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Assessment of eggshell color and yolk fatty acid profiles in two laying hen strains housed with or without access to legume or aromatic plant species outdoor

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Abstract

Plant species vary regarding aspects including taste, palatability, and bioactive compounds. Consequently, the intake of different plant species by laying hens results in the consumption of associated nutrients and the expectation is the modification of shell color and yolk fatty acid profiles of eggs. Here, we investigated the effect of housing environment on shell color and yolk fatty acid profiles of eggs obtained from Lohmann Sandy (LS) and Lohmann LSL Classic (LW) strains. Housing environments included deep litter (DL), free access to outdoor *Mentha piperita* (*M. piperita*), *Petroselinum crispum* (*P. crispum*), and *Medicago sativa* (*M. sativa*). 260 four-week-old birds were randomly distributed to DL and outdoor plant-associated groups, with four and three replicates, respectively, and 10 birds per replicate. Range accessibility was granted at 12 weeks of hen age for outdoor plant-associated groups. Shell color and yolk fatty acid profiles were analyzed at 25 weeks of hen age. There was greater variability among the housing environments regarding the shell color: redness (a*) and yellowness (b*) at the broad end, brightness (L*), a*, b*, and shell color (ΔE*) at Centre and pointed end ($P<0.01$; $P<0.05$). The overall ΔE* was also significantly highest in eggs collected from *M. sativa* and *P. crispum* hens and lowest in eggs from *M. piperita* hens. Palmitic (C16:0), stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6), and alpha-linolenic (C18:3n3) fatty acids were significantly higher in eggs obtained from *M. sativa* and *M. piperita* than DL and *P. crispum* hens, each category with similar values ($P<0.01$; $P<0.05$). While the total saturated fatty acids were highest and lowest in *M. sativa* and *P. crispum*, respectively, total unsaturated fatty acids were highest and lowest in *P. crispum* and *M. sativa*, respectively ($P<0.01$; $P<0.05$). No differences were observed in myristic acid (C14:0) and arachidonic acid (C20:4 n-6) among the housing environments ($P>0.05$). Additionally, all the yolk fatty acid profiles were similar between the laying hen strains ($P>0.05$). It was concluded that most of the yolk fatty acid profiles could be modified when hens are granted free access to outdoor *M. sativa* and *M. piperita*. Genetic influence on fatty acid profiles needs further studies. *M. sativa* and *P. crispum* may have the potential to modulate the shell color intensity.

Keywords*: aromatic plants, eggshell color, fatty acid profiles, free-range system, laying hen*

Introduction

Cage-free systems such as floor or litter or deep litter and free-range systems are becoming popular among consumers, owing to the perception that they offer a welfare-friendly environment to laying hens. In particular, in the free-range production system, it is recommended that hens have free access to outdoor vegetated areas (Sossidou et al., 2015; Hammershøj & Johansen, 2016), which can modify egg composition through the consumption of different plant species and small organisms such as worms and insects.

In addition, the demand for cage-free eggs has come along with the growing interest in functional foods that can play a critical role in preventing certain human diseases (Ferrier et al., 1995; Golzar Adabi et al. 2013). The proficiency in the utilization of eggs as functional foods is well-established (Surai and Sparks, 2001), where the approach of manipulation of layer diet can modify yolk fatty acid contents. There is evidence that intake of plants via supplementation of forage materials to poultry feed or free access to vegetated outdoor areas can modify yolk fatty acid compositions (Lopez-Bote et al., 1998; Karsten et al., 2010; Hammershøj & Steenfeldt, 2012; Mugnai et al., 2014; Hammershøj & Johansen, 2016; Kop-Bozbay et al., 2021). However, the nutritional relevance of plant species is an important factor, which is associated with the amount of plants hens can consume and consequently, influence ingested fatty acids, resulting in enriched yolk fatty acids (Sossidou et al., 2015; Hammershøj & Johansen, 2016). Furthermore, it can be argued that some plant species comprise nutrients that can modify yolk fatty acids or modulate the transfer of ingested fatty acids to yolk. While there is vast information regarding the potential of pastures such as *Medicago sativa* in enriching yolk fatty acid contents (Hammershøj & Johansen, 2016), the reverse is true for aromatic plants. These plants have been highly studied as additives in layer feed with inadequate information about their potential as outdoor forage materials.

