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Quality traits of table eggs as affected by management practices and storage periods

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Abstract

This study evaluated the external and internal quality characteristics after various storage periods (SP) of eggs from different management practices (MP) regarding layer hen housing and feeding systems. One hundred eggs collected from farms with Lohman Sandy hens raised in i) a cage system where the standard diet was offered *ad libitum* (CAB), ii) a free-range production system with free access to both the standard diet and alfalfa pasture for grazing (FRG), or iii) a village poultry system where hens were fed on insects, grass, vegetables, or kitchen waste, as well as a restricted standard diet (VCR) were stored at 0, 7, 14, 21, and 28 d at +4 °C and 60% relative humidity. Data were analysed in a three MP × five SP factorial arrangement with 20 eggs each. SP was analysed using polynomial regression. The CAB eggs had a higher weight loss and shell ratio compared to the FRG and VCR eggs. The yolk index, albumen index, Haugh unit (HU), and yolk colour of FRG and VCR eggs were higher than CAB eggs. The yolk ratio of the CAB group was higher than that of the other groups, whereas the albumen ratio was lower. The egg weight loss increased linearly, whereas the albumen ratio and pH increased cubically during storage. The egg weight loss, shell thickness, and yolk index were related to the interaction between MP and SP. Shell ratio and all internal quality traits, except for the pH of yolk and albumen, reflected only the effect of the MP; the association became more significant as the SP increased. Our results suggest that shell ratio, surface area, and all internal quality traits, except for yolk colour, change during storage.

Keywords: Free-range eggs, village eggs, cage eggs, layer hen
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Introduction

Recently, there has been a growing interest in nutritional trends and animal welfare issues, leading consumers to choose healthy, high-quality eggs produced in alternative systems. These alternative systems (cage-free, antibiotic-free, and alternative feeding; Bray and Ankeny, 2017) aim to improve animal welfare to obtain better quality products. In cage-free systems, chickens are housed with perches and litter and have access to vegetated pasture. In village poultry systems, hens are housed in environmentally-controlled housing that is fenced to protect them from theft, predators, and unfavourable environments. The animals are not allowed to consume any foreign matter containing pesticides or other harmful substances. During the day they range freely around the poultry house to collect food, and additional feed can be given in the morning and/or evening. Cages are multi-story systems where the animals do not have access to the outdoors.

Environmental factors such as housing system (Vlčková *et al*., 2019; Carvalho *et al*., 2022; Čobanović *et al*., 2022; Sokołowicz *et al*., 2022; Alig *et al*., 2023a,b), feeding strategy (Moreira *et al*., 2012; Sokołowicz *et al*., 2018; Popova *et al*., 2020; Ahizo *et al*, 2021; Kop-Bozbay *et al*., 2021; Anene *et al*., 2023), and storage period (Vlčková *et al*., 2019; Drabik *et al*., 2021; Carvalho *et al*., 2022; Sokołowicz *et al*., 2022) can affect egg quality traits. Research indicates that enhancing hen welfare in poultry systems has an impact on the physical quality characteristics of eggs, such as yolk colour, albumen quality, Haugh Unit (HU), shell quality, and size, without resulting in a substantial rise in egg defects. Indeed, Alig *et al*. (2023a) found that brown laying hens produced eggs with intense yolk colour in a free-range system, lower albumen height and HU in the cage-free system, and stronger eggshells in both the cage-free and free-range systems. Additionally, eggs from barren colony cages exhibited more unfavourable characteristics. In another study, Alig *et al*. (2023b) reported that egg quality was superior for white-laying hens in caged systems. In contrast, only yolk colour was improved in cage-free systems. Similarly, Sokołowicz *et al*. (2018) found that free-range and organic systems produced heavier eggs with more intense yolk colour compared to litter systems, whereas alternative housing systems had no effect on albumen height and HU.

Scientists note that there can be substantial differences in the feeding regime and diet used in different housing systems, which can greatly affect the quality characteristics of eggs. For example, Ghanima *et al*. (2020) claimed that differences in feeding had a more direct impact on egg quality parameters than the housing system. Some studies have reported that performance was affected by feed restriction but had no marked effect on egg quality (Moreira *et al*., 2012; Ahizo *et al*., 2021). However, Anene *et al*. (2023) showed that restricted feed at 115 g per day in laying hens increased albumen height and HU in eggs compared to *ad libitum* feeding. Alig *et al*. (2023a) found that access to pasture in free-range hens provided nutritional advantages resulting in darker yolks and better shell quality.

