PRESERVATION OF EGG QUALITY USING GRAPE POMACE CAKES AS A NATURAL ANTIOXIDANT IN THE DIETS OF LAYING HENS ENRICHED IN OMEGA 3 FATTY ACIDS

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Abstract

For the antioxidant effect evaluation of a winery by-product - the grape pomace cakes, a 4-week trial was conducted on 180 Lohmann Brown laying hens, aged 52 weeks. The layers were assigned to three groups: a control group (C) and two experimental groups (E1 and E2), with 60 hens/ group, housed in standard Zucami type cages (2 hens/cage). Compared to the control formulation, the experimental formulation included 5% flax meal, enriched in polyunsaturated fatty acids and as antioxidants 100 mg vitamin E/kg feed (E1); 2% grape pomace cakes (E2). Forty eggs/group were randomly collected in the end of the trial, 10 eggs/group being stored at +20°C and 10 eggs/group at +4°C, for 14 and 28 days in order to determine their fatty acids content and the quality preservation over time. After 14 days, eggs stored at +4°C has significantly (P <0.05) higher values of the Haugh unit, compared to those from 28 days, with comparable values for both experimental groups and control group. In contrast, the eggs stored at room temperature (+20°C), has lower values of the Haugh unit in both storage periods (14 and 28 days), compared to those stored at +4°C, without significant differences between groups (P>0.05), although experimental groups included flax meal. The results regarding the freshness of the eggs stored in the fridge (+4°C) for 14 days showed a 20% AA eggs in E2 group, while in the other groups were no AA eggs. Only group E2 had 10% eggs, stored at +20°C for 14 days, with grade A freshness, compared to 0% for groups C and E1. The grape pomace cakes had comparable effects with vitamin E on the preservation of egg quality over time, thus it can be affirmed that the grape pomace cake diet is effective in maintaining the quality of eggs enriched in polyunsaturated fatty acids over time.

Key words: antioxidants; grape pomace cakes; eggs; quality

INTRODUCTION

Current researches in poultry are increasingly focused on the use of nutritional strategies to improve the nutritional value of animal products [19], by increasing the content of protein, polyunsaturated fatty acids, amino acids, xanthophylls, vitamins or minerals.

Today it is well known that polyunsaturated fatty acids (PUFAs) can play an important role in preventing the occurrence of cardiovascular disease, high blood pressure, diabetes, cancer, arthritis, other inflammatory and autoimmune disorders [11], [16], [17]. The main polyunsaturated fatty acids (PUFA) are linoleic acid and alpha-linolenic acid. Therefore, ensuring adequate amounts of PUFA fatty acids cannot be lacking in nutritional recommendations.

Obtaining animal products rich in PUFAs on tne nutritional way through the use of plant raw materials rich in these acids can lead to lipid oxidation processes [15] and the quality of eggs enriched with fatty acids may decrease over time. In order to minimize these processes and to prevent lipid rancidity, in general, it is added to the ratios of birds produced with antioxidant properties. Antioxidants can be obtained synthetically, such as butylated hydroxy- anizole (BHT), tertiary butyloxy hydroxy quinone (TBHQ),

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or may be plant-based. Antioxidants such as vitamin E, vitamin C, carotenoids or raw plant materials rich in polyphenols with derivatives (extracts, oils, sows) can play a beneficial role in slowing down the lipid oxidation process.

The use of vegetable by-products plays an important role in modern agriculture because they are low in price of the synthetic compounds used conventionally, and depending on the nutrients and contained biofactors, they can significantly contribute to improving the characteristics of the feed, the quality of the animal feed and the environmental protection.

MATERIAL AND METHODS

Animals and experimental design

The experiment was conducted in the experimental halls of the National Institute of Animal Biology and Nutrition (IBNA-Balotesti, Romania) based on a protocol approved by the ethics commission of the institute. For 4 weeks, a number of 180 laying hens, Lohmann Brown hybrid, aged 52 weeks, divided into 3 groups, one of which control (C) and two experimental (E1 and E2), each with 60 chicks /group, were housed in 90 standard Zucami cages (2 chiks / cage), allowing daily recordings of the eggs production. Allotments was made according to the live weight of the chickens, so that there were no significant differences between the groups, with an average weight of 1668.40 ± 36.10 g.

