Quality of the eggs obtained from hens fed diet formulations rich in polyunsaturated fatty acids and with grape seeds meal as antioxidant

Margareta Olteanu[1†], Rodica Diana Criste[1], Tatiana Dumitra Panaite[1], Mariana Ropotă[1], Monica Mitoi[2], P. A. Vlaicu[1] and Cristina Şoica[1]

[1]National Research Development Institute for Animal Biology and Nutrition, Balotesti, Ilfov, Romania; [2]Institute of Biology Bucharest, Romania

SUMMARY

The 4-week investigations were conducted on 99 Tetra layers aged 67 weeks, assigned to three groups: control (C), experimental 1 (E1) and experimental 2 (E2). The layers received a basal diet with corn, soybean meal and sunflower meal, enriched in polyunsaturated fatty acids via 7% flax meal. The diets of the experimental groups differed from diet C by the inclusion of grape meal, as natural antioxidant, 2% for E1 and 3% for E2. The use of 7% flax meal enriched the diet in omega-3 polyunsaturated fatty acids (14.17% in average) and with omega-6/omega-3 ratio of 3.07 in average. The yolk of the resulting eggs was also enriched in omega-3 polyunsaturated fatty acids, 4.91% in average, and with omega-6/omega-3 ratio of 4.25 in average. The use of grape meal in the experimental diets improved the oxidative status of the eggs collected in the end of the experiment from the experimental groups compared to group C, the antioxidant capacity being 3.62% in group E2. The cholesterol level in the yolk of the eggs from the experimental groups was significantly ($P \leq 0.05$) lower than in the control group, by 13.57% in group E1, and by 16.27% in group E2.

Keywords: eggs, PUFA, cholesterol, antioxidants, grape meal, quality

INTRODUCTION

Animal nutrition is currently striving to improve the quality of animal products with beneficial effects on consumer health state and quality of life.

† Corresponding author e-mail address: margareta.olteanu@ibna.ro

The acknowledged positive effects of the omega-3 polyunsaturated fatty acids on human health prompted the production of foods with high feeding value (Riediger et al., 2009, Huang, 2010, Shapiro et al., 2010, Turner et al., 2011, Chowdhury et al., 2014; Nobili et al., 2016). The eggs are staple food for a large category of consumers, being an almost perfect nutritional model. Eggs enrichment in polyunsaturated fatty acids is closely related to the fatty acids profile of layer diets (Van Elswyk et al., 1998; Simopoulos, 2000). The inclusion of feed ingredients high in polyunsaturated fatty acids, such as oleaginous included as seeds, meals or oil extracts, and sea algae, can enrich the eggs in omega-3 polyunsaturated fatty acids (Criste et al., 2009). Fats are, however, promoting rancidity in the feeds, therefore lipid oxidation in the eggs (Ren et al., 2013), which deteriorates egg quality and decrease their acceptance by the consumers.

The use of antioxidants in animal feeds became compulsory due to their capacity to inhibit oxidation by their reaction with the free radicals. Because consumer perception of food quality changed, there is an increasing interest in replacing the synthetic antioxidants with natural ones in animal feeding.

Winery by-products (grape pomace, grape seeds and peals) are a natural source of antioxidants with high levels of polyphenols. The abundancy of polyphenols in these by-products (Radovanovic et al., 2009; Granato et al., 2010), gives them antioxidant properties (Xia et al., 2010; Georgiev et al., 2014; Ky et al., 2014). Recent in vivo and in vitro studies on monogastric animals confirmed the beneficial effects of these bioreactive compounds due to their antioxidant and antimicrobial activity (Hu et al., 2013; Yu şi Ahmedna, 2013; Juśkiewicz et al., 2015). The inclusion of these winery by-products in diet formulations would enhance not just the antioxidative stability of the animal products, but would also limit the use of synthetic additives. They would also help meeting consumer demand for healthy and quality products (Brenes et al., 2016).

The purpose of our work was to study the effect of different levels of grape meal, used as antioxidant in layer diets high in polyunsaturated fatty acids, on egg quality.

