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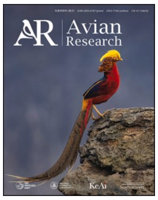

Reproductive resilience and trade-offs: Egg component allocation under nutritional constraints in Japanese Quail

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ABSTRACT

Under natural conditions, organisms regularly experience seasonal or unpredictable fluctuations in their diet that may shape the quantity and quality of their reproduction. While these dietary shifts are known to influence overall reproductive output, their specific effects on the proportional composition of egg components remain unexplored. To partially simulate the natural conditions, we conducted two trials using adult Japanese Quails (*Coturnix japonica*) to examine how dietary restriction and unpredictable feeding influence the proportional composition of egg components. In the first trial, quails were assigned to one of four treatments: full-fed (control) or restricted feeding at 20% (DR20), 30% (DR30), and 40% (DR40) below daily intake. In the second trial, quails were assigned to one of three treatments: full feeding (control), constant 40% restriction (DR40), or unpredictable feeding schedule (UNPR). Each trial lasted for 14 days, and birds were housed individually. Severe dietary restrictions (DR30, DR40) reduced albumen and shell mass, shell thickness, and egg volume. However, yolk mass, yolk color, egg shape index, and shell density remained unaffected across all dietary levels. Severe restrictions also increased the yolk ratio while reducing the albumen ratio, suggesting that birds may evolutionarily prioritize yolk deposition to sustain reproductive functions. However, reduced albumen content due to maternal nutritional stress may also limit essential nutrients, and disrupt the embryo's osmoregulation, which ultimately affects offspring quality. Reduced shell contents at the severe restriction levels can affect the egg's ability to provide physical protection, regulate gas exchange and control water loss, thereby influencing embryonic development. In contrast, moderate restriction (DR20) and unpredictable feeding (UNPR) had no effect on any of the egg traits. These findings suggest that quails strategically allocate resources among egg components under dietary constraints, indicating potential trade-offs in reproductive investment. The resilience observed under moderate restriction and unpredictability underscores the species' adaptive capacity in fluctuating environments.

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1. Introduction

Reproductive output in birds is determined based on dietary conditions, genetic traits, and the age of hens (Xin et al., 2011; Sharma et al., 2022). Among these factors, dietary condition holds a major role as it directly influences the bird's overall health, metabolic processes, and reproductive system and ensures optimal egg development, eggshell strength, and overall vitality (Houston, 1997; Wang et al., 2017). For example, when the perceived dietary sources are changed, birds may temporarily or permanently alter the time of egg laying, clutch size, and/or quality of eggs laid (Davies and Deviche, 2014; Regan et al., 2020). High-quality eggs are characterized by their size, shape, strength, color, and the resulting size and vigor of the hatchlings (Krist, 2011; Loffredi et al., 2021). All these egg traits are the function of dietary conditions, where both permanent and temporary changes can lead to significant effects (Houston, 1997; García-Campa et al., 2020; Kowalska et al., 2021).

Dietary restriction (DR) and unpredictable feeding are laboratory interventions that mimic resource depletion or fluctuation in natural environments. In contrast, DR is commonly used in commercial poultry production, mainly in growing birds, to delay sexual maturity, alleviate metabolic disorders, and extend reproductive lifespan (Walsh and Brake, 1997; Lu et al., 2021; Ebeid et al., 2022; Anene et al., 2023). Birds under moderate dietary restriction show higher egg production, with fewer defective or damaged eggshells, and exhibit improved fertility and hatchability compared to those fed ad libitum (Hocking et al., 2002). However, severe and prolonged restrictions can lead to declining egg production (Olawuni et al., 1992; Mahrose et al., 2022; Anene et al., 2023). Dietary unpredictability, an increasingly unavoidable phenomenon due to the unfolding environmental variability, may affect metabolic regulation but could also impose stress due to perceived uncertainty (Pravosudov et al., 2001; Fokidis et al., 2012).

To cope with the above conditions, birds may adjust their reproductive strategies, shifting resource allocation among different egg components and the body. In Japanese Quails (*Coturnix japonica*), dietary restriction (up to 30%) does not reduce egg production or quality but leads to lower body mass, likely due to improved feed conversion efficiency (Gebhardt-Henrich and Marks, 1995; Hassan et al., 2003; Fidan and Kaya, 2014). Further studies in this species also indicated that moderate restriction may enhance egg production and quality, whereas severe restriction reduces most egg traits (Mahrose et al., 2022). Time-restricted feeding in laying hens also reduced egg mass and laying pattern compared to full feeding (Saibaba et al., 2021). Similarly, both permanent and temporary dietary restriction feeding have been found to decrease yolk content, whereas total egg mass and eggshell mass remained unchanged (Anene et al., 2023). On the other hand, birds may adjust nutrient investment among egg components in response to dietary availability, which has crucial implications for egg and offspring quality (Hsu et al., 2016; Fortuna et al., 2023). Depending on nutritional conditions, they may allocate more nutrients to the yolk, albumen, or shell. Alternatively, if egg size is reduced due to feed scarcity, one egg component may be reduced disproportionately without a compensatory shift in allocation. However, the effects of different dietary conditions on the proportional investment in different egg components remain largely unexplored.

Although all egg components are indispensable for embryonic development, they may not contribute equally to offspring quality; hence, birds may prioritize some over others. For example, the yolk contains all energy and other nutrients necessary for embryonic development (Ho et al., 2011; Liu et al., 2021; Ding et al., 2023), including fatty acids, cholesterol, vitamins, immunoglobulins, and maternally deposited hormones (Ricklefs, 1984; Darras, 2019; Hossain et al., 2024). However, this nutritional richness depends on the critical function of other components, such as the albumen, shell, and membranes (Willems et al., 2014; Starck, 2021; Gu et al., 2023). In addition to providing sterile embryonic fluid, albumen is the primary source of protein for tissue formation and growth in the developing embryo (Muramatsu et al., 1990; Willems et al., 2014). The eggshell also serves as a crucial barrier between the embryo and the external environment, providing physical protection and regulating gas exchange to support the successful development of embryos (Attard and Portugal, 2021; Gu et al., 2023). All these components may be influenced by nutritional availability, but not to the same extent. Preferential investment in one trait may be costly to the others, reflecting a resource allocation trade-off.

Recently, we reported that a key regulatory pathway, the mechanistic target of rapamycin (mTOR), mediates resource allocation into fitness traits, including body mass, egg production, and egg mass under different dietary conditions in Japanese Quails (Reda et al., 2024a, 2024b). Accordingly, severe restriction levels (30% and 40%) decreased body mass, egg production, and total egg mass. In contrast, moderate DR (20%) had no detectable impact on egg production, despite a reduction in body mass, suggesting a selective allocation of resources to reproduction (Reda et al., 2024b). Additionally, daily unpredictable feeding showed no effect on egg number or egg mass (Reda et al., unpublished data). The present study further investigates how different dietary regimens affect the relative proportions of different egg components.

We hypothesized that egg components compete for similar nutrients while being constrained by overall egg size and structural requirements. To test this, we conducted two trials, investigating how various dietary conditions affect the proportion of yolk mass, albumen mass, shell mass, shell thickness, and other physical properties in Japanese Quail eggs. First, we applied a gradient of constant dietary restrictions (20%, 30%, and 40% of the daily feed requirement for each individual) for two weeks. Second, we assessed the effects of temporal variation in feed availability by implementing an unpredictable feeding regimen, whereby birds received, on average, slightly above their feed requirement for two weeks, but in daily portions randomly fluctuating between 30% and 170% of their daily needs. We predicted that increasing dietary restriction and unpredictability would uniformly reduce all egg components. Alternatively, birds could prioritize investing in yolk at the expense of albumen and shell, representing a trade-off in resource allocation.

