Evaluation of oxidation stability and antioxidant activity in eggs enriched in ω – 3 polyunsaturated fatty acids

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SUMMARY

A 6-week experiment was conducted on 168 Tetra SL laying hens (26 weeks) assigned to 4 groups (C, E1, E2 and E3). The basal diet for all 4 formulations included corn, wheat, soybean meal, rice bran and rapeseeds meal. The experimental diets differed from the control (C) diet by the inclusion of 7% flaxseeds meal (E1, E2, E3), of a vitamin supplement (E1) and of grape seeds powder (3% for E2 and 1.5% for E3). The concentration of ω - 3 polyunsaturated fatty acids (ω - 3 PUFA) in the compound feeds given to the experimental groups was about 4 times higher than in the control diet. The yolk of the eggs collected from the experimental groups had significantly (P≤0.05) higher concentration of ω - 3 PUFA, while ω – 6/ω – 3 PUFA ratio was about 3 times lower than in the eggs from the control group. The peroxide value was significantly (P≤0.05) lower in the yolks of groups E1 (0.136±0.058 mEq O_2/ kg) and E2 (0.074±0.069 mEq O_2/ kg) compared to groups C (0.230±0.055 mEq O_2/ kg) and E3 (0.263±0.111 mEq O_2/ kg). At the same time, the yolks from groups E1 and E2 had a higher antioxidant capacity than those from groups C and E3. The supplement of 3% grape seeds powder and of vitamin E (100 mg/kg CF) inhibited the onset of lipid peroxidation in the yolks of the eggs enriched in polyunsaturated fatty acids (ω – 3 PUFA).

Keywords: eggs, ω - 3 PUFA, grape seeds powder, peroxide value, conjugated dienes and trienes, antioxidant capacity

INTRODUCTION

The egg is a valuable food because it is a highly valuable source of proteins, vitamins, minerals and lipids, such as phospholipids and polyunsaturated fatty acids (Meluzzi et al., 2000; Anton et al., 2006). The

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production of eggs enriched in polyunsaturated fatty acids (PUFA) uses feeds rich in these nutrients, because there is a positive correlation between their dietary levels and those from the yolk (Leeson et al., 1998). Many authors consider that higher concentrations of ω – 3 polyunsaturated fatty acids (ω - 3 PUFA), of α-linolenic acid (ALA) particularly, occur when the diet formulations for laying hens include flax under various forms: seeds (Bean and Leeson, 2003; Criste et al., 2009); oil (Grobas et al., 2001; Milinsk et al., 2003; Souza et al., 2008); flaxseeds meal (Aziza et al., 2013; Panaite et al., 2016).

Although the interest for foods rich in ω - 3 PUFA is increasing (Siro et al., 2008), their presence leads to the onset of the process of oxidation of the double bonds C=C. As the number of double bonds from the PUFA increases, there is the possibility of forming complex mixtures of hydroperoxides, which are easily breakable and which are hard to detect (Frankel, 1998). Hydroperoxides are the main primary products of oxidation, indicators of the changes appearing in the early stages of oxidation. They accumulate during the initial stage and during the stage of propagation of oxidation (Shahidi et al., 2002; Fennema et al., 2007 b). Monitoring the concentration of hydroperoxides allows evaluating the state of lipid degradation (Shahidi et al., 2002). After the concentration of hydroperoxides reaches the peak level, the subsequent decrease is presumed to be the result of hydroperoxides decomposition in a variety of secondary oxidation products such as volatile substances, dihydroperoxides, ketones, hydroxyl- compounds, dimers and polymers, etc. (Frankel, 2005). Papuc et al., (2016) show that during the formation of the free radicals, the double bonds from the molecules of higher unsaturated fatty acids rearrange and, as a result, conjugated dienes and trienes appear. The concentration of the primary and secondary products of oxidation can be measured, thus providing indications as to the oxidative status of the particular food item. The oxidative deterioration of the lipids affects directly the flavour, colour and feeding value. Furthermore, the radicals formed during this process can also deteriorate the membrane of the foods of animal origin, the enzymes, vitamins and proteins (Shahidi and Wanasundara., 1996).

