Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 1, 2021
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SHELF LIFE OF EGGS FROM HENS FED DIETS RICH IN POLYUNSATURATED FATTY ACIDS AND ANTIOXIDANTS UNDER THE EFFECT OF DIFFERENT STORAGE TIME AND TEMPERATURES

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Abstract

We investigated the effectiveness of diets enriched with natural sources of polyunsaturated fatty acids and antioxidants on shelf life, internal and external quality parameters of eggs in different storage time and temperature. The diets given were as follow: basal diet (C), a diet containing mixture of rapeseed and grapeseed meal (T1) and a diet containing mixture of flaxseed and sea buckthorn meal (T2). A total of 108 eggs were selected (36 eggs/ group) and were separated into 6 batches with 6 eggs in each. The experimental design was consisted of 3 storage periods (0, 14 and 28 days) and 2 storage temperatures (5°C, 21°C). Each egg was weighed and broken, and the physiochemical properties of eggs such as albumen, yolk and eggshell weight, eggshell thickness and breaking strength and the most important freshness parameters Haugh Unit (HU), albumen and yolk pH were determined using an Egg Analyzer TM, manufactured by Orka Technology Ltd. The egg, albumen, shell weight and HU significantly decreased with increasing storage time and temperature, especially for eggs stored at 21°C for 28 days from C group. The albumen and yolk pH significantly ($P<0.05$) increased with increasing storage time and temperature in all samples, but those from group C were significantly ($P<0.05$) higher compared with eggs from T1 and T2 groups. The interaction effects between the storage time and temperature were significant for all determined parameters, which can conclude that storage time and temperature are the major factors affecting egg quality, but temperature is a more sensitive determinant of egg quality deterioration compared with the storage period. Eggs from groups fed diets rich in polyunsaturated fatty acids and antioxidants significantly ($P<0.05$) delayed egg deterioration in both storage temperatures.

Key words*: egg quality, shelf life, storage time, temperature.*

INTRODUCTION

For many years the most important external and internal egg quality characteristics have been shown to be, and still are, egg weight, shell characteristics, yolk and albumen ratio and freshness parameters, given by albumen and yolk pH and Haugh Unit (HU) (Samli et al., 2005). All these parameters have a major impact on shelf life of eggs. According to Directive CE 13/2000 and CE 557/2007, regarding the marketing standards for eggs, defines shelf life as the period from the collection of the eggs to their consumption, during which time the product is in a satisfactory state of quality in terms of chemical, physical, microbiological and sensory attributes. Egg quality consists of different aspects, each of which can be related to internal or external egg quality. The internal egg quality relates in general to the quality of the albumen and yolk (Bamelis, 2003). Albumen quality is not only an important indicator for egg freshness, but it is also important for the egg breaking industry because albumen and yolk have different markets (Scott & Silversides, 2000). The quality of eggs including their weight, shell characteristics and internal components are affected by a wide range of factors of which the genotype, nutrition, age of hens, temperature, humidity, the presence of carbon dioxide (CO_2), and storage time are the most important factors in terms of maintaining egg quality. Storage time and temperature appear to be the most crucial factors affecting albumen quality and HU. It has been reported (Silversides & Budgell, 2004) that pH is a useful tool for describing the changes in albumen quality over time during storage. Albumen pH increases with the loss of CO_2 from the egg. An increase in pH has been reported by extending the storage time from 2

