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The effects of *Rumex patientia* leaf powder in laying hens' diets on egg physical parameters and yolk color

Cristina-Camelia Matache[1], Tatiana Dumitra Panaite*[1], Gabriela Maria Cornescu[1], Mara-Ioana Munţiu- Rusu[1] and Vasile Bunduc[2]
*corresponding author: tatiana.panaite@ibna.ro

[1]National Research Development Institute for Animal Biology and Nutrition (IBNA), no. 1, Calea Bucuresti, 077015 Balotesti, Ilfov, Romania, e-mail address: secretariat@ibna.ro
[2]SC Avicola Lumina SA, Street Tulcei no. 3, 907175, Lumina, Constanta, Romania, e-mail address: office@avicolalumina.ro.

ABSTRACT

The experiment studied the effects of different levels of *Rumex patientia* spp. leaf powder inclusion in laying hens' diets on their physical parameters and yolk color intensity. The study was conducted on 168 Hy-line laying hens (30 weeks aged), assigned in 3 treatment groups (C, E1 and E2) and housed in an experimental hall equipped with Big Dutchman cages, placed on 3 tiers with digestibility cages (3 treatments x 28 replicates x 2 birds per cage). Feed and water were administrated *ad-libitum*. During the entire 5 weeks experimental period (one week of accommodation), performance parameters were recorded: average daily feed intake(g/day/hen), feed conversion rate (g feed/g egg), laying rate intensity (%) and average egg weight (g). Eggs yolk color is generated by carotenoids as a nutritional response, which depends on their amount, type, and ratio. One of the most important aspects in how consumers rate the quality of food is color. The experimental results demonstrated that an inclusion rate of 1.5% and 3% *Rumex patientia* species leaves powder improved the yolk color intensity without negative effects on production performances and egg quality parameters.

Keywords: egg quality; laying hens; performance; *Rumex patientia* species leaves; yolk color.

INTRODUCTION

Nowadays, scientists are currently looking into readily available, inexpensive natural compounds with high nutrient content that improve animal health without affecting their productivity.

Considered an excellent protein quality source, eggs are already regarded as functional food, obtained easily by dietary nutritional modulation beneficial to improve consumers' health, without affecting poultry welfare.

Due to their significance in preventing and lowering cataracts incidence and age-related macular degeneration, eggs are a significant natural source of carotenoids in the human diet, particularly the xanthophylls lutein and zeaxanthin (Mrowicka et al., 2022). The dietary carotenoids influence the yolk´s colour depending on their amount, type, and ratio (Kojima et al., 2022). One of the most important aspects in how consumers´ evaluates the food quality is colour; when considering eggs, the yolk colour, along with shell colour, taste, and appearance, represents one of the most important factors that influences consumers´ behaviour, preferences, and preferences for eggs. (Berkoff et al., 2020).

Laying hens are unable to synthesize pigments through their own biochemical processes, therefore the egg yolk colour is determined by the presence and utilization of dietary pigments (Spasevski et al., 2018).

The hens' diet is supplemented with various carotenoids to achieve the desired egg yolk coloration, especially when low carotenoid feeding ingredients are used. Because many synthetic pigments have been shown to have negative effects on human health, producers placed a lot more emphasis on food colours obtained by using natural pigments (Li et al., 2012).

Genus *Rumex*, family *Polygonaceae* includes about 200 species of very common annual, biennial, and perennial herbal worldwide distributed. These wild plants are harvested mostly from their natural habits and offers a viable economic alternative as an accessible and an affordable source of nutrients for farm animals, therefore *Rumex* spp. is widely used as food, or as a medicinal herb (Li et al., 2022). Idris et al. (2019) revealed that according to proximal chemical assessment, the vitamins, minerals, and essential oils content found in the root and leaves of various Rumex spp, the plant can be taken into consideration an appropriate supplemental diet ingredient. To date, chemical analyses of these wild herb reported important bioactive components: 56 quinones were isolated from 17 *Rumex* spp., 57 flavonoids, 25 tannins, 6 stilbenes, 22 naphthalene's, 6 terpenes, 3 diterpene alkaloids, 14 lignans and 79 other components (Li et al., 2022). Also, using this phytoadditive into monogastric diets could represent a method of biological control of this wild herb (Ringselle et al., 2019). In the literature search we found several studies concerning antibacterial activity of *Rumex* species – such as *R. nervosus L*., *R. crispus L*. *R. japonicus L*., *R. alveollatus L*.– used for inhibitory activity against microorganisms (Ulukanli et al., 2005; Elzaawely et al., 2005; Mohammadi-Sichani et al., 2012).

