THE EFFECTS OF DIETARY MULBERRY LEAF POWDER IN LAYING HENS' DIETS ON INTERNAL AND EXTERNAL EGG QUALITY PARAMETERS AND YOLK COLOR

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Abstract

The experiment studied the effects of different levels mulberry leaf powder (ML) inclusion in laying hens' diets on their physical parameters and yolk color intensity. The study was conducted on 120 Hy-line laying hens (age 27 weeks), divided in 3 groups C (0%), E1 (1.5%), E2 (3%), 40 laying hens/group, 10 cages/group; 4 laying hens/cage, accommodated into Zucammi-type digestibility cages. Feed and water access were provided ad-libitum. During the entire 4-wks experimental period, performance parameters were recorded: laying intensity (%), average egg weight (g), eggs classification (%). Concerning production parameters, no significant differences ($p \geq 0.05$) were registered. At the beginning, at 2nd and 4th weeks' period, 18 eggs/group were collected to determine the internal and external eggs' quality parameters. The color intensity recorded a significant increase ($p \leq 0.05$) in the experimental groups (1st and 2nd egg collection). The eggshell thickness (mm) was significant higher ($p \leq 0.05$) on E2 group (1st and 2nd egg collection) compared to E1 and C groups. Also, 18 eggs/group were colected to assess shelf-life characteristics after 14 and 28 days storage period. Significant differences ($p \leq 0.05$) were recorded for average egg weight on E1 group compared to C and E2 groups (at 14 days storage period). The experiment demonstrated that an inclusion rate of 1.5% and 3% mulberry leaves powder can significantly increase yolk color intensity without negative effects on production performances.

Key words: *egg quality, yolk color, laying hens, phytoadditiv, mulberry leaves*

INTRODUCTION

Poultry production represents among the most important livestock sectors, taking into consideration that provides the cheapest animal protein, as eggs and meat, for human consumption in the shortest period of time. The feeding costs accounts for 60 to 75% of the total poultry production cost. The unavailability and consequently high costs of conventional poultry feed have seriously affected poultry production. Therefore, finding economically cost-effective and locally available food alternatives from conventional food sources could help reduce the cost of poultry feed.

Mulberry leaves powder can be considered as possible feed alternatives for poultry with a high nutritional value (Venkatesh et al., 2015; Siddiqui et al., 2022), with a rich content of protein (15-35%), minerals (2,42-4,71%) and metabolizable energy (1.130-2.240 kcal/kg) (Sadddul et al., 2004).

Therefore, mulberry leaves could provide an affordable and accessible protein source for laying hens and broilers feeding (Al-Kirshi et al., 2009; Guha and Reddy, 2013; Mwai et al., 2021). Mulberry leaves contain β-carotene, a vitamin A precursor and also xanthophyll, which can serve as an excellent source of egg yolk pigmentation (Srivastava et al., 2006; Siddiqui et al., 2022). According to Srivastava et al. (2006), due to the β-carotene content of, it can be converted by poultry into vitamin A and xanthophile. The yolk color intensification it is a real benefit

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The manuscript was received: 05.10.2022
Accepted for publication: 02.11.2022

for the poultry industry, but also for the consumers (Mahmoud et al., 2010; Meliandasari et al., 2015).

In poultry, using dietary mulberry leaf powder has improved laying hens' performances, product quality and oxidative activity by extending the shelf - life of eggs, an important factor for farmers (Siti et al., 2019). Olteanu et al. (2015) found that dietary mulberry leaf powder supplementation improved poultry meat quality, while Chen et al. (2015) and Lin et al., (2017) suggested that by supplementing the laying hens' diet with mulberry leaf extract, alters the antioxidant profile of eggs. Also, Kamruzzaman et al., (2014), stated that mulberry leaves have an important effect in reducing yolk cholesterol, without affecting the feeding rate, growth performances and/or the eggs' quality characteristics. Al-kirshi et al., (2010), by using an extract of mulberry leaves in poultry feed, noticed a Haugh unit improvement but a weight loss of eggshell and yolk.

The purpose of this study was to evaluate the effects of different levels of mulberry leaf powder (ML) inclusion on the internal and external quality parameters of eggs and their color.