According to the Lohmann Brown hybrid breeding guide, the lighting of the experimental room was ensured according to a program with up to 16 hours of light daily, the temperature was maintained in the range of 22-24ºC, and the relative humidity at 60-70% throughout the entire experimental period. Feed and water were administered *ad-libitum*.

Feed

The structure of each diet was calculated based on the chemical composition determinated in the raw feed materials using a mathematical model of the composition of the feed diets for birds [1]. The birds received a basic diet based on: corn, soy meal, and corn gluten. The diets of experimental groups E1 and E2 were different from the diet of group C by including 5% flaxseed and the nature of the antioxidants used. Thus, to the diet of experimental group E1, vitamin E was added in proportion of 100 mg / kg DM feed, and to the diet of experimental group E2 were added grape seed cake in proportion of 2% (table 1).

Table 1 Compound feeds formulation

Items	C	E 1	E 2
Corn	60.35	55.35	55.35
Flaxseed	-	*5*	*5*
Grape seed cake	-	-	*2*
Soy meal	25	25	23
Gluten	1	1	1
Sunflower oil	2	2	2
Monocalcium phosphate	1.25	1.25	1.25
Calcium carbonate	8.9	8.9	8.9
Salt	0.3	0.3	0.3
Methionine	0.15	0.15	0.15
Choline	0.05	0.05	0.05
Premix A6	1	-	1
Premix A6 + 75 ppm vit E (100 ppm vit E in furaj)	-	1	-
Total	100	100	100
Content per kg diet: vitamin A: 13,500 IU; vitamin D3: 3,000 IU; vitamin E: 27 mg; vitamin K3: 2 mg; vitamin B1: 2 mg; vitamin B2: 4.8 mg; pantothenic acid: 14.85 mg; nicotinic acid: 27 mg; vitamin B6: 3 mg; vitamin B7: 0.04 mg; vitamin B9: 1 mg; vitamin B12: 0.018 mg; vitamin C: 25 mg; manganese: 71.9 mg; iron: 60 mg; copper: 6 mg; zinc: 60 mg; cobalt: 0.5 mg; iodine: 1.14 mg; selenium: 0.18 mg.			

Sampling

To determine the shelf time, at the end of the experiment, from all three groups (C, E1, E2) were randomly collected:

- 20 eggs / group that were stored at room temperature (+20ºC), for 14 days (10 eggs/group), respectively 28 days (10 eggs /group), in total 60 eggs;

- also 20 eggs / group that were stored in a refrigerator, at a temperature of +4ºC for 14 days (10 eggs / lot), respectively 28 days (10 eggs/lot), in total 60 eggs.

After 14 days, respectively 28 days, both on the eggs stored in the refrigerator and on the ones stored at room temperature were made determinations regarding the Haugh Unit and the degree of freshness.

Chemical analysis

To determine the profile of fatty acids from raw feed materials, compound feeds and eggs, firstly, the fat was extracted from the egg yolk samples, which were then subjected to saponification through reflux boiling, in an acidified methanol solution (2% H_2SO_4 in methanol) to obtain methyl esters (FAME) from fatty acids. It was followed by the resumption of methyl esters of fatty acids in hexane and the concentration on rotavapor, according to SR CEN ISO / TS 17764-1: 2008. The samples thus processed were subjected to gas chromatographic analysis, according to SR CEN ISO / TS 17764-2: 2008 using a Perkin Elmer-Clarus 500 chromatograph, fitted with a system for injection into the capillary column, with high polarity stationary phase (BPX70: 60m x 0.25mm inner diameters and 0.25µm thick film); or high polarity cyanopril phases, which have similar resolution for different geometric isomers (THERMO TR-Fame: 120m x 0.25mm ID x 0.25µm film).

The concentration of polyphenols in grape seed cake was determined according to the method described by [8], modified. The equipment used was a Thermo Scientific UV-VIS Spectrophotometer. The results were expressed in mg equivalent gallic acid / g fresh substance (mg EAG / g sample).