2. Materials and methods

The experiment was performed in accordance with the EU Directive "Legislation for the protection of animals used for scientific purposes" and after approval by the Ethical Committee for Animal Use of the University of Debrecen, Hungary (Protocol No. 5/2021/DEMAB).

2.1. Animals and housing

Experiments were conducted in the Animal House of the Institute of Animal Science, Biotechnology, and Nature Conservation at the University of Debrecen (Hungary). Adult (nine-week-old) female Japanese Quails were housed in an individual cage system (18.5 cm long × 21 cm wide × 18.5 cm high) in a condition-controlled room with a temperature of 25 ± 2 °C and relative humidity of 60–75%. This age of the birds represents a period shortly after the onset of full sexual maturity when birds show stable egg production while have not yet been exposed to age-related reproductive decline or stress associated with prolonged egg-laying (Huss et al., 2008; Ball and Balthazart, 2010). A standardized lighting regimen was implemented, operating on a 12:12 light-to-dark cycle, which was controlled using light timers. Lights were turned on each morning between 8:00 a.m. The feeders were immediately removed, and the remaining feed was measured and replenished with fresh feed. The basal diet for quails was formulated on a soybean-corn-wheat basis to meet the specific nutrient requirements of breeder quails according to the National Research Council (1994), as outlined in Table 1. Prior to the actual experiment, the birds underwent a one-week acclimation period to adapt to individual housing conditions.

Table 1
Composition and analyzed nutrient contents of the breeder basal diet.

Feed ingredients	Inclusion rate (%)
Soybean meal (46% CP)	34.88
Corn	30.37
Wheat	20.00
Sunflower oil	6.79
Limestone	5.64
MCP	1.29
Salt	0.38
DL-Methionine	0.15
Vitamin and mineral premix[a]	0.50
Analyzed nutrient content (%)	
Metabolizable energy	3327.58 kcal/kg (13.93 MJ/kg)
Crude protein	18.20
Lysine	1.12
Tryptophan	0.54
Phenylalanine	0.84
Methionine	0.28
Methionine + cysteine	0.75
Threonine	0.64
Leucine	1.40
Isoleucine	0.69
Valine	0.78
Arginine	1.33
Calcium	2.50 (estimated)
Available Phosphorus	0.35 (estimated)
Sodium	0.15 (estimated)

NE vitamin A, 2×10^5.

NE vitamin D_3, 4.9 g/kg vitamin E, 200 mg vitamin K_3, 150 mg vitamin B_1, 500 mg vitamin B_2, 1.2 g Ca-d-Pantothetane, 400 mg vitamin B_6, 2 mg vitamin B_{12}, 11 mg biotin, 2.502 g niacin, 60 mg folic acid, 300 g choline *chloride*, 13.2 g Zn, 1.92 g Cu, 9.612 g Fe, 13.2 g Mn, 180 mg I, 42 mg Se, 12 mg Co.

[a] 1 kg premix contained: 10^6.

During the acclimation period, quails were provided with ad libitum feed every morning (8:00 to 9:00), and their feed intake was recorded at 24-h intervals following the previous feeding. Food was provided using a 200 g capacity plastic feeder attached to the cage mesh. The feeder was designed to avoid spillage by allowing birds to insert their heads and feed without difficulty, while preventing them from scratching or spilling feed outside the feeder. Average daily feed intake was calculated from the daily measured feed intake, which was used to set the different dietary restrictions/unpredictable treatments (Table 2). In addition to monitoring feed intake, the initial and final live body mass of each bird were recorded during the acclimation phase using a digital balance (±0.1 g) to monitor any trend, but no directional change was observed.

2.2. *Experimental design*

Two consecutive trials were conducted using quantitative feed manipulation and unpredictable feeding, with the same housing and feed conditions applied. For the first trial, 32 adult quails with similar body mass (Table 2) were selected and randomly distributed into four treatment groups that consisted of 20% restriction (DR20, $n = 8$), 30% restriction (DR30, $n = 8$), 40% restriction (DR40, $n = 8$), and the control group ($n = 8$) received ad libitum feed. Feeding was administered individually, and the trial was conducted for 14 days, from July 21 to August 3, 2021. In the second trial, 24 adult birds with similar body masses (Table 2) were assigned into three treatment groups: the control ($n = 8$) received a daily amount of more than their average feed intake individually, the unpredictably fed birds (UNPR, $n = 8$) received merely more than total feed intake during the experimental period as the controls, but a randomly variable daily amount of feed between 30% and 170% of their respective daily feed intake, and the restricted group (DR40, $n = 8$) received a constant 60% of their respective feed intake. The trial was also conducted for 14 days, from November 02 to November 15, 2022 (Fig. 1). The 14-day treatment period was applied based on Japanese Quails' rapid reproductive cycle, where the critical phase of follicular development spans 3–4 days prior to ovulation. Therefore, dietary intervention can effectively affect ovarian development and egg components (yolk, albumen and shell), making the 14-day treatment period practical and biologically relevant for observing the effects of dietary conditions (Reda et al., 2024b).

Table 2
Average daily feed intakes (ADFI (g)) and average body mass (g) of female Japanese Quails during pre-treatment and the entire treatment period.

Treatment	Initial ADFI[a]	Trial ADFI[b]	Initial body mass	Final body mass
Control	30.70 ± 0.80	31.14 ± 0.90	283.78 ± 4.73	283.56 ± 4.85
DR20	29.70 ± 1.11	23.27 ± 0.76	279.69 ± 9.07	246.49 ± 7.70
DR30	30.08 ± 1.41	20.16 ± 1.55	280.22 ± 5.43	232.10 ± 5.65
DR40	29.28 ± 0.95	17.85 ± 0.54	281.14 ± 5.61	225.86 ± 6.08
UNPR	29.21 ± 0.66	27.83 ± 0.81	281.01 ± 12.18	254.36 ± 9.86

[a] Initial ADFI is the daily individual intake within seven days before starting the experiment, which is applied to calculate the restriction levels.

[b] Trial ADFI is the average intake from day one to day 14 during the feeding trial. Data are presented as means ± SE for the control ($n = 16$), DR20 ($n = 8$), DR30 ($n = 8$), DR40 ($n = 16$), and UNPR ($n = 8$) groups. Abbreviations: control, full fed; DR20, DR30, DR40, 20–40 % restriction, respectively, UNPR, unpredictable feeding. Values are in mean ± SEM.

2.3. *Feed analysis*

The nutrient composition of the experimental diets (Table 1) was analyzed by the Agricultural Laboratory Center at the University of Debrecen. Samples were finely ground to ≤1 mm particle size (≤0.5 mm for amino acid analysis) following the MSZ EN ISO 6498:2012 standard. Dry matter content was analyzed by oven drying at 103 °C for 30 min (MSZ ISO 6496:2001). Crude protein was measured using the Kjeldahl method (MSZ EN ISO 5983–2:2009), while crude fiber was analyzed via acid and alkaline digestion (MSZ EN ISO 6865:2001). Crude fat was extracted using the Randall method (MSZ EN ISO 11085:2015), and crude ash content was determined after incineration in a muffle furnace (MSZ EN ISO 5984:1992). Amino acid composition was assessed using acid hydrolysis, oxidation, and ion-exchange chromatographic analysis (MSZ EN ISO 13903:2005).