MATERIAL AND METHOD

The experiment was conducted for 4 weeks in IBNA Balotești-Animal Physiology and Nutrition Laboratory, on 120 HyLine laying hens (27 weeks). 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 1 (E1) and experimental 2 (E2), with 40 birds/group, 10 cages/group; 4 birds/cage, respectively. No mortality was registered and all hens were healthy throughout the study period. The layers had free access to the feed and water. 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) mulberry leaves powder (table 1). According to the literature, mulberry leaves contain 15–35% crude protein, 8–10% carbohydrates, 6.8-7.4 % lipids, 12–18% minerals (Al-Kirshi et al., 2009; Venkatesh et al., 2015; Wang et al., 2018), 13-20% crude fibres, β-caroten (Srivastava et al., 2006), tannins, vitamins, sterols (beta-sitosterol, campesterol), malic acid, citric acid, polifenols (0,751 mg/mL) for black mulberry, (0,437 mg/mL) for white mulberry and 1.130-2.240 Kcal/kg metabolizable energy (Saddul et al., 2004). Before being included into the compound feed structure, the mulberry leaves were dried, finely ground and then added into the feed.

Table 1 Diet formulation and chemical analysis results

Specifications	Inclusion rate of mulberry leaves powder		
	Con	1.5% ML	3% ML
Corn, %	45.57	43.90	42.00
Wheat, %	18.00	18.00	18.00
Sunflower meal, %	4.90	4.10	4.00
Soyabean meal, %	11.10	11.40	11.20
Gluten, %	5.00	5.00	5.00
Vegetal oil, %	3.40	40	4.70
Monocalcium phosphate, %	1.10	1.10	1.10
Calcium carbonate, %	9.30	9.30	9.30
Salt, %	0.20	0.20	0.20
Methionine, %	0.11	0.12	0.12
Lysine, %	0.27	0.27	0.29
Choline, %	0.05	0.05	0.05
Premix*, %	1.00	1.00	1.00
Calculated analysis			
Metabolizable energy, kcal/kg	2750	2750	2750
Crude protein, %	16.39	16.02	16.87
Ether extract, %	5.18	5.60	6.67
Crude fiber, %	4.64	4.38	5.88

Note: Con - basal die; 1.5% ML - 1.5% mulberry leaf powder; 3% ML - 3% mulberry 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 experiment, the parameters monitored were as follows: laying intensity rate (%), egg weight (g) and eggs classification in regard to Regulation (EC) No. 852/2004 on the general rules on food hygiene, as amended and supplemented, and Directive 2000/13/EC (table 2).

The laying percentage was calculated as a daily average report between the number of layed eggs and hens. The egg production and eggs weight were measured and registered daily to evaluate the productive parameters.

During the entire experimental period, a total of 168 eggs were collected to evaluate the internal and external egg parameters, of which: 6 eggs at the beginning of the study, 18 eggs/group at 2 weeks period, another 18 eggs/group at 4 weeks period, respectively. For the assessment of shelf-life 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 ± 2^0C room temperature to determine if dietary mulberry leaf powder (1.5 vs. 3%) influenced the internal and external eggs' parameters throughout time. Digital Egg Tester DET-6500 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 color. The breaking load, for eggshell strength is measured at a pressing speed of 0,5 mm/s. The yolk color is calculated based on the DSM's YolkFan™ and the ZEN-NOH YOLK COLOR CHART 2019, evaluated by sensory inspection in diffused light. (to determine an accurate color measurement, DET6500 uses natural light, because artificial light varies much between regions depending on its light source).

The Haugh unit (HU) score was estimated based on the albumen height and egg weight, as follows: $HU = 100 \times \log (H - 1.7W^{0.37} + 7.6)$, where H = albumen height (mm), W= egg weight (g).

The heigher 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 scale (precision 0,001). The egg shell thickness, with the concave side down, was measured using a digital micrometer (Mitutoyo Absolute).