The antioxidants are usually added to layer diets to minimize lipid oxidation and to maintain the quality of the eggs enriched in ω – 3 PUFA (Qi and Sim, 1998; Galobart et al., 2001). The capacity to donate a hydrogen atom to a free radical is essential for the antioxidant activity (Buettner, 1993, Antolovich et al., 2002). The antioxidants act through a variety of mechanisms among which the control of substrate (lipids and oxygen) oxidation, control of pro-oxidants and inactivation of the free radicals. The consumers are very interested in the use of natural, plant antioxidants, which are regarded as being safe.

Several scientists reported that the peel of grapes, the grape seeds and the grape seeds extracts have strong activities against the effects of the free oxygen radicals, acting as free radical scavengers. The winery by-products are rich in polyphenols (Katalinić et al., 2010) and flavonoids (Yilmaz and Toledo, 2004), which are antioxidants.

The purpose of our study was to evaluate the stability to oxidation of the yolk lipids from the eggs enriched in ω – 3 PUFA, produced by the use in layer diets of flaxseeds meal as source of polyunsaturated fatty acids and of grape seeds powder and vitamin E as sources of antioxidants.

MATERIAL AND METHODS

Experimental design

A 6-week experiment was conducted on 168 Tetra SL laying hens (26 weeks) assigned to 4 groups (C, E1, E2 and E3) homogenous in terms of body weight, housed in an experimental hall with controlled environmental conditions (average temperature 21.94±1.96 °C and humidity 56.83±6.38 %), in agreement with Tetra SL management guide. The light regimen (16h light/24 h) was provided with light bulbs. The layers had free access to the water and feed.

Diets

The basic formulation of the compound feeds was the same (Table 1) for all groups (C, E1, E2 and E3). Table 1 shows that the difference between the control group and the experimental groups was the inclusion of 7% flaxseeds meal (E1, E2, and E3), of a vitamin E supplement (E1) and of grape seeds powder (E2, E3).

The flaxseeds meal was used in the feed formulations for groups (E1, E2, E3) as source of ω – 3 PUFA, mainly of ALA. The vitamin E supplement (100 mg/kg CF) was used in the formulation for group E1 as antioxidant, this vitamin being usually used in high-fat broiler diets. The powder of grape seeds that were mechanically degreased was used as natural antioxidant in the formulations for groups E2 (3%) and E3 (1.5%).

Collected samples and chemical analysis

Two samples each were collected from the flaxseeds meal, the grape seeds powder and the compound feeds, which were assayed for the fatty acids concentration. The fatty acids from the compound feeds were determined by gas chromatography, which presumes the transformation of the fatty acids from the sample into methyl esters followed by their separation in the chromatographic column, identification by comparison with standard chromatograms and determination of the percentage of the fatty acids from the sample (Panaite et. al 2016). Two samples of

compound feed/batch were collected upon manufacture and after 14 and 28 days of storage and assayed for the fat degradation indices. The methods used to determine the fat degradation indices were: the volumetric method (according to STAS 12266-84) for the acidity index and peroxide value and the quantitative method of Kreiss.

Table 1. Compound feeds formulation

Ingredients	Control group (C)	Experimental group 1 (E1)	Experimental group 2 (E2)	Experimental group 3 (E3)
Corn, %	20	20	20	20
Wheat, %	28.25	26.6	24.18	24.47
Rice bran, %	10	10	10	10
Soybean meal, %	18.72	13.28	15.32	13.36
Rapeseeds meal, %	8	8	4.68	8
Oil, %	3.53	3.29	3.9	3.84
Flaxseeds meal, %	-	7	7	7
Grape seeds meal, %	-	-	3	1.5
Methionine, %	0.08	0.16	0.18	0.16
Lysine, %	-	0.17	0.16	0.16
Calcium carbonate, %	8.72	8.74	8.78	8.74
Monocalcium phosphate, %	1.27	1.32	1.36	1.33
Salt, %	0.38	0.39	0.39	0.39
Choline, %	0.05	0.05	0.05	0.05
Premix A6 (IBNA), %	1	-	1	1
A6 (100 mg vit. E/kg.NC), %	-	1	-	-
Total	100	100	100	100
Calculated				
Dry matter, (DM %)	88.27	89.19	89.59	89.45
Metabolisable energy, (kcal/kg)	2,780	2,780	2,780	2,780
Crude protein, %	17.50	17.50	17.50	17.50
Ether extractives, %	4.89	5.31	6.02	5.91
Fibre, %	4.43	4.75	5.47	5.23
Calcium, %	3.90	3.90	3.90	3.90
Phosphorus, %	0.67	0.65	0.62	0.64
Lysine, %	0.87	0.84	0.84	0.84
Methionine, %	0.36	0.41	0.42	0.41
Methionine + Cystine, %	0.70	0.70	0.70	0.70