Eggs are highly resistant to long-term storage periods due to their unique structure. However, during storage, the egg content undergoes physicochemical changes that can negatively impact egg quality, such as deteriorating albumen quality and HU. During prolonged storage periods, the pH value of albumen and yolk increases, and the weight of albumen decreases due to the release of carbon dioxide from the breakdown of carbonic acid in the albumen (Eke *et al*., 2013; Vlčková *et al*., 2019). The HU is a criterion for assessing internal egg quality and is one of the most important changes observed during storage period. In fresh eggs, the HU score is approximately 80 and decreases with a longer storage period (Vlčková *et al*., 2019; Drabik *et al*., 2021).

The egg's external and internal quality traits can be affected by both genetics, such as age and strain of layer, and non-genetic factors (Alsobayel and Albadry, 2011), such as housing system, feeding regime, and storage conditions and periods (Alsobayel and Albadry, 2011; Soltani and Omid, 2015; Yamak *et al*., 2020). Therefore, the changes in the egg quality traits that start deteriorating right after oviposition (Yamak *et al*., 2020) depend on egg production conditions and storage periods. Therefore, the egg has to be stored to maintain its quality until consumed or processed into a product (Yamak *et al*., 2020). A reduction in the internal egg quality is caused by enhanced interaction between lysozyme and ovomucin as pH increases during egg storage (Soltani and Omid, 2015). Eggs produced in a cage system are generally cleaner than free-range or village-type eggs because the eggs from hens that have outdoor access can be contaminated with mud, faeces, and dust. A better understanding of how laying hen management practices (housing system and feeding regimen that impact laying performance) and storage periods can affect table egg quality is crucial for scientific and commercial activities. Nevertheless, there is a lack of knowledge regarding the effect of changing egg storage periods on the quality traits of eggs from laying hens under different MP. Accordingly, in this study, the effects of different MP (*ad libitum* feeding in a cage system, *ad libitum* feeding with grazing in a free-range system, and restricted feeding with vegetable waste in a village chicken production system) and SP (1, 7, 14, 21, or 28 d) on certain internal (yolk index, albumen index, HU, yolk colour, yolk ratio, albumen ratio, yolk pH, and albumen pH) and external (egg weight, egg weight loss, shape index, shell ratio, shell thickness, and shell surface area) egg quality traits were examined using eggs collected from Lohmann Sandy hens.

Material and Methods

This observational experiment was conducted at the Eskişehir Osmangazi University, Faculty of Agriculture, Animal Science Laboratory. Ethics committee approval was not requested from the local ethics committee of the University for this study because of non-animal-based research. However, we followed theoretical or national ethical rules and other scientific purposes throughout the research (Gross and Tolba, 2015).

Laying hen farms were selected according to the statement of the owner farmer to gather information on i) genotype of hens, ii) housing system, iii) general flock health, iv) flock age, v) feeding strategies, vi) feed intake, and vii) egg-laying rate. Consequently, three farms representing the housing system and feeding management (management practices, MP) as well as layer hens (Lohman Sandy layer at an average of 50 w of age) were enrolled. Farms that used the same commercial layer feed (hereinafter referred to as the standard diet) based on a maize and soybean meal (160 g crude protein and 2750 kcal metabolizable energy per kg diet) were selected. The first farm had a cage system where the standard diet was offered *ad libitum* (CAB). The second farm had a free-range production system with free access to the standard diet and alfalfa pasture for grazing (FRG). The third farm had a village chicken production system where hens were fed on insects, grass, vegetables, or kitchen wastes, as well as the restricted standard diet (VCR). According to the statement of the owner farmer, in the CAB, FRG, and VCR farming systems, the daily standard diet intake of hens was 115, 95, and 60 g per day, respectively. The standard diet restrictions were approximately 50% of *ad libitum* intake. The hens in three farms had unrestricted access to water.