Also, the genetic structure of hens can influence some fatty acids (Sarıca et al., 2009; Sözcü et al., 2021). In particular, this effect is linked to genotype or strain differences in the plant intake, ingestion of nutrients from different plants, and their transfer to egg compartments (Lorenz et *al.*, 2013) in outdoor laying production systems or systems where there is dietary manipulation through the addition of forage materials to layer diets.
Shell color is a critical egg quality trait, with color preference, varying among consumers and regions. Thus, shell color has a genetic basis, with greater variability among genotypes and strains as reported by Lordelo et al. (2020). However, there is little information regarding the effect of outdoor plant species on shell color, warranting further studies.
This study examined the eggshell color quality and yolk fatty acid compositions from two laying hens housed with or without free access to *Mentha piperita*, *Petroselinum crispum*, and *Medicago sativa* vegetated areas. It was hypothesized that access to different outdoor aromatic plant species and a legume pasture may boost laying hens differently with bioactive compounds, and this could lead to the enrichment/improvement of yolk fatty acid profiles and the modification of the eggshell color intensity.

Materials and methods

Ethical approval

The egg samples used in the study were obtained from a long-term study involving two commercial laying strains housed in four housing environments, carried out at Ayhan Şahenk Agricultural Application and Research Centre of Niğde Ömer Halisdemir University, Niğde province, Türkiye. The Niğde Ömer Halisdemir University animal experiments local ethics committee approved the animal use protocol (approval number: 2021/04), and the management of birds followed the guidelines for the regulation of animal experiments by the Ministry of Food, Agriculture and Livestock, Türkiye.

Bird`s housing and management

Reports (Tainika et al., 2024a, b) provide details on birds, housing, and management from three to 52 weeks of hen age. Briefly, Lohmann LSL Classic (LW, white eggshell color) and Lohmann Sandy (LS, cream eggshell color) strains were under the same housing conditions regarding stocking densities, photoperiod, number of drinkers and feeders, etc. The study followed the vaccination program as required in the region.
There were a total of 26 replicate pens at the same poultry house, each with 10 hens. The birds were designated for the outdoor plant-based housing environment (18 replicate pens), with free access to the respective outdoor pens cultivated with either *Mentha piperita* (*M. piperita*), *Petroselinum crispum* (*P. crispum*), or *Medicago sativa* (*M. sativa*). For range accessibility, the pop holes were opened every day between 8:30 a.m. and 3:30 p.m. from 12 weeks of hen age. The other eight replicate pens were for completely indoor (deeplitter, DL) housed birds.
Specifically, during the egg-laying period, the birds were fed standard feed *ad libitum* composed of 17 % CP; 2700 Kcal ME/kg; 3.9 % Ca, and 0.4 % P).

Data collection

One hundred and fifty-six eggs (78 eggs per strain; 6 eggs per replicate pen) were used to assess the eggshell color and yolk fatty acid profiles at 25 weeks of hen age.

Eggshell color determination: In the laboratory, all the eggs were first weighed (0.01 g precision weighing scale) and their shell color was determined as follows. Color differences (shell color, ΔE*) were calculated using the L*, a*, and b*. $\Delta E^* = (L^2+a^2+b^2)^{½}$. The lightness (L*), redness (a*), and yellowness (b*) of the shell were determined with a Minolta CR400 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). ΔE* was determined as the average of three L*, a*, and b* measurements that were recorded at the center, blunt edge, and pointed edge of the egg.

Assessment of fatty acid profiles: To determine the egg yolk fatty acid compositions, Folch et al. (1957) and Samman et al. (2009) procedures were followed. The yolk fatty acid profiles were expressed as a percentage of total fatty acids.

Statistical analysis: During data analysis, the normality assumptions of the data were examined using the Kolmogorov-Smirnov test, and tests for homogeneity of variance were conducted using the Levene test, confirming that the assumptions were met. Accordingly, an analysis of variance was applied to the data. Intra-group multiple comparisons utilized Duncan`s multiple comparison test at $P<0.05$. The statistical software package SPSS 21 was used for data analysis. The following statistical model was used for the analysis of the data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$$

In the model, Y_{ijk}: observation value, μ: population mean, α_i: the effect of the i. housing environment, β_j: the effect of the j. strain, $\alpha\beta_{ij}$: the effect of interaction and e_{ijk}: the effect of random error $e \sim N(0, \sigma^2)$.

Results

The results for eggshell color are indicated in Table 1. As expected, shell color differed between strains. Also, there was a significant variation regarding the shell color: redness (a*) and yellowness (b*) at the broad end, lightness (L*), a*, b*, and shell color (ΔE*) at the center and pointed end (P<0.01; P<0.05) among the housing environments. It was identified that the overall ΔE* was significantly highest in eggs collected from *M. sativa* and *P. crispum* hens and lowest in eggs from *M. piperita* hens.

Table 1. Effect of strain and housing environment on shell color in laying hens at 25 weeks of age.

