

Article

Comparative Assessment of Egg Quality Across Farming Systems and Stages of Laying Cycle

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Abstract

The aim of this study was the evaluation of egg quality between three different farming systems. Eggs collected from intensive (IS), extensive (ES), and dual-purpose systems (DPSs) at three stages of the production cycle (age) were analyzed for both external and internal traits. ISs represent high-input systems while ESs and DPSs represent low-input systems. Both the farming system and age had significant effects on quality characteristics. Eggs from the ES displayed a greater egg weight (64.3 ± 0.20 g) and shell weight (6.6 ± 0.03 g). Eggs from the IS farms displayed a higher Haugh unit score (83.2 ± 0.50), darker colored yolks (12.0 ± 0.06), and a lower incidence of blood spots (0.2 ± 0.05). The age and farming system influenced the fatty acid profile of eggs across farming systems. In particular, DPS eggs showed higher levels of unsaturated ($62.2 \pm 0.20\%$) and lower levels of saturated ($37.8 \pm 0.10\%$) fatty acids. The effect of age was also significant on the oxidation stability of yolks. The interaction effect (farming system $\times$ age) had significant effects on most traits. However, the farming system alone accounted for a greater portion of the variation in most egg quality parameters.

Keywords: production system; egg production; yolk; albumen; fatty acid profile; oxidation stability



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1. Introduction

Egg production is a fundamental component of the farming industry, playing a crucial role in the food supply chain [1]. Eggs are a rich source of high-quality protein and essential nutrients, making them indispensable in the human diet [2]. Maintaining a high egg quality is critical not only for consumer satisfaction and health but also for ensuring food safety, a longer shelf life, and marketability [3]. Therefore, the importance of maintaining the egg quality cannot be overstated. Key parameters such as shell strength, yolk color, and albumen consistency are widely recognized as indicators of the egg quality [4].

In addition to nutritional and technological aspects, consumer expectations have changed considerably in recent years. A growing demand for eggs produced under animal welfare-friendly and environmentally sustainable systems has emerged worldwide [5]. Conventional intensive systems, while efficient and capable of meeting large-scale demand, raise concerns about animal welfare and environmental impact [6]. As a response, alternative systems such as extensive and dual-purpose production have been developed [7]. Extensive systems typically provide outdoor access and lower-input conditions, while dual-purpose systems are designed to balance egg and meat production, reducing the need to cull one-day-old male chicks [7]. Despite their potential advantages, dual-purpose systems remain relatively understudied, particularly in terms of egg quality outcomes [8].

Egg quality is influenced by a complex interplay of factors that include genetics, nutrition, and flock management, as well as pre-laying and post-laying conditions [9,10]. For instance, the shell quality and shape can determine marketability and transport efficiency [11], while internal characteristics such as freshness, yolk pigmentation, and the presence of defects strongly affect consumer choice [12–16]. The nutritional composition, especially the fatty acid profile and ω -6/ ω -3 ratio, has also gained increasing attention due to its relevance for human health [17,18]. These diverse quality parameters are all affected, directly or indirectly, by the production system and the age of laying hens. Although numerous studies have explored the impact of the hen age and various production systems on the egg quality, systems like dual-purpose ones remain understudied due to their niche application.

Hence, this study aimed to evaluate the egg quality across three farming systems (intensive, extensive, dual-purpose) at three stages of the laying cycle (early, middle, late) with a focus on effect sizes to report the relative influence of each factor, as part of a broader effort to align poultry farming practices with evolving consumer expectations.

2. Materials and Methods

2.1. Farming Systems and Egg Samplings

A total of six commercial laying-hen farms were included in this study. Two farms operated under an intensive farming system (IS), two under an extensive system (ES), and the remaining two were characterized as dual-purpose systems (DPSs). ESs are characterized by smaller-scale poultry houses with decreased labor and basic infrastructure. In ESs, the number of housed hens was less than 10,000, with a stocking density of 2–4 birds per 9 square meters. In ISs, the number of hens reached 100,000 and the stocking density on average was 6–9 birds per square meter. In DPSs, a total of 500 birds were raised. The stocking density on average was 6 birds per square meter. Hens from the IS were housed exclusively indoors, whereas ES and DPS hens had outdoor access. Commercial layer breeds were used in ISs (Lohmann brown, Dekalb white) and ESs (Lohmann Brown, Isa Brown) while the Sasso silver dual-purpose breed was used in DPSs. Daily egg collection was carried out automatically in ISs and manually in ESs and DPSs. Feed in ISs was composed mainly of corn, wheat, palm oil, and soybean meal supplemented with vitamins, minerals, and some amino acids to meet the nutritional demands. Diet in ESs was based mainly on grazing, while for the DPSs, a combination of grazing and pellet feed was applied. The pellet was used as a supplement and consists of corn, wheat, oat, soybean flakes, minerals, and some vitamins.

Eggs were collected on laying day, placed on egg trays in insulated boxes, and shipped to the laboratory. Furthermore, the egg collection was performed according to the production stage, as is expressed from the age of flocks (3 sampling $\times$ 3 systems $\times$ 2 farms). The schedule of egg collection took place at 32, 61, and 92 weeks of age (early, middle, and late stages of production cycle). Those sampling activities were performed for each system and led to the collection of 100 eggs. In total, 1800 eggs were collected and analyzed for

their external and internal quality characteristics. Moreover, 50 yolks were also collected in the same manner for egg yolk fatty acid profile analysis, resulting in 900 yolk samples. Additionally, 30 yolks from each system were used for the oxidation stability analysis, on 0, 7, and 28 days of storage, resulting in 180 analyzed yolk samples.