The albumen and yolk pH was measured using a portable pH meter (Five Go F2-Food kit with LE 427IP67, Sensor Metler Tolledo, Greifensee, Switzerland)

In addition, the egg color (parameters L*, a* și 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 ob-server angle of 2°/10° using the CIE-Lab system (Commission Internationale de l'Eclaraige).

Statistically analysis: The analytical data were compared by variance analysis (ANOVA) procedure using a linear model ($Yij = \mu + Aj + eij$) at SPSS version 20 (Inc., Chicago IL, USA). The difference between the means was considered significant at $p < 0.05$. The results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSIONS

The results concerning the influence of dietary mulberry leaf powder inclusion on average egg weight, laying percentage and egg classes are presented within table 2. These results obtained indicate that 1.5% mulberry leaf powder inclusion in experimental group E1 and, 3% mulberry leaf powder inclusion in experiemtal group E2, had no negative influence on laying performances and egg weight compared to C group. Egg production (%), average egg weight (g) and egg mass production (g/layer/day), registered no significant differences ($p \geq 0.05$) between groups. Therefore, the inclusion of different levels of dietary mulberry leaf powder found no significant differences ($p \geq 0.05$) on egg production, egg classification and production parameters of the laying hens (egg weight, laying intensity). When using mulberry leaf extract 0, 0.5, 1, or 2% for 12 weeks, Lin et al. (2017) showed that the effects of mulberry leaf powder did not influence the laying hens' performance parameters. On the other hand, Yanan Ding et al. (2021) included fermented mulberry leaves powder (FMLP) in broilers' diet for 56 day study length period: 0%, 3%, 6%, 9%, vs 3% unfermented mulberry leaf powder (UMLP). A highly statisticaly significant ($p < 0.01$) increased average daily gain (11.44%, 10.46%) was registered, as well as an descreased FCR (by 15.88%, 10.59%) on 3% FMPL comare to C control.

Table 2 The influence of mulberry leaves powder dietary inclusion on the laying percentage and egg classification (average values/group)

Egg's production and classification	Inclusion rate of mulberry leaves powder			SEM	*P*-value
	Con	1.5% ML	3% ML		
Egg weight, (g)	59.70	59.74	60.18	0.695	0.907
Laying intensity rate, (%)	79.30	86.34	83.27	4.01	0.498
Eggs classification *					
Total eggs (no.)	866	957	876	nd	nd
XL (>73 g), %	0.35	0.31	0.35	nd	nd
L (63-73 g), %	22.06	25.81	22.06	nd	nd
M (53-63 g), %	72.52	71.89	72.52	nd	nd
S (<53 g), %	5.54	2.40	5.54	nd	nd

Note: Con - basal diet; 1.5% ML - 1.5% mulberry leaf powder; 3% ML - 3% mulberry leaf powder; *European Council Directive (2006) [36].; SEM, standard error of the mean; *P*-value, means significantly differences ($p \le 0.05$).

The results regarding the influence of the mulberry leaf powder dietary inclusion on the egg yolk pigmentation intensity are presented within Table 3.

As expected the color intensity parameter registered a significant increase ($p < 0.05$) on E1 and E2 groups after 2 and 4 weeks of the experiment length duration.

When using mulberry powder Lokaewmanee et al., (2009) noticed a significant higher ($p < 0.05$) yolk color in all experimental groups (10.73 to 10.86) compared to C group (10.34), the 2% mulberry leaf powder inclusion showed the highest value.

Also, many other observations, such as better yolk color, increased beta-carotene and vitamin K, have been reported by many researchers when mulberry leaf powder was added to laying hens diet (Tateno et al., 1999 a,b; Sudo et al., 2000).