More than 14 *Rumex* spp. can be consumed fresh or cooked as vegetables and are considered a valuable functional food in certain world countries, but there is little knowledge about using *Rumex* spp. in poultry diets.

According to Teleuţă et al., (2008), *Rumex patientia* is widespread in Eastern Europe, especially in Romania, Bulgaria, Bosnia and Herzegovina, Moldova, North Macedonia and Serbia, not only as traditional food ingredient but also as feed for sheep and goats. Despite its utilization in human and small ruminants' nutrition, we noticed a significant gap information concerning *Rumex* spp. usage in monogastric.

Consequently, our knowledge of *Rumex* spp. being largely based on very limited data, the purpose of this study was to test two different levels of *Rumex patientia* spp. into laying hens' diet and their effects on egg physical parameters and yolk colour intensity.

MATERIALS AND METHODS

The experiment was conducted for 5 weeks using 168 HyLine laying hens (30 weeks aged) within IBNA Balotești-Animal Physiology and Nutrition Laboratory. Experimental procedures were approved by the Ethical Committee of the National Research Development Institute for Biology and Animal Nutrition, in accordance with the Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, law 43/11.04.2014, Directive 2010/63/EU). The layers were assigned to 3 groups: control (C), experimental group 1 (E1) and experimental group 2 (E2), 56 birds/group, 28 cages/group; 2 birds/cage, respectively.

Feed and water access was provided ad-libitum. Control group was administrated a standard diet (16,39% PB; 2750 Kcal EM/kg compound feed), and the experimental diets included: 1.5 % (E1) and 3% (E2) Rumex patientia spp. leaves powder (table 1). *Rumex patientia* leaves were first dried, finely ground and then added into the compound feed.

The following productive parameters were registered throughout the entire experiment: average daily feed intake (ADFI; g/hen/day); feed conversion rate (FCR; kg feed/kg egg); laying rate intensity (LRI; %); average egg weight (AEW; g). Daily, the average feed intake parameter was calculated as the difference between the feed amount administrated and the feed leftovers unconsumed. The feed conversion rate production parameter was expressed as kg of feed consumed per kg of egg produced. Throughout the entire experimental period, the egg production was recorded and egg collection was realized at 11.00 a.m., daily. The laying rate intensity parameter was calculated as the eggs number laid divided by the number of laying hens.

Table 1. Diet formulation and proximal analysis results

Specifications	Inclusion rate of Rumex leaves powder		
	C	E1 1.5 % RL	E2 3% RL
Corn, %	45.57	44.63	43.8
Wheat, %	18	18	18
Sunflower meal, %	4.9	5	5
Soyabean meal, %	11.1	10.3	9.5
Rumex leaves powder, %	-	1.5	3
Gluten, %	5	5	5
Vegetal oil, %	3.4	3.5	3.6
Monocalcium phosphate, %	1.1	1.1	1.1
Calcium carbonate, %	9.3	9.3	9.3
Salt, %	0.2	0.2	0.2
Methionine, %	0.11	0.12	0.12
Lysine, %	0.27	0.3	0.33
Choline, %	0.05	0.05	0.05
Premix*, %	1	1	1
Calculated analysis			
Metabolizable energy, kcal/kg	2800	2855.44	2856.52
Crude protein, %	16.39	15.94	15.93
Ether extract, %	5.18	5.6	6.67
Crude fiber, %	4.64	4.38	5.88
Lysine, %	0.83	0.84	0.84
Methionine, %	0.41	0.41	0.41
Met.+cist, %	0.72	0.72	0.70
Calcium, %	3.90	3.90	3.90
Phosphorus, %	0.67	0.67	0.66