One hundred freshly-laid, day-old eggs of similar weight (59.96 ± 7.29 g) were collected from the three farms with different MP without any visible shell defects. Then, the eggs were weighed and placed in sterile carton trays (viol) with 20 pockets labelled with the relevant farm (CAB, FRG, or VCR), pointed out numerically, and immediately transported to the laboratory. A total of 300 eggs were used in 15 groups (3 MP × 5 SP), with 20 eggs examined in each. The 20 eggs for each MP were stored for 0, 7, 14, 21, or 28 d in chambers in a controlled cabin (Microtest, MIT-120) at +4 °C and relative humidity of 60%.

In this study, external (egg weight, shape index, egg weight loss, shell ratio, shell thickness, and shell surface area) and internal (yolk index, albumen index, Haugh Unit, yolk colour, yolk ratio, albumen ratio, albumen pH, yolk pH) egg quality parameters were determined after 0-, 7-, 14-, 21-, and 28-d storage periods. The fresh eggs for each MP for SP of 0 d were measured within four hours of being laid. All eggs were weighed on day 0 (initial weight) and the relevant analysis day (final weight) using a 0.01 g precision balance. Egg weight loss was calculated as a percentage of the final egg weight compared to the initial weight [(initial weight - final weight/initial weight) × 100]. The shape index was calculated from the length and width values of the egg [(width/height) × 100] measured with a digital calliper (Dasqua Ip54-0.01 mm). The eggs were delicately cracked onto a smooth, levelled glass surface resting on a metal stand with a reflective mirror. The eggshell was weighed and the eggshell ratio (%) was calculated as the eggshell weight × 100/egg weight. The eggshell membranes were eliminated, and the thickness of the eggshell (mm) was calculated at three specific points (blunt, equator, and pointy end) utilizing a digital calliper. Eggshell surface area (mg/cm^2) was calculated from egg weight using the formula developed by Nordstorm and Ousterhout (1982).

The yolk and albumen heights of the broken-out eggs were measured using a tripod micrometre (Mitutoyo-0.01 mm). The yolk diameter, albumen length, and albumen width were measured with a digital calliper. The yolk index [(height/diameter) × 100] and albumen index [(height/(length + width)) × 100] were calculated. The Haugh unit score (HU) was calculated from egg weight and albumen height [HU = 100 Log (albumen height (mm) – 1.7 × egg weight$^{0.37}$ (g) + 7.57]. The yolk colour was determined using a colour spectrum fan (ROCHE), which includes different shades of yellow from 1 to 15. The yolk and albumen were delicately placed in a sterile container and weighed with a precision balance. Then, the yolk and albumen ratios (%) were calculated as the [yolk weight × 100/egg weight] and [albumen weight × 100/egg weight], respectively. The pH of the yolk and albumen were measured using a digital pH meter (Hanna HI2002-02).

The Statistical Package for the Social Sciences (SPSS) software program (version 21.0, SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. Normality and homogeneity of variance were analysed using the Kolmogorov–Smirnov test and Levene's test, respectively. Data expressed as percentages were subjected to arcsine transformation before the analysis, whereas actual percentages are reported. Responses to the studied quality characteristics for eggs from the MP groups in each SP were assessed using the general linear model procedure that included the main effects of MP (CAB, FRG, and VCR), SP (0, 7, 14, 21, and 28 d), and the interaction between these factors, the random effect of the farm, and robust standard errors. In the model, SP was introduced as a quantitative factor, which was analysed using polynomial regression and as a random effect. The difference between means was determined using Duncan's multiple comparison test and deemed significant at the P <0.05.

Results and Discussion

The eggs from CAB birds had a higher shell ratio than those from FRG and VCR (P <0.05; Table 1). The albumen index, HU, and yolk colour score of FRG and VCR eggs were higher than CAB eggs (P <0.05; Table 2). The yolk ratio of the CAB group was higher than that of the other groups, whereas the albumen ratio was lower (P <0.05; Table 2). As observed in this study, housing and feeding strategies had a marked effect on the internal and external quality of the eggs (Kop-Bozbay *et al*., 2021; Alig *et al*., 2023a,b; Anene *et al*., 2023). When these two management practices are combined, it is important to evaluate the effect of laying hens on egg quality obtained from different MP, as well as the stability of quality traits during the storage period. Egg quality should be a priority for producers, as problems with egg quality can incur substantial costs for the industry. Since consumers demand high-quality products, as the age of the eggs they buy increases (under refrigerator conditions), they will prefer the eggs from the MP that produces the least effect on product quality. Therefore, it is crucial to elucidate the factors that affect egg quality.