*1kg premix IBNA (A6) contains: = 1,350,000 IU/kg vit. A; 300,000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg Vit. B1; 480 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; 2500 mg/kg Vit. C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium;

The polyphenols content and the antioxidant capacity of the flaxseeds meal and grape seeds powder were determined by the National Institute of Biology – Romanian Academy. The polyphenol content of the methanol extracts has been determined according to the method described by

Mihailović et al., (2013), modified. The determination of the antioxidant capacity of the methanol extracts has been done using the DPPH method.

The stability at oxidation of the lipids from the eggs enriched in polyunsaturated fatty acids was determined on 72 eggs (18 eggs/group) collected during the final experimental week. Six yolk samples (3 eggs/sample) were formed for each group, put into 15 mL plastic tubes and frozen. The frozen samples were thawed before being assayed for the fatty acids concentration, peroxide value, concentration of conjugated dienes and trienes and the total antioxidant capacity.

The fatty acids methyl esters (FAME) were determined according to standard ISO 5508:2002, using 1 g fat extracted from dried yolk (65°C). The fatty acids methyl esters were determined with a Perkin Elmer- Clarus 500, gas chromatograph having flame ionization detector (FID) and capillary column BPX70, with high or medium polar stationary phase, 60 m long, inner diameter 0.25mm, and film thickness 0.25µm. H_2O was used as carrier, and analytical purity air was used as burning gas. The amount of fatty acids methyl esters from the fat sample is calculated with the relation between the sample area, standard area and dilution. The result is expressed in grams (g) fatty acids per 100 g fat.

Evaluation of yolk oxidative stability

Yolk fat was extracted using the method described by Folch et al., (1957). 2 g of yolk sample were homogenized with 10 mL methanol and 20 mL chloroform and stirred for one hour. The resulting solution was filtered into a separation funnel, to which 7.5 mL 0.88% KCl solution was added and the phases separated. The lower layer was collected in a 100 mL Berzelius beaker. It was left to evaporate at room temperature until the next day. The beakers with samples were weighed and the difference from the initial weight of the beakers represented the amount of fat extracted from the sample.

The peroxide value (PV) determination relied on the capacity of the peroxides to oxidize the ferrous ion at low pH, with xylenol orange (FOX). Oxidation is quantified using a complex, xylenol orange, which forms with the ferrous ion a violet-blue complex, which can be determined spectrophotometrically, with peak absorption at 560 mn (Nouroozzadeh et al., 1994). From the extracted fat we prepared samples of 0.01- 0.05 g in a glass vial, in which 9.9 mL chloroform-methanol (7:3, v/v) mixture was added and mixed. After adding 50 µL xylenol orange, 10 mM, and 50 µL $FeCl_2$ solution (1000 mg/kg), we homogenized again, and after 5 minutes the absorbance was read at 560 nm, using a JASCO V-530 spectrophotometer. The standard curve was obtained using $FeCl_3$ solution (10 mg/kg). The peroxide index was expressed as mEq O_2/ kg fat.

The concentration of conjugated dienes and trienes was determined by UV molecular absorption spectrometry, method also used by other authors (Gutteridge et al., 1990; Halliwell et al., 1993). The procedure we used was in agreement with the method described by Pegg, (2005). Part of the fat collected in Berzelius beakers, using the extraction procedure described above, was retaken on 10 mL isooctane for dissolution. If the fat didn't dissolve, more isooctane was added gradually up to a maximum volume of 50 mL. Absorbance was read, for the clear solutions, at 233 nm (conjugated dienes) and at 268 nm (conjugated trienes), using a JASCO V-530 spectrophotometer. The concentration of conjugated dienes was expressed in μmol/g fat, and that of conjugated trienes in absorbance units (A_{268} nm).