to 30 days (Abdel-Nour et al., 2011). Moreover, laying hens nutrition is an important factor for controlling egg internal and external components quality and can successfully enrich the egg in some minor components of interest for human nutrition. Some meals were investigated in many studies to assess their effect on egg quality of the laying hens as reported previously (Sărăcilă et al., 2017; Vlaicu et al., 2017a; Gheorghe et al., 2019; Turcu et al., 2019). Others (Lokaewmanee et al., 2014) reported that dietary plant extracts improved significantly the eggshell quality and breaking strength. Sharma et al. (2009) observed increased eggshell thickness (by 10.0%) and breaking strength (by 15.2%) in hens fed diet supplemented with herbal products. Panaite et al. (2019) reported that diet supplemented with sea buckthorn mixture, significantly ($P<0.05$) decreased albumen and yolk pH after 4-week storage, compared with control diet. Also, it was reported (Mridula et al., 2012) that the HU from hens fed 10% flaxseed was higher that control eggs, whereas in contrast Najib & Al-Yousef (2010), reported no effect on HU and yolk index at 10% flaxseed inclusion rate. Recently, Panaite et al. (2020) reported that a mixture of 10% rapeseed meal with 2.5% flaxseed meal had no effect on HU, but significantly ($P<0.05$) affected albumen pH. No impact on egg internal and external quality parameters were also reported in other studies (Bozkurt et al., 2012; Swiatkiewicz et al., 2013; Vlaicu et al., 2017b), when hens were fed enriched diets. Very few authors reported the results of enriched diets on shelf life of eggs under the effect of different storage temperatures in time to provide more evidence for preserving conditions of enriched egg, in order to extend the shelf life.

With this regard, the aim of this study was to investigate the effect of diets enriched with natural sources of polyunsaturated fatty acids and antioxidants, given by mixture of rapeseed with grapeseed meal and a mixture of flaxseed with sea buckthorn meal in laying hens, in order to put into evidence the evolution of their effect on shelf life, internal and external quality characteristics of eggs under the effect of different storage conditions, as time and temperature, for 28 days.

MATERIALS AND METHODS

Experimental design: The eggs used in this study were obtained from one hundred twenty, 50- to 56-week-old Tetra SL LL hens, which were included in a laying trial at the experimental facilities of the Department of Chemistry and Animal Nutrition Physiology from National Research-Development Institute for Animal Biology and Nutrition, Balotesti, Romania. The study was conducted on external and internal egg components of laying hens which were fed 6 weeks (42 days) with diets enriched with some natural sources of polyunsaturated fatty acids and antioxidants. The laying hens were housed in an experimental hall having identical conditions, equipped with Big Dutchman three-tier cages (2 hens/cage; 20 cages/group) dimensioned according to the sanitary-veterinary norms regarding the minimum standards for protection and handling of laying hens, monitored by a Viper Touch computer (temperature of 21°C to 23°C, humidity of 67 to 70% and ventilation 2 to 3%) with *ad libitum* access to feed and water. Lighting was provided for 16 h light (incandescent lighting, 10 lx) and 8 h darkness cycle. Laying hens individually weighed were randomly divided into three experimental groups (C, T1 and T2). For the elaboration of the diet feed formulations used in this experiment, we considered the objective of the experiment, the species, the hybrid, the age and the nutritional requirements of the Tetra SL laying hens (Tetra-SL LL commercial Layer Management Guide, 2007). The layer diets were isonitrogenous and isocaloric and formulated according to recommendations for dietary need of laying hens for various nutrients. Experimental diets were prepared every two weeks to avoid oxidation. Control group (C) was fed a basal diet; while the other two groups were fed diets rich in polyunsaturated fatty acids and antioxidants as follow: 9% rapeseed meal and 3% grapeseed meal (T1) and a diet with 9% flaxseed meal and 3% buckthorn meal (T2). Each hen was fed daily 120 g of a basal diet C, T1 or T2, through individual feed troughs once daily at 08:30 and water was administered using automatic feeders. The basic structure of diets was the same for all three experimental groups,

characterized by 2750 kcal/kg metabolizable energy and 16.50% crude protein.

Egg sampling. A total of 108 eggs were collected after 6-weeks experimental period when the hens were 56 weeks old to evaluate the shelf life on external and internal quality parameters influenced by the storage time and temperature. The fresh eggs for the initial determinations (day 0) were collected from each treatment (n = 6) and measured within 2 h of being laid. The effect of diets on storage time and temperature was determined from a total of 24 eggs/group divided in four batches of 6 eggs. The samples of the 6 eggs from each group were labelled according to date of production and stored in unchangeable conditions, in chambers in a refrigerator (5°C) and at room temperature (21°C) for 14 and 28 days. Relative humidity was regulated at 50 to 60% for all samples.