The shape index ensures a lower percentage of egg breakage during collection, grading, and packaging, as well as higher acceptance in the market. In this study, all eggs had an ideal shape index. No differences were observed based on the impact of the MP or SP, as previously reported for the effects of MP or feeding strategy (Tabidi, 2011; Anene *et al*., 2023) and SP (Batkowska *et al*., 2016).

The SP affected all external egg quality characteristics except for the shape index (Table 1). A higher shell ratio was observed in fresh eggs than in those stored for varying periods (P <0.05). As SP increased, the albumen index decreased compared to fresh eggs (P <0.05; Table 2). The HU was highest in fresh eggs and decreased with the increase in storage period (P <0.05). On day 7 of storage, there was an increase in the yolk ratio and a decrease in the albumen ratio compared to 0, 14, 21, and 28 d (P <0.05). The yolk pH value was higher than the other groups after 7 d and 21 d of storage (P <0.05). The albumen pH of fresh eggs was lower than those in all SP (P <0.05).

Table 1. External quality traits of eggs depend on management practices (MP) and the storage period (SP)[1]

MP	SP	Egg weight, g	Egg weight loss, %	Shape index	Shell ratio, %	Shell thickness, mm	Shell surface area, mg/cm²
CAB	0	59.88	nd	75.87	14.62	0.429^{ab}	71.39
	7	61.24	0.62^{f}	75.20	14.49	0.401^{c}	72.21
	14	59.81	1.04^{e}	76.76	14.19	0.399^{cd}	70.80
	21	60.13	1.52^{d}	76.69	13.86	0.387^{cde}	70.83
	28	58.86	2.16^{a}	76.31	14.05	0.385^{cde}	69.44
FRG	0	60.08	nd	76.75	13.28	0.427^{ab}	71.56
	7	59.58	0.55^{f}	77.18	13.12	0.386^{cde}	70.86
	14	61.02	0.90^{e}	77.34	13.08	0.377^{f}	71.89
	21	59.45	1.46^{d}	77.19	13.07	0.398^{cd}	70.26
	28	58.75	1.90^{b}	73.82	13.23	0.372^{f}	69.51
VCR	0	60.09	nd	77.01	13.72	0.440^{a}	71.54
	7	59.80	0.47^{f}	76.21	13.02	0.380^{ef}	71.09
	14	60.97	0.93^{e}	73.66	12.78	0.376^{f}	71.83
	21	60.25	1.39^{d}	76.99	12.93	0.420^{b}	71.00
	28	59.51	1.75^{c}	77.31	13.00	0.372^{f}	70.19
Management practices (MP)							
CAB		59.98	1.07	76.17	14.24^{a}	0.400	70.93
FRG		59.77	0.96	76.46	13.16^{b}	0.392	70.82
VCR		60.12	0.91	76.24	13.09^{b}	0.398	71.13
Storage period (SP), d							
0		60.02	nd	76.54	13.88^{a}	0.432	71.49^{a}
7		60.21	0.55	76.20	13.54^{b}	0.389	71.39^{a}
14		60.60	0.95	75.92	13.35^{b}	0.384	71.51^{a}
21		59.94	1.46	76.96	13.29^{b}	0.401	70.70^{ab}
28		59.04	1.94	75.81	13.43^{b}	0.376	69.71^{b}
SEM		0.201	0.042	0.368	0.060	0.0020	0.171
Main effect of							
MP		NS	***	NS	***	NS	NS
SP		NS	***	NS	**	***	**
MP×SP		NS	*	NS	NS	***	NS

CAB: *ad libitum* feeding in cage system; FRG: *ad libitum* feeding with grazing (free access to chicory vegetated area) in a free-range system; VCR: restricted feeding with vegetable waste in village chicken production system; nd: not determined; SEM: standard error of the mean