The total antioxidant capacity was determined using the spectrophotometric method described by Prieto et al., (1999). The method relies on the reduction of Mo (VI) to Mo (V), by the sample analyts and the subsequent formation of a green phosphate/Mo (V) complex, at acid pH. 1 g egg yolk was weighed in centrifuge tubes of 30-50 mL. 10 mL 80% methanol solution was added and stirred for one hour in darkness. The mixture was centrifuged for 15 minutes, at 10,000 rpm, and the supernatant was collected. 0.2 mL sample solution was pipetted in 15 mL tubes, over which 4 mL ammonium phosphor-molibdate reagent solution was added and incubated at 95 °C for 90 minutes. The mixture was left to cool and the absorbance was read at 695 nm on a JASCO V-530 spectrometer, compared to a blank. The results were expressed as mM ascorbic acid equivalent.

Statistics

The analytical data were compared using variance analysis (ANOVA), with Stat View for WINDOWS (SAS, version 6.0). The difference of the mean values was considered significant for $P \leq 0.05$. The results were expressed as mean± SD for all measurements.

RESULTS AND DISCUSSION

Table 2 shows that the flaxseed meal has a high content of ω-3 polyunsaturated fatty acids. Aziza et al., (2013) and Olteanu et al., (2016), reported higher concentrations of ω-3 PUFA in the flaxseed meal (43.23 % and 44.50 g/100 g, respectively), and a lower concentration of ω-6 PUFA (20.06 % and 22.43 g/100 g, respectively). As expected, the grape seeds powder had the highest content of polyphenols, which gave it a higher antioxidant capacity than the flaxseeds meal (Table 2). The grape seeds powder had a higher content of polyphenols, therefore a higher antioxidant capacity than the grape seeds meal used by Olteanu et al., (2016).

Table 2. Chemical composition of the flaxseeds meal and of the grape seeds meal

Item	Flax meal	Grape seeds meal
Fatty acids profile (g/100 g dry matter)		
∑SFA	11.07	13.48
∑MUFA	18.71	21.34
∑UFA	88.93	86.05
∑PUFA, of which:	70.23	64.71
∑ PUFA ω-3	42.93	1.47
∑ PUFA ω-6	27.30	63.23
PUFA ω – 6/ ω - 3	0.64	42.91
Antioxidant capacity		
Polyphenols concentration (mg/g)	3.328 ± 0.05	90.415 ± 8.53
Antioxidant capacity (mM Trolox/g)	9.896 ± 0.18	493.074 ± 49.26

As shown in Table 3, the polyunsaturated fatty acids concentration of the compound feeds given to the experimental groups was about 4 times higher than in the compound feeds given to the control group. All the compound feeds given to the experimental groups, supplemented with 7% flax meal had an ω – 6/ω – 3 ratio lower than that of the control group (Table 3), the lowest ratio being recorded in group E1 (Table 1). Dubois et al., (2007) showed that the flaxseeds have a good ω – 6/ω – 3 ratio due to the high concentration of ω – 3.

Table 3. Fatty acids concentration (g% total fatty acids) in the compound d feeds depending on the level of saturation

Item	C	E1	E2	E3
∑SFA	14.78	14.12	14.07	13.49
∑UFA	84.37	85.83	85.81	86.33
∑MUFA	32.73	31.77	30.91	30.96
∑PUFA of which:	51.64	54.06	54.90	55.37
∑PUFA ω - 3	1.78	7.41	7.07	7.19
∑ PUFA ω - 6	49.86	46.65	47.83	48.17
PUFA ω – 6/ ω - 3	28.02	6.30	6.77	6.70

Where: SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

The evaluation of compound feeds quality preservation in time showed that even though the compound feeds given to the experimental groups had important amounts of lipids in their composition, after 14 days from manufacturing, the peroxide value and the acidity index were comparable between the four groups (Table 4). This is due to the supplemental vitamin E (E1) and to the grape seeds powder (E2, E3) which delayed the process of lipid degradation during the first two weeks after manufacturing. After 28 days from manufacturing the compound feeds for groups E2 and E3, the peroxide value was higher than 1.2 mg $Na_2S_2O_3$/g, and the Kreiss reaction

produced a ,,doubtful" result (TAble 4). Even though Singh et al. (2011a, b) have shown that the flax is stable for a longer period at room temperature, the compound feed, being a complex mixture, is more vulnerable to lipid oxidation.