MATERIAL AND METHODS

The 4-week feeding trial was conducted on 99 Tetra layers aged 67 weeks, assigned to three groups: control (C), experimental 1 (E1) and experimental 2 (E2), with 33 layers per group, assigned according to layer live weight. The layers were housed in standard Zucami cages (2 layers/cage), in an experimental hall with 21.5^{0}C average temperature and 60-70% relative humidity throughout the experimental period. The lighting

programme (16h daily) was according the TETRA management guide. The layers had free access to the feed and water.

The diets were formulated according to the chemical composition of the feed ingredients, using a mathematical model for poultry diets formulation (Burlacu et al., 2002). The layers received a basal diet with corn, soybean meal and sunflower meal, enriched in polyunsaturated fatty acids via 7% flax meal (Table 1). The diets of the experimental groups differed from diet C by the inclusion of grape meal, as natural antioxidant, 2% for E1 and 3% for E2, as shown in Table 1.

Table 1. Diet formulation

Item	C	E1	E2
Corn	57.94	55.94	55.58
Soybean meal	16.91	16.91	18.33
Sunflower meal	4.05	4.05	2
Flax meal	*7*	*7*	*7*
Grape meal		*2*	*3*
Oil	0.5	0.5	0.51
Methionine	0.19	0.19	0.19
Lysine	0.13	0.13	0.1
Carbonate	10.48	10.48	10.48
Phosphate	1.37	1.37	1.38
Salt	0.38	0.38	0.38
Choline	0.05	0.05	0.05
Premix*	1	1	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

Samples of the grape pomace, flax meal and feeds were collected and assayed for the basic chemical composition: dry matter (DM) using gravimetric method, crude protein (CP) using the Kjeldahl method, ether extractives (EE) using the extraction in organic solvents method, crude fibre (CF) using the method with intermediary filtration and ash (Ash) using gravimetric method from Regulation (CE) no. 152/ 2009. The gross energy was determined by calculation from the chemical analysis (dry matter, protein, fibre, ether extractives, nitrogen-free extractives and ash) using the equations of Burlacu et al. (2002). The indices of fat quality (peroxide value, fat acidity, Kreiss reaction) were determined according to STAS 12266-84 on samples collected upon manufacture of the compound feeds and after 14 and 28 days of storage.

The bioproductive parameters were monitored throughout the experimental period: average daily feed intake (g/layer/day), laying percentage, average egg weight (g/day), feed conversion ratio (g feed/ g egg mass). In the end of the feeding trial we collected 18 eggs from each group and measured the physical parameters of egg quality: weight, yolk colour intensity, Haugh unit and egg freshness, using an Egg Analyzer™, type 05-UM-001; eggshell thickness with the Egg Shell-Thickness Gauge and eggshell breaking strength with the Egg Force Reader. After concluding the physical measurements we for med 6 yolk samples/group (3 eggs/sample) and assayed them for the fatty acids profile, cholesterol concentration, concentration of total polyphenols a nd antioxidant capacity.

Fatty acids were determined by gas chromatography, whose working principle is the transformation of the fatty acids from the sample into methyl esters, followed by their separation in chromatographic column, identification by comparison with standard chromatograms and percent determination of the fatty acids esters from the sample. For the preparation of the fatty acids methyl esters (FAME) in agreement with the standard SR CEN ISO/TS 17764-1:2008. The analysis of the methyl esters was done according to standard SR CEN ISO/TS 17764-2:2008. Cholesterol was determined by gas chromatography according to AOAC 994.10 (2016).

To determine the polyphenols concentration and the antioxidant capacity of the feeds and egg samples, we first extracted the phenol compounds in acidified methanol (methanol:HCl=80:20). The polyphenols content of the methanol extracts was determined according to the method of Mihailovic et al. (2013), using a UV-VIS Thermo Scientific spectrophotometer, and the results were expressed in mg equivalents gallic acid /g fresh matter (mg GAE/g sample). The antioxidant capacity of the methanol extracts was determined by the DPPH method proposed by Marxen et al. (2007), using a UV-VIS Analytik Jena Specord 250 Plus spectrophotometer with thermostat carousel, and the results were expressed in Trolox equivalents/g fresh matter (mM TE/g sample).