2.4. *Egg trait measurements*

Eggs were collected daily. Each egg was marked individually and immediately weighed using an analytical balance (±0.01 g). Egg width (along the maximum breadth) and length (along the longitudinal axis) were measured using a 200 mm digital caliper. The egg shape index was expressed as the percentage of egg width to length. Egg volume was measured by liquid displacement in a graduated cylinder. Eggs were carefully broken with the blunt end of a knife, and the eggshells were washed, air-dried, and weighed using an analytical balance (±0.01 g). The eggshell ratio was calculated as the percentage of eggshell mass to the egg mass. Eggshell thickness was measured at three regions (equator and both ends of the egg) using a digital caliper (±0.001 mm) (Das et al., 2010). The egg yolk and albumen were separated into a clean container, and the mass of both components was measured (±0.01 g). Eggshell, yolk, and albumen ratios, expressed in percentages in figures, were calculated as the ratios of the eggshell, yolk, and albumen masses to the whole egg mass, respectively. The egg yolk-to-albumen ratio was calculated by dividing the yolk mass by the albumen mass. Egg surface area (cm^2) was calculated as $(3.68 \times \text{width} - 0.62 \times \text{length}) \times \text{length}$, following the method specified in Narushin et al. (2022). Eggshell density was determined by dividing the eggshell mass (mg) by the egg surface area (cm^2) following Vits et al. (2005). Yolk color was determined using a DSM-Firmenich YolkFan™ (DSM-Firmenich, Switzerland) with a scale range from 1 to 16 units (1 = pale yellow to 16 = orange red).

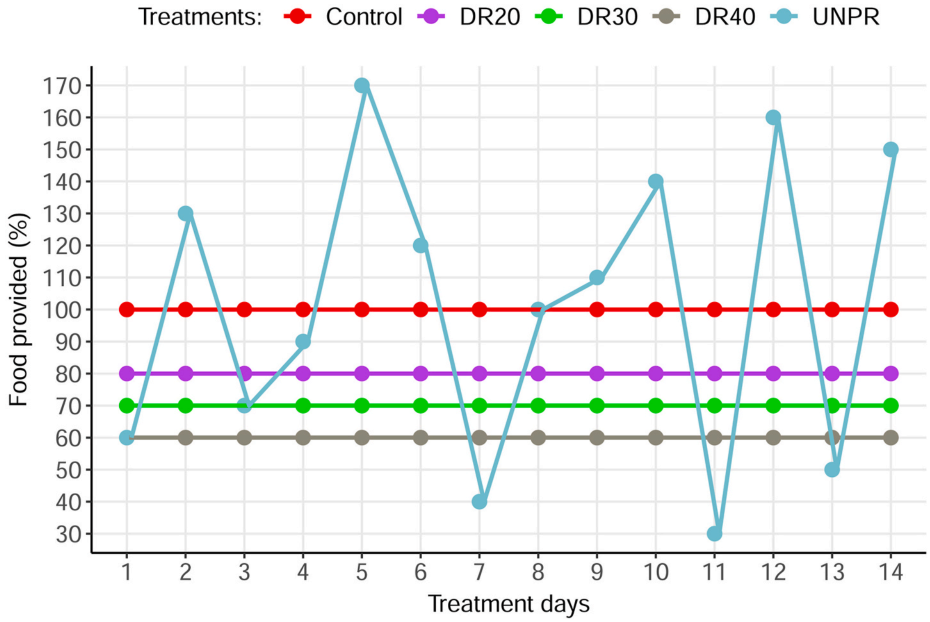

Fig. 1. Schematic representation of the experimental design showing feed given to each group in percentage of daily feed intake over the 14 days experimental period. Birds were either fully fed (control), subjected to a constant 20% (DR20), 30% (DR30) or 40% (DR40) dietary restriction or assigned to an unpredictable feeding regimen (UNPR), with daily intake fluctuating between 30% and 170% of individual daily need.

2.5. *Statistical analysis*

All data were analyzed using R v. 4.3.2 "Eye Holes" (https://www.r-project.org/). Mixed-effect models from various family distributions were employed to account for the repeated measures and the unbalanced nature of the data arising from varying sample sizes across treatments, differences in the number of observations per subject, and potential experimental variations. To analyze egg mass, yolk mass, albumen mass, shell masse, egg volume, length, width, egg shape index, egg surface area, and shell density, a polynomial mixed-effect model of the Gaussian family (Demidenko, 2013) was used to incorporate the polynomial nonlinear pattern of the variables across days as a random effect. The function lmer from the lme4 package v. 1.1.31 (Bates et al., 2015) was used to define fixed and random effects and estimate model parameters. Furthermore, to analyze the eggshell thickness and yolk-to-albumen ratio, Generalized Additive Mixed-Effects Models (GAMM) from the Gaussian family were employed, using the gamm function from the mgcv package v. 1.8.42 (Wood, 2017) to capture nonlinear patterns across treatment days. In this model, the smooth functions were included as part of the fixed effects, whereas the fluctuating components of the smooth function were treated as random effects. Generalized Linear Mixed-Effect Models (GLMM) from the beta family distribution were used to analyze shell ratio, albumen ratio, and yolk ratio and presented them as percentages in graphs to make a clear presentation of the proportions. The GLMM was fitted using glmmTBM function from the glmmTBM package v. 1.1.8 (Brooks et al., 2017) to account for the proportional nature of the response variables, which were constrained between 0 and 1 (ratio). In the saturated models of all variables, the treatment and treatment period were considered fixed effects, while individual bird identity and nonlinear components of the polynomial and additive models were treated as random effects. The Cumulative Link Mixed Model (CLMM) was employed to analyze the effect of treatment and treatment period on yolk color due to the ordinal nature of the data (score). The model was fitted using the clmm function from the ordinal package (Christensen, 2023). A logit link function was applied to model the ordinal response.

As the data were composed from two trials, 'trial' was added as an additional factor in the saturated models. During model fitting, the variable 'trial' did not significantly contribute to the models fitted to analyze yolk and albumen mass, albumen ratio, egg volume, length, width, egg shape index, and egg surface area. Therefore, it was excluded from the final models for these variables. Estimated marginal means for treatment groups were computed using the emmeans package (Lenth et al., 2018), where pairwise comparisons were adjusted for multiple testing using the Tukey method, with statistical significance set at $p <$ 0.05 (two-tailed). Bars on graphs represent means ± SEM. We used powerSim from the simr package to conduct post-hoc power analysis based on the actual data and the mixed-effect models used (Kumle et al., 2021). The power to detect a treatment effect over the 14-day treatment period for all traits is above 82%, indicating that the sample size across time points was adequate.

3. Results

3.1. *Mass of egg components*

Severe dietary restriction levels (DR30 and DR40) led to a decline in daily and overall egg mass compared to the control group (DR30: $p =$ 0.012, DR40: $p < 0.001$; Fig. 2A and B; Table 3). However, egg mass did not respond to the unpredictable feeding treatment ($p = 0.998$). Egg yolk mass varied across the experiment days, with the severely restricted groups showing reduction starting on day five (Fig. 2C; Table 3). However, the overall egg yolk mass showed no significant difference among the treatment groups (Fig. 2D; Table 3). Albumen mass changed over time in response to dietary treatment, with differences emerging between groups as the experiment progressed (Table 3). Specifically, quails in the DR30 and DR40 groups exhibited a decline in albumen mass starting from day five of the treatment period (Fig. 2E). Among them, DR40 birds had the lowest overall albumen mass compared to the control ($p = 0.002$), DR20 ($p = 0.011$), and UNPR ($p = 0.016$). In contrast, the DR20 and UNPR groups showed no significant change in albumen mass throughout the experiment (Fig. 2F).