Table 3 Effects of dietary mulberry leaf powder supplementation on egg yolk intensity pigmentation (average values/group*)

Parameter	Inclusion rate of mulberry leaves powder							SEM	*P*-value
	Initial	after 2 week's			after 4 week's				
		Con	1.5% ML	3% ML	0	1.5	3		
Yolk color (DSM score)	4.39^{b}	4.69^{b}	6.15^{a}	6.83^{a}	5.01^{b}	6.39^{a}	6.67^{a}	0.271	0.001
L*	44.60^{a}	44.23^{ab}	44.60^{a}	42.76^{c}	44.68^{a}	44.69^{a}	43.02^{bc}	0.273	0.001
a*	0.11^{c}	1.15^{b}	1.51^{a}	1.63^{a}	1.14^{b}	1.40^{a}	1.49^{a}	0.102	0.001
b*	16.16c	17.62^{bc}	19.40^{a}	19.94^{a}	18.74^{ab}	17.21^{c}	17.24^{bc}	0.339	0.001

Note: Con - basal die; 1.5% ML - 1.5% mulberry leaf powder; 3% ML - 3% mulberry 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); *Results are given as the means of 18 yolks/group; ^{a,b,c} Means within a row with different superscripts differ significantly, $p \le 0.05$; SEM - standard error of the mean

Within table 4 the effects of mulberry leaves dietary inclusion on internal and external egg parameters are presented. Whole egg weight and albumen weight parameters registered statistical significant values ($p < 0.05$) at initial period compared to the 2nd and 4th weeks. As for the shell weight, no significant differences ($p > 0.05$) between groups were observed either, with the exception of C group at 2 weeks, which were significantly ($p < 0.05$) lower than the values recorded after 4 weeks both for group C and group E2.

Similar results were obtained by Olteanu et al. (2012), using 3 and 6% mulberry leaf powder dietary inclusion.

Mulberry leaves demonstrated an important effect on lowering yolk cholesterol without affecting the feed rate, production performances and/or egg quality characteristics (Kamruzzaman et al., 2014).

Contrary to our results, Al-kirshi et al., (2010), discovered that, using dietary mulberry leaf extract in poultry feeding, eggshell and yolk weight parameters decreased significantly ($p < 0.05$) while the Haugh unit value increased.

Eggshell thickness parameter showed a significant improvement ($p < 0.05$) on E2 group (2nd experimental week), compared to the other experimental groups. In a similar study, Huang et al., (2021) found that by adding 30 mg/kg or 60 mg/kg of mulberry leaf powder or mulberry leaf extract into laying hens diets (60 weeks) a significant ($p<0.05$) improvement of eggshell quality, eggshell thickness and breaking strenght were noticed.

Also, positive results regarding yolk weight, eggshell weight, eggshell thickness and breaking strength, egg freshness and yolk color were obtained by Lin et al., (2017) using 0.5, 1 and 2% mulberry leaves powder in laying hens feeding (22 weeks of age).

On the other hand, Xue-dong et al., (2013) noticed a significant decreasing ($p<0.05$) of egg production rate and egg weight parameters in a laying hens study (48 weeks of age) when adding 5, 10 and 15% mulberry leaves powder.

Table 4 Effects of dietary mulberry leaf powder supplementation on the external and internal egg quality parameters (average values/group*)