The antioxidant capacity in the methanol extracts was determined by the DPPH method proposed by [7] using a UV-VIS Analytik Jena Specord 250 Plus spectrophotometer with thermostatic carousel. The results were expressed in mM Trolox equivalents /g sample (mM TE / g sample).

The determinations regarding the Haugh Unit and the degree of freshness were made with the Analyzer TM device, type 05-UM–001.

Statistical analysis

The effects of treatments were tested using the StatView program, variance analysis (ANOVA and t test), the results being presented as mean values ± standard error, the differences being considered statistically significant at $P \leq 0.05$

RESULTS AND DISCUSSIONS

The flaxseed added in the diets of laying hens with the purpose of enriching eggs in PUFAs was characterized by a content of 12.19 g saturated fatty acids (SFA), 66.93g polyunsaturated fatty acids (PUFA), of which 44.50g represented by omega-3 PUFAs, respectively 22.43g / 100g fat omega-6 PUFAs. There are a number of studies in the literature that present the results of using flax in poultry diets [2], [3], [13] and its by-products [10], [12], [18] in poultry feed, as a raw material rich in PUFAs.

However, the diets rich in PUFAs, over time, are exposed to oxidative degradation with the occurrence of the rancid phenomenon. Among the natural sources, with a high level of polyphenols, which confer antioxidant capacity are also the winery by-products: grapes marc, seeds and peels of grapes, as well as cake, respectively meals from grapes. The abundance of bioactive polyphenols from these by-products [6], [14] is of real interest for animal nutrition, as they can be used as natural antioxidants, as an alternative to synthetic antioxidants.

The grape seed cakes, used as an antioxidant in the present study to prevent this phenomenon were characterized by a content of: polyphenols 0.631 mg EAG / g; flavonoids 5.0651mg / ml and antioxidant capacity 28.4678 mM Trolox equivalents / g sample, results comparable to those presented by researchers [4], [9], [12].

Due to flaxseed, raw feed material rich in PUFAs added in the ratios of experimental groups, it was found that the values of these acids increased by 0.27% in the diet of E1 group (with the addition of vitamin E), respectively by 7.00% in the diet of E2 group, compared to the diet of C group (table 2). This difference between the experimental groups E1 and E2 is due to the contribution of PUFAs brought by the cakes from grape seeds added in the E2 diet.

Table 2 Content of saturated, unsaturated fatty acids and their ratios in the tested feeds (g / 100g fat)

Item	C	E1	E2
SFA	13.43	13.94	13.83
MUFA	31.16	30.61	27.14
PUFA	55.11	55.26	58.97
UFA	86.27	85.87	86.11
PUFA / SFA	4.10	3.96	4.26
SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polysaturated fatty acids; UFA-unsaturated fatty acids.			

Omega-3 fatty acids are part of the long-chain PUFAs family and are represented by alphalinolenic acid (ALA), eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), all of which have good well-known health effects.

Alpha linolenic acid (18: 3n3) is considered essential fatty acid because it cannot be synthesized by the body, the main source of obtaining is by food.

Table 3 Content of saturated, unsaturated fatty acids and their of egg yolk fat (g / 100g fat)

Items	C	E 1	E 2
SFA	36.06 ±0.72	35.64 ±1.05	34.93 ±1.83
MUFA	37.85 ±1.52	36.66 ±1.69	37.25 ±1.96
PUFA	26.03 ±1.23	27.62 ±0.86	27.74 ±0.41
α-Linolenic fatty acid (18:3n3)	$0.32^{b,c}$ ±0.03	1.57^{a} ±0.06	1.79^{a} ±0.17
Docosahexaenoic acid (22:6n3)	$0.96^{b,c}$ ±0.12	2.27^{a} ±0.02	2.79^{a} ±0.29
SFA / UFA	0.56 ±0.02	0.55 ±0.02	0.54 ±0.04
PUFA / MUFA	0.69 ±0.06	0.75 ±0.06	0.74 ±0.05
SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polysaturated fatty acids; UFA-unsaturated fatty acids; Omega-3 – omega-3 fatty acid; Omega-6 – omega-6 fatty acid. a, b, c significany differences (P≤0.05) between groups C, E1, E2			