Note: C – Control diet; 1.5% RL - 1.5% *Rumex patientia* spp. leaf powder; 3% RL - 3% *Rumex patientia* spp. leaf powder;

*1kg premix contains: 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg Vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg Vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium

During the entire experimental period, a total of 168 eggs were collected to evaluate the internal and external egg parameters. At the beginning of the study 6 eggs were measured, then, at 2 weeks and 4 weeks period, 18 eggs/group were analyzed to evaluate the internal and external egg parameters. For the assessment of storage period, a total of 54 eggs, 9 eggs/group/period, were collected at the end of the experiment, and kept for 14 and 28 days, at 20 ± 1.2^{0}C room temperature to determine if dietary *Rumex* spp. leaf powder (1.5 vs. 3%) influenced the internal and external eggs' parameters during the time exposure.

Digital Egg Tester DET-6500 (NABEL Co., Ltd., Kyoto, Japan) was used to evaluate eggs' components quality parameters: whole egg weight (g), eggshell breaking strength (kgF), eggshell thickness (mm), albumen height (mm), yolk height (mm), Haugh unit (value), freshness egg, (%), yolk diameter (mm), yolk index, yolk colour. The breaking load, for eggshell strength is measured at a pressing speed of 0,5 mm/s. The Haugh unit (HU) score was estimated based on the albumen height and egg weight, as follows: HU = 100 x log (H-1.7W0.37+7.6), where H = albumen height (mm), W= egg weight (g).

The higher the HU value the better the egg quality (AA: ≥ 72, A: 71-60, B: 59-31, C: ≤ 30). The yolk index is determined as follows: YI: YH/YD, where YH= yolk height (mm), YD= yolk diameter (mm).

The egg white, egg yolk and egg shell were sampled separately and weighed with Kern&Sohn GmbH, EMB 3000-1 Balingen, Germany, 0.1 g precision. The egg shell thickness, with the concave side down, was measured using a digital micrometer (Mitutoyo 547-400S ABSOLUTE Digimatic Thickness Gauge 0-12mm / 0-.47" Mitutoyo, Japan). The albumen and yolk pH were measured using a portable pH meter (Five Go F2-Food kit with LE 427IP67, Sensor Mettler Tolledo, Greifensee, Switzerland).

The yolk colour is calculated based on the DSM's YolkFanTM evaluated by sensory inspection in diffused light (to determine an accurate colour measurement, DET6500 uses natural light, because artificial light varies much between regions depending on its light source). In addition, the egg yolk colour (parameters L*, a* and b*) was determined using the portable colorimeter 3nh YS3020 (Shenzhen Threenh Technology Co., Ltd, Beijing, China) with customized aperture (8mm/4mm/1×3mm), 2.6s measuring time, high accuracy of 0.04, with an observer angle of 2°/10° using the CIE-Lab system (Commission Internationale de l'Eclaraige).

The analytical data were compared by variance analysis (ANOVA) procedure using a linear model (Yij =μ + Aj + eij) at SPSS version 20 (Inc., Chicago IL, USA), where, yij = response variable, μ = the overall mean, aj = the effect treatment, εij = the random error terms. The difference between the means was considered significant at $p < 0.05$. The results were expressed as mean and standard error of mean (SEM).

RESULTS AND DISCUSSION

The results concerning the influence of two different levels of dietary *Rumex patientia* leaves powder inclusion on productive parameters were presented in table 2. The parameters were measured at 2 weeks and 4 weeks, respectively. There were no statistical differences ($p \geq 0.05$) registered between groups. On the other hand, statistically significant differences ($p \leq 0.01$) were noticed on the average daily feed intake for all groups at 2 weeks compared to 4 weeks period collection.

Table 2. The influence of *Rumex patientia* spp. on laying hen productive performances during the experiment (average values/group).