[a,b,c,d,e,f] Means within a column lacking a common superscript differ (P <0.05). NS: P >0.05, *: P <0.05, **: P <0.01, ***: P <0.001

[1]The values are the means of the 20 eggs

The egg weight loss measures the egg's ability to maintain freshness during storage. In the present study, it was found that weight loss was lower in eggs obtained from cage-free systems (FRG and

VCR), in contrast to the findings of Zhang *et al*. (2016) and Carvalho *et al*. (2022). This can be explained by the absence of differences in fresh egg weights across all three production methods. The increase in storage period leads to a linear increase in egg weight loss due to the loss of water and carbon dioxide caused by the increase in shell pore size with increasing storage period (Eke *et al*., 2013). Similarly, studies have reported a decrease in egg weight during storage (Tomczyk *et al*., 2019; Vlčková *et al*., 2019; Carvalho *et al*., 2022; and Sokołowicz *et al*., 2022). Additionally, a strong interaction was observed between the MP and SP factors for egg weight loss. There was a general increase in the weight loss of eggs of all three MP during the storage period. However, the rate of increase varied for the three MP, and CAB eggs showed the highest weight loss at each measurement. These results are in agreement with the results reported by other researchers (Samiullah *et al*., 2017; Ahizo *et al*., 2021). The egg weight loss increased linearly, affecting the shell surface area quadratically, whereas the albumen ratio changed cubically during storage (*P* <0.001).

Table 2. Internal quality parameters of eggs depend on management practices (MP) and storage period (SP)[1]

MP	SP	Yolk index	Albumen index	Haugh unit	Yolk colour	Yolk ratio, %	Albumen ratio, %	Yolk pH	Albumen pH
CAB	0	42.70^{ghi}	10.82	89.73	7.40	28.28	57.10	6.32	8.34
	7	45.30^{defg}	9.37	85.61	7.65	29.02	56.50	6.41	8.75
	14	46.22^{bcde}	9.15	84.03	7.60	29.21	56.61	6.33	8.87
	21	43.52^{fgh}	8.69	79.82	7.15	28.35	57.79	6.26	8.81
	28	40.54^{i}	8.40	77.30	6.95	29.14	56.80	6.42	8.86
FRG	0	47.64^{bcd}	12.27	92.43	10.90	26.87	59.85	6.26	8.31
	7	48.96^{ab}	10.22	89.34	11.35	28.86	58.02	6.42	8.80
	14	45.93^{cdef}	9.43	84.99	11.10	26.97	59.95	6.27	8.48
	21	46.38^{bcde}	10.83	88.00	11.00	26.21	60.72	6.27	8.71
	28	41.96^{hi}	8.99	81.73	10.80	27.47	59.40	6.41	8.82
VCR	0	47.65^{bcd}	10.45	89.41	11.45	27.12	59.16	6.28	8.38
	7	48.55^{abc}	10.80	88.31	11.20	29.42	57.56	6.40	8.79
	14	51.06^{a}	10.22	85.97	11.10	27.06	60.16	6.34	8.75
	21	47.79^{bcd}	10.54	87.44	11.45	27.05	60.02	6.31	8.61
	28	44.09^{efgh}	8.80	79.62	11.15	27.37	59.62	6.40	8.84
Management practices (MP)									
CAB[1]		43.66	9.29^{b}	83.30^{b}	7.35^{b}	28.80^{a}	56.96^{b}	6.35	8.73
FRG		46.18	10.35^{a}	87.30^{a}	11.03^{a}	27.27^{b}	59.59^{a}	6.33	8.63
VCR		47.83	10.16^{a}	86.15^{a}	11.27^{a}	27.61^{b}	59.30^{a}	6.35	8.67
Storage period (SP), day									
0		46.00	11.18^{a}	90.52^{a}	9.92	27.42^{b}	58.70^{a}	6.29^{b}	8.34^{b}
7		47.60	10.13^{b}	87.76^{b}	10.07	29.10^{a}	57.36^{b}	6.41^{a}	8.78^{a}
14		47.74	9.60^{b}	85.00^{c}	9.93	27.75^{b}	58.91^{a}	6.31^{b}	8.70^{a}
21		45.90	10.02^{b}	85.09^{c}	9.87	27.20^{b}	59.51^{a}	6.28^{b}	8.71^{a}
28		42.20	8.73^{c}	79.55^{d}	9.63	28.00^{b}	58.61^{a}	6.41^{a}	8.84^{a}
SEM		0.270	0.137	0.439	0.117	0.138	0.157	0.007	0.023
Main effect of									
MP		***	***	***	***	***	***	NS	NS
SP		***	***	***	NS	***	***	***	***
MP×SP		*	NS	NS	NS	NS	NS	NS	NS