Evaluation of egg quality. Characteristics were evaluated in individual eggs for external and internal quality traits. The external characteristics of eggs were egg weight (g), shell weight (g), shell strength (kgF) and shell thickness (μm), whereas internal quality parameters include albumen weight (g), yolk weight (g), albumen pH, yolk pH and Haugh unit. The albumen ratio, yolk ratio and shell ratio respectively were calculated with appropriate formulas (Lokaewmanee & Meesri, 2015).

$$Albumen\ ratio = \frac{albumen\ weight}{egg\ weight} \times 100$$

$$Yolk\ ratio = \frac{yolk\ weight}{egg\ weight} \times 100$$

$$Shell\ ratio = \frac{shell\ weight}{egg\ weight} \times 100$$

Egg weight was measured by weighing egg individually using sensitive balance. All egg parameters were measured automatically by an egg multi-tester Egg Analyzer TM, type 05-UM-001, manufactured by Orka Technology Ltd.

Statistical Analysis. The results were statistically analysed to determine the quality traits of eggs over time. All the numerical results obtained were subjected to Matlab & Simulink libraries. The model included the main effects of the storage times and temperatures and the two-way interactions between these factors. To investigate the effects of storage time and temperature on egg quality parameters among the eggs separated into three storage times and two storage temperatures, ANOVA software was used. To this end we use the linear regression model and we obtain the slope (a) and intercept (b) estimates of the model. We also compute the determination coefficient (R^2) in order to show how the variability of our parameters is explained by the linear regression model. Coefficient of determination (R^2) indicates the proportionate amount of variation in the response variable y explained by the independent variables x in the linear regression model. The larger the R^2 is the more variability is explained by the linear regression model. The level of significance was selected at $p<0.05$.

RESULTS AND DISCUSSIONS

The results regarding the effect of storage time and temperature on quality traits of eggs are shown below. With several exceptions, both storage time and temperature significantly ($P<0.05$) affected almost all parameters of internal egg quality. Albumen weight linearly decreased from 0 to 14 days stored at 5°C in all groups, after which the weight was maintained. Significant differences ($P<0.05$) were registered among 0 vs. 14 and 28 days respectively, in all groups (Table 1). Yolk weight, from samples stored at 5°C for first 14 days, registered significant ($P<0.05$) differences only in T1 samples, after that the weight was maintained constant until 28 days. Dramatic deterioration was observed in both albumen and yolk ratio due to the storage time of 28 days at 5°C. The highest albumen ratio was obtained in T1 eggs (59.29%) followed by T2 (57.46%) and C (56.52). In contrast, to the albumen ratio, the T1 yolk ratio, registered the lowest value (22.48%) compared with C (24.38%) and T2 (24.43%). After 28 days of storage at 5°C, albumen weight from C group was significantly ($P<0.05$) lower compared with T1 samples. Similarly, Samli et al. (2005) reported that albumen ratio decreases with 5.40% after a storage period of 35 days at room temperature (15-18°C), in table eggs from 50-weeks old laying hen. Contrary, Panaite et al.

(2020) reported significant increase of yolk weight at 0 days, from eggs enriched, laid by hens fed rapeseed meal, compared with that fed flaxseed meal. Further, albumen weight from eggs stored at 21°C for 28 days, significantly (P<0.0113) decreased in C samples after 14 and 28 days, while those from T1 significantly (P<0.0066) decreased only after 28 days (Table 2). The albumen weight from T2 samples, were not affected by time or temperature. As it was expected, albumen ratio was affected significantly (P<0.05) in C samples (57.63%) compared with T1 (58.57%) and T2 (58.81%) egg, respectively. Albumen weight at 14 and 28 days of storage at 21°C was significantly (P<0.05) lower compared with both T1 and T2 groups. This positive effect could be justified by the antioxidant effect of grapeseed and sea buckthorn meals added in the hens feed, which delayed the protein deterioration.