2.2. Egg Quality Analysis

The internal and external quality traits of eggs were evaluated by taking specific measurements. External characteristics included egg weight (g) (EW), width and length (cm), egg shape index (ESI), shell weight (g) (SW), and shell thickness (mm) (ST). As for the internal traits, values for egg yolk color (YC), number of blood spots (BSs) in the yolk, albumen height (mm) (AH) and albumen weight (g) (AW), and Haugh units (HUs) were recorded.

Each egg was weighed, and then its length and width were measured with a Vernier caliper. Subsequently, the egg was opened to measure albumen height with a digital Haugh tester (ORKA Food Technology LLC, West Bountiful, UT, USA) and to visually evaluate yolk color using the DSM Yolk Fan (DSM-Firmenich, Kaiseraugst, Switzerland). Blood spots in the yolk were visually counted. HU scores and the ESI were calculated manually by the formula presented in Table 1 [19]. The ESI was measured by taking into account the values from egg length and width. Yolk and albumen were weighed, after their separation. The SW recorded after rinsing the eggshells to remove adhering albumen and leaving them for 24 h at room temperature. The ST measurement was also carried out using a Vernier caliper. All external and parts of the internal quality trait analyses were completed following the receipt of eggs in the laboratory on the same day.

Table 1. Equation for some examined quality traits.

Trait	Formula
HU	$100 \times \text{LOG}_{10} (\text{H} - 1.7 \times \text{W}^{0.37} + 7.6)$
ESI	$(\text{egg width} / \text{egg length}) \times 100$

HU = Haugh unit score; ESI = egg shape index %; H = height of albumen measured in millimeters; W = weight of egg in grams.

2.3. Fatty Acid Profile Analysis

Transesterification of yolk fatty acids was performed by direct methylation [20]. In detail, 0.04 g of yolk was added to a screw cap 15 mL glass tube, followed by the addition of 0.8 mL of methanol (99.9%) and 2.4 mL of methanolic-HCl. Subsequently, the tubes were incubated in a water bath at 95 °C for 1 h and were vortexed every 15 min. Then, 6.4 mL of deionized water containing 0.88% NaCl and 2.4 mL of hexane was added. Tubes were vortexed and centrifuged (1 min at 1500 g). The supernatant was collected and analyzed via gas chromatography using an Agilent chromatograph 6890N, equipped with a flame ionization detector and a capillary column DB-23, 60 m–0.25 mm i.d., 0.25 µm film thickness (Agilent Technologies, Santa Clara, CA, USA). Fatty acids were quantified as the percentage of the displayed fatty acid methyl esters.

2.4. Oxidation Stability Analysis (TBARS Assay)

Yolk oxidation stability analysis was implemented according to the method of Vyncke [21]. Ten yolks were used for each day of analysis (days 0, 7, and 28 to simulate typical shelf storage conditions). Five grams of egg yolk (5 g) were weighed and transferred to a 100 mL beaker. Additionally, 25 mL of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% propyl gallate, 0.1% EDTA) was added, and the mix was left at room temperature for 25 min and then filtered through a fiber filter (Ahlstrom-Munksjö, Tampere, Finland). An aliquot of 5 mL of the clear filtrate was transferred to a 15 mL Pyrex glass

test tube with a Teflon-lined screw cap. Subsequently, 5 mL of thiobarbituric acid (TBA) was added, and the tubes were left in the dark overnight. The absorbance was measured at 532 nm using a UV-VIS double beam spectrophotometer (Halo DB-20S, Dynamica Scientific, Ltd., Livingston, UK). To calculate the value of oxidation, a standard curve was prepared with 1,1,3,3 tetramethoxypropane (TEP), and the result was expressed as mg of malondialdehyde equivalents per kg (MDA), which is the product of oxidation reactions.

2.5. Statistical Analysis

Data for all examined traits, except the trait blood spots (BSs), were analyzed within the methodological frame of mixed linear models with the analysis of variance (ANOVA) method [22]. All models involved the fixed effects (main and interaction) of the factors “farming system” (FS: IS, ES, DPS) and “age” of the flock (age: early, middle, late). To account for the variability introduced by the individual farms, factor “farm” with 6 levels was included in the corresponding models as a random effects factor, nested within the three FSs. Specifically, the general form of the models was

$$Y = FS + \text{Farm}(\text{FS}) + \text{Age} + \text{FS} \times \text{Age} + \text{error},$$

where Y corresponds to the examined traits (except BSs).

Regarding the analysis of oxidation stability (MDA concentration), the same model as previous was applied including an additional fixed effect (main and interactions) of the factor “storage time” (S: 0, 7, and 28 days). Specifically, the general form of this model was

$$Y = FS + \text{Farm}(\text{FS}) + \text{Age} + S + \text{FS} \times \text{Age} + \text{FS} \times S + \text{Age} \times S + \text{Age} \times \text{FS} \times S + \text{error},$$

where Y corresponds to the MDA concentration.

