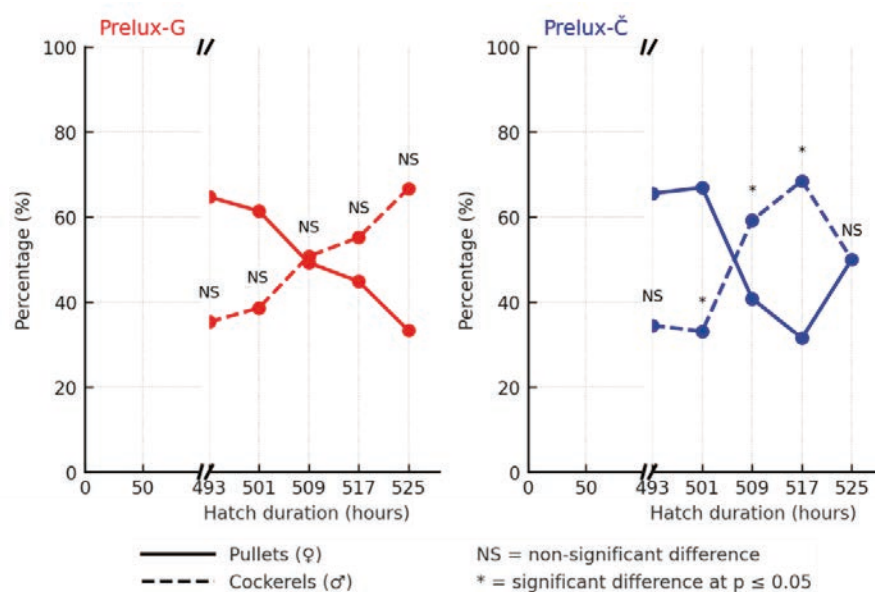

Figure 2: Percentage of cockerels and pullets by hatch duration and genotype

This result is primarily due to the small sample size of chicks hatched at this time, as the test is less sensitive with a small number of observations.

In relation to Prelux-Č, significantly more Prelux-Č cockerels than pullets hatched in the following time intervals — 501 to 509 hours and 509 to 517 hours (Figure 2). By 501 hours after the start of incubation, 41.90% of pullets and only 19.20% of cockerels had hatched, while by 509 hours 90.91% of pullets and 84.42% of cockerels had hatched. These results indicate a pronounced hatching dynamic between the sexes, with Prelux-Č pullets hatching earlier than Prelux-Č cockerels. Up to 501 hours, a larger proportion of pullets (24.12% of all Prelux-G pullets) hatched than cocks (15.38% of all Prelux-G cocks). After this time, however, more cockerels hatched than pullets. By 509 hours, 93% of all pullets and 89.47% of all cockerels had hatched. The statistical insignificance of hatching time in Prelux-G cockerels and pullets contrasts with the significant differences observed in Prelux-Č hybrids. This suggests that although sex-specific hatching patterns exist, their dynamics may vary between the different hybrid strains. These results are consistent with recent studies showing that genetic factors and specific characteristics of hybrid lines can influence hatching timing. Genetic make-up plays a crucial role in sex-specific hatching patterns in poultry, with male chicks often exhibiting different hatching dynamics than females due to differences in hormonal regulation and developmental processes (Cook & Monaghan, 2004). The observed differences in hatching dynamics between the two Prelux strains can be attributed to inherent genetic character-

istics of the hybrids. Further research focusing on the hormonal and genetic mechanisms underlying these differences is needed to better understand the causes of these differences.

3.5 EXTERNAL CHARACTERISTICS OF THE EGGS AS PREDICTORS FOR THE SEX OF THE CHICKS

The data presented in Table 4 illustrate the odds ratios (OR) describing the relationship between the probability of hatching a male chick (cock) versus a female chick (pullet) based on the external characteristics of the eggs. Theoretically, assuming statistical significance ($p \leq 0.05$) — which was not observed in this study — and holding all other model variables constant, an increase in egg weight by 1 g would reduce the probability of hatching a male chick in Prelux-G hybrids by a factor of 0.98. This relationship is indicated by the negative sign of the parameter β (Table 4). However, since the p-values in Table 4 exceed the conventional threshold of 0.05, it can be concluded that external egg traits do not serve as reliable predictors of chick sex in any of the Prelux hybrids studied.