Statistical analysis

The experimental results are shown as average values ± standard error, the statistical processing being done with StatView software, variance analysis (ANOVA and t test), the differences being considered significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

The three compound feeds included flax meal (C, E1, E2) as source of omega-3 polyunsaturated fatty acids and grape meal as natural antioxidant (E1, E2). Table 2 shows that the flax meal had: 56.41 g omega-3 polyunsaturated fatty acids /100g fat, with omega-6/omega-3 ratio of 0.27,

similar to data published by Aziza et al. (2013); Criste et.al. (2015); Panaite et al. (2016). The grape meal had 11,43% protein and 3.97% ash, values comparable with those reported by Mironeasa et al. (2010) and Elagamey et al. (2013). The polyphenols content was 3.865 mg GAE/g sample, ues comparable with those reported by Elagamey et al. (2013) and an antioxidant capacity of 34.998 mM TE/g sample.

Table 2– Chemical composition of the flax and grape meals*

Item	Flax meal	Grape meal
DM, %	90.94	86.32
CP, %	35.55	11.43
EE, %	10.79	0.81
Fibre, %	9.60	39.24
Ash, %	5.60	3.97
Σ SFA, g/ 100 g fat	10.20	17.88
Σ MUFA, g/ 100 g fat	18.21	19.72
Σ PUFA (g/ 100 g fat) of which	89.80	81.94
- omega-3, g/ 100 g fat	56.41	2.38
- omega-6, g/ 100 g fat	15.18	59.84
- omega-6 / omega-3	0.27	25.10
Polyphenols, mg GAE/g	0.987	3.865
Antioxidant capacity, mM TE/g	3.422	34.99

*Chemical composition on dry matter (DM) basis; DM=dry matter; CP=crude protein; EE= ether extractives; Fibre=fibre; Ash=ash; Σ= sum; SFA =saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; GAE=gallic acid equivalents; mM TE=.miliMols Trolox equivalents

The nutrients determined in the compound feeds samples (Table 3) showed that all 3 compound feeds had in average: 16.88% crude protein and 15.03 MJ/kg gross energy.

Table 3. Compound feeds content of main nutrients and of gross energy

Item	DM %	OM %	CP %	EE %	Fibre %	NFE %	Ash %	GE MJ/kg
C	87.72	74.55	16.88	3.38	3.80	50.49	13.17	15.08
E1	89.61	76.26	16.61	3.16	4.48	52.01	13.35	15.01
E2	89.82	76.28	17.15	2.97	4.60	51.57	13.53	15.01

The content of omega-3 polyunsaturated fatty acids (Table 4) revealed values of 13.91 – 14.64 g/100g fat, higher than 8.68 – 8.81 g /100g fat reported by Panaite et al., (2016) for 5% dietary flax meal, while omega-6/omega-3 ratio was 2.93 – 3.16, close to the values of 4.73-4.87 reported by Panaite et al., (2016), but lower than 6.78 reported by Aziza et al., (2013) for 10% dietary flax meal.

Table 4. Total fatty acids content of the tested compound feeds according to the level of saturation (g/ 100 g fat)

Item	C	E1	E2
SFA	14.09	14.74	14.10
MUFA	27.83	27.70	28.32
PUFA	58.08	57.55	57.58
Omega-3	13.96	13.91	14.64
Omega-6	44.12	43.65	42.95
Omega-6 / Omega-3	3.16	3.14	2.93

SFA =saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; Omega-3 - omega 3 polyunsaturated fatty acids; Omega-6 - omega 6 polyunsaturated fatty acids

We also determined the oxidative status of the compound feeds, i.e. the concentration of the polyphenols, with an average value of 1.25 mg GAE / g compound feed (Figure 1) and the antioxidant capacity, which was higher in the experimental groups, by 1.10% in group E1, and by 1.47% in group E2, compared to group C (Figure 2), with a good correlation between these two parameters, $R^2 = 0.8599$ (Figure 3).