The least significant difference (LSD) criterion was used for testing the differences among treatments’ mean values. In all cases, the normality and the homoscedasticity of the models’ residuals were checked, and no serious violations were detected. Due to the high skewness of the data of BSs, this variable was deemed unsuitable for analysis using parametric tests such as ANOVA and LSD (various attempts to transform the raw data were unsuccessful, since the normality and the homoscedasticity of the model’s residuals were not satisfied). To evaluate the effects of FS and age groups, the non-parametric Kruskal-Wallis (K-W) test was employed. For post hoc pairwise distribution comparisons, the Mann-Whitney (M-W) U test was used to identify specific group differences. A spearman correlation was assessed further to investigate the relationship of FS and age in affecting BS. In all non-parametric tests, the observed significance level (*p*-value) was computed with the Monte Carlo simulation method utilizing 10,000 resampling circles [23]. With this method, the inferential conclusions are safe and valid even in cases where the methodological assumptions of the tests are not satisfied (random samples, independent measurements, symmetrical distributions, and absence of “heavy” outliers). Given the large sample size, statistical significance alone might not adequately reflect the practical biological importance of the findings [24]. Therefore, effect size indices (i.e., partial eta squared index for ANOVA models [25]) were reported along with *p*-values to offer a more meaningful interpretation of the results. According to the American Statistical Association (ASA) statement on *p*-values, the assessment of the effect sizes is more important than the statistical significance (*p*-values).

In all hypothesis testing procedures, the significance level was predetermined at $\alpha = 0.05$ ($p \leq 0.05$). All statistical analyses were carried out with SPSS IBM Statistics software (v.27) enhanced with the module Exact Tests (for the implementation of Monte

Carlo simulation). One of the authors (Georgios Menexes) developed special SPSS syntax code in order to analyze the data.

3. Results

3.1. Egg Quality Traits Analysis

The effects of the FS and age on external egg quality traits were significant for all traits studied (Table 2). Eggs from ESs displayed a higher EW (64.3 ± 0.20 g). The evaluation of the ESI shows that eggs produced under intensive conditions were slightly rounder ($77.4 \pm 0.20\%$) in comparison to other systems. Eggshells from the ES were heavier (6.6 ± 0.04 g). The SW had a proportional, small increase as age increased, reaching 6.6 ± 0.03 g at the late stage of production, while ST reduced with the advancement of age (0.37 ± 0.01 mm). The interaction effect (FS $\times$ age) was also significant (Table S1). EW and SW increased notably for the DPS during the middle and late stages. The ESI fluctuated with the progress of age in the ES and DPS while it showed a constant increase in the IS. In addition, ST had a more stable decrease also in the IS. Effect size indices showed that the FS had a stronger influence than age, especially for shell thickness ($\eta^2 = 0.620$) and the egg shape index ($\eta^2 = 0.386$), while age mainly affected the egg weight ($\eta^2 = 0.359$) and shell traits. Although FS $\times$ age interactions were significant, they contributed less to variation, indicating that the FS and age had a greater influence on these traits.

Table 2. Effect of FS and age on external quality traits.

Trait	FS			Age			Significance			Effect Size Index		
	IS	ES	DPS	Early	Middle	Late	FS	Age	FS $\times$ Age	η^2 (FS)	η^2 (Age)	η^2 (FS $\times$ Age)
EW (g)	60.1 ± 0.20^a	64.3 ± 0.20^b	$61.0 \pm 3.1.0.20^c$	57.4 ± 0.20^a	63.3 ± 0.20^b	63.6 ± 0.20^b	**	**	**	0.384	0.359	0.083
ESI (%)	77.4 ± 0.20^b	76.4 ± 0.20^a	76.5 ± 0.20^a	77.6 ± 0.20^a	75.9 ± 0.20^b	76.6 ± 0.20^c	**	**	**	0.386	0.032	0.060
SW (g)	6.2 ± 0.03^b	6.6 ± 0.04^a	6.3 ± 0.03^b	6.1 ± 0.03^a	6.4 ± 0.03^b	6.6 ± 0.03^c	**	**	**	0.373	0.108	0.076
ST (mm)	0.44 ± 0.01^a	0.43 ± 0.01^a	0.37 ± 0.01^b	0.46 ± 0.01^a	0.41 ± 0.01^b	0.37 ± 0.01^c	**	**	**	0.620	0.118	0.041

Mean $\pm$ standard error for external quality characteristics of the examined eggs. Different superscripts (a–c) indicate statistically significant differences at $\alpha = 0.05$ ($p \leq 0.05$) among groups of FS or among age classes, according to the LSD criterion. **: $p < 0.05$; FS = farming system; IS = intensive system; ES = extensive system; DPS = dual-purpose system; EW = egg weight; ESI = egg shape index; SW = shell weight; ST = shell thickness; FS $\times$ Age = interaction effect; η^2 = partial eta squared (effect size index).

The farming system (FS) and age also exerted marked effects on internal egg quality traits. Table 3 presents the impact of the FS, age, and their combination on the internal quality of eggs. Eggs from ISs had a significantly higher mean AH (7.2 ± 0.06 mm) and subsequently higher HU scores (82.3 ± 0.50). More intense YCs (12.0 ± 0.06) were also observed for the IS. Heavier yolks were obtained from eggs originating from the DPS (17.7 ± 0.10 g), while greater AWs were measured for ESs (36.9 ± 0.20 g). As age progressed, yolk and albumen weights increased. The effect size indices (η^2) highlighted the FS as the dominant factor for YC ($\eta^2 = 0.923$), YW ($\eta^2 = 0.639$), AW ($\eta^2 = 0.628$), and HU ($\eta^2 = 0.603$), while age showed stronger effects on the YW ($\eta^2 = 0.408$) and HU ($\eta^2 = 0.292$). Regarding the significance of the interaction effect (Table S2), the AH and HU in the ES and DPS exhibited a greater reduction from the early to late stages of the production cycle, while a smaller reduction rate was present in the IS. Also, in the IS, the means of YC showed small variations within age groups in comparison to the ES and DPS, where a great variation was seen in all ages. The YW in ISs had a small increase from early to middle and a greater increase from middle to late stages, whereas the reverse was observed in the ES and DPS. Despite significant FS $\times$ age interactions, their contribution to the total variation was small, emphasizing that the FS and age acted mainly as independent sources of variation.