Table 1. Effects of storage time and temperature on albumen and yolk weight stored at 5°C and regression analysis coefficients (a), (b) and (R^2)

Item	Albumen weight				Yolk weight			
			5°C					
	C	T1	T2	p	C	T1	T2	p
0 days	39.27^{a}	39.79^{a}	37.37^{a}	ns	16.38	15.89^{a}	16.51	ns
14 days	35.17^{b}	35.46^{b}	35.16^{b}	ns	15.63	14.71^{b}	15.79	ns
28 days	34.01^{bA}	35.04^{bB}	34.92^{b}	*	15.00	14.04^{b}	15.31	ns
ratio	56.51	59.37	57.47	ns	24.40	22.54	24.44	ns
SEM	0.641	0.593	0.433	-	0.350	0.277	0.312	-
p	<0.001	<0.0001	0.0146	-	ns	0.0036	Ns	-
			Time					
(a)	38.93	39.81	37.37	-	16.71	15.93	16.79	-
(b)	-0.16	-0.09	-0.09	-	0.04	0.04	0.04	-
R^2	0.87	0.99	0.99	-	0.59	0.93	0.99	-
			Temperature					
(a)	38.11	39.4	37.11	-	16.99	16.11	16.88	-
(b)	-0.16	-0.11	-0.11	-	0.03	0.04	0.04	-
R^2	0.64	0.87	0.99	-	0.78	0.73	0.87	-

$^{a,\ b}$Different lowercase letters indicate significant differences among the means in each column on different storage temperature. ^{AB} Different uppercase letters indicate significant differences among the means in each row between groups; *significant at P<0.05; ns - not significant. C - control diet; T1- diet supplemented with rapeseed and grapeseed meal mixture; T2- diet supplemented with flaxseed and sea buckthorn meal mixture; SEM – standard error of the mean.

Table 2. Effects of storage time and temperature on albumen and yolk weight stored at 21°C and regression analysis coefficients (a), (b) and (R^2)

Item	Albumen weight				Yolk weight			
			21°C					
	C	T1	T2	p	C	T1	T2	p
0 days	39.27^{a}	39.79^{a}	39.46	ns	16.38	15.89^{a}	16.51	ns
14 days	35.08^{bA}	38.35^{B}	38.44^{B}	*	15.32	14.34^{b}	15.47	ns
28 days	35.02^{bA}	37.25^{bB}	37.79^{B}	*	15.11	14.13^{b}	15.21	ns
ratio	57.62	58.56	58.82	ns	25.47	23.47	25.78	ns
SEM	0.645	0.399	0.437	-	0.356	0.0272	0.433	-
p	0.0113	0.0066	ns	-	ns	ns	ns	-
			Time					
(a)	38.92	39.26	39.10	-	16.55	16.39	16.62	-
(b)	-0.19	-0.17	-0.17	-	-0.03	-0.04	-0.03	-
R^2	0.89	0.80	0.99	-	0.89	0.99	0.99	-
			Temperature					-
(a)	37.94	38.4	36.61	-	16.62	16.62	16.42	-
(b)	-0.21	-0.17	-0.17	-	-0.04	-0.04	-0.04	-
R^2	0.68	0.67	0.99	-	0.99	0.99	0.98	-

$^{a,\ b}$Different lowercase letters indicate significant differences among the means in each column on different storage temperature. ^{AB}Different uppercase letters indicate significant differences among the means in each row between groups; *significant at P<0.05; ns - not significant. C - control diet; T1- diet supplemented with rapeseed and grapeseed meal mixture; T2- diet supplemented with flaxseed and sea buckthorn meal mixture; SEM - standard error of the mean.