As it can be observed from table 3, the eggs collected from experimental groups E1 and E2 have a significantly higher ($P < 0.05$) α-linolenic acid content, compared to eggs from the C group, registering an increase by 7.09 times, in group E1 (with vitamin E added in feed), respectively by 5.59 times, in group E2 (with cakes of grape seeds added in feed). The total PUFAs registered an increase by 6.11%, in group E1, respectively by 6.57%, in group E2, compared to the value registered in the C group. In a review regarding on the possibilities of enriching the eggs with omega-3 fatty acids, taking into account 26 studies carried out between 1991-2011, which used as sources of omega-3 fatty acids , oilseeds, fish oils and / or micro-algae, [5] show an increase in ALA and DHA content of eggs, observing many variations, being influenced by the type of supplement used and the level of inclusion of the source in the diets.

In order to determine the effect of the natural antioxidant presence - the grape seeds cakes, compared with the vitamin E, in the preservation in time of the quality of the eggs rich in PUFAs the Haugh Unit was determined and their degree of freshness.

From the graphical representations (figures 1 and 2) it can be observed that with the increase of the storage period of the eggs from 14 days to 28 days, for both cases at room temperature (+20ºC) and in the refrigerator (+4ºC) the Haugh Units values has decreased.

However, by comparing the storage conditions, it can be observed that in the case of eggs stored in the refrigerator (+4ºC) the values of the Haugh Unit were significantly ($P<0.05$) higher, compared to those stored at room temperature (+20ºC), regardless of the storage period. Thus, in the case of E1 group (with vitamin E added in feed), there were increases with 102.11%, at 14 days of storage, respectively by 61.27%, at 28 days of storage. And in the case of E2 group (with grape seeds cakes added to feed), there were increases of 86.66%, at 14 days of storage, respectively by 35.98%, at 28 days of storage.

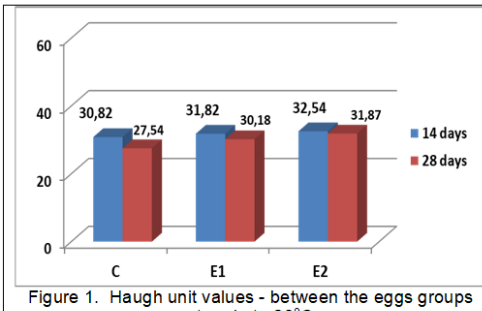

Figure 1. Haugh unit values - between the eggs groups stored at +20^{0}C

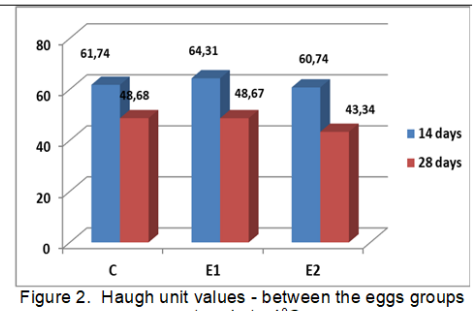

Figure 2. Haugh unit values - between the eggs groups stored at +4^{0}C

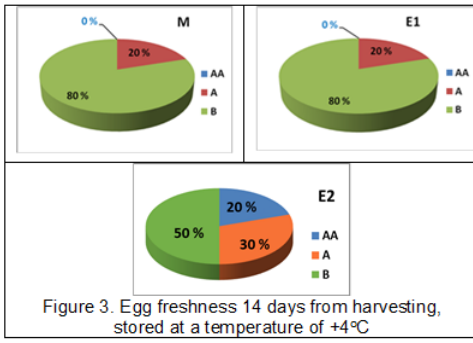

Figure 3. Egg freshness 14 days from harvesting, stored at a temperature of +4°C

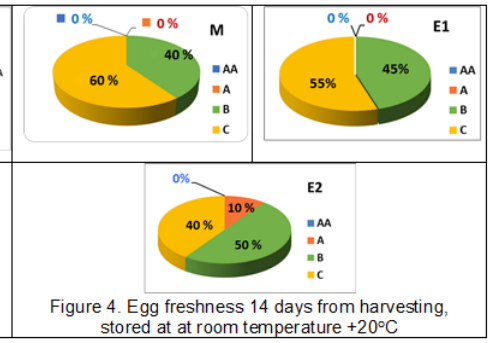

Figure 4. Egg freshness 14 days from harvesting, stored at at room temperature +20°C