Furthermore, dietary treatments affected eggshell mass over time (Table 3), with noticeable variation beginning on day four of the experiment (Fig. 2G). The severe restriction group (DR40) scored lower shell mass compared to all the other groups (control: $p < 0.001$, DR20: $p = 0.012$, UNPR: $p < 0.001$; Fig. 2H). The DR30 group showed a reducing but marginal effect on shell mass compared to the control and UNPR

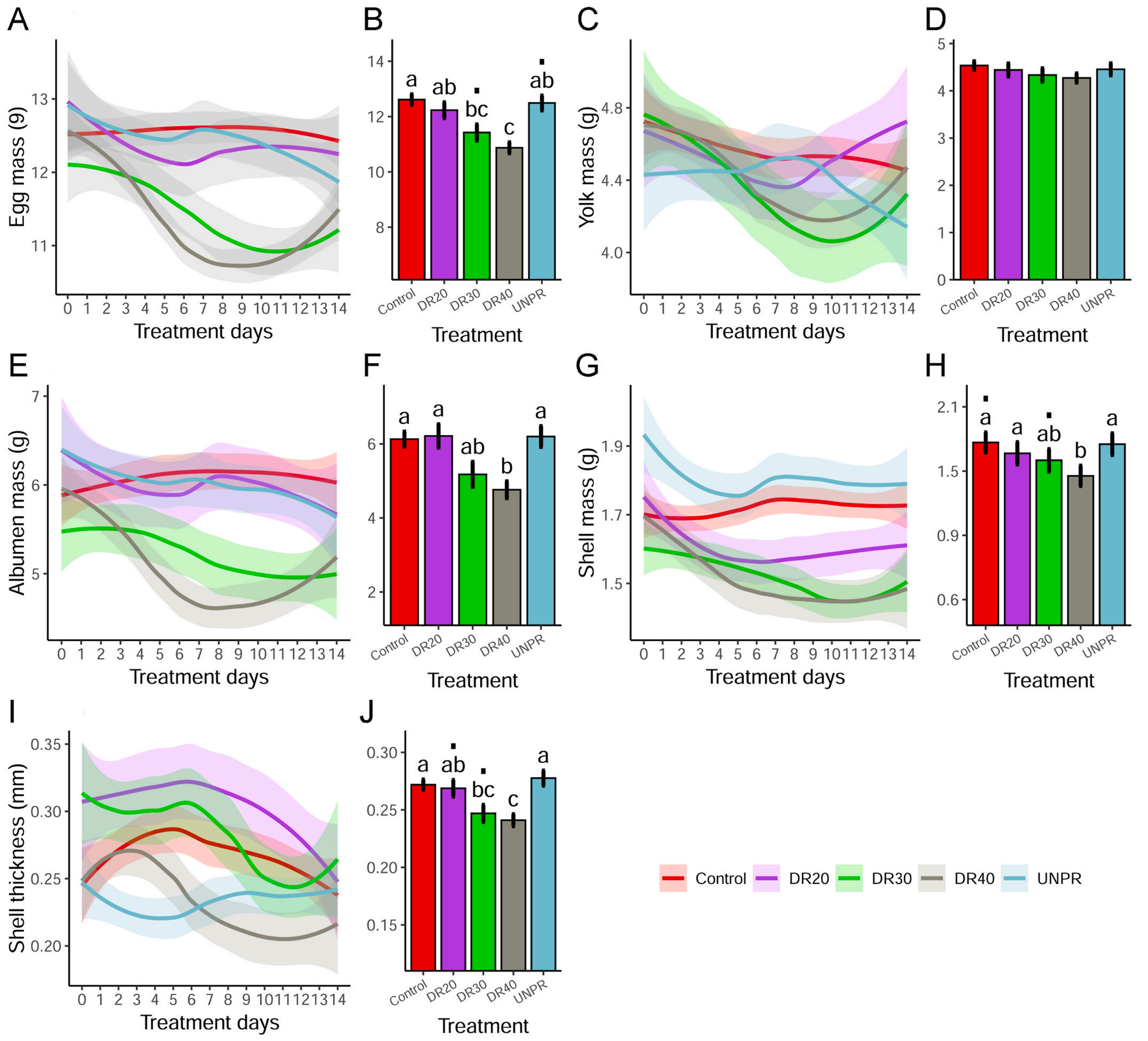

Fig. 2. Effect of dietary treatments on components of Japanese quail eggs across the 14 treatment days. (A) Daily egg mass, (B) average egg mass, (C) Daily yolk mass, (D) average yolk mass, (E) daily albumen mass, (F) average albumen mass, (G) daily shell mass, (H) average shell mass, (I) daily shell thickness, and (J) average shell thickness. Data are presented as means ± SE for the control ($n = 16$), DR20 ($n = 8$), DR30 ($n = 8$), DR40 ($n = 16$), and UNPR ($n = 8$) groups over a 14-day period. The treatment means shown in the bar graphs are adjusted marginal means derived from the mixed-effects models, rather than unadjusted raw averages. Tukey's test was applied to compare adjusted marginal means at $p < 0.05$ significance level. Means followed by a common letter are not significantly different at $p < 0.05$. Dots on the top of letters indicate $p < 0.1$ on mean comparison. The corresponding coloured shaded areas represent the 95% confidence intervals. Abbreviations as in Fig. 1. The explanations apply also to Figs. 3 and 4.

groups ($p < 0.1$). Similarly, eggshell thickness changed over time in response to the dietary treatment (Fig. 2I; Table 3). Shell thickness was lower in the DR40 and DR30 restriction levels compared to the control (DR30: $p = 0.014$, DR40: $p < 0.001$) and the UNPR (DR30: $p = 0.021$, DR40: $p < 0.001$) groups (Fig. 2J). Unpredictable feeding did not have a significant effect on any of the egg components (Fig. 2).

3.2. *Relative proportion of egg components*

The relative proportions of egg components changed in response to dietary treatments, with distinct patterns emerging over time (Fig. 3; Table 4). Yolk ratio increased under severe dietary restriction (Fig. 3A). On average, the DR40 group had a higher yolk ratio than the control ($p < 0.001$), DR20 ($p < 0.001$), and UNPR ($p = 0.045$) groups, while no significant differences were observed among the other groups (Fig. 3B). In contrast, the albumen ratio was significantly reduced in the severely restricted groups (Fig. 3C). The DR40 scored the lowest albumen ratio compared to the control ($p < 0.001$), DR20 ($p < 0.001$), and UNPR ($p < 0.001$) groups, while the DR30 group showed marginal reduction compared to the control group ($p = 0.099$, Fig. 3D). Shell ratio remained unchanged across all treatment groups (Fig. 3E and F). Dietary restriction also affected the yolk-to-albumen ratio (Table 4). The DR40 group exhibited the highest yolk-to-albumen ratios, significantly exceeding values in the control ($p < 0.001$), the DR20 ($p = 0.002$), and the UNPR ($p = 0.011$) groups, whereas no significant differences were observed among the other groups (Fig. 3G and H).

Table 3

ANOVA outputs derived from models indicating the effect of dietary treatment, treatment days, and their interaction on mass of egg components and shell thickness.