Table 3. Effect of FS and age on internal quality traits.

Trait	FS			Age			Significance			Effect Size Index		
	IS	ES	DPS	Early	Middle	Late	FS	Age	FS × Age	η^2 (FS)	η^2 (Age)	η^2 (FS × Age)
AH (mm)	7.2 ± 0.06 ^a	6.5 ± 0.06 ^b	5.6 ± 0.05 ^c	7.5 ± 0.06 ^a	6.1 ± 0.06 ^b	5.7 ± 0.06 ^c	**	**	**	0.539	0.261	0.054
HU (score)	83.2 ± 0.50 ^a	76.7 ± 0.50 ^b	71.8 ± 0.43 ^c	86.5 ± 0.50 ^a	74.4 ± 0.50 ^b	70.8 ± 0.50 ^c	**	**	**	0.603	0.292	0.074
YC (score)	12.0 ± 0.06 ^a	8.9 ± 0.06 ^b	6.6 ± 0.05 ^c	8.1 ± 0.05 ^a	10.1 ± 0.06 ^b	9.4 ± 0.06 ^c	**	**	**	0.923	0.335	0.267
YW (g)	16.5 ± 0.10 ^a	16.3 ± 0.10 ^a	17.7 ± 0.10 ^b	14.9 ± 0.10 ^a	17.4 ± 0.10 ^b	18.2 ± 0.10 ^c	**	**	**	0.639	0.408	0.094
AW (g)	35.4 ± 0.20 ^a	36.9 ± 0.20 ^b	33.5 ± 0.20 ^c	33.8 ± 0.20 ^a	35.6 ± 0.20 ^b	36.5 ± 0.20 ^c	**	**	**	0.628	0.087	0.033

Mean ± standard error for internal quality characteristics of the examined eggs. Different superscripts (a–c) indicate statistically significant differences at $\alpha = 0.05$ ($p \leq 0.05$) among groups of FS or among age classes, according to the LSD criterion. **: $p < 0.05$. FS = farming system; IS = intensive system; ES = extensive system; DPS = dual-purpose system; AH = albumen height; HU = Haugh unit score; YC = yolk color; YW = yolk weight; AW = albumen weight; FS × Age = interaction effect; η^2 = partial eta squared (effect size index).

The incidence of BSs in egg yolks differed significantly among FSs (Table 4). Eggs from the ES (0.6 ± 0.07) showed a higher frequency of BSs compared with both the IS (0.2 ± 0.05) and DPS (0.5 ± 0.05). Pairwise comparisons confirmed significant differences between the IS and both the ES and DPS ($p < 0.001$), while no significant difference was found between the ES and DPS ($p = 0.966$). No significant differences were observed among age groups; however, the interaction effect was significant (Table S3), showing greater variations for the DPS and ES across all age groups.

Table 4. Effect of FS on BS incidence in egg yolk.

Pairwise Comparison (FS)			M-W p -Value
IS-ES	0.2 ± 0.05 ^a	0.6 ± 0.07 ^b	<0.001
IS-DPS	0.2 ± 0.05 ^a	0.5 ± 0.05 ^b	<0.001
ES-DPS	0.6 ± 0.07 ^a	0.5 ± 0.05 ^a	0.966

Blood spot incidence (mean ± standard error). Different superscripts (a,b) indicate statistically significant differences at $\alpha = 0.05$ ($p \leq 0.05$) among groups of FS or among age classes, according to the LSD criterion. FS = farming system; M-W = Mann-Whitney test; IS = intensive system; ES = extensive system; DPS = dual-purpose system.

The correlation analysis (Table 5) further revealed that age was only weakly related to the BS incidence within systems. In the ES, the correlation was slightly negative ($\rho = -0.065$), suggesting no meaningful change in BS frequency with increasing age. In the IS, the relationship was negligible ($\rho = 0.026$), while in the DPS alone, the correlation was significant and weakly positive ($\rho = 0.110$), pointing to a slight tendency for the BS incidence to rise with age, though the effect was minor.

Table 5. Spearman correlation coefficients (ρ) between age and blood spot (BS) incidence within each farming system.

FS	ρ (Spearman)	p -Value
ES	−0.065	0.171
IS	0.026	0.563
DPS	0.110	0.007

FS = farming system; IS = intensive system; ES = extensive system; DPS = dual-purpose system.

3.2. Fatty Acid Profile

The egg yolk FA composition as individual fatty acids and as different lipid classes is presented in Tables 6 and 7, respectively. The FS and age significantly influenced the FA composition of egg yolks (Table 6). Specifically, oleic acid (C18:1) and palmitic acid