Yolks weight significantly (P<0.0272) decreased only in T1 samples, as in the case of those stored at 5°C. In terms of yolk ratio T1 eggs (23.46%) maintained the lowest value compared with those from C (25.41%) and T2 (25.79%), which had close average values (Table 2). There were no significant (P>0.05) differences among the groups in terms of albumen or yolk weight. Previously, Lokaewmanee & Meesri (2015) reported drastically (P<0.0001) decreased of albumen (65.53% to 46.40%) ratio and significantly increased (P<0.05) yolk ratio (27.37% to 34.99%) in lutein enriched eggs after only 21 storage days at 30°C. These changes in egg quality traits as albumen and yolk ratio have been reported also by others (Tabidi, 2011; Samli et al., 2005) and were attributed to water loss by evaporation through the pores in the shell and the escape of CO_2 from albumen.

Shell quality, same as weight, was not affected by temperature (5°C) or by storage time. But there were observed some variations in terms of regression coefficients (Table 3). Shell thickness was also not affected, were noted some slightly lower values for all samples. In terms of shell ratio T2 registered the highest value (14.01%) versus T1 (13.61%) and C (13.03%) samples. In contrast, shell strength, increased under the influence of time. The T2 samples had the highest values after 28 days storage time (4.31) compared with C (3.89) and T1 (3.98), but the differences were not statistically (P>0.05).

Table 3. Effect of storage time and temperature on shell weight, thickness and strength stored at 5°C and regression analysis coefficients (a), (b) and (R^2)

Item	Shell weight			Shell thickness			Shell strength		
	5°C								
	C	T1	T2	C	T1	T2	C	T1	T2
0 days	8.83	8.84	8.96	0.35	0.35	0.35	3.63	3.63	3.67
14 days	8.35	8.83	8.84	0.34	0.34	0.34	3.87	3.95	3.97
28 days	8.29	8.33	8.74	0.34	0.32	0.33	3.89	3.98	4.31
SEM	0.127	0.180	0.248	0.005	0.006	0.005	0.242	0.148	0.151
p	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Time								
(a)	9.03	8.81	9.83	0.34	0.32	0.35	3.72	3.58	3.82
(b)	-0.03	-0.01	-0.01	0	0	0	0.01	0.01	0.01
R^2	0.67	0.87	0.99	0.57	0.59	0.69	0.57	0.54	0.59
	Temperature								
(a)	8.97	8.76	9.58	0.35	0.34	0.33	3.77	3.59	3.82
(b)	-0.04	-0.01	-0.01	0	0	0	0.01	0.01	0.01
R^2	0.89	0.64	0.99	0.99	0.99	0.99	0.69	0.77	0.99

ns - not significant. C- control diet; T1- diet supplemented with rapeseed and grapeseed meal mixture; T2- diet supplemented with flaxseed and sea buckthorn meal mixture; SEM - standard error of the mean.

Shell weight from samples analysed under the influence of temperature (21°C) and time (28 days) from C group, significantly (P = 0.0216) decreased after 14 days of storage, while those from T1 and T2, were not affected (Table 4). Shell thickness maintained close values between all groups at all storage times. Similarly, with samples analysed at 5°C for 28 days, shell strength of samples under the influence of temperature (21°C) significantly (P = 0.0130) increased in T1 samples at 28 days compared with those from 0 days. Also, T2 and C samples registered higher values for shell strength after 28 days of storage, but without significance between them. In line with our results, (Lokaewmanee & Meesri, 2015) reported that egg shell quality was not influenced by lutein enriched diet under the effect of storage time and temperature. Silversides & Scott (2001) reported that the weight of the shell increased with age of the hen until 45-weeks, but when considered as percentage of the egg, the shell decreased with increasing age of the hen. Also, Olteanu et al. (2017), reported increased eggshell weight at the end of the trial, when diets rich in PUFA and antioxidants were given to laying hens. As in our study, the changes in egg shell traits are unclear. Contrary to these findings, Samli et al. (2005), reported that shell weight, and other egg parameters significantly (P<0.001) decreased with increased storage time and

temperature in commercial eggs from old laying hens. In the literature is a lack of reports on internal and external quality characteristics of eggs from hens fed diets rich in polyunsaturated fatty acids and antioxidants, in different storage conditions.