Dependent variable[a]	Term	NumDF	DenDF	*F*-value	*p*-value
Egg mass	treatment	4	48.06	6.44	<0.001
	poly (day, 14)	14	490.50	9.37	<0.001
	treatment × poly (day, 14)	56	490.36	3.27	<0.001
Yolk mass	treatment	4	43.94	0.67	0.615
	poly (day, 14)	14	488.18	3.05	<0.001
	treatment × poly (day, 14)	56	487.86	1.06	0.363
Albumen mass	treatment	4	48.31	6.68	<0.001
	poly (day, 14)	14	491.71	3.62	<0.001
	treatment × poly (day, 14)	56	491.49	2.04	<0.001
Shell mass	treatment	4	47.62	9.44	<0.001
	trial	2	557.61	18.71	<0.001
	poly (day, 2)	1	47.66	17.76	<0.001
	treatment × poly (day, 2)	8	557.21	5.66	<0.001

Dependent variable[a]	Term	df	*F*-value	*p*-value
Shell thickness	treatment	4	9.95	<0.001
	trial	1	174.03	<0.001
	s (day)	2.06	15.48	<0.001

[a] Fitted models: for yolk mass and albumen mass: lmer(~ treatment × poly (day, 14) + (1|birdID), family = Gaussian ()); for shell mass: lmer (~ treatment × poly (day, 2) + trial + (1|birdID), family = Gaussian ()); for shell thickness: gamm (~ s (day) + treatment + trial, random = list (birdID = ~1)). Abbreviations: poly (day, 14): linear term of the effect of day in polynomial mixed-effect model of degree 14; poly (day, 2): linear term of the quadratic effect of day; s (day): smoothed term of restriction period in the additive mixed-effect of day.

3.3. Egg length, width, surface area, shell density, shape index and yolk color

Dietary restriction had notable effects on egg volume ($p < 0.001$), length ($p = 0.003$), width ($p = 0.002$), and egg surface area ($p = 0.001$) (Table 5). Post-hoc analyses revealed that the severe restriction (DR40) induced significant reductions in egg volume ($p = 0.023$; Fig. 4A and B), length ($p = 0.001$; Fig. 4C and D), width ($p < 0.001$; Fig. 4E and F), and surface area ($p < 0.001$; Fig. 4I and J) compared to the full-fed (control) group. The DR30 resulted in lower egg length compared to the control group ($p = 0.035$; Fig. 1C and D). However, the DR30 group did not show a notable difference in the remaining traits compared to the control or the other treatment groups. None of the traits differed between the moderate restriction (DR20) and control group (Fig. 4).

The two-way ANOVA of the mixed model revealed a significant treatment effect on eggshell density ($p = 0.011$; Table 5). However, post-hoc comparisons did not identify significant pairwise differences between groups (all $p > 0.05$; Fig. 4K and I), suggesting that individual group means did not differ significantly after adjusting for multiple comparisons. Treatment did not affect the egg shape index (Fig. 4G and H; Table 5). While the yolk color score significantly increased during the experiment (becoming more orange over time) ($p < 0.001$; Fig. 4M), it was not affected by any of the treatments (Fig. 4N; Table 6). Unpredictable feeding had no effect on any egg traits (Fig. 4).

4. Discussion

Reproduction in female birds imposes a substantial nutritional demand. The size and composition of eggs not only determine the investment required for reproduction but also serve as key indicators of reproductive success (Anene et al., 2023; Zhang et al., 2023). Dietary scarcity, whether through consistent nutritional limitation and unpredictable fluctuations, can reduce both the quality and quantity of laid eggs. However, there is a knowledge gap regarding how these nutritional conditions affect egg components and the proportional allocation of resources among them. Therefore, our study focuses on elucidating the effect of nutritional availability on different egg components. To investigate this, we applied different levels of constant quantitative dietary restriction or temporal dietary variability. Our findings reveal three key results: (1) severe, but not moderate, dietary restriction affected albumen mass, shell mass, shell thickness, yolk ratio, albumen ratio, yolk to albumen ratio, egg volume, length, width, and surface area; (2) none of the treatments influenced yolk mass, shell ratio, egg shape index, shell density, and yolk color; (3) daily unpredictable feeding had no significant effect on any measured egg traits, despite the group consuming about 11% less feed than the control group in the two-week trials relative to their daily feed intake.

In the current study, severe dietary restriction levels altered the content of most egg traits, which may be mediated by hormonal and molecular mechanisms. According to the previous studies, dietary restriction, a simulation of natural nutritional deficiency in the lab, can affect birds' reproduction through multiple interconnected physiological and molecular pathways. One of the primary effects is suppression of the hypothalamic-pituitary-gonadal (HPG) axis, which plays a critical role in regulating avian reproductive function (Richard-Yris et al., 1987; Ottinger et al., 2005; Valle et al., 2015). For instance, studies on House Finches (*Haemorhous mexicanus*), laying Geese (*Anser cygnoides*), and Japanese Quail demonstrated that nutritional limitation impairs both hypothalamic and gonadal function, disrupting processes important for reproduction, such as sexual maturation, ovarian follicle development, and egg production (Ottinger et al., 2005; Valle et al., 2015; Liu et al., 2019). Deficient dietary condition further suppresses gonadotropin-releasing hormone (GnRH), leading to reduced release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Bruggeman et al., 1998; Ottinger et al., 2005; Davies et al., 2015), which in turn negatively affects reproductive performance (Prastiya et al., 2022).

In addition to neuroendocrine mechanisms, the nutrient-sensing pathway governed by insulin-like growth factor 1 (IGF-1)/mechanistic target of rapamycin (mTOR) axis also mediates the reproductive response to nutritional cues. Under restricted nutritional conditions, both hepatic and local IGF-1 secretion can be reduced, leading to downregulation of mTOR activity. Moreover, intracellular amino acid deficiency inhibits mTOR activation and its downstream effectors. This suppression further negatively affects egg production and quality (Tosca et al., 2008; Hertamawati et al., 2019; Hao et al., 2021; Reda et al., 2024b). Moreover, dietary restriction can impede hepatic synthesis of yolk constituents, including very low-density lipoproteins and vitellogenin, and onsite synthesis of albumen constituents (Walzem et al., 1999; Richards et al., 2003).

Egg mass has been significantly reduced under severe dietary restriction, whereas moderate restriction (DR20) and unpredictable feeding had no noticeable effect. The effects on egg mass are a reflection of changes in all egg components (Arnold and Green, 2007; Mytiai et al., 2021). While numerous studies have reported reduced egg size under nutritional deficiency (Olawuni et al., 1992; Reda et al., 2024b), the relative proportions of egg components remain largely unexplored. Albumen and yolk, which together account for more than 85% of the total egg mass, comprise nearly all of the egg's interior content and serve as key indicators of egg quality (Sun et al., 2019; Malfatti et al., 2021). Both components determine the quality of hatchlings (Finkler et al., 1998; Nelson et al., 2010). Our study showed that severely restricted feeding treatments significantly reduced albumen mass (Fig. 2B), whereas none of the restriction levels affected yolk mass (Fig. 2A). Consequently, the yolk ratio was notably higher in the DR40 group (Fig. 3A), indicating that yolk mass was less affected relative to the total egg mass (Reda et al., 2024b). However, we cannot rule out the possibility that yolk could be affected with increased restriction level and time.