(C16:0) were the predominant FAs, followed by linoleic acid (C18:2) and stearic acid (C18:0). With respect to differences among systems, IS eggs contained higher levels of C16:0 ($24.2 \pm 0.07\%$), C18:0 ($14.0 \pm 0.10\%$), and C24:0 ($1.4 \pm 0.02\%$), while in contrast, ES eggs were richer in C16:1 ($1.85 \pm 0.03\%$) and C18:2 ($16.2 \pm 0.14\%$). Furthermore, DPS eggs exhibited elevated concentrations of C18:1 ($38.3 \pm 0.13\%$), α -LA ($1.2 \pm 0.02\%$), and DHA ($2.5 \pm 0.03\%$). In terms of age-related trends, higher proportions of C14:0, C18:2, and DHA were observed at the early stage of production ($3.1 \pm 0.06\%$, $16.8 \pm 0.13\%$, and $2.2 \pm 0.03\%$, respectively), whereas C16:0, C16:1, and C18:1 increased towards the late stage. The interaction effects of FS $\times$ age were significant but moderate (Table S4); for instance, specific variations were observed, such as higher C16:1 in the ES at later stages and persistently higher α -LA and DHA in the DPS across all ages. Finally, effect size indices identified the FS as the dominant factor for traits such as C18:2 ($\eta^2 = 0.772$), DHA ($\eta^2 = 0.632$), and C18:1 ($\eta^2 = 0.576$), while age mainly influenced C14:0 ($\eta^2 = 0.320$).

Table 6. Fatty acids (%) in yolk and the effects of age, FS, and their interaction.

FS				Age			Significance			Effect Size Index		
FA	IS	ES	DPS	Early	Middle	Late	FS	Age	FS $\times$ Age	η^2 (FS)	η^2 (Age)	η^2 (FS $\times$ Age)
C14:0	2.1 ± 0.06^a	2.5 ± 0.06^b	1.7 ± 0.06^c	3.1 ± 0.06^a	1.8 ± 0.06^b	1.5 ± 0.06^c	**	**	**	0.544	0.320	0.176
C16:0	24.2 ± 0.07^a	23.2 ± 0.07^a	22.8 ± 0.07^b	23.2 ± 0.07^a	23.1 ± 0.07^a	23.9 ± 0.07^b	**	**	**	0.458	0.073	0.096
C16:1	1.77 ± 0.03^a	1.85 ± 0.03^a	1.74 ± 0.03^c	1.77 ± 0.03^a	1.67 ± 0.03^b	1.92 ± 0.03^c	**	**	**	0.038	0.050	0.054
C18:0	14.0 ± 0.10^b	13.7 ± 0.10^a	12.7 ± 0.10^c	13.1 ± 0.10^a	14.0 ± 0.10^b	13.2 ± 0.10^c	**	**	**	0.272	0.060	0.056
C18:1	34.8 ± 0.14^a	35.1 ± 0.14^a	38.3 ± 0.13^b	34.6 ± 0.13^a	36.1 ± 0.14^b	37.4 ± 0.14^c	**	**	**	0.576	0.223	0.222
C18:2	15.7 ± 0.14^a	16.2 ± 0.14^b	15.1 ± 0.13^c	16.8 ± 0.13^a	15.4 ± 0.14^b	14.9 ± 0.13^c	**	**	**	0.772	0.117	0.174
α -LA	0.7 ± 0.05^a	0.7 ± 0.03^a	1.2 ± 0.02^b	0.9 ± 0.03^a	0.9 ± 0.03^a	0.7 ± 0.03^b	**	**	**	0.440	0.070	0.137
C20:4	3.9 ± 0.02^a	4.1 ± 0.03^b	3.4 ± 0.03^c	3.7 ± 0.03^a	3.9 ± 0.03^b	3.7 ± 0.03^a	**	**	**	0.468	0.011	0.087
DHA	1.6 ± 0.03^a	2.1 ± 0.03^b	2.5 ± 0.03^c	2.2 ± 0.03^a	2.1 ± 0.03^b	1.8 ± 0.03^c	**	**	**	0.632	0.109	0.074
C24:0	1.4 ± 0.02^a	1.3 ± 0.03^a	0.84 ± 0.03^b	1.2 ± 0.03^a	1.3 ± 0.03^b	1.1 ± 0.02^c	**	**	**	0.394	0.075	0.136

Fatty acids (%) of yolks (mean $\pm$ standard error) and the contents of polyunsaturated, monounsaturated, total saturated, and total unsaturated (%) fatty acids. Different superscripts (a–c) indicate statistically significant differences at $\alpha = 0.05$ ($p \leq 0.05$) among groups of FS or among age classes, according to the LSD criterion. **: $p < 0.05$. FA = fatty acid; FS = farming system; IS = intensive system; ES = extensive system; DPS = dual-purpose system; FS $\times$ Age = interaction effect, η^2 = partial eta squared (effect size index); C14:0 = myristic; C16:0 = palmitic; C16:1 = palmitoleic; C18:0 = stearic; C18:1 = oleic; C18:2 = linoleic; α -LA = alpha linolenic; C20:4 = arachidonic; C24:0 = lignoceric; DHA = docosahexaenoic acid.

Table 7. Effects of FS and age on yolk lipid classes (% of total identified fatty acids).