Egg parameters	initial	Inclusion rate of mulberry leaves powder						SEM	*P*-value
		after 2 weeks			after 4 weeks				
		Con.	1.5% ML	3% ML	Con.	1.5% ML	3% ML		
Weight egg and its components									
Egg weight, (g)	61.66^{a}	59.44^{b}	59.58^{b}	59.83^{b}	59.56^{b}	59.54^{b}	59.65^{b}	0.335	0.000
Albumen weight (g)	40.95^{a}	38.68^{b}	39.11^{b}	39.04^{b}	38.31^{b}	38.64^{b}	38.55^{b}	0.353	0.000
Yolk weight (g)	13.74	14.05	13.66	13.78	14.09	13.79	13.87	0.221	0.783
Shell weight (g)	6.97^{abc}	6.71^{c}	6.81b^{c}	7.01^{abc}	7.16^{ab}	7.11^{abc}	7.23^{a}	0.096	0.001
Eggshell breaking strength (kgF)	3.98	3.93	4.51	4.53	4.09	4.26	4.13	0.176	0.090
Eggshell thickness, (mm)	0.39 ^{b}	0.38^{b}	0.40^{ab}	0.44^{a}	0.38 ^{b}	0.37 ^{b}	0.39^{b}	0.009	0.000
Albumen quality parameters									
Albumen pH (value)	8.46	8.53	8.61	8.61	8.61	8.56	8.47	0.055	0.225
Albumen height (mm)	7.53	7.23	7.37	7.03	6.53	6.43	7.29	0.345	0.178
Haugh unit (value)	85.02	83.47	84.93	83.10	79.91	79.38	85.27	2.65	0.531
Freshness egg, (%) AA	88.89	83.33	94.44	88.89	83.33	88.89	100.0	nd	nd
Freshness egg, (%) A	5.56	5.56	0.00	11.11	5.56	0.00	0.00	nd	nd
Freshness egg, (%) B	5.56	11.11	5.56	0.00	11.11	11.11	0.00	nd	nd
Freshness egg, (%) Total	100	100	100	100	100	100	100	nd	nd
Yolk quality parameters									
Yolk pH (value)	6.51^{a}	6.21^{c}	6.15^{c}	6.22^{bc}	6.33^{abc}	6.25^{bc}	6.42^{ab}	0.048	0.000
Yolk height (mm)	18.73^{a}	18.21^{a}	18.63^{a}	18.24^{a}	15.99^{b}	16.01^{b}	16.95^{ab}	0.428	0.000
Yolk diameter (mm)	38.85	39.01	37.84	41.48	40.26	43.18	40.86	2.30	0.713
Yolk index	0.48^{a}	0.47^{ab}	0.50^{a}	0.47^{ab}	0.40^{b}	0.40^{b}	0.44^{ab}	0.016	0.000

Note: Con - basal die; 1.5% ML - 1.5% mulberry leaf powder; 3% ML - 3% mulberry leaf powder;
*Results are given as the means of 18 yolks/group; ^{a,b,c} Means within a row with different superscripts differ significantly, $p \leq 0.05$

The mulberry leaf powder dietary inclusion (1.5 vs. 3%) into experimental diets (E1, E2) had no influence ($p<0.05$) on the egg white quality parameters (pH and Haugh units).The Haugh unit recorded higher values within the first two experimental weeks, and slightly lower within the last two experimental weeks, but without significant differences between groups and periods ($p>0.05$).

However, mulberry leaf powder dietary inclusion had a beneficial effect concerning the freshness degree of our samples. Thereby, on E2 group, registered 100% AA category eggs collected after 4 weeks feeding. Al-kirshi et al., (2010) showed that 10, 15 and 20% mulberry leaves inclusion in laying hens diets (26 weeks of age) the feeding rate, eggshell and yolk weight decreased significantly ($p<0.05$), and Haugh unit values increased significantly ($p>0.05$).

In our study significant differences ($p\leq0.05$) were recorded for yolk pH, yolk height and yolk index. The values were significant higher ($p\leq0.05$) on yolk pH at initial compared to values registered after 2 weeks period on all 3 groups, and compared to E1 group after 4 weeks period. The yolk height parameter was significant higher ($p\leq0.05$) on initial, after 2 weeks period on all groups and after 4 weeks period on E2 group, compared to C and E1 groups (after 4 weeks period). Yolk index parameter was significant higher ($p \leq 0.05$) on initial period compared to two weeks period compared to C and E1 groups (after 4 weeks period).

In a study, Panja et al. (2013), observed that 0.5, 1, 1.5 and 2% mulberry leaves inclusion did not affect poultry performances but egg quality. Contrarily, Xue-dong et al. (2013) observed a significat decrease ($p<0.05$), of egg production rate and egg weight on a study conducted on laying hens (48 weeks of age) when including 5, 10 and 15% mulbery leaves.