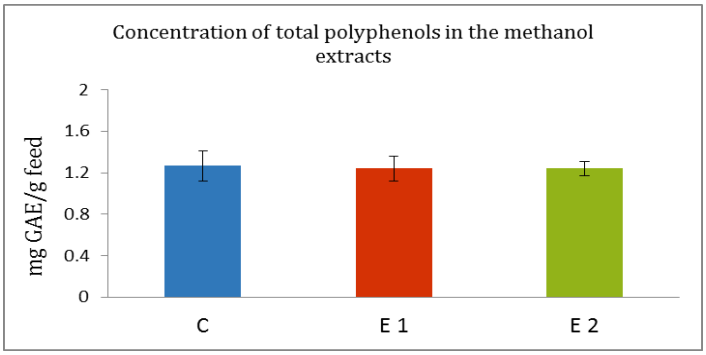

Figure 1

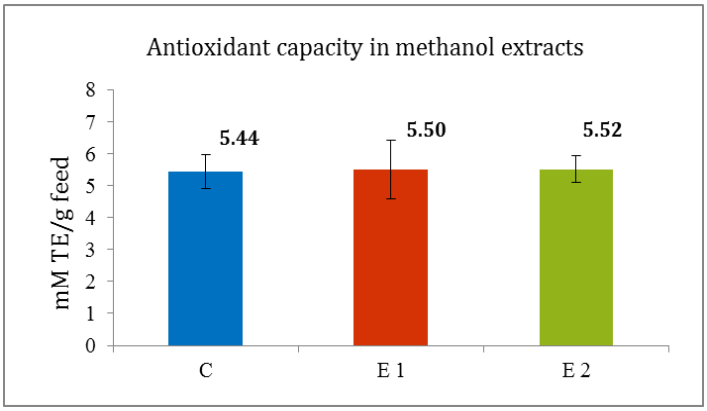

Figure 2

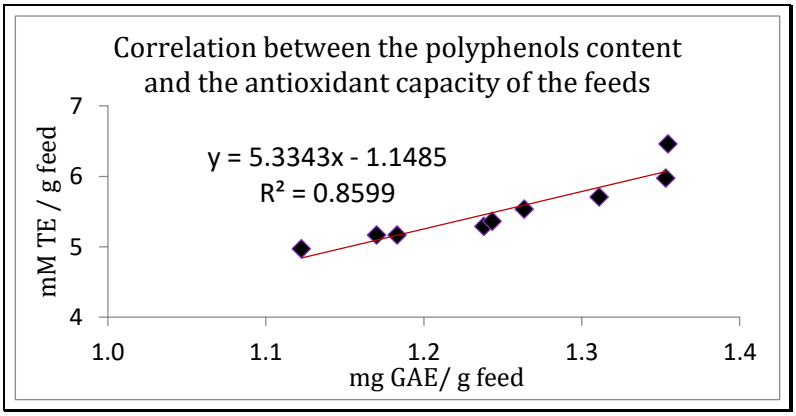

Figure 3

The physical measurements of the eggs (Table 5) showed significant (P≤0.05) differences in egg yolk weight, which was 4.18% higher in group E2 than in group C. Egg yolk colour intensity was 6.38% (P≤0.05) higher in group E2 than in group C. Similar results were reported by Aziza et al. (2013) for egg yolk weight and eggshell weight.

Table 5. Egg properties (average values/group/experimental period)

Item	C	E1	E2
Albumen weight, g	37.55 ± 0.57	38.02 ± 0.47	37.04 ± 0.39
Yolk weight, g	16.49 ± 0.29	16.00±0.24 [c]	17.18±0.26 [b]
Eggshell weight, g	8.63±0.30	9.07 ± 0.27	9.25 ± 0.18
Eggshell thickness, mm	0.326±0.007	0.333±0.007	0.338±0.006
Eggshell breaking strength, kf	3.14 ± 0.24	3.61 ± 0.16	3.59 ± 0.26
Colour intensity	4.39 ± 0.18	4.11 ± 0.12 [c]	4.67 ± 0.11 [b]