Table 4. Evolution of the lipid degradation indices in the compound feeds

Item		C	E1	E2	E3
Peroxide value (mg $Na_2S_2O_3$/g)	Initial	0.42	0.53	0.51	0.6
	14 days from manufacture	0.56	0.55	0.62	0.66
	28 days from manufacture	1.03	1.25	1.44	1.35
Fat acidity (mg KOH / g)	Initial	15.72	14.43	14.37	15.88
	14 days from manufacture	18.59	18.54	17.28	19.66
	28 days from manufacture	39.31	40.86	39.06	40.91
KREISS reaction	Initial	Negative	Negative	Negative	Negative
	14 days from manufacture	Negative	Negative	Negative	Negative
	28 days from manufacture	Negative	Negative	Doubtful	Doubtful

Layer diets supplementation with oleaginous feeds increased the total PUFA concentration (linoleic acid and α-linolenic acid) in the egg yolk (Leeson et al., 1998), which was also noticed in this experiment. The yolks of the eggs from the experimental groups had a concentration of ω – 3 PUFA significantly (P≤0.05) higher than those from the control group (Table 5). The concentration of ω – 6 PUFA was higher (P≤0.05) in the yolk from the control group eggs than in the yolk from the experimental groups eggs (Table 5). ω – 6/ω – 3 ratio was significantly (P≤0.05) higher, by about 3 times, in the yolks from the control group eggs than in the yolk from the experimental groups eggs (Table 5).

Table 5. Fatty acids concentration (g% total fatty acids) in the egg yolk depending on the level of saturation

Item	Initial	Average of the determinations			
		C	E1	E2	E3
ΣSFA	34.60±1.112^{b}	34.43±0.814^{a,e}	33.91±1.025	33.61±0.923	32.85±0.916^{b}
ΣUFA	64.62±0.850cde	65.45±0.838^{e}	65.93±1.041^{a}	66.22±0.8^{a}	66.99±0.906^{ab}
ΣMUFA	56.62±0.668^{bcde}	33.96±0.944^{a}	33.94±1.156^{a}	33.29±0.903^{ae}	34.44±0.914^{ad}
ΣPUFA, of which:	7.99±0.338^{bcde}	31.49±0.303 ^{acde}	31.99±0.300^{abde}	32.92 ±0.255^{abc}	32.55±0.512^{abc}
-ΣPUFA ω - 3	1.71±0.07^{cde}	1.56±0.19^{cde}	4.58±0.21^{abe}	4.39±0.24^{abe}	4.13±0.20^{abcd}
-ΣPUFA ω - 3	28.62±0.45 ^{bc}	29.93±0.3 ^{acde}	27.41±0.18^{abde}	28.54±0.30^{bc}	28.42±0.49^{bc}
-PUFA ω – 6/ ω - 3	16.75±0.55 ^{bcde}	19.23±2.39 ^{acde}	5.99±0.27^{ab}	6.50±0.41^{ab}	6.88±0.35^{ab}

*Where: a,b,c,d, significant differences (P≤0.05) from C, E1, E2, E3.
SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

Lipid degradation was evaluated by the peroxide value. Frankel, (2005), noticed that, if there is no antioxidant, the higher is the proportion of unsaturated fatty acids in the fat, the higher is the peroxided value. The peroxide values of the egg yolk from groups E1 and E2 were significantly

(P≤0.05) lower than in groups C and E3 (Table 6). This shows that although all the diets for the experimental groups included flax meal (7%), the addition of vitamin E (100 mg vit. E/kg. CF) for group E1 and 3% grape seeds powder for group E2, delayed lipid peroxidation within the yolk. The yolk from group E2 had the lowest peroxide value (Table 6), which proves the higher efficiency of the 3 % grape seeds powder as natural antioxidant, compared to the lower concentration (1.5%) grape seeds powder used for group E3.

Table 6. Peroxide value of the yolk

Item	Peroxide value (mEqO2/kg)
Group C	0.230 ± 0.055^{bcd}
Group E1	0.136 ± 0.058^{ad}
Group E2	0.074 ± 0.069^{ad}
Group E3	0.263 ± 0.111^{ac}

* Where: a,b,c,d, significant differences (P≤0.05) from C, E1, E2, E3.