FS				Age			Significance			Effect Size Index		
FA	IS	ES	DPS	Early	Middle	Late	FS	Age	FS $\times$ Age	η^2 (FS)	η^2 (Age)	η^2 (FS $\times$ Age)
MUFA	36.6 ± 0.20^a	36.9 ± 0.20^a	40.0 ± 0.20^b	36.4 ± 0.20^a	37.8 ± 0.20^b	39.3 ± 0.20^c	**	NS	**	0.584	0.186	0.152
PUFA	22.0 ± 0.20^b	22.7 ± 0.20^a	22.2 ± 0.20^b	23.4 ± 0.20^a	22.3 ± 0.20^b	21.2 ± 0.20^c	**	**	**	0.441	0.104	0.177
UFA	58.6 ± 0.20^a	59.6 ± 0.20^b	62.2 ± 0.20^c	59.8 ± 0.20^a	60.0 ± 0.20^a	60.5 ± 0.20^b	**	**	**	0.644	0.021	0.109
SFA	41.4 ± 0.10^a	40.4 ± 0.10^a	37.8 ± 0.10^b	40.2 ± 0.10^a	39.9 ± 0.10^a	39.5 ± 0.10^c	**	**	**	0.153	0.030	0.146
ω -3	1.9 ± 0.07^a	2.8 ± 0.07^b	3.5 ± 0.06^c	2.9 ± 0.06^a	2.9 ± 0.07^a	2.4 ± 0.07^b	**	**	**	0.561	0.039	0.058
ω -6	19.3 ± 0.30^a	19.4 ± 0.30^a	17.3 ± 0.30^b	19.7 ± 0.30^a	18.1 ± 0.30^b	18.2 ± 0.30^b	**	**	**	0.810	0.025	0.072
ω -6/ ω -3	12.2 ± 0.20^a	7.6 ± 0.20^b	5.5 ± 0.20^c	8.5 ± 0.20^a	7.8 ± 0.20^b	8.8 ± 0.20^a	**	**	NS	0.573	0.017	0.009
AI	0.57 ± 0.01^a	0.56 ± 0.01^a	0.48 ± 0.01^b	0.60 ± 0.01^a	0.51 ± 0.01^b	0.49 ± 0.01^c	**	**	**	0.586	0.293	0.212
TI	1.2 ± 0.01^a	1.1 ± 0.01^b	0.9 ± 0.01^c	1.01 ± 0.01^a	1.01 ± 0.01^a	1.01 ± 0.01^a	**	NS	**	0.676	0.03	0.227

Fatty acid composition (%) of yolks expressed as mean $\pm$ standard error. Different superscript letters (a–c) indicate significant differences at $\alpha = 0.05$ ($p \leq 0.05$) among groups of FS or among age classes, according to the LSD criterion; **: $p < 0.05$. FA = fatty acid; FS = farming system; IS = intensive system; ES = extensive system; DPS = dual-purpose system; FS $\times$ Age = interaction effect; η^2 = partial eta squared (effect size index); MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = total saturated fatty acid; UFA = total unsaturated fatty acid; ω -3 = omega 3 fatty acids; ω -6 = omega 6 fatty acids; AI = atherogenicity index; TI = thrombogenicity index, NS = not significant.

The lipid classes (Table 7) provided additional insights into the effects of the FS, age, and their interaction. Significant differences were observed between FSs for most lipid classes. Eggs from the DPS had the highest MUFA ($40.0 \pm 0.20\%$, $\eta^2 = 0.584$) and ω -3

contents ($3.5 \pm 0.06\%$, $\eta^2 = 0.561$), resulting in a more favorable ω -6/ ω -3 ratio (5.5 ± 0.20 , $\eta^2 = 0.573$) and lower AI (0.48 ± 0.01 , $\eta^2 = 0.586$) and TI (0.9 ± 0.01 , $\eta^2 = 0.676$) values. In contrast, eggs from the IS contained higher SFA ($41.4 \pm 0.10\%$, $\eta^2 = 0.153$) and a greater ω -6/ ω -3 ratio ($12.2 \pm 0.20\%$), while those from the ES had the highest PUFA content ($22.7 \pm 0.20\%$, $\eta^2 = 0.441$) and intermediate values for other indices. Age effects were evident: MUFA and UFA increased in the late stages, whereas PUFA, SFA, ω -6, and AI declined.

The FS $\times$ age interaction was also significant, reflecting specific variations across systems and stages (Table S5). MUFA and UFA remained consistently higher in the DPS across all ages, while AI and TI were lowest for the DPS in every age group. PUFA, although highest in ESs' early stage of production, decreased in late stages, with the IS exhibiting the greatest PUFA content later. The SFA content was higher in the IS and DPS during early stages, but IS eggs showed the highest SFA in the late stage. Eggs from the ES and DPS maintained a higher ω -3 across all ages, while ω -6 levels were consistently higher in the IS and ES. Effect size indices confirmed the FS as the dominant factor affecting PUFA, MUFA, UFA, ω -3, ω -6, and ω -6/ ω -3 ($\eta^2 = 0.441$ – 0.810), whereas age had a smaller influence, and the interaction contributed notably to variation in PUFA, SFA, ω -3, ω -6, AI, and TI.

3.3. Oxidation Stability Analysis

Yolk oxidation stability results are presented in Table 8 (TBARS assay). The mean content of the formed by-product of the reaction, MDA (and MDA equivalents), is presented across FS, age, and S (storage time).

Table 8. Mean MDA content comparison between FS, age, and S.

Trait MDA (mg/kg)	FS ($\eta^2 = 0.006$)			Age ($\eta^2 = 0.014$)			S ($\eta^2 = 0.300$)		
	IS	ES	DPS	Early	Middle	Late	0	7	28
	0.12 ± 0.01^a	0.12 ± 0.01^a	0.12 ± 0.01^a	0.12 ± 0.01^{ac}	0.10 ± 0.01^a	0.13 ± 0.01^c	0.07 ± 0.01^a	0.10 ± 0.01^b	0.18 ± 0.01^c

Mean $\pm$ standard error for oxidation stability expressed as mg/kg of malondialdehyde (MDA). Different superscripts (a–c) indicate statistically significant differences at $\alpha = 0.05$ ($p \leq 0.05$) among groups of FS, age, and S, according to the LSD criterion. FS = farming system; η^2 = partial eta squared (effect size index); IS = intensive system; ES = extensive system; DPS = dual-purpose system; S = storage time.