		Broad end					Center				Sharp end				
	F	EW	*L**	*a**	*b**	Δ*E**	*L**	*a**	*b**	Δ*E**	*L**	*a**	*b**	Δ*E**	A
F1	DL	55.36	84.45	4.56^{b}	13.14^{b}	86.43	84.90^{ab}	4.42^{b}	13.23^{b}	86.87^{b}	84.45^{a}	5.05^{b}	13.63^{b}	84.92^{a}	86.07^{ab}
	Pc	55.47	85.78	3.42^{a}	11.50^{a}	87.23	86.04^{b}	3.26^{a}	11.36^{a}	87.41^{b}	86.35^{c}	3.68^{a}	11.72^{a}	86.63^{c}	87.09^{b}
	Mp	55.24	84.78	4.01^{ab}	11.62^{a}	86.41	83.75^{a}	3.77^{a}	11.44^{a}	85.30^{a}	84.98^{ab}	4.31^{a}	12.02^{a}	85.35^{ab}	85.69^{a}
	Ms	55.86	85.74	3.55^{a}	11.31^{a}	87.25	85.78^{b}	3.45^{a}	11.37^{a}	87.29^{b}	86.12^{bc}	3.96^{a}	11.71^{a}	86.46^{bc}	87.00^{b}
	P	0.741	0.066	<0.001	<0.000	0.186	<0.005	<0.001	<0.000	<0.002	<0.006	<0.000	<0.000	<0.007	<0.010
F2	LS	55.90	76.89	8.41	20.63	80.16	87.32	8.09	20.42	80.50	87.15	9.15	21.31	77.88	79.51
	LW	55.05	93.37	-0.54	3.34	93.44	92.88	-0.54	3.49	92.96	93.64	-0.53	3.44	93.66	93.35
	P	<0.049	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000
	SEM	0.20	0.70	0.38	0.72	0.57	0.67	0.37	0.71	0.55	0.70	0.41	0.74	0.67	0.58
	HE × HS	0.628	<0.036	<0.001	0.102	0.113	<0.039	<0.002	<0.031	0.102	<0.018	0<.001	<0.036	<0.021	<0.028

Abbreviations: F: Factor; F1: Housing environment (HE); F2: Hen strain (HS); DL: deep litter, *Pc*: *Petroselinum crispum*, *Mp*: *Mentha piperita*, *Ms*: *Medicago sativa*, P: P value; A: Average ΔE*; LS: Lohmann Sandy, LW: Lohmann LSL Classic, SEM: standard error of mean, ΔE*: shell color, L*: lightness, a*: redness, b*: yellowness, ×: interactions between different factors. EW: Egg weight, g; Means within the same column with different letter superscript significantly differ (P<0.05).

The data for the yolk fatty acid compositions are shown in Table 2. There was no significant effect of hen strain on yolk fatty acid profiles (P>0.05). However, housing environment affected the yolk fatty acid compositions: DL and *P. crispum* eggs had significantly lower palmitic (C16:0), stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6), and alpha-linolenic (C18:3n3) acids than *M. piperita* and *M. sativa* eggs (P<0.01). On the other hand, myristic (C14:0) and arachidonic (C20:4 n-6) fatty acid compositions were not different among the housing environments (P>0.05). The housing environment significantly influenced the total saturated fatty acids (SFA) and total unsaturated fatty acids (UFA) (P<0.05): while the *M. sativa* eggs had the highest and lowest total SFA and total UFA, the *P. crispum* eggs had the lowest and highest total SFA and total UFA. There was a significant housing environment × hen strain interaction effect on palmitic (C16:0), stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6), and alpha-linolenic (C18:3n3) acids.

Table 2. Effect of strain and housing environment on yolk fatty acid composition (%) in laying hens at 25 weeks of age.

Factor		Palmitic (C16:0)	Stearic (C18:0)	Myristic acid (C14:0)	Arachidonic acid (C20:4 n-6)	Oleic acid (C18:1n9c)	Linoleic acid (C18:2n6)	Alpha linolenic acid (C18:3n3)	Total saturated fatty acids	Total unsaturated fatty acids
F1	DL	18.62^{a}	5.57^{a}	2.53	1.44	38.18^{a}	14.63^{a}	1.42^{a}	32.39^{ab}	67.61^{bc}
	Pc	18.27^{a}	5.63^{a}	2.66	1.40	39.23^{a}	14.10^{a}	1.39^{a}	32.12^{a}	67.88c
	Mp	21.19^{b}	6.05^{b}	2.62	1.39	42.26^{b}	15.74^{b}	1.53^{b}	32.86^{bc}	67.14^{ab}
	Ms	21.23^{b}	6.00^{b}	2.58	1.39	41.42^{b}	15.55^{b}	1.54^{b}	33.18^{c}	66.82^{a}
	P	<0.000	<0.001	0.218	0.701	<0.000	<0.000	<0.000	<0.015	<0.015
F2	LS	19.89	5.77	2.61	1.40	40.23	15.02	1.47	32.68	67.32
	LW	19.58	5.82	2.58	1.42	39.99	14.94	1.46	32.56	67.44
	P	0.893	0.319	0.564	0.613	0.776	0.718	0.902	0.957	0.957
	SEM	0.259	0.054	0.024	0.016	0.365	0.151	0.015	0.129	0.129
	HE × HS	<0.000	<0.001	0.866	0.211	<0.000	<0.000	<0.000	<0.008	<0.008