Figure 3 shows the results regarding the freshness of the eggs kept in the refrigerator for 14 days. It can be seen that in group E2, were obtained 20% of AA eggs while in the other there were no AA eggs. In the case of eggs kept for 14 days, but at room temperature (+ 20°C), no eggs with in AA class were obtained, but only 10% eggs with A class (figure 4).

The determinations made on eggs stored for 28 days in the refrigerator (figure 5) show that also in E2 group (with grape seed cakes added to feed) a percentage of 35% eggs with freshness grade A was obtained.

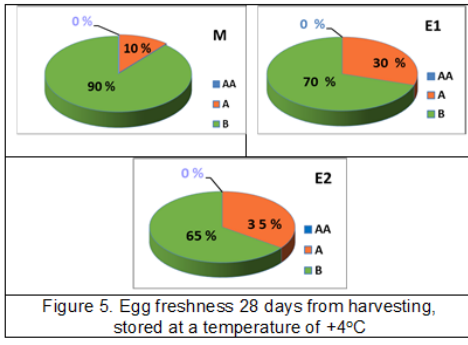

Figure 5. Egg freshness 28 days from harvesting, stored at a temperature of +4°C

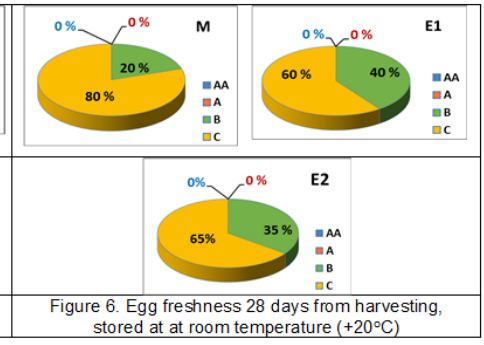

Figure 6. Egg freshness 28 days from harvesting, stored at at room temperature (+20°C)

Eggs stored at room temperature (+ 20°C) for 28 days (figure 6) were not obtained eggs with freshness degree AA, but only eggs with freshness grade B, 40%, in E1 group and of 35% in E2 group, respectively eggs with degree of freshness C in percentage of 60%, in E1 group (with vitamin E added in feed) and of 65%, in E2 group (with grape seed cakes added in feed).

CONCLUSIONS

• The inclusion of 5% flaxseed in the diets of the experimental groups resulted in increases in the values of polyunsaturated fatty acids (PUFA) in both feeds and eggs;

• The recorded values of the Haugh Unit in the case of eggs collected from the experimental groups, kept in the refrigerator, for 14 days, were significantly ($P<0.05$) higher than in those kept 28 days;
• The data regarding the freshness of the eggs kept in the refrigerator, for 14 days, revealed that only in the group with 2% grape seeds cakes there were eggs with freshness degree AA;
• The results show that the ratio for laying hens with grape seed cake was effective in maintaining the quality of eggs enriched in polyunsaturated fatty acids in time.