The content of MDA measured (mg/kg) was similar for all FSs, and no significant difference was found between them. Age had an effect on MDA as the differences between both the early and middle stages compared to the late were significant. The differences between the means of S groups were statistically significant, showing an increasing trend of MDA accumulation as the duration of storage time extended. On day 0, the lowest content of MDA was recorded (0.07 ± 0.01), following an increasing trend. On day 28, the highest content in MDA was recorded (0.18 ± 0.01). According to the analysis of S $\times$ age $\times$ FS interaction (Table S6), the differences between the FSs for day 7 were significant between the IS and ES in the early stage, while in the late stage, the opposite was observed. Finally, on day 28, the highest concentration of MDA was recorded for the IS, during the early stage, for the DPS, during the middle stage, and for the ES, during the late stage of the production cycle. The storage time mainly influenced to the variation in MDA content ($\eta^2 = 0.300$).

4. Discussion

This study evaluated the influence of the FS and flock age on the external and internal quality traits of eggs. These traits are critical not only for industry standards but also for consumer perceptions of egg quality, which are often shaped by marketing and personal beliefs. The findings provide a comprehensive understanding of how production systems

and hen age shape these quality parameters, highlighting the need for evidence-based communication to align consumer expectations with scientific outcomes. Six commercial laying farms were included in this study. The ES and DPS represent low-input systems (LI) while the IS represents a high-input (HI) production system. This design allowed for extensive within-farm data collection. However, replication at the FS level was rather limited, which should be taken into consideration when interpreting differences across FSs.

Egg quality parameters and their evaluation in association with production systems have been studied by many researchers, with opinions being mixed. The effect of the FS on EW was significant in the current study, complying with the results of other studies on intensive and free-range systems [26,27]. The FS affected the SW and EW, with eggs originating from ESs and DPSs displaying higher means, while the highest ESI was recorded for the IS. Age also influenced the EW, SW, and ST. Eggs produced during the late stage of the production cycle appeared to be heavier, with the same effect noted for SW. The ST decreased as age advanced, as also shown in previous studies [28]. The significant interaction effects of hen age $\times$ FS show that in ISs, there is a tendency for smaller variation in the means of the examined external traits within age groups (EW, SW, ST, ESI), while ESs and DPSs show more variability, potentially due to environmental stressors or diet. The ESI fluctuated with the age in the ES and DPS while it showed a constant increase in the IS, where the conditions might contribute to the production of rounder eggs. Similar interaction effects have been reported by previous studies [29]. Typically, the ESI percentage ranges from 72 to 76 for oval-shaped eggs, whereas eggs with an ESI < 76 and ESI > 79 are considered sharp or incredibly round, respectively [19]. This measurement indicates the ability of eggs to fit into the trays in a transportation truck and the likelihood of breaking during transfer. Generally, the differences were small and do not risk the marketability of eggs.

The effect of the FS on freshness, as described by HUs, was significant. This finding is in contrast to Wiseman et al. (2024), who stated that the FS did not affect the HU score [11]. Many studies have reported significant differences in HU score, with eggs produced under LI systems displaying lower means [30–32], while other studies have reported higher values in HUs from free-range eggs [33,34]. Specifically, each FS includes various practices of handling eggs after oviposition. Eggs laid on the ground in deep litter systems until collection appear to show a smaller HU score; as the porous shell absorbs ammonia components, its albumen height tends to decrease and thus lowers its overall quality [9,35]. The means obtained in this study for all FSs align with the industry expected standards for egg marketability, falling within the typical range (Grade A or higher) [11]. In contrast, the same effect on albumen was assumed to appear for conventional systems by other authors in the field [36]. The interaction effect demonstrates that eggs produced in LI systems exhibit a higher rate of AH and HU reduction across age groups. This finding further supports the vulnerability of albumen consistency when eggs are laid outdoors. In this research, the HU score and AH were primarily influenced by the FS. Yolks appear more orange to red in eggs originating from the IS. This is an attribute influenced mainly by the feed, as in IS farms, the diet consists primarily of corn, soybean meal, wheat, and some feed additives that may contain natural pigments (canthaxanthin, lutein), which contribute to a more intense yolk color [37]. YC is the most important inner characteristic for consumers, with preferences showing variation nationwide [12,13]. A significant interaction effect was also shown for the YC, where the variation across age groups observed in the ES and DPS is related to changes in the type of feed due to grazing (variation in nutrient intake). YW was affected by the FS, age, and their interaction, being more influenced by the first. Yolks from eggs of the DPS were the heaviest, whereas the eggs from the ES had the most considerable AW in this study. The different feed conversion rate and onset of reproductive

maturation of the flocks used in each FS may explain the various upward trends of AW and YW throughout the production cycle.

Data from the literature show that the frequency of BSs correlates with eggshell color, suggesting that darker-themed eggshells are more difficult to check for internal defects during candling [38]. In addition, research on eggshell pigments shows identical reflection properties of protoporphyrin, the shell's main pigment that defines its color, and hemoglobin, which is present in blood [39]. Age is also considered a factor for the presence of BSs. Progress of age influences the presence of these traits, as concluded by previous authors [40], which was not evident in the current study. Eggs produced in the DPS displayed significantly higher means of BSs in the yolk in all age groups. In the case of the IS of the current study, candling application and immediate sorting of eggs might be responsible for the less frequent observations of BSs, whereas in the small-scale and lower-input FS, this practice is not included in the management scheme. The presence of BSs is undesirable to consumers and is considered a defect; therefore, it reduces the marketability of eggs [16,41].