Specification		ADFI (g/hen/day)	FCR (kg feed/kg egg)	LRI (%)	AEW (g)
2 weeks	C	109.89	1.82	82.69	60.15
	E1	110.79	1.82	84.31	60.71
	E2	109.27	1.80	86.73	60.62
4 weeks	C	101.71	1.68	82.76	61.09
	E1	103.52	1.72	84.56	61.32
	E2	108.16	1.79	88.68	61.56
1 to 4	C	105.80	1.75	82.72	60.62
weeks	E1	107.16	1.77	84.43	61.01
	E2	108.71	1.79	87.71	61.09
Mean		107.22	1.77	84.96	60.91
SEM		1.03	0.02	1.23	0.22
Main effect					
Period					
2 weeks		109.98[a]	1.81[a]	84.58	60.49
4 weeks		104.46[b]	1.73[b]	85.33	61.32
1 to 4 weeks		107.22[ab]	1.77[ab]	84.96	60.91
Group					
C		105.80	1.75	82.72	60.62
E1		107.16	1.77	84.43	61.01
E2		108.71	1.79	87.71	61.09
p-value					
period		0.01	0.01	0.76	0.06
group		0.52	0.55	0.25	0.64
period * group		0.32	0.27	0.94	0.93

Note: C – Control diet; 1.5% RL - 1.5% *Rumex patientia* spp. leaf powder; 3% RL - 3% *Rumex patientia* spp. leaf powder; ADFI - average daily feed intake (g/hen/day); FCR - feed conversion rate (kg feed/kg egg); LRI - laying rate intensity (%); AEW - average egg weight (g).); a,b,Means within column with different superscripts differ significantly, SEM, standard error of the mean; P-value, means significantly differences ($p \leq 0.05$).

The FCR parameter registered a better statistically significant ratio for all groups ($p \leq 0.01$) at 4 weeks period collection compared to 2 weeks period collection. Concerning laying intensity rate and average egg weight, no significant differences ($p \geq 0.05$) were observed neither on 2 weeks, nor on 4 weeks period. Also, no statistical differences ($p \geq 0.05$) were noticed between the period and group interaction.

There are scarce information concerning *Rumex* spp. inclusion effects on laying hens' productive performances. However, we found broiler studies of *Rumex nervosus* leaves meal (RN) dietary inclusion of 1000, 3000 and 5000 mg/kg which registered a significant improvement of body weight ($p = 0.016$) on the 10 days age (Azzam et al., 2020). When RN was added up to 3000

mg/kg, again a significant increased body weight (p = 0.003) was noticed on 14 days of age compared to control group. Feed conversion ratio registered a significant value when adding 1000 mg/kg RN (p = 0.016) in broilers' diet (Azzam et al., 2020). Qaid et al. (2021) supplemented with 1, 3, and 5 g of RNL/kg on broiler chicken diets infected with *Eimeria tenella* to evaluate its effect on performance indices, carcass trait, and meat quality. Within the second week after infection, RNL dietary inclusion compensated for the growth losses caused by *Eimeria tenella* and a significant improvement (p < 0.05) was noticed for body weight gain, feed conversion rate, cumulative feed intake, production efficiency factor compared to an infected/unmedicated group.

Table 3. The influence of *Rumex patientia* spp. on egg weight and its components during the experiment (average values/group)