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REFERENCES

[1] Burlacu Gh., Cavache A., Burlacu R., 2002: The productive potential of feeds and its use, Ed. Ceres, p 218-224.
[2] Cherian G., Quezada N., 2016: Egg quality, fatty acid composition and immunoglobulin Y content in eggs from laying hens fed full fat camelina or flax seed, Journal of animal science and biotechnology, nr. 7(1), p. 15.
[3] Criste R.D., Panaite T.D., Ciurescu C., Ropota M., Rachieru D., 2009: Effects of moderate (5%) levels of linseed in layer diets, Archiva Zootechnica, nr 12(3): p. 11-21.
[4] Deng Q., Penner M.H., Zhao Y., 2011: Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins, Food Research International, nr 44: p 2712–2720.
[5] Fraeye I., Bruneel C., Lemahieu C., Buyse J., Muylaert K., Foubert I., 2012: Dietary enrichment of eggs with omega-3 fatty acids: A review, Food Research International, nr 48: p 961-969.
[6] Granato D., Castro I.A., Katayama F.C.U., 2010: Assessing the association between phenolic compounds and the antioxidant activity of Brazilian red wines using chemometrics, LWT-Food Sci. Technol., nr 43: p 1542–1549.
[7] Marxen K., Vanselow K.H., Lippemeier S., Hintze R., Ruser A., Hansen U.P., 2007: Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements, Sensors, nr 7: p 2080-2095
[8] Mihailović V., Matić S., Mišić D., Solujić S., Stanić S., Katanić J., Stanković N., 2013: Chemical composition, antioxidant and antigenotoxic activities of different fractions of Gentiana asclepiadea L. roots extract, EXCLI Journal, nr 12: p 807-823.
[9] Olteanu M., Criste R.D., Panaite T.D., Ropota M., Mitoi M., Varzaru I., Untea A.E., 2014: Study of the feeding value and antioxidant capacity of winery by-products, potential natural antioxidants for farm animal diet formulations, Archiva Zootechnica, nr 17(2): p 55-69.
[10] Olteanu M., Criste R.D., Panaite T.D., Ropotă M., Mitoi M., Vlaicu P.A., Soica C., 2017: Quality of the eggs obtained from hens fed diet formulations rich in polyunsaturated fatty acids and with grape seeds meal as antioxidant, Archiva Zootechnica, nr 20(1): p 37-49.
[11] Pahlavani M., Razafimanjato F., Ramalingam L., Kalupahana N.S., Moussa H., Scoggin S., Moustaid-Moussa N., 2017: Eicosapentaenoic acid regulates brown adipose tissue metabolism in high-fat-fed mice and in clonal brown adipocytes, J. Nutr. Biochem., nr 39: p 101–109.
[12] Panaite T.D., Criste R.D., Ropota M., Criste V., Vasile G., Olteanu M., Mitoi M., Socoliuc R., Vlaicu A., 2016: Determination of the feeding value of food industry by-products, Sci. Papers: Anim. Sci. Series, nr 66: p 106-111.
[13] Panaite T.D., Ropota M., Turcu R., Olteanu M., Corbu A.R., Nour V., 2017a: Flaxseeds: Nutritional Potential and Bioactive Compounds, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca: Food Science and Technology, nr 74(2): p 65-73.
[14] Radovanovic A., Radovanovic B., Jovancicevic B., 2009: Free radical scavenging and bacterial activities of southern Serbian red wines, Food Chemistry, nr 117: p 326–331.
[15] Ren Y., Perez T.I., Zuidhof M.J., Renema R.A., Wu J., 2013: Oxidative stability of omega-3 polyunsaturated fatty acids enriched eggs, J. Agric. Food Chem., nr 61(47): p 11595-11602.
[16] Shahidi F., Ambigaipalan P., 2018: Omega-3 polyunsaturated fatty acids and their health benefits, Annu. Rev. Food Sci. Technol, nr 9: p 345-381.
[17] Tortosa-Caparrós E., Navas-Carrillo D., Marín F., Orenes-Piñero E., 2017: Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome, Crit. Rev. Food Sci. Nutr., nr 57(16): p 3421-3429.
[18] Vlaicu P.A., Panaite T.D., Dragotoiu D., Ropota M., Bobe E., Olteanu M., Criste R.D., 2017: Feeding quality of the meat from broilers fed with dietary food industry by-products (flaxseed, rapeseeds and buckthorn meal, grape pomace), Scientific Papers. Series D. Animal Science, nr LX: p 123-130.
[19] Zhang W., Xiao S., Samaraweera H., Lee E.J., Ahn D.U., 2010: Improving functional value of meat products, Meat Science, nr 86: p 15-31.