The analysis also showed statistically significant differences between the three FSs regarding the egg FA profile. Higher means of C16:0 and C18:0 in the ISs result from cereal-based mixtures and seed oils in the feed. Moreover, the higher levels of DHA that were found in the DPS and ES suggest that these fatty acids and their precursors are abundant in grasses. Several studies indicate higher levels of DHA in extensive free-range systems and a higher ratio of ω -6/ ω -3 for conventional systems [40,42,43], in agreement with the current study. This observation depends on the type of feed that is given to the hens. AI differences were not significant between the IS and ES but were notably lower for the DPS. The FA profile was also influenced by age, which was shown in previous studies that involved different age groups in their designs [44–46]. The ratio of ω -6/ ω -3 is thought to be significantly lower in flocks of older ages [47], an observation that was not evident in this study. It might be possible that this finding is related to the expression and synthesis of enzymes in the livers of hens, which are responsible for yolk formation and reduce sharply at older ages [48]. In contrast, Peng et al. did not find any significance of age in the yolk fatty acid composition [42]. AI and TI ratios were affected by the FS. The IS showed a significantly higher TI. Lower ratios of AI and TI may reduce the atherogenic and thrombogenic potential, as is suggested by Chen and Liu (2020) [49]. The effect size indices also show that the FS mainly influences the percentages of these FAs. According to the significant interaction effect for the FA profile, it is evident that the means of these traits show less variation for ISs in all age groups, where more controlled feeding schemes are applied. Conversely, a greater variation is observed for the ES-DPS, where it is suggested that the availability of feed components, responsible for the FA profile, likely depends on the availability of grasses. Although the main effects of the FS, age, and their interaction were significant, according to the effect size indices, the variability in this trait is mostly driven by the FS. It is confirmed that production systems include different types of feeding and feed composition, which alter the FA profile in produced eggs.

Regarding the oxidation stability analysis, when using the TBARS assay, no significant differences were found between the FSs. The concentration of MDA increased as the storage time extended, and a steeper increase in MDA was observed between days 7 and 28 of storage. This time period was chosen to simulate typical shelf storage conditions. A previous study (Mierlita, 2020) found a significant influence of both the FS and S on the MDA content [43]. The significant interaction effect ($S \times \text{age} \times \text{FS}$) on MDA content explains the enhanced lipid peroxidation in eggs stored for 7 and 28 days in different age groups between FSs. It is suggested that those differences might relate to the yolk PUFA content and yolk characteristics. PUFAs in yolk are considered substrates of oxidation

reactions, and due to their chemical structure, those fatty acids are more susceptible to oxidation (presence of multiple double bonds), resulting in greater accumulation of MDA over time [50]. It is concluded that a longer storage time (on shelf) enhances the progression of oxidation reactions and is the most determinant factor on oxidation stability.

The findings highlight the significance of each factor and their combined effect on egg quality. The variations in egg quality that are observed between the production systems may support the diversification of products based on consumer needs. However, consumer perceptions of egg quality may be influenced by marketing and welfare concerns. Food industries may need to establish standards to protect consumers from misleading or confusing terms about quality based on product origin. Moreover, there is a need to provide clear evidence-based communication to the consumer side regarding egg quality traits and farming system characteristics and practices, to allow for informed purchasing decisions and enhanced trust in the egg industry.

5. Conclusions

The claim that eggs originating from low-input poultry FSs are of superior quality requires further backing by scientific evidence. The objective characterization of egg quality differs from consumer perceptions as these are mostly driven by marketing trends or personal values. To resolve the gap between consumer expectations and egg quality, consumer education and accurate product labeling based on scientific findings are needed. This study highlights that the egg quality cannot be attributed exclusively to a specific production system, as no approach consistently yielded superior outcomes across all measured parameters. It is concluded that the external and internal quality of eggs relate not only to production processes but also to the age of the flock, as physiological changes occur during the life of hens. These two factors not only act independently, but there is also a significant interaction between them. In this study, the effect of the production system explains most of the variability and appears to be stronger than the effects of the stage of the production cycle (age of flock) and interactions.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/app151910693/s1>, Table S1. Interaction effect of FS and age on external traits; Table S2. Interaction effect of FS and age on internal traits; Table S3. Interaction effect, determined with Mann-Whitney test, between FSs within age groups on BS incidence; Table S4. Interaction effect of FS and age for fatty acids in yolk; Table S5. Interaction effect of FS and age on yolk lipid classes; Table S6. Mean MDA content for the $S \times \text{age} \times \text{FS}$ interaction.

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Abbreviations

The following abbreviations are used in this manuscript:

ESI	Egg Shape Index
HU	Haugh Unit
AH	Albumen Height
EW	Egg Weight
SW	Shell Weight
ST	Shell Thickness
YC	Yolk Color
YW	Yolk Weight
AW	Albumen Weight
BS	Blood Spot
HI	High Input
LI	Low Input
ES	Extensive System
IS	Intensive System
DPS	Dual-Purpose System
FA	Fatty Acid
AI	Atherogenicity Index
TI	Thrombogenicity Index
FS	Farming System
TCA	Trichloroacetic Acid
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substance
MDA	Malondialdehyde
PUFA	Polyunsaturated Fatty Acid
ω -6	Omega 6
ω -3	Omega 3
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
MUFA	Monounsaturated Fatty Acid
UFA	Unsaturated Fatty Acid
SFA	Saturated Fatty Acid

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