Table 4. Effect of storage time and temperature on shell weight, thickness and strength stored at 21°C and regression analysis coefficients (a), (b) and (R^2)

Item	Shell weight (g)			Shell thickness			Shell strength		
				21°C					
	C	T1	T2	C	T1	T2	C	T1	T2
0 days	8.83	8.84	8.96	0.35	0.35	0.35	3.63	3.63[a]	3.67
14 days	8.24[a]	8.55	8.73	0.34	0.35	0.35	3.98	3.99	3.92
28 days	8.07[b]	8.21	8.56	0.33	0.34	0.34	4.10	4.41[b]	4.32
SEM	0.160	0.148	0.204	0.006	0.005	0.006	0.180	0.140	0.165
p	0.0216	ns	ns	Ns	ns	ns	ns	0.0130	ns
				Time					
(a)	8.65	8.79	8.89	0.35	0.35	0.35	3.63	3.64	3.64
(b)	-0.01	-0.01	-0.01	0.00	0.00	-0.01	0.02	0.02	0.02
R^2	0.99	0.98	0.99	0.92	0.93	0.99	0.97	0.99	0.99
				Temperature					
(a)	8.66	8.75	8.75	0.35	0.35	0.35	3.72	3.72	3.71
(b)	-0.03	-0.01	-0.01	0.00	-0.01	-0.01	0.03	0.02	0.02
R^2	0.86	0.86	0.99	0.71	0.99	0.99	0.87	0.89	0.99

[ab]Different letters indicate significant differences among the means in each column on different storage temperature. ns- not significant. C- control diet; T1- diet supplemented with rapeseed and grapeseed meal mixture; T2- diet supplemented with flaxseed and sea buckthorn meal mixture; SEM – standard error of the mean.

As storage time and temperature increased, egg weight significantly (P<0.001) decreased in all groups under 21°C, within 28 days storage time, compared with those stored at refrigerator (5°C). After 14 days at refrigerator, egg samples from C group had a significantly (P<0.05) lower weight compared with those from T1. At the end of storage time (28 days) at 5°C, both T1 and T2 egg weight were significantly higher compared with C eggs. The interaction effects between storage time and temperature were significant for egg weight kept at 21°C, but the interaction effects on egg weight was not significantly decreased by storage from 0 to 14 days at 5°C, significant interaction was observed after 14 days of storage. When the storage temperature was increased to 21°C, however, the egg weight dramatically decreased from 64.09 to 59.02 in C group, from 64.18 to 59.83 in T1 and from 64.17 to 59.38 in T2 (Figure 1A). These results are in agreement with those of Jin et al. (2011) and Samli et al. (2005), who reported significant egg weight reductions of approximately 3% within 10 days of storage at 29°C. Similar weight losses were also reported by Akyurek and Okur (2009). The storage period and temperature significantly affected all freshness parameters of internal egg quality given by HU, albumen and yolk pH (P<0.001). However, the albumen and yolk pH significantly increased with storage time of 28 days and temperature at 21°C. The interaction effects between storage time and temperature were significant for HU, yolk pH and albumen. Significant deteriorations were observed in HU due to storage time of 14 days and temperature of 21°C (Figure 1B). However, the eggs stored at refrigerator (5°C) tended to have higher HU compared with those from initial day. Similarly, previously was reported that by supplementing laying hens' diets with flaxseed-rosehip mixture, or flaxseed-grapeseed mixture, HU increases due to the effect of antioxidants added to delay the yolk lipid oxidation and protein denaturation from albumen (Sărăcilă et al., 2017). Storage at temperatures higher than 5°C for more than 14 days caused considerable deterioration in HU. In all treatments HU decreased significantly (P<0.05) from 86.28 (0 days) to 72.67 (14 days) to 32.12 (28 days) in C group, from 86.70 (0 days) to 75.27 (14 days) to 43.68 (28 days) in T1 eggs, while those from T2 decreased from 86.54 to 75.15 and at day 28 to 44.22 when preserved at 21°C. With respect to the effect of storage time and temperature on the physiochemical properties of eggs, we observed a significant (P<0.05)