CAB: *ad libitum* feeding in cage system; FRG: *ad libitum* feeding with grazing (free access to chicory vegetated area) in the free-range system; VCR: restricted feeding with vegetable waste in village chicken production system; nd: not determined; SEM: standard error of the mean

[a,b,c,d,e,f,g,h,i] Means within a column lacking a common superscript differ (*P* <0.05). NS: *P* >0.05, *: *P* <0.05, **: *P* <0.01, ***: *P* <0.001

[1]The values are the means of the 20 eggs

The egg weight loss (Table 1), shell thickness, and yolk index of eggs (Table 2) were affected by the interaction between MP and SP (*P* <0.001). The greatest weight loss was detected in eggs from CAB at 28 d of storage, whereas the lowest was detected at the storage of 7 d in all MP groups (*P* <0.05). The fresh eggs obtained from VCR had the highest shell thickness value, whereas the FRG and VCR groups had the lowest shell thickness at 14 and 28 d (*P* <0.05). The yolk index value was highest in VCR eggs at 14 d and lowest in CAB eggs at 28 d of storage (*P* <0.05). A significant interaction between dietary protein sources and SP for the changes in egg weight loss and albumen quality was reported by Wang *et al*. (2015). CAB, FGR, and VCR hens consumed different feedstuffs, including protein sources, which may explain why the interaction effect was significant for the related traits. Meaningful interactions between the SP and MP factors for these variables appear to be caused by one measure for each MP; more extended MP relating to feeding strategies needs to be observed to determine whether these interactions were genuinely significant. Although information concerning the effects of interaction between the MP and SP on the internal quality of stored shell eggs is lacking (Lewko and Gornowicz, 2011; Czarnowska-Kujawska *et al*., 2021), explaining the interaction between the MP and SP is crucial for improving the MP with suitable feeding strategies (Jones *et al*., 2014; Kop-Bozbay *et al*., 2021).

The quality of eggshells is dependent on the mineral intake of laying hens, specifically calcium and phosphorus. A decrease in mineral intake, caused by lower daily consumption of a standard diet due to grass consumption in FRG hens (Kop-Bozbay *et al*., 2021) and restricted feeding in village hens, may decrease shell weight. Studies by Mahrose *et al*. (2022) in quails and Ahizo *et al*. (2021) in laying hens reported that the shell ratio was reduced by feed restriction, which supports this finding. Furthermore, the laying hens in FRG and VCR had a larger free-range area, which allowed for greater mobility. As a result, minerals obtained through compound feed, grazing, or coprophagic behavior may be used for bone development and preservation rather than eggshell formation (Philippe *et al*., 2020).

The housing system may also affect shell quality. For example, Čobanović *et al*. (2022) found that eggs from free-range systems had the lowest shell weight, shell ratio, and shell thickness. The shell thickness observed in the present study was similar in all three MP environments, with a minimum of 0.39 mm and a maximum of 0.40 mm. Anene *et al*. (2023) reported that restricted feed intake in hens did not have a negative effect on shell thickness. The current study used eggs of similar weight, and there was no difference in shell thickness in the MP groups, confirming the finding that egg weight and shell thickness are related (Fathi *et al*., 2019). The shell ratio, thickness, and surface area decreased during storage, a widely reported phenomenon (Alsobayel and Albadry, 2011; Martinez *et al*., 2021; Sokołowicz *et al*., 2022). Additionally, a statistically significant effect of MP and an interaction with the storage period on shell thickness was observed. Shell thickness decreased rapidly during the first week of storage in all MP eggs and remained fairly constant until 28 d in CAB eggs. It was lower in FRG and VCR eggs than in CAB at 28 d.