Specification		Egg weight and components					
		Whole egg weight (g)	**Albumen (g)**	**Yolk (g)**	**Egg shell (g)**	**Shell thickness (mm)**	**Breaking strength (kgF)**
Initial		61.14	40.17	13.98	7.00	0.393	4.61
2 weeks	C	59.59	38.09	14.40	7.11	0.370	4.24
	E1	59.75	38.51	14.31	6.93	0.371	4.52
	E2	60.03	38.62	14.48	6.93	0.364	4.31
4 weeks	C	61.81	40.46	14.46	6.89	0.361	4.31
	E1	61.48	39.86	14.69	6.93	0.359	3.85
	E2	61.14	39.21	14.73	7.20	0.378	4.97
Mean		60.71	39.27	14.44	7.00	0.376	4.40
SEM		0.11	0.13	0.09	0.03	0.002	0.07
Main effect							
Period							
initial		61.14^{a}	40.17^{a}	13.98^{b}	7.00	0.402^{a}	4.61
2 weeks		59.79^{b}	38.41^{b}	14.39^{a}	6.99	0.369^{b}	4.36
4 weeks		61.48^{a}	39.85^{a}	14.63^{a}	7.01	0.366^{b}	4.38
Group							
Initial		61.14	40.17^{a}	13.98	7.00	0.393^{a}	4.61^{ab}
C		60.70	39.27^{b}	14.43	7.00	0.381^{ac}	4.27^{b}
E1		60.61	39.19^{b}	14.50	6.93	0.365^{b}	4.19^{b}
E2		60.59	38.92^{b}	14.61	7.07	0.371^{bc}	4.64^{a}
p-Value							
period		≤0.001	≤0.001	0.05	0.22	0.000	0.89
group		0.92	0.56	0.31	0.74	0.029	0.03
period * group		0.16	0.04	0.03	0.78	0.065	0.00

Note: C – Control diet; 1.5% RL - 1.5% *Rumex patientia* spp. leaf powder; 3% RL - 3% *Rumex patientia* spp. leaf powder; a-b,Means within column with different superscripts differ

significantly; SEM, standard error of the mean; P-value, means significantly differences ($p \leq 0.05$).

Table 3 showed the influence of *Rumex patientia* spp. on egg weight, its components, and eggshell traits (thickness and breaking strength force). The whole egg and albumen weight were highly significant ($p \leq 0.001$) on initial and on the 4*th* week period compared to the 2nd week collection period. The yolk weight for all groups were significantly higher ($p \leq 0.05$) on the 2nd collection week period and on the 4th week period of the experiment compared to the initial period. Concerning eggshell thickness highly significant ($p \leq 0.001$) thinner shells were observed during the 2nd week and the 4th week period of the experiment compared to the initial period. Also, albumen weight was significant higher ($p \leq 0.05$) at initial group compared to C, E1, E2 groups. The eggshell thickness value was significant higher ($p \leq 0.029$) at initial compared to E1, E2, while the C group values were significant higher ($p \leq 0.05$) compared to E1 group. Breaking strength force registered significant higher ($p \leq 0.03$) values at initial and E2 compared to C, E1 groups. Eggshell thickness and breaking strength force, like other egg quality parameters, depend greatly of various environmental factors, laying hen age/strain/nutrition. Lack of data was noticed when searching similar studies to compare our results.

In Table 4 are presented the internal quality parameters of eggs during the entire experimental period. Some authors (Dong et al., 2017; Nematinia and Mehdizadeh, 2018) consider pH albumen to be a superior parameter, a better indicator of egg freshness compared to the Haugh unit. In the same time, the higher values of pH albumen depend mainly on the buffering capacity of the albumen and the temperature (Akter et al., 2014).

In this study, the pH values of the albumen parameter registered a significantly higher values ($p \leq 0.001$) when measured at 2nd week compared to 4th experimental week. Within groups' comparison, the pH albumen measured registered significant higher values ($p \leq 0.05$) for experimental (E1, E2) groups compared to C group reported for the both periods. The same observation was noted for yolk diameter parameter which registered a significant higher value ($p \leq 0.05$) for E1, E2 groups compared to initial and C group both for 2nd and 4th experimental week.

For Haugh units were no significant differences ($p \geq 0.05$) between the periods or the groups. The values of Haugh units measured during the experiment were similar with those obtained by Menezes et al., (2012) which noticed that the poultry age had a major influence on Haugh units registered values.