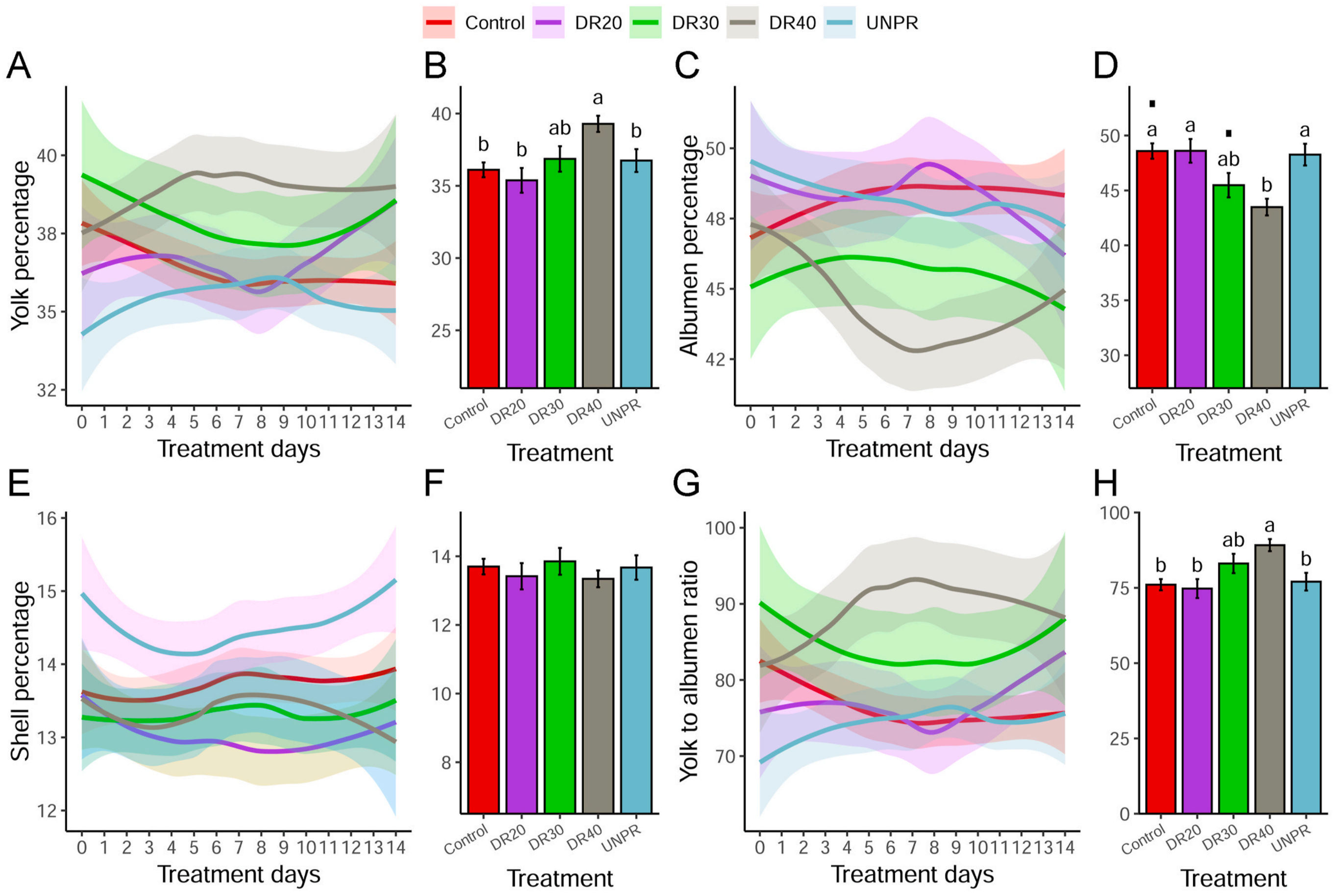

Fig. 3. Variations in proportions of egg components as affected by dietary treatments across 14 treatment days. (A) Daily yolk ratio, (B) average yolk ratio, (C) daily albumen ratio, (D) average albumen ratio, (E) daily shell ratio, (F) average shell ratio, (G) daily yolk-albumen ratio, and (H) average yolk-albumen ratio. Yolk, albumen, and shell ratios are calculated against the total egg mass and are presented as percentages for clarity of proportions.

Table 4
ANOVA outputs derived from models indicating the effect of dietary treatment, treatment days, and their interaction on ratios of egg components.

Dependent variable [a]	Term	df	χ^2	*p*-value
Yolk ratio	treatment	4	22.05	<0.001
	trial	1	11.07	<0.001
	poly (day, 2)	2	0.24	0.887
	treatment × poly (day, 2)	8	20.69	0.008
Albumen ratio	treatment	4	25.49	0.012
	poly (day, 2)	2	6.85	0.033
	treatment × poly (day, 2)	8	34.17	<0.001
Shell ratio	treatment	4	3.72	0.445
	trial	1	17.11	<0.001
	poly (day, 2)	2	4.47	0.107
	treatment × poly (day, 2)	8	8.62	0.374

Dependent variable	Term	df	*F*-value	*p*-value
Yolk to albumen ratio	treatment	4	6.84	<0.001
	trial	1	5.07	0.025
	s (day)	1	0.69	0.406

[a] Fitted models: yolk and shell ratio, glmmTMB (∼ treatment × poly (day, 2) + trial + (1|birdID), family = beta_family ()); albumen ratio, glmmTMB (∼ treatment × poly (day, 2) + (1|birdID), family = beta_family ()); yolk to albumen ratio, gamm (∼ s (day) + treatment + trial, random = list (birdID = ∼1).

In contrast, the albumen ratio was lower in the DR40 group (Fig. 3B), reflecting a disproportionate reduction relative to the total egg mass. As a result, the yolk-to-albumen ratio, which captures the relative importance of both components, was the highest in the DR40 group (Fig. 3D), confirming that the treatment affected albumen more than the yolk. The composition of these two egg components may have contributed to the difference, as the yolk primarily consists of water, fats, proteins, and other essential nutrients, whereas albumen is composed mainly of water and proteins (Hossain et al., 2024). These differences were also reflected in the dry matter content of both components in another study (Wu et al., 2007): albumen dry content significantly decreased with reduced nutrient density, whereas the dry content of the yolk showed no variation. Our results align with previous research: in chicken, albumen and yolk masses remained unchanged under dietary restriction up to 20% (Moreira et al., 2012), and albumen was found to be more sensitive to reductions in nutritional density than yolk (Wu et al., 2007).

The allocation of nutritionally and physiologically costly compounds between yolk and albumen is controlled by nutritional availability and reproductive strategy, which reflects an evolutionary trade-off (Chen et al., 2023; Javůrková and Mikšík, 2023). Under limited dietary availability, yolk and albumen may have competing demands for essential nutrients. Birds may prioritize nutrient allocation to the yolk over albumen under nutritional constraints, potentially compromising egg integrity and hydration, the primary function of albumen. Accordingly, maternal investment in yolk has been shown to determine the hatchling phenotype (Nelson et al., 2010; Javůrková and Mikšík, 2023).

Yolk is a primary source of major nutrients and a crucial egg component for offspring viability (Ho et al., 2011; Liu et al., 2021; Ding et al., 2023). Preferential yolk deposition may be crucial for ensuring successful embryonic development, viable hatchling and post-natal performance even under compromised other components. Offspring with enough supply of yolk contents receive the required energy,

Table 5
ANOVA outputs derived from models indicating the effect of dietary treatment, treatment days, and their interaction on egg size variables and eggshell density.