Significant (P≤0.05) differences in the level of dienes and trienes, secondary products of lipid oxidation, were determined between the experimental groups (E1, E2, E3) and the control group (Tables 7 and 8). The yolk of E3 group eggs had a significantly (P≤0.05) lower concentration of conjugated dienes compared to the control group (by 12.92%) (Table 7). Although the diet of the control group was not enriched in fatty acids, the yolk of the eggs coming from this group had a significantly (P≤0.05) higher level of conjugated dienes compared to the experimental groups (E1, E2, E3). This proves the beneficial action of the grape seeds powder (E2 and E3) and of the vitamin E (E1) on the oxidative stability of the lipids from the eggs enriched in ω – 3 PUFA. The method used to determine the concentrated of conjugated dienes in the egg yolk was also used by Predescu et al. (2013), for refrigerated eggs. The study proved that during refrigeration, the eggs undergo processes of lipid oxidation as shown by the increased concentration of primary products of oxidation (hydroperoxides, conjugated dienes and trienes) and of the secondary products of peroxidation.

Table 7. Concentration of conjugated dienes (CD) in the yolk

Item	CD value *(μmol/g)*
Group C	8.178 ± 0.283^{bcd}
Group E1	7.250 ± 0.086^{a}
Group E2	7.122 ± 0.097^{a}
Group E3	7.121 ± 0.135^{a}

* Where: a,b,c,d, significant differences (P≤0.05) from C, E1, E2, E3.

Table 8 shows the absorbance of the conjugated trienes (CT, A_{268} nm) from the egg yolk. The yolk of the control group eggs displayed a higher absorbance than those from the experimental groups (Table 8). The lowest absorbance was noticed in the yolk from E3 group, close to that of E2 group (Table 8). This shows the efficiency of the grape seeds powder to delay the peroxidation of yolk lipids.

Table 8. Absorbance of the conjugated trienes (CT) from the yolk

Item	$A_{CT\ (268\ nm)}$
Group C	3.292
Group E1	3.148
Group E2	3.126
Group E3	3.122

The yolk of E3 eggs (1.5% grape seeds powder) had an antioxidant capacity significantly (P≤0.05) lower than the other groups (Table 9). This result, corroborated with the peroxide values (Table 6) shows that the proportion of 1.5% grape seeds powder was too low to slow down yolk lipid degradation. The highest antioxidant capacity was determined in the yolk of the eggs from groups E1 (100 mg vit. E/kg CF) and E2 (3 % grape seeds powder). These results are due to the antioxidant activity of the grape seeds powder (E2) and of vitamin E (E1). Şahin et al., (2010) showed that the addition of resveratrol, phenolic compound from the grape seeds, improved the antioxidant status of the quail eggs. Papuc et al., (2008) have shown that the oxidation of the unsaturated fatty acids from the foods can be slowed down by the addition of plants rich in compounds with antioxidant activity.

Table 9. Antioxidant capacity of the yolk

Item	Antioxidant capacity (*mM ascorbic acid equivalent*)
Group C	5.795 ± 1.78^{cd}
Group E1	7.866 ± 0.71^{d}
Group E2	8.672 ± 3.67^{ad}
Group E3	6.103 ± 0.36^{abc}

* Where: a,b,c,d, significant differences (P≤0.05) from C, E1, E2, E3.

CONCLUSIONS

The interest in foods with higher ω-3 PUFA levels is increasing, but the presence of these fatty acids favours the process of double bonds oxidation. The compound feeds given to the layers from the experimental groups (with 7% flax meal), had a higher concentration of ω-3 PUFA than the control group. The dietary experimental compound feeds determined the

production of eggs enriched in ω-3 PUFA. The addition of 3% grape seeds powder and of vitamin E (100 mg/kg CF) delayed the onset of lipid degradation within the eggs enriched in polyunsaturated fatty acids (ω-3 PUFA). Within this context, both the peroxide value and the antioxidant capacity have shown better results in the yolk of E1 and E2 groups. The 1.5% grape seed powder didn't have the efficiency of the higher concentration.

The experimental results support the possibility of using specific levels of grape seeds powder in the layer diets high in ω-3 polyunsaturated fatty acids, to improve compound feeds and egg quality by inhibiting lipid oxidation.

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