Abbreviations: F: Factor; F1: Housing environment (HE); F2: Hen strain (HS); DL: deep litter, *Pc*: *Petroselinum crispum*, *Mp*: *Mentha piperita*, *Ms*: *Medicago sativa*, P: P value; LS: Lohmann Sandy, LW: Lohmann LSL Classic, SEM: standard error of the mean, ×: interactions between different factors, Means within the same column with different letter superscript significantly differ (P<0.05).

Discussion

The results for the strain differences regarding shell color were expected because the LS strain lays cream-shell eggs, and the LW strain lays white-shell eggs, which aligns with the genetic influence on the coloration of shell (Samiullah et al. 2015). Furthermore, Lordelo et al. (2020) reported shell color differences among Amarela, Branca, Pedres, and Preta breeds and Tetra brown commercial hybrid laying hen.

In the present study, the effect of housing environment on shell color would reveal that the specific ingested bioactive compounds obtained from intake of specific outdoor plants can modify the physiological and biochemical characteristics of various pigments in laying hens differently and consequently, the extent of shell pigment deposition (Samiullah et al. 2015). Additionally, the present results confirmed that the extent of pigment deposition across the egg is not uniform and might be modulated by the bird's responsiveness to dietary manipulation with free access to different outdoor vegetated areas. Moreover, in the present study, the housing system and strain interaction effect on shell color highlights the pattern of changes in the coloration of eggshells between hybrids under different housing systems.

In this study, the major fatty acids in egg yolks were oleic (C18:1n9c), linoleic (C18:2n6), and palmitic (C16:0), which is similar to results reported by some authors (Lopez-Bote et al., 1998; Sarıca et al., 2009; Lordelo et al., 2020).

The variation in palmitic (C16:0), stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6), and alpha-linolenic (C18:3n3) fatty acids would indicate that these fatty acids were receptive to dietary manipulation with free access to different outdoor forage plants. However, the responsiveness was much higher with access to *M, sativa* and *M. piperita* than with *P. crispum*. These results would suggest that (i) the intake of *M. sativa* and *M. piperita* was higher than *P. crispum* and consequently, a higher intake of bioactive compounds that modified the above fatty acids and (ii) *M. sativa* and *M. piperita* might be associated with a higher concentration of specific bioactive components that would modulate the above yolk fatty acids (Mugnai et al., 2014; Dal Bosco et al., 2016).

Indeed, some authors have reported variability in fatty acid compositions based on the differences in outdoor plant species (Karsten et al., 2010; Lopez-Bote et al., 1998; Popova et al., 2020; Mierliță, 2020; Islam et al., 2021). Anderson (2011) also found more unsaturated fatty acids and higher levels of n-3 fatty acids in free-range eggs than in cage eggs. On the contrary, the lack of differences in myristic (C14:0) and arachidonic (C20:4 n-6) fatty acids would suggest that these specific fatty acids did not respond to dietary manipulation with free access to different plant compositions.

Several authors have also reported changes in total SFA and total UFA due to the housing environment (Popova et al., 2020; Mierliță, 2020; Islam et al., 2021).

In the present study, the lack of strain differences in the fatty acid compositions would agree with Lordelo et al (2020), who found similar fatty acid profiles in eggs obtained from Amarela, Branca, Pedres, and Preta breeds and tetra brown commercial hybrid laying hen. In contrast, several authors reported genetic influence on some fatty acid compositions. For instance, Sarıca et al. (2009) identified significant differences in some fatty acids in two local Turkish breeds and a commercial laying hen strain. Sözcü et al. (2021) found that the yolk saturated fatty acids profile, except C15:0, the total yolk SFAs, and yolk UFAs, except C18:1c, C18:2c, and C20:3n6 differed between Atabey and Atak-S genotypes. The contradiction in results is associated with different factors including strains, feeding, housing system and environment, study region, age of hens at which fatty acid analysis was conducted, etc.

In the present study, the significant housing environment × strain interaction effect on some yolk fatty acids would indicate a pattern of changes in these fatty acid compositions as laying hens react to the different housing environmental circumstances.

Conclusions

This study demonstrated that eggs may be enriched with the major fatty acids with free access to outdoor areas vegetated with *M. piperita* and *M. sativa* plant species. In addition, *M. sativa* and *P. crispum* plant species seem to have the potential to modify the shell color intensity.

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