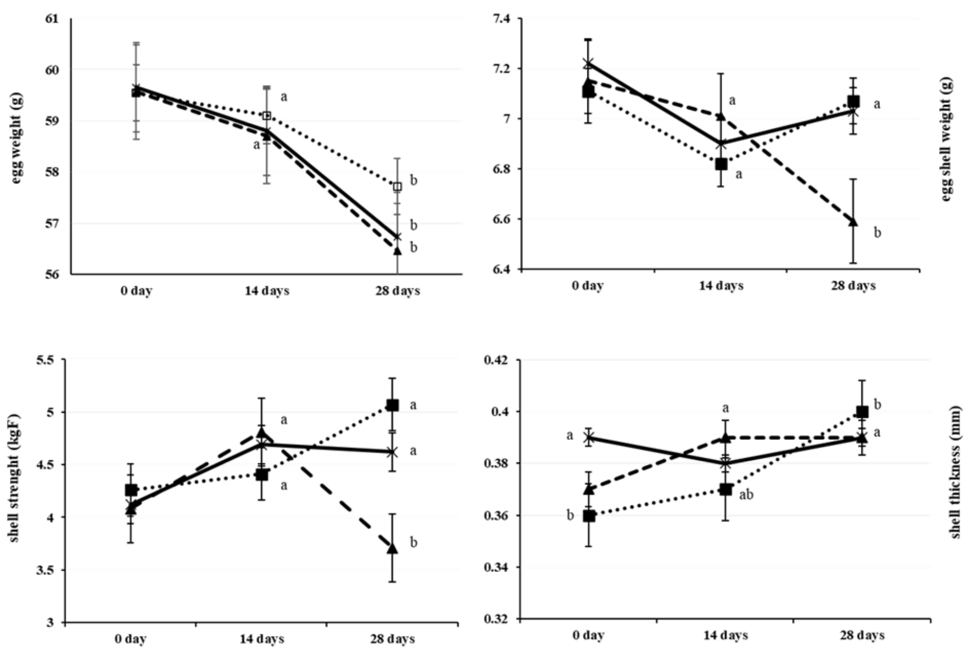

Figure 1. Shelf time evolution (0–28 days) of egg weight and shell quality according to the dietary treatments: control (▲), experimental E1 (■) and experimental E2 (×); [a–b] Mean values within a line not sharing the same superscripts are significantly different at $P \geq 0.05$

As presented within Figure 1 the egg weight parameter for all experimental groups decreased significantly ($p \leq 0.05$) from 0 day up to 28 days of shelf-life period. Concerning eggshell weight, a significant decrease ($p \leq 0.05$) was noticed only on C group. Eggshell strength recorded no differences ($p \geq 0.05$) on E1 and E2 groups, except for C group which registered a significantly lower ($p \leq 0.05$) quality of this parameter on 28th days of shelf-life period. A significantly increase ($p \leq 0.05$) eggshell thickness was noticed on E1 group for 28th days of shelf-life period.

As expected, albumen weight values were significantly lower ($p \leq 0.05$), decreasing gradually for all groups, correlated with pH albumen and Haugh units evolution values (Figure 2).

For yolk quality parameters evaluation as observed within Figure 3, yolk weight significantly increased ($p \leq 0.05$) on 28th days of shelf-life. The same trend was noticed for pH yolk values, for all groups. Egg yolk index values significantly decreased ($p \leq 0.05$) for each shelf-life period, on all groups. Similar results were registered by Akyurek and Okur (2009), Lee *et al.* (2016), Dong *et al.* (2017) who noticed that white and yolk quality were highly affected ($p \leq 0.05$) during the storage period for 28 days at 21°C.