Dependent variable[a]	Term	NumDF	DenDF	F-value	p-value
Volume	treatment	4	47.65	5.03	0.002
	poly (day, 14)	14	493.34	2.95	<0.001
	treatment × poly (day, 14)	56	492.92	1.44	0.025
Length	treatment	4	48.13	4.10	0.006
	poly (day, 14)	14	491.06	6.71	<0.001
	treatment × poly (day, 14)	56	490.88	2.53	<0.001
Width	treatment	4	48.08	4.79	0.002
	poly (day, 14)	14	491.22	6.47	<0.001
	treatment × poly (day, 14)	56	491.02	2.36	<0.001
Egg shape index	treatment	4	47.83	1.48	0.223
	poly (day, 14)	14	469.56	1.22	0.258
	treatment × poly (day, 14)	53	469.37	1.12	0.273
Egg surface area	treatment	4	48.16	5.25	0.001
	poly (day, 14)	14	490.94	9.15	<0.001
	treatment × poly (day, 14)	56	490.77	3.01	<0.001
Eggshell density	treatment	4	48.32	3.65	0.011
	trial	1	48.12	18.48	<0.001
	poly (day, 2)	14	495.69	1.49	0.109
	treatment × poly (day, 14)	56	495.22	0.58	0.994

[a] Fitted models: for volume, length, width and surface area: lmer (∼ treatment × poly (day, 14) + (1|birdID), family = Gaussian ()); for shell density: lmer (∼ treatment × poly (day, 14) + trial + (1|birdID), family = Gaussian.

hormones, minerals, vitamins, and immunoglobulins that support embryonic development, post-natal growth and immunity function (Moran Jr, 2007; Liu et al., 2021). Therefore, under nutritional deficiency, birds may evolutionarily prioritize yolk deposition, including lipids and proteins, to sustain reproductive functions. This is achieved by reallocating available resources or mobilizing body reserves, reflecting an adaptive trade-off strategy (Deeley et al., 1975; Hughes, 2015). However, maintaining yolk mass under scarce nutritional conditions cannot be attained without a cost to other components. The birds' preferential investment in yolk may reduce the allocation of resources to albumen and other egg components.

On the other hand, the protein content of albumen is synthesized shortly in the oviduct, making it highly dependent on dietary amino acids and protein availability (Fisher, 1969; Moran Jr, 1987; Christians and Williams, 2003). Under limited resource conditions, not only the dietary nutrients, but also birds may preferentially allocate nutrients mobilized from body reserves toward yolk deposition rather than albumen, while albumen proteins are more immediate and dependent on circulating amino acids. Albumen provides sterile embryonic fluid and is the primary source of proteins for tissue formation and growth in the developing embryo (Muramatsu et al., 1990; Willems et al., 2014). It has been reported that partial removal of albumen had negative consequences on chick weight at hatch. Replacing the removed albumen with saline solution increased uptake of yolk, possibly as compensation for the removed albumen proteins (Muramatsu et al., 1990; Everaert et al., 2013; Willems et al., 2014). Therefore, a reduction in albumen mass due to maternal nutritional stress could limit the availability of essential nutrients and antibodies, compromising the embryo's osmotic balance and immunity, and potentially reducing offspring performance.

The lack of effect of unpredictable feeding on all egg components suggests that these traits may only be affected by sustained nutritional deficiency. Birds in the unpredictable feeding group consumed, on average 11% less feed than in the control group over the 14-day trial. This group received between 30% and 170% of their daily requirement. When provided less than their daily requirement, birds mostly consumed the entire feed. When offered more than their requirement, they consumed slightly above their daily needs and did not finish all the daily excess feed. As a result, their overall intake was lower than that of the control group, yet their reproductive performance remained unaffected. Our unpublished results showed that daily unpredictable feeding did not affect egg number or mass but significantly impacted the body mass of laying birds (Reda et al., unpublished data). Although stress due to unpredictability is expected to impair reproduction, birds can buffer short-term dietary fluctuation without compromising any egg traits. This may represent an adaptive strategy to cope with fluctuating environmental conditions, where food availability varies unpredictably. Under moderate nutritional constraints, birds preferentially invest resources into reproduction at the expense of body reserve or mobilize internal reserve (Li et al., 2011; Cardona et al., 2019). Such preferential allocation may underscore the species' resilience in the face of unpredictably changing conditions. However, it remains possible that a longer exposure duration or more severe unpredictability could alter these responses, highlighting a significant gap in the current literature that warrants further investigation.

Eggshell quality is another critical egg component affected by dietary conditions. The eggshell plays a crucial role in protecting the embryo and preventing the egg from infection, dehydration, and mechanical damage (Narushin and Romanov, 2002; Hincke et al., 2012; Ketta and Tůmová, 2018). Therefore, birds invest a significant portion of available resources, including minerals and organic nutrients, in eggshell formation (Nakano et al., 2003). In our study, shell mass and shell thickness decreased with the severity of restriction, but the unpredictably fed group remained comparable to the control group. However, the shell ratio remained unchanged, indicating that the reduction in shell traits was proportional to egg mass in response to the restriction treatments. Reduced shell thickness resulting from nutritional limitation affects the shell's ability to provide physical protection, regulate gas exchange and control water loss, influencing embryo development. During incubation, the embryo mobilizes minerals from the shell, which leads to eggshell thinning, resulting in faster water loss. This process is exacerbated due to severe dietary restriction, leading to excessive water loss, less protection, increased embryo vulnerability, mechanical damage and microbial invasion, and ultimately affects hatchability and hatchling quality (Castilla et al., 2010; Veldsman et al., 2020; Biesek, 2023).

Research on eggshell traits under dietary restriction has provided inconsistent results. Some studies report decreased shell thickness beyond 30% restriction (Mahrose et al., 2022), while others suggest no effect even at 40% restriction (Ali et al., 2007) or a surprising increase in shell traits with increasing levels of moderate DR (up to 30%) (Olawuni et al., 1992). One proposed explanation is that eggs retained longer in the uterus due to dietary limitation may accumulate more mineral deposition (Wilson et al., 2017; Chen et al., 2021). However, reduced nutrient intake can also lead to mineral reallocation toward somatic maintenance, potentially compromising eggshell integrity (Moreira et al., 2012). Our findings on eggshell density support the idea that birds maintain structural integrity even under restriction (Fig. 4). Maintaining shell density despite the reduced shell thickness at the severe restriction levels may be a compensatory mechanism to preserve the embryo from adverse effects such as a semi-permeability barrier for gas exchange and microbial invasion. Studies suggested that the protective capacity of an eggshell is not simply affected by its thickness but also by its shell density, the reflection of mineral content, which plays a crucial role in maintaining structural integrity and sustaining embryo viability and hatchability (Tatara et al., 2016; Ketta and Tůmová, 2018). Therefore, severe deficiency may push birds to either reduce eggshell density to conserve resources or invest more in shell density at the expense of body condition, possibly as an adaptive strategy to protect embryos. Future studies should explore how long-term dietary manipulations influence these trade-offs.