[b,c] significant differences (P≤0.05) from E1, E2

Compound feeds enrichment in polyunsaturated fatty acids using flax medal had a positive influence on egg quality (Figure 4). Thus, ALA concentration in the yolk ranged between 1.04±008 (E2) and 1.22±0.04 (E1), compared to 0.86 g/100 g fat (Aziza et al., 2013), or 1.77 - 2.01 g/100 g fat (Panaite et al., 2016). The concentration of the docosapentanoic acid ranged between 0.17±0.09 (E2) and 0.23±0.06 (E1), compared to 0.02 g/100 g fat (Aziza et al., 2013), or 0.18 – 0.22 g/100 g fat (Panaite et al., 2016). The concentration of the docosahexaenoic acid ranged between 3.12±0.40 (E2) and 3.65±0.48 (E1), compared to 0.86 g/100 g fat (Aziza et al., 2013), or 2.62 – 2.95 g/100 g fat (Panaite et al., 2016).

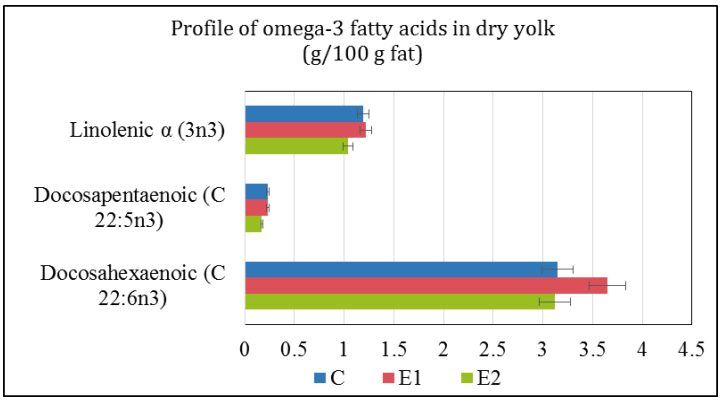

Figure 4

The content of omega-3 polyunsaturated fatty acids (Table 6) showed values ranging between 4.54±0.18 (E2) and 5.35±0.12 (E1), higher than 1.74 g/100 g fat (Aziza et al., 2013) and close to 5.05 – 5.17 g/100 g fat (Panaite et al., 2016); omega-6/omega-3 ratio ranged between 3.80±0.17 (E1) and 4.60±0.19 (E2), close to 4.92 – 5.12 (Panaite et al., 2016) and much below 9.37 (Aziza et al., 2013).

Table 6. Total fatty acids concentration in yolk fat, depending on the level of saturation (g/ 100 g fat) (average values/group)

Item	C		E1		E2	
SFA	36.18	±0.47	36.03	±0.57	37.19	±1.57
MUFA	38.02	±1.02	38.37	±1.33	37.47	±1.71
PUFA	25.76	±1.02	25.52	±1.08	25.31	±1.26
Omega-3	4.84	±0.13	5.35	±0.12	4.54	±0.18
Omega-6	20.91	±0.44	20.17	±0.41	20.77	±0.47
Omega-6 / Omega-3	4.34	±0.16	3.80	±0.17	4.60	±0.19

SFA =saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; Omega-3 - omega 3 polyunsaturated fatty acids; Omega-6 - omega 6 polyunsaturated fatty acids; [a,b,] significant differences (P≤0.05) from C, E1, E2

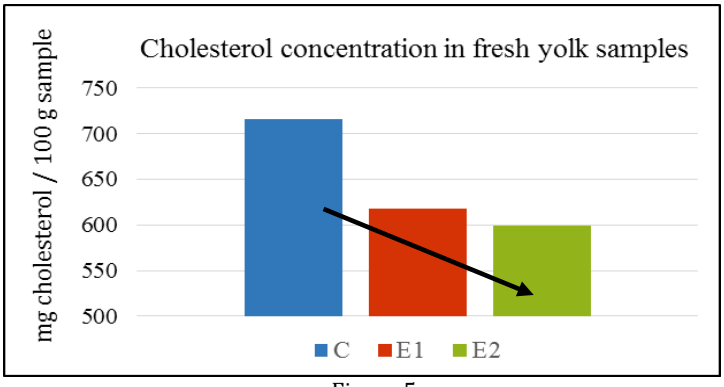

Figure 5

The cholesterol concentration determined on the eggs collected in the end of the trial (Table 5) were significantly (P≤0.05) different in the experimental groups: 13.57% lower in the eggs from group E1 and 16.27% lower in the eggs from group E2 than in the control group

The influence of the winery by-products on the cholesterol level of the eggs has also been proven by Su et al., (2008) and by Hu et al., (2013) on layers, which improved layer performance and increased egg cholesterol level.