These results are consistent with previous studies showing that external egg traits such as weight, shape and shell pigmentation have limited predictive power for determining chick sex (Jia et al., 2023). Recent studies show that such associations, when present, are often breed- or line-specific; for example, White Leghorn eggs showed a statistical correlation between size

Table 4: Multivariate logistic regression: correlation between the external characteristics of the hatching eggs and the sex of the chicks

Genotype	Parameter	DF	PE (ß)	SE (ß)	Wald χ²	p-value	OR (exp ß)
Prelux-Č	Constant	1	1.551	15.292	0.010	0.9192	
	Egg width	1	0.469	0.637	0.542	0.4616	1.59
	Egg height	1	−0.109	0.287	0.146	0.7021	0.89
	Shape index	1	−0.143	0.274	0.274	0.6006	0.86
	Egg weight	1	−0.087	0.081	1.153	0.2829	0.91
	Shell color	1	0.013	0.013	1.113	0.2913	1.01
Prelux-G	Constant	1	19.641	25.402	0.597	0.4394	
	Egg width	1	0.655	0.666	0.968	0.3251	1.92
	Egg height	1	−0.396	0.432	0.841	0.3589	0.67
	Shape index	1	−0.336	0.364	0.854	0.3552	0.71
	Egg weight	1	−0.011	0.050	0.055	0.8139	0.98
	Shell color	1	0.022	0.016	1.821	0.1771	1.02

DF = Degrees of freedom; PE (β) = Parameter estimate (β); SE (β) = Standard error (β); OR exp (β) = Odds ratio exp (β)

traits and chick sex (Anjana et al., 2024). The biological mechanisms of sex determination in birds are extremely complex and are primarily influenced by genetic and hormonal factors rather than morphological characteristics of the eggs (Ioannidis et al., 2021; Zhang et al., 2023). Some studies have hypothesised that egg size may be indirectly related to sex ratio due to differences in resource allocation during egg formation. However, this remains speculative and is not supported by relevant data (Simmons, 2000). The present study confirms the assumption that the external egg characteristics alone are not sufficient to accurately predict the sex of Prelux hybrids.

In parallel, considerable progress has recently been made in sexing technologies. Optical and spectral approaches, including near-infrared spectroscopy (Schreuder et al., 2024) and hyperspectral imaging in combination with machine learning (Ji et al., 2024; Ahmed et al., 2025), have achieved high accuracies even before incubation or at the earliest embryonic stages. Morphological characteristics of developing embryos, obtained by imaging and computer vision, are also promising (Zhang & Jacobs, 2025). In addition to optical methods, molecular diagnostic techniques such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) (Van der Hofstadt et al., 2025) offer rapid and highly specific sex identification. Importantly, these methods are already in industrial use: Several *in ovo* sexing technologies have been in commercial use since 2024, particularly in the United States and Europe (Associated Press, 2024). Taken together, these developments contextualise our non-significant findings and highlight that the future of practical, ethical chick sexing is likely to depend on advanced biochemical or optical solutions rather than external egg measurements. Other experimental approaches are also discussed in the literature, such as parthenogenesis (Olsen, 1968; Parker et al., 2010) or genetic editing for self-sorting traits (Quansah et al., 2013; Bruijnis et al., 2015; Doran et al., 2017). However, these methods are either impractical due to the low viability of embryos or face ethical and legal challenges. In short, while *in ovo* sex determination is the most practical solution today (He et al., 2019; Ching et al., 2023; Matsumoto et al., 2024), the future is likely to be characterised by the further development of ethical, accurate and non-destructive alternatives.

4 CONCLUSIONS

The results of this study show that the morphological characteristics of the hatching eggs, the hatching dynamics and the sex of the chicks are strongly influenced by the genotype. The eggs of the Prelux-Č hybrids were significantly narrower, lighter in colour and less pigmented than those of the Prelux-G hybrids, which was associated with an earlier hatching time and a lower chick weight. In addition, the duration of the hatching window differed between genotypes, with a longer hatching window (64 hours) observed in Prelux-Č than in Prelux-G (56 hours), suggesting a genotype-specific influence on the synchronisation of hatching. A pronounced sex-specific pattern in hatching time was only observed in Prelux-Č, where pullets hatched significantly earlier than cockerels; no such differences were observed in the Prelux-G hybrid. The study also showed that the duration of egg storage prior to incubation significantly affected hatching time in Prelux-Č, with longer storage leading to delayed hatching, whereas this effect was not statistically significant in Prelux-G. Importantly, the analysis revealed no significant correlation between external egg traits — such as egg size, shape index, shell colour or weight — and the sex of emerging chicks in either hybrid, confirming the limited predictive value of these morphological indicators. These results emphasise the complex interplay between genetic, physiological and environmental factors in determining hatching dynamics and highlight the limitations of using external egg traits for sex prediction. The results suggest that genotype-specific differences in hatching window and sex-specific hatching dynamics may have important implications for hatchery management. Shorter and better synchronised hatching windows, as observed in Prelux-G, may reduce the risk of early hatched chicks suffering from food and water deprivation, and thus improve post-hatch viability. Conversely, hybrids with a wider hatching window, such as Prelux-Č, may require adapted collection schedules or management protocols to minimise welfare concerns and performance variation. From a breeding perspective, the observed sex-specific hatching patterns could be relevant for programmes to improve the uniformity of day-old chicks. The inclusion of hatching synchrony as a selection criterion could contribute to better flock performance, reduce early mortality, and improve the efficiency of rearing systems. Although the present study focussed on Prelux hybrids, the results underline the importance of considering the genetic background when developing hatchery strategies. Hatcheries managing multiple genotypes could benefit from genotype-tailored incubation regimes, that could optimise hatchability and chick quality in different lines.

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