increase in albumen pH with increasing storage time and temperature (Figure 1C). The albumen pH was not affected by storage time at 5°C. At 14 days, albumen pH was higher in eggs stored at 21°C, compared with those stored at 5°C. After 28 days, albumen alkalizing was accelerated by increasing storage temperature (21°C) with the interaction of storage period (28 days), registering significantly higher values compared with those stored at 5°C. There were no differences ($P>0.05$) among the groups stored in different conditions. Generally, the increase in albumen pH occurs due to the dissociation of carbonic acid (H_2CO_3), forming water and carbon dioxide (Figueiredo et al., 2013). The pH determination of the albumen is a suitable measure to evaluate the freshness of the eggs, since there is less influence of the strain and age of the bird on the pH compared with other quality measurements (Silversides and Scott, 2001). The albumen pH of the freshly laid egg usually ranges from 7.6 to 8. However, the albumen pH increases with the storage period of the egg, reaching 9.5 while under the high storage temperature the pH can reach over 10 (Alleoni & Antunes, 2001), after that the albumen alkalinization starts to occur due to protein degradation.

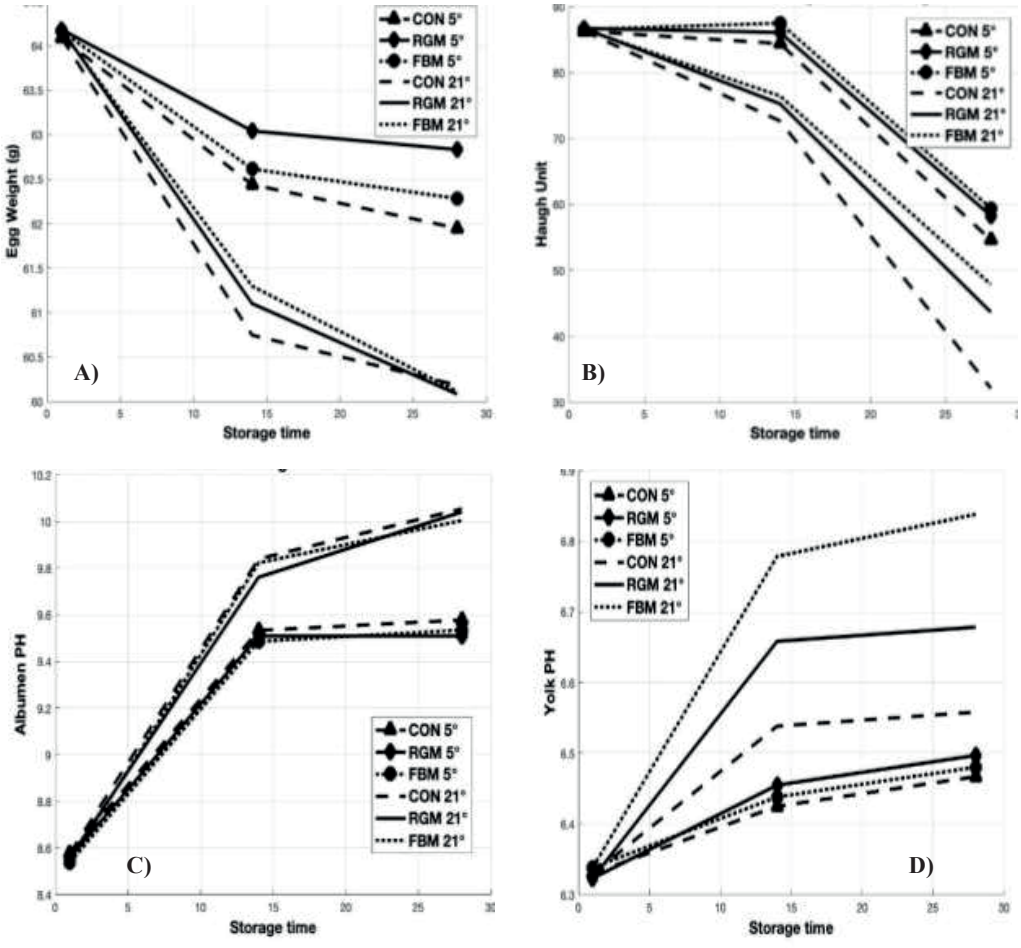

Figure 1. Relationship between storage time (0, 14 and 28 days) and temperature (5°C and 21°C) on freshness parameters: A) egg weight(g); B) Haugh Units (HU); C) albumen pH; D) yolk pH

The yolk pH value as albumen pH increased with increasing storage time (Figure 1D). The yolk pH values from eggs stored at 5°C, increased linearly from 0 to 28 days refrigeration, without significant differences among the groups. On the other hand, eggs stored at 21°C significantly ($P<0.05$) differed among the groups after both 14 and 28 days of storage. Responsible for differences in both T1 and T2 yolk samples at 28 days of storage time, could be the high fatty acid content in the added meals from rapeseed (40.26 to 43.19%) and grapeseed meal (64.71 to 66.60%) in T1 and flaxseed meal (70.23 to 78.80%) and sea buckthorn meal (27.33 to 30.44%) in T2 (Panaite et al., 2016; Vlaicu et al., 2017; Cornescu et al. 2018). It was reported that the increase in yolk pH (usually 6.0) has little variation (6.35 to 6.85) even after long storage periods (Oliveira and Oliveira, 2013). It has been reported that during storage, CO_2 escapes through the eggshell pores. So, the increase in albumen pH over time may be also due to the loss of CO_2 and/or a change in the