Table 4. The influence of *Rumex patientia* spp. on internal quality parameters of eggs during the experiment (average values/group)

Specification		Albumen			Yolk			
		pH-albumen	Albumen height	Haugh Units	pH-yolk	Yolk height	Yolk diameter	Yolk index
Initial		8.50	8.04	88.25	6.45	17.77	38.06	0.49
2 weeks	C	8.48	7.49	85.31	6.52	17.46	38.24	0.46
	E1	8.62	7.11	82.77	6.44	17.59	39.40	0.45
	E2	8.65	7.89	88.46	6.52	18.17	40.23	0.45
4 weeks	C	8.33	7.81	87.59	6.44	18.08	39.00	0.46
	E1	8.54	7.43	84.62	6.52	18.02	39.66	0.46
	E2	8.56	7.82	87.63	6.59	18.26	39.20	0.47
Mean		8.53	7.66	86.38	6.50	17.91	39.11	0.46
SEM		0.02	0.14	0.93	0.02	0.13	0.20	0.01
Main effect								
Period								
Initial		8.50^{ab}	8.04	88.25	6.45	17.77	38.06	0.49^{a}
2 weeks		8.59^{a}	7.50	85.51	6.49	17.74	39.29	0.45^{b}
4 weeks		8.48^{b}	7.69	86.61	6.52	18.12	39.29	0.46^{b}
Group								
Initial		8.50^{ab}	8.03	88.25	6.45	17.77	38.06^{b}	0.49^{a}
C		8.41^{b}	7.65	86.45	6.48	17.77	38.62^{b}	0.46^{b}
E1		8.58^{a}	7.27	83.70	6.48	17.81	39.53^{a}	0.45^{b}
E2		8.61^{a}	7.86	88.04	6.56	18.21	39.71^{a}	0.46^{b}
p-Value								
period		0.01	0.51	0.59	0.50	0.18	1.00	0.21
group		≤0.001	0.25	0.20	0.22	0.36	0.05	0.05
period * group		0.81	0.81	0.79	0.23	0.73	0.22	0.86

Note: C – Control diet; 1.5% RL - 1.5% *Rumex patientia* spp. leaf powder; 3% RL - 3% *Rumex patientia* spp. leaf powder; a,b,Means within column with different superscripts differ significantly; SEM, standard error of the mean; P-value, means significantly differences ($p \leq 0.05$).

For the yolk index parameter, the value registered before the start of the experiment was significantly ($p \leq 0.05$) higher compared to those obtained for all groups (C, E1, E2) both at 2nd and 4th experimental weeks. It is important to highlight that the yolk index parameter offers information about the degree of egg freshness (≥ 0.38 extra fresh eggs, 0.28 - 0.38 fresh eggs, ≤ 0.28 regular eggs), but the value will decrease simultaneously with the storage period and temperature .

Regarding the storage time evaluation (Figure 1), the main parameters analysed were egg weight, egg freshness measured by the Haugh unit (the reference parameter used to evaluate eggs' freshness) and albumen and yolk pH. Data shown in Figure 1 indicates that the storage period increasing to 28

days in room temperature conditions (up to 20 ± 1.2°C) resulted in a significant deterioration of egg quality.