Figure 6 shows that the use of grape meal in the diet formulations for groups E1 and E2 increased slightly the antioxidant capacity of the methanol extracts from the yolk of these groups, by 0.20% in group E1, and by 3.62% in group E2, compared to group C. these results are similar to those reported by Bunduc et al. (2016) and by Olteanu et al. (2016) who, using grape meal as antioxidant in diets high in polyunsaturated fatty acids, reported an antioxidant capacity of the methanol extracts from egg yolk ranging between 100.11 - 126.35 mM Trolox equivalents/ g sample.

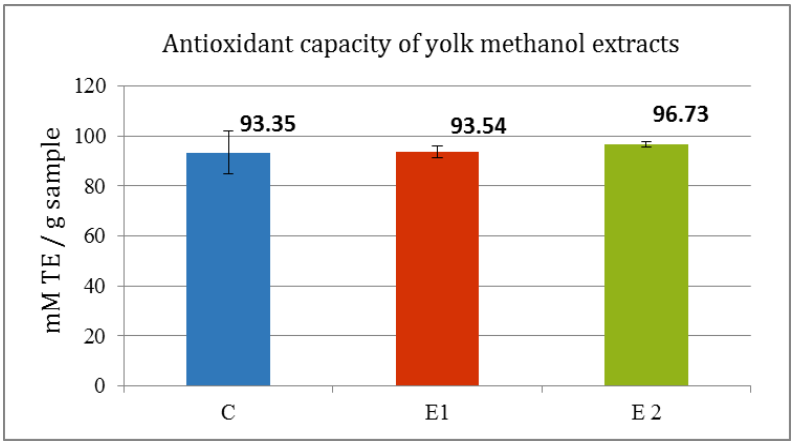

Figure 6

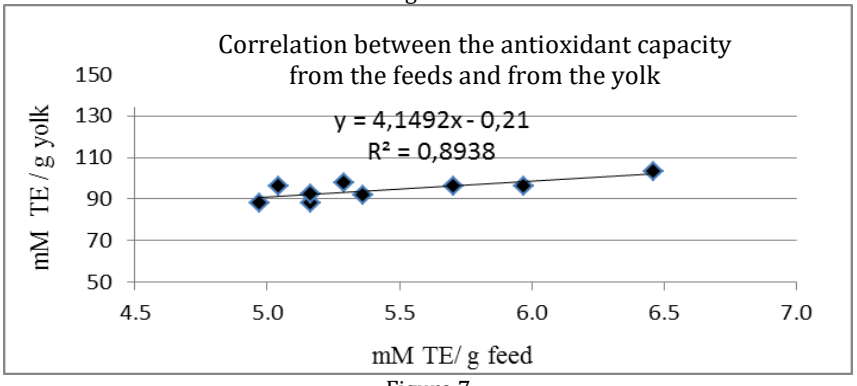

Figure 7

The values of the antioxidant capacity in the yolk and in the feeds are properly correlated (Figure 7), with $R^2 = 0.8938$.

Conclusions

The use of 7% flax meal in the compound feeds enriches both the diets and the yolks in omega-3 fatty acids. The use of rape meal as antioxidant in the diet formulations for the experimental groups (2% for E1 and 3% for E2), maintained the oxidative status of the feeds rich in polyunsaturated fatty acids throughout the feeding trial and improved the oxidative status of the eggs collected in the end of the trial (3.62% higher in the yolk from E2 than in the yolk from C).

All these conclusions show that the oxidative status of the eggs enriched in polyunsaturated fatty acids can be improved, while decreasing the cholesterol level, by using 3% grape meal as natural antioxidant in layer diets.

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