bicarbonate buffer system (Biladeau and Keener, 2009). Although the pH of the egg albumen and yolk increased along with the storage time and temperature, the changes of albumen pH were not as large as those of the yolk pH. Previous studies reported similar effects on yolk pH, which was significantly affected by storage time (Samli et al., 2005; Akyurel and Okur, 2009; Jin et al., 2011). Increasing storage time and temperature diluted the egg albumen resulting in breakdown of the protein structures of the albumen and vitelline membrane (Jones, 2007) which accelerates to the passage of some components of the albumen pass through the yolk membrane, reducing egg weight and viscosity (Ahn et al., 1999). In this study, storage time up to 28 days and temperature (21°C) significantly ($P<0.05$) affected almost all of the internal and external egg quality parameters. As shown in the presented tables, we calculated the correlation coefficients of storage time and temperature on egg quality traits. Storage time and temperature were negatively correlated with albumen, yolk and shell weight but were positively correlated with egg weight. Among these coefficients, high temperature showed the highest negative correlation with albumen weight, shell weight and thickness in C and T1 after 28 days storage time, which means that temperature, was a more sensitive determinant of egg quality deterioration than storage time. Moreover, temperature was an absolute factor in determining the internal egg quality because the HU dramatically decreased after being stored for 14 to 28 days at storage temperatures up to 21°C. Similar interaction effects also occurred for the albumen pH and yolk pH with increasing storage time and temperature.

CONCLUSIONS

The quality characteristics of eggs were not adversely affected when eggs were stored in refrigerators for 14 days, but room temperature significantly affect some egg quality characteristics by increasing weight loss, yolk weight, yolk pH, albumen pH and by reducing HU during storage for different time intervals. It can be concluded that egg from hens fed diets rich in polyunsaturated fatty acids and antioxidants, should be kept in refrigerators up to 28 days and at room temperature up to 14 days, after that the alteration in albumen and yolk starts to occur, but still maintain relatively good internal quality characteristics for human consumption up to 28 days.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0473, within PNCDI III - PC3.

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