Egg shape index (width/length), the measure of sharpness and roundness, showed no variation across the dietary treatments (Fig. 4G

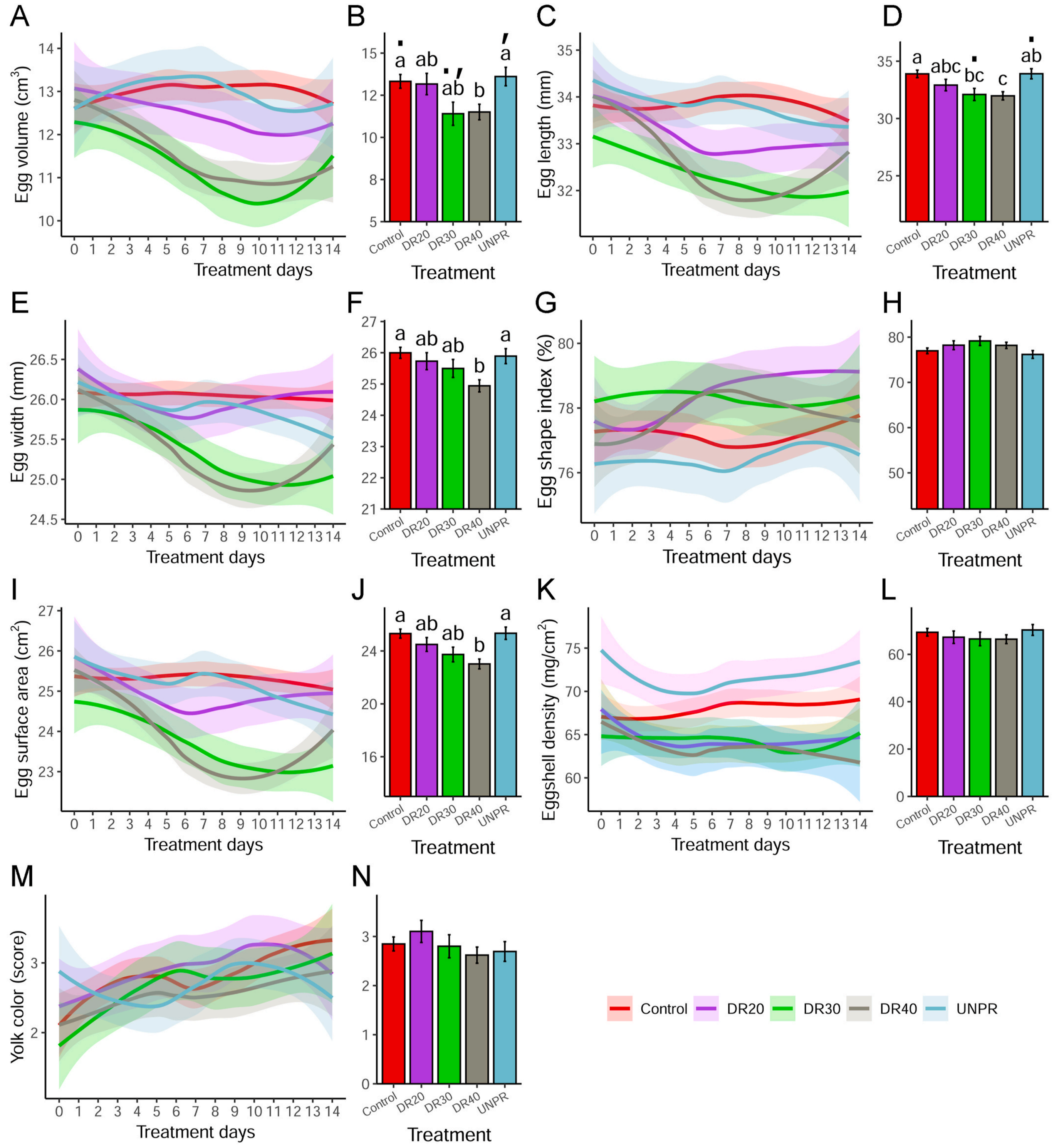

Fig. 4. Effect of dietary availability and variability on egg size traits across 14 days treatment period. (A–B) egg volume, (C–D) egg length, (E–F) egg width, (G–H) egg shape index, (I–J) egg surface area, (K–L) eggshell density, and (M–N) yolk color.

and H). Our finding aligns with previous findings in Japanese Quails (Mahrose et al., 2022), although some studies have reported an increase in shape index under dietary restriction (Ali et al., 2007). While the shape index in our study showed weak negative correlations with yolk, albumen, and shell mass, the biological significance of these associations remains unclear. Shape index may affect the mechanical resistance to external mechanical forces (Altuntaş and Şekeroğlu, 2008).

Furthermore, neither dietary restriction nor unpredictable feeding affected yolk color, suggesting that other factors are more influential (Hammershøj et al., 2010; Cahyadi et al., 2019; Franco et al., 2020). Yolk color is primarily determined by the dietary intake of carotenoids and the genetic background of the species (Biard et al., 2009). Maternally allocated carotenoids act as potent antioxidants that protect the developing embryo from oxidative damage and support the development of immunocompetence (Peluc et al., 2012). In our study, even the severe dietary restriction had no effect on yolk color, which, similar to

Table 6
Summary of the Cumulative Link Mixed Model (CLMM) on the effect of dietary treatments and treatment period on egg yolk color.

Factors[a]	Estimates	SE	χ^2	*p*-value
day	0.096	0.02	5.43	<0.001
DR20	0.534	0.41	1.28	0.199
DR30	−0.142	0.42	−0.34	0.737
DR40	−0.520	0.32	−1.65	0.099
UNPR	−0.645	0.39	−1.63	0.103

[a] Fitted model: clmm (~ treatment + day + trial + (1|birdID), link = "logit").

the pattern observed in yolk mass, may suggest that birds prioritize carotenoid deposition into yolk even under limited dietary availability. Alternatively, the level of carotenoids present in the restricted diets may have been sufficient to maintain the naturally pale coloration of quails' egg yolk. In our study, yolk color significantly increased over time in all treatment groups (Fig. 4M), reinforcing the importance of age over quantitative dietary availability. The average yolk color in our study (2.64, pale yellow) is consistent with previous reports in this species (Zita et al., 2013; Hrnčár et al., 2014; Ondrušíková et al., 2018). Even though the dietary carotenoid content is not far from poultry diet (National Research Council, 1994), the yolk color is significantly paler, suggesting a genetic role (Wen et al., 2022).

5. Conclusions

This study investigated how dietary restriction and unpredictable feeding differentially influence egg components in Japanese Quails. Our findings showed that while severe dietary restriction reduces albumen and shell traits, yolk mass remains stable, leading to an increased yolk proportion. This pattern indicates a potential adaptive strategy, where birds prioritize nutrient allocation towards yolk deposition to support embryo development under challenging nutritional conditions. This may be due to the yolk's relative importance over the other egg components for embryonic development. As the primary source of nutrients and hormones to the embryo, the yolk plays a critical role in determining offspring viability. The reduced albumen content in response to nutritional deficiency can also compromise embryonic viability, as it is a crucial source of protein and is essential for maintaining osmotic balance. The reduced shell mass and thickness in severe dietary restriction conditions can lead to excessive water loss, abnormal gas exchange, increased embryo vulnerability, and ultimately lower hatchling quality. In contrast, moderate dietary restriction (up to 20%) had no significant effect on any egg traits, suggesting that quails can tolerate some degree of restriction without compromising reproductive output. Furthermore, birds subjected to unpredictable feeding maintained egg traits, implying resilience to short-term fluctuations in feed availability as long as total intake is sufficient. These results provide important insights into how birds allocate resources under nutritional stress, reflecting trade-offs between structural investment and reproductive investment. Future research should explore the long-term effects of these feeding strategies and their potential impacts on offspring fitness and survival.

CRediT authorship contribution statement

Gebrehaweria K. Reda: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sawadi F. Ndunguru:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Renáta Knop:** Writing – review & editing, Methodology, Conceptualization. **James K. Lugata:** Writing – review & editing, Methodology, Conceptualization. **Brigitta Csernus:** Writing – review & editing, Methodology, Conceptualization. **Gabriella Gulyás:** Writing – review & editing, Methodology, Conceptualization. **Csaba Szabó:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Ádám Z. Lendvai:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Levente Czeglédi:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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