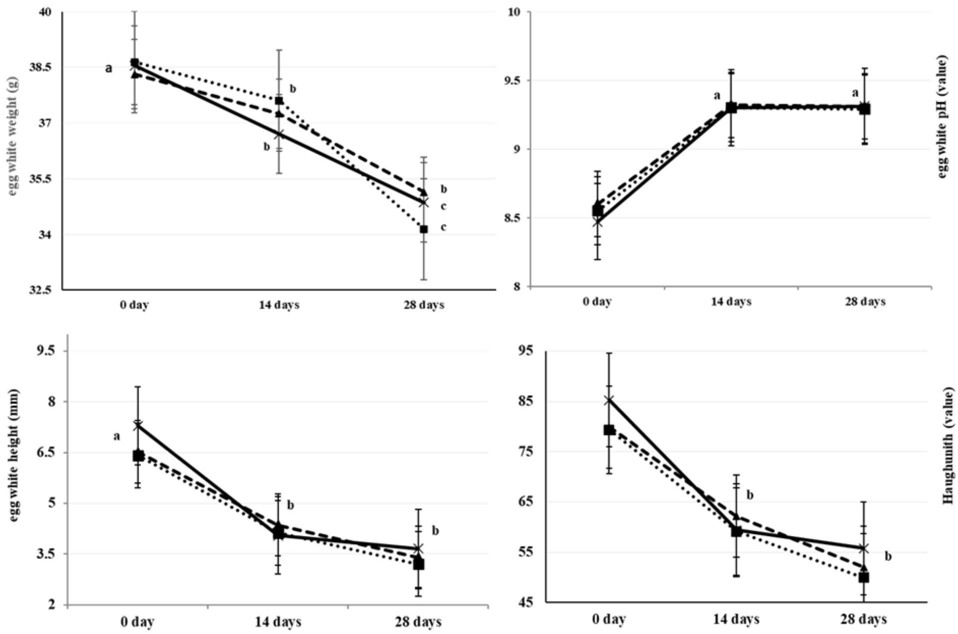

Figure 2. Shelf time evolution (0–28 days) of albumen quality according to the dietary treatments: control (▲), experimental E1 (■) and experimental E2 (×); a–b Mean values within a line not sharing the same superscripts are significantly different at P ≥ 0.05

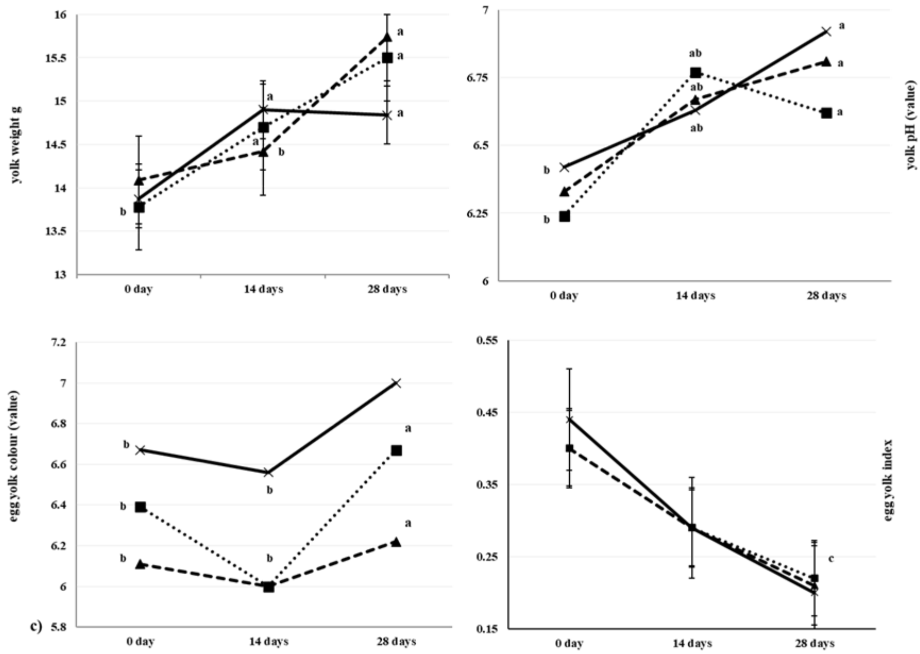

Figure 3. Shelf time evolution (0–28 days) of yolk egg quality according to the dietary treatments: control (▲), experimental E1 (■) and experimental E2 (×); a–b Mean values within a line not sharing the same superscripts are significantly different at P ≥ 0.05.

CONCLUSIONS

In conclusion, the mulberry leaves powder inclusion in laying hens feeding had a positive and beneficial influence on yolk pigmentation intensity within both experimental groups, without any negative effects on the internal and external egg quality parameters. Mulberry leaves could provide an alternative to synthetic colorant usually used in poultry production for egg yolk pigmentation.

ACKNOWLEDGEMENTS

The paper was made within project P_40_441 - GALIMPLUS – “Development of innovative feeding solutions for gallinaceans, with the purpose to obtain affordable foods with improved feeding qualities”, financing contract no.144/13.10.2016. Project co-financed from the European Fund for Regional Development through the Operational Program Competitiveness 2014-2020

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