The HU is a measure of egg protein quality based on albumen height and is widely regarded as the most reliable indicator of egg freshness and predictor of egg internal quality. In this study, cage-free (FRG and VCR) eggs had higher HU than found in previous studies (Englmaierová *et al*., 2014; Popova *et al*., 2020; Alig *et al*., 2023a,b), but Hu was similar to findings by Samiullah *et al*. (2017) and Dikmen *et al*. (2017). Higher albumen content and index were found in these groups, supporting our conclusion. Samli *et al*. (2005) reported that HU and pH of albumen were the most important parameters influenced by the SP. This difference may be due to variations in feeding strategy (*ad libitum* or restricted feeding), vegetation type (grass, legumes, other plants, or their mixture), and percentage of pasture utilization. Saibaba *et al*. (2021) and Anene *et al*. (2023) also reported a similar finding that HU was higher in eggs obtained from hens fed restricted feed. Furthermore, Anene *et al*. (2023) demonstrated an increase in the albumen index and a decrease in the yolk index in eggs from feed-restricted hens. This suggests that the feeding strategy has a greater impact on HU than the housing system. As the egg ages, the HU decreases due to moisture loss and the degradation of proteins within the albumen (da Pires *et al*., 2021). This decrease in HU with storage period is consistent with the literature (Batkowska *et al*., 2016; Vlčková *et al*., 2019; Drabik *et al*., 2021; Martinez *et al*., 2021; Sokołowicz *et al*., 2022). Eggs with HU values below 70 are not recommended for consumption due to reduced nutritional quality and the possible presence of pathogenic microorganisms (Qi *et al*., 2020). Our study found that HU decreased to 79.55 after 28 d of storage, likely due to a decrease in albumen height during the storage period. As a result, both the albumen index and yolk index decreased in our study. At the start of storage, CAB eggs had the lowest yolk index. Additionally, the decrease in yolk index during the storage period was higher for CAB eggs compared to FRG and VCR eggs.

In the FRG group, animals had access to pasture and met some of their daily dry matter requirements from green plants. This led to a decrease in their standard feed consumption (Kop-Bozbay *et al*., 2021). Similarly, in the VCR system, chickens were fed a restricted amount of commercial feed and consumed vegetable waste. As CAB hens fed *ad libitum* consumed more feed, excess dietary lipids may have accumulated in the yolk, resulting in a higher yolk ratio. Anene *et al*. (2023) reported that eggs from restricted-fed hens had a lower yolk ratio than those from *ad libitum*-fed hens, but the albumen ratio was unaffected. The increase in yolk content and decrease in albumen content in CAB eggs may be related to the higher shell content detected in CAB eggs. The changes in albumen and yolk weights of all MP eggs related to the storage period can be explained by the diffusion of water from the vitelline membrane into the yolk (Hidalgo *et al*., 2008). Therefore, the detected differences could be attributed to factors such as the nutritional and welfare conditions of the laying hen (Philippe *et al*., 2020). Batkowska *et al*. (2016) and Sokołowicz *et al*. (2022) reported that the yolk ratio increased and the albumen ratio decreased over time in eggs obtained from different rearing systems, regardless of the rearing system. However, in the present study, the yolk ratio decreased and the albumen ratio increased only after 7 d of storage. There was no change at 28 d of storage. This may be an indication that our storage conditions were suitable and eggs obtained from three different MP can be stored for longer periods.

Egg yolk colour is directly related to the amount of carotenoids in the yolk (Nour *et al*., 2017). The colour of the yolk is directly affected by pigments, and hens that have access to green vegetation produce darker yolks. This explains why FRG and VCR hens had darker yolks. Indeed, FRG and VCR hens, unlike CAB hens, had access to green areas containing pigments and xanthophylls important in the formation of yellow colour (Alig *et al*., 2023a,b). Similarly, Galic *et al*. (2019) and Alig *et al*. (2023a,b) found that eggs produced in caged housing systems were lighter in colour. However, in the present study, the storage period had no effect on yolk colour intensity, in agreement with Carvalho *et al*. (2022) and Sokołowicz *et al*. (2022).