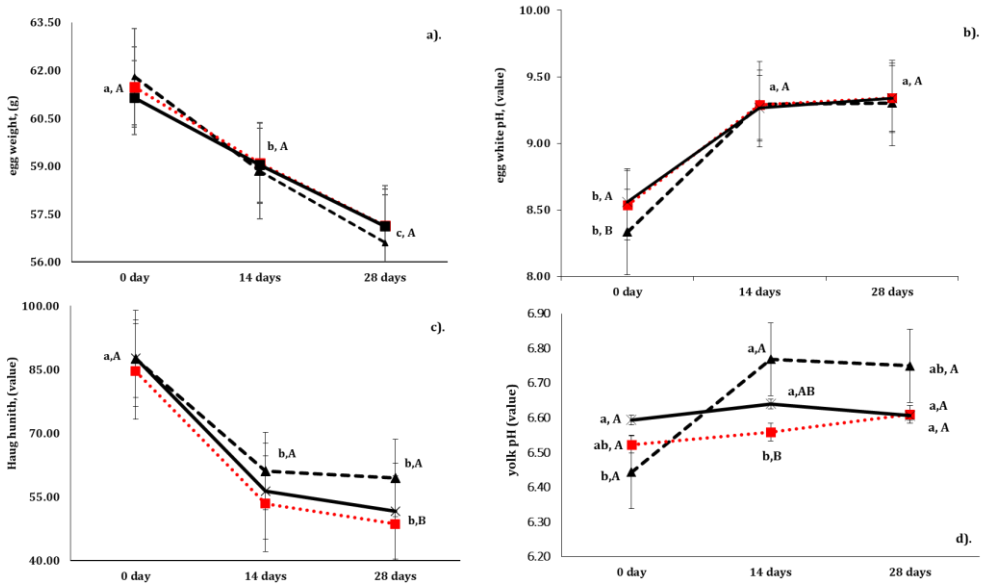


Figure 1. Storage time evolution (0, 14 and 28 days) on freshness parameters: a) egg weight (g); b) albumen pH; c) Haugh Units (HU) and d) yolk pH, according to the dietary treatments: control (▲), experimental E1 (■) and experimental E2 (×); a,b,c - Mean values within a period not sharing the same superscripts are significantly different at P ≥ 0.05; A,B,C - Mean values between a group not sharing the same superscripts are significantly different at P ≥ 0.05

Thus, the effects storage period and temperature interaction were significant for egg weight loss parameter. The data presented in fig. 1a shown a significant decreasing ($p \leq 0.0001$) during time (0 to 28 storage days), of the egg weight recorded for all groups (C, E1, E2), but without statistical significance ($p = 0.225$; $p = 0.912$; $p = 0.600$) between groups for reference periods (0, 14 and 28 days). In the study carried out by Samli et al., (2005) it is shown that the weight of the egg decreased significantly ($p<0.001$) after 5 and 10 days respectively, storage period temperature at 29°C.

To assess the albumen quality (fig. 1b), the study's' results shown, that pH albumen values increased significant ($p < 0.0001$) between 0 day and 14 days for all groups, remaining constant until day 28. No significant differences were recorded between experimental and control groups for any of the analysed periods (both at 14 and 28 days of storage). Similar results were obtained by Jin et al., (2011), when the pH albumen increased from 8.01 to 9.72 at the storage temperature of 29°C.

Significant pH changes were noticed for egg yolk (fig. 1d). In relation to the storage period (from 0 to 28 days), it is observed that after 14 days of storage at 20 ± 1.2°C, the values registered on experimental groups were significantly lower ($p \leq 0.05$) compared to C group. Concomitantly, group C registered a rapid increasing of pH yolk determined at 14 days, consistently maintained until the 28th day, while for the experimental groups, the yolk quality depreciation was slower, which led to the hypothesis that the *Rumex patientia* powder inclusion in hens 'diets delayed the depreciation rate of yolk, probably due to the high bioactive components (antioxidants, polyphenols, xanthophylls) of *Rumex patientia* (unpublished data).

Similar effects on yolk or albumen pH significantly affected by storage time was reported by other researchers (Samli et al., 2005; Akyurel and Okur, 2009; Garcia et al., 2010; Caner and Yüceer, 2015).

As expected, Haugh unit values decreased statistically ($p < 0.0001$) simultaneously with the storage period at 20°C for all groups (C, E1, E2) from 0 to 28 days (fig. 1c). If at 14 days no significant differences were recorded between all groups ($p = 0.460$), at 28 days the experimental groups (E1, E2) showed significantly ($p \leq 0.0001$) lower values compared to group C. Similar results were reported by Jin et al., (2011), where the Haugh unit decreased from 91.3 to 72.63 during 10 days of storage to 21°C. The storage period and temperature seem to be the most important factors affecting the albumen quality or the Haugh unit (Yeasmin et al., 2014; Liu et al., 2016; Nematinia and Mehdizadeh, 2018). Numerous studies have reported the pH of egg albumen increases simultaneously with water loss carbon dioxide (CO2) through the shell causing the rupture of electrostatic complex between lysozyme and ovomucine (Giunchi et al., 2008; Sari et al., 2016) and the osmotic exchanges between albumen and yolk through the vitelline membrane (Eke et al., 2013; Yimenu et al., 2017). Consequently, during the storage period, according to the information provided by Kumbar ´ et al., (2015), there is noticed an increasing of egg acidity, albumen liquefaction, air chamber and distension and flaccidity of yolk membrane.