Feeding strategies, such as feeding regime and access to green vegetation did not affect albumen and yolk pH. However, the pH values of both the yolk and albumen increased with the storage period. The observed increase in yolk pH (Chung and Lee, 2014; Wang *et al*., 2015; Drabik *et al*., 2021; Sokołowicz *et al*., 2022) and albumen pH (Carvalho *et al*., 2022; Sokołowicz *et al*., 2022) is consistent with the effects of the storage period in eggs from different housing systems. In addition, Jin *et al*. (2011) found that pH increased rapidly during the first days of storage and then the increase in pH decreased, as confirmed in the present study. During the storage period, albumen protein decomposes and water and carbon dioxide are progressively lost from the egg, decreasing albumen height and increasing albumen and yolk pH, resulting in lower HU (Philippe *et al*., 2020). In the present study, in line with the literature, albumen and yolk pH values increased with the storage period.

The regression results of the SP on the egg's external and internal quality traits were significant as polynomials (linear, quadratic, or cubic) with low R^2 values, except for egg weight loss, shell thickness, HU, and yolk pH (Table 3). In contrast, the egg weight and shape index results were not significant. The SP had a linear regression equation that can be used to predict the response for egg weight loss, shell surface area, and yolk colour. The regression equation that can be used to predict the response for shell ratio and yolk index was quadratic, and the corresponding equation for shell thickness, albumen index, HU, yolk ratio, albumen ratio, yolk pH, and albumen pH was cubic.

Table 3. Best fit regression equations, coefficients of determination, polynomial contrasts, and significance levels of the relationship between the storage period and the egg's external and internal quality parameters

Variable	Regression equation[1]	R^2	L	Q	C
External quality parameters of eggs					
Egg weight loss, %	$y = -0.456 + 0.4782x$	0.889***	***	NS	NS
Shell ratio, %	$y = 14.382 + 0.0771x^2 - 0.5777x$	0.039	**	*	NS
Shell thickness, mm	$y = 0.5683 - 0.0067x^3 + 0.0649x^2 - 0.1939x$	0.299*	***	***	***
Shell surface area, mg/cm²	$y = 72.234 - 0.425x$	0.041	***	NS	NS
Internal quality parameters of eggs					
Yolk index	$y = 42.386 - 0.8991x^2 + 4.4638x$	0.183	***	***	NS
Albumen index	$y = 14.787 - 0.1858x^3 + 1.7049x^2 - 5.0833x$	0.108	***	NS	*
Haugh unit	$y = 99.505 - 0.4693x^3 + 4.0318x^2 - 12.385x$	0.223*	***	NS	*
Yolk colour	$y = 10.113 - 0.0767x$	0.003	*	NS	NS
Yolk ratio, %	$y = 21.532 + 0.3635x^3 - 3.3402x^2 + 8.9151x$	0.072	NS	NS	***
Albumen ratio, %	$y = 64.151 - 0.3662x^3 + 3.2919x^2 - 8.422x$	0.063	*	NS	***
Yolk pH	$y = 5.8148 + 0.0316x^3 - 0.2784x^2 + 0.7227x$	0.208*	**	NS	***
Albumen pH	$y = 7.2482 + 0.0527x^3 - 0.5118x^2 + 1.5622x$	0.180	***	**	***

[1]Regression equations and coefficients of determination (R^2) are based on 100 observations
L, Linear; Q, Quadratic; C: Cubic. NS: *P* >0.05, *: *P* <0.05, **: *P* <0.01, ***: *P* <0.001

Conclusion

The results of the present study indicate that i) except for egg weight loss, shell thickness, and yolk index, the studied egg quality traits were statistically unrelated to the interaction between MP and SP,

ii) shell ratio and all internal quality traits, except for the pH of yolk and albumen, reflected only the effect of the MP, and iii) the association became more marked as the SP increased, suggesting that shell ratio, shell surface area, and all internal quality traits, except for yolk colour, change during the storage period. The results provide recommendations for consumers and the egg industry on strategies to optimize desirability and profitability. Further research is required to determine the reasons behind the varying effects of the three different MP environments.

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Conflict of Interest Declaration

The author declares that they have no conflict of interest.

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