Table 5 shows the influence of *Rumex patientia* spp. on yolk color intensity during the entire experiment. Using a DSM YolkFan™, a highly significant increase value ($p \leq 0.0001$) was noticed on the 2nd and the 4th weeks of egg collection compared to initial period. An increasing tendency ($p < 0.05$) of yolk color intensity was noticed between the experimental groups and initial. Concerning L* parameter (lightness from black to white, on a scale of 0 to 100) statistically significant differences ($p \leq 0.001$) were registered on C group compared to E1, E2, initial, which confirms an intense darkening of egg yolk, increasing simultaneously within the period trial, and also between groups. The a* parameter (corresponding with red color spectrum) registered highly significant different positive values between all periods ($p \leq 0.0001$).

Also, the same significant ($p \leq 0.56$) positive values of a* parameter was noticed for C, E1, E2 groups compared to initial. The b* parameter (corresponding with yellow color spectrum) had significant positive values ($p \leq 0.05$) for the 2nd and the 4th period compared to initial. If at 2 weeks the registered values for b* parameter of E1 group were higher compared to groups C and E2 (possibly, due to the high variability of b* parameter values) after 4 weeks a uniformity of the yolk color intensity was noticed.

Table 5. The influence of *Rumex patientia* spp. on yolk color intensity during the experiment (average values/group)

Specification		Yolk colour DSM	L*	a*	b*
Initial		4.14	44.60	0.11	16.16
2 weeks	C	6.06	45.29	1.02	17.54
	E1	5.94	44.91	1.17	19.64
	E2	6.44	44.65	1.10	17.92
4 weeks	C	6.39	45.83	1.56	18.54
	E1	6.06	44.63	1.21	18.07
	E2	6.50	44.56	1.33	18.43
Mean		5.78	44.93	1.07	18.04
SEM		0.08	0.09	0.04	0.10
Main effect					
Period					
Initial		4.43^{b}	44.60	0.11^{c}	16.16^{b}
2 weeks		6.15^{a}	44.95	1.09^{b}	18.37^{a}
4 weeks		6.32^{a}	45.01	1.37^{a}	18.35^{a}
Group					
Initial		4.14	44.60^{b}	0.11^{b}	16.16^{c}
C		5.72	45.56^{a}	1.29^{a}	18.04^{b}
E1		6.00	44.77^{b}	1.19^{a}	18.86^{a}
E2		6.47	44.60^{b}	1.22^{a}	18.18^{b}
p-Value					
period		0.0001	0.76	0.0001	0.92
group		0.058	0.001	0.56	0.00
period*group		0.770	0.18	0.03	0.00

Note: C – Control diet; 1.5% RL - 1.5% *Rumex patientia* spp. leaf powder; 3% RL - 3% *Rumex patientia* spp. leaf powder; L*= luminosity; a*= index saturation of color intensity in green (negative) or red (positive); b*= index saturation of color intensity in blue (negative) or yellow (positive); a,b,c Means within column with different superscripts differ significantly, $p \leq 0.05$; SEM - standard error of the mean

In general, prior research is limited to *Rumex patientia* utilization in laying hens feeding. Therefore, taking into consideration that the plant is also known as the spinach of high-altitude cold desert (Singh et al., 2013) we compared

our results with those studies when the yolk intensification color was obtained using spinach. As in case of Beyzi et. al (2022) who included 1 and 2% dried spinach leaves on laying hens diets observing an intense yolk pigmentation at both doses.

CONCLUSION

Rumex patientia spp. utilization in laying hens' diet (1.5 and 3%) improved yolk color intensity but further studies with dietary higher inclusion levels would be required. The inclusion levels tested did not influenced negative the production parameters, nor the internal and external physical egg. *Rumex patientia* spp. could represent an environmentally friendly, cheap, available natural source of pigments and a potential alternative to synthetic colorants, without harmful effects on laying hens' welfare.

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