USE OF HIGH LEVEL (4%) DIETARY GRAPE MEAL IN LAYING HENS DIETS

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Abstract

The use in poultry diets of feed ingredients rich in polyunsaturated fatty acids requires the use of antioxidants to prevent fatty acids oxidation. The purpose of our experiment was to assess the use of a high level (4%) of dietary grape meal as antioxidant in layer diets rich in polyunsaturated fatty acids, on layer performance and egg quality. The 4-week trial used 99, Tetra layers aged 64 weeks assigned to three groups (C, E1 and E2). The basal diet (C) consisted of corn, sunflower meal and soybean meal. The experimental diets (E1 and E2) had additionally 7 % flax meal, while E2 included 4% grape meal as antioxidant. All diets had similar levels of protein and energy, 16.79 % CP and 15.13 MJ / kg gross energy. The diet formulations for the experimental groups had a higher level of α linolenic acid (omega-3) (13.96 g/100 g fat, in E1, and 15.13 g/100 g fat, in E2) than the formulation for group C, 1.25 g/100 g fat. The ratio of PUFA omega-6/omega-3 in the fat of the compound feeds for the experimental groups decreased by 93.34% for E1 and by 93.57% for E2, compared to group C. The grape meal, with 3.86 mg gallic acid equivalent/g as polyphenols content, and an antioxidant capacity of 34.99 mM Trolox equivalent/g, increased the antioxidant capacity in the methanolic extracts from the eggs harvested from the experimental groups: 96.72 mM Trolox equivalent /g yolk in E1, 100.10 mM Trolox equivalent /g yolk in E2, compared to 91.78 mM Trolox equivalent /g yolk in group C.

Key words: eggs; PUFA; antioxidants; grape meal

INTRODUCTION

Because the consumers are nowadays increasingly concerned with food quality, the production of high feeding quality foods is an increasing concern of the farmers. For instance, the acknowledged positive effects of the omega-3 polyunsaturated fatty acids on human health [11], [22], [23], [26] require finding ways to incorporate them in human diets. Various studies reported in the literature [5], [7], [8], [16], [24] are proving the direct influence of the structure and nature of the feeds given to animals on the feeding value of the animal products.

The egg is one of the most demanded staples, so that the production of highly nutritious eggs, enriched in polyunsaturated fatty acids, is closely related to the fatty acids profile of layer diets. The inclusion of ingredients rich in polyunsaturated fatty acids, such as seeds, meals of oily extracts from oleaginous plants, enriches the eggs in omega-3 fatty acids. However, these ingredients are propitious to rancidness, implicitly to egg lipid oxidation [21], which damage egg quality and make the consumers reluctant to accepting them. It is therefore necessary to use dietary antioxidants, particularly natural ones, whose high concentration of polyphenols block oxidation by their reaction with the free radicals.

The studies conducted over the past decade have shown that winery by-products, due to their antioxidant capacity associated to the polyphenols and flavonoids content [9], [20], can be used in layer nutrition. Among these by-products are the grape marc, which is produced in large amounts [10], [13], [25] grape seeds extracts [1], [12] or the grape seeds oils [3], [4].

The purpose of our experiment was to test the effect of using 4% grape meal included in high-polyunsaturated fatty acids compound feeds, on layer performance and egg quality.

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MATERIAL AND METHOD

The experiment was conducted for 4 weeks on 99 TETRA layers, aged 64 weeks, assigned to three groups control (C), experimental 1 (E1) si experimental 2 (E2), with 33 layers per group. The layers were assigned to the groups according to their live weight, so as not to have significant differences between groups. The hens had free access to the feed and water. According to the TETRA management manual, the light regimen was of 16 h daily, the temperature was maintained in the range of 22-24^{0}C, with a relative humidity of 60-70% throughout the experimental period.

Each diet formulation was calculated based on the determinations of chemical composition of the feed ingredients, using a mathematical model for poultry diet formulation [2]. The basic diet consisted of corn, wheat, sunflower meal and soybean meal. The diets for the experimental groups E1 and E2 differed from diet C by the inclusion of 7% flax meal, as ingredient rich in polyunsaturated fatty acids, while E2 diet also included 4% grape meal as antioxidant.

Throughout the entire experimental period we monitored the following bioproductive parameters average daily feed intake (g/day/bird), the feed conversion ratio (f feed/g egg mass), the laying percentage and the average egg weight (g/zi). Egg samples (18 eggs/group) were collected at the beginning and end of the trial, and the quality physical parameters of the egg were determined. After the physical measurements, 6 average egg samples per group were formed (3 eggs/sample), to determine yolk nutrient content; the fatty acids and cholesterol yolk content; the total polyphenol concentration and the antioxidant capacity of the methanol yolk extracts.

The physical parameters of the eggs were represented by albumen, yolk and eggshell weight, albumen and yolk pH, using InoLab Ph-meter; yolk colour intensity, the Haugh unit, and egg fresh ness, using Egg AnalyzerTM, tip 05-UM-001; eggshell thickness, using the Egg Shell Tchickness Gauge and eggshell breaking strength, using the Egg Force Reader.

Standardised methods were used to determine the main nutrients from the feed ingredients, compound feeds and yolk:

-The dry matter (DM)-by the gravimetric method / *Regulation (CE) nr. 152/2009 and standard SR ISO 6496:2001*, with Sartorius scale and BMT drying closet, ECOCELL;

-The crude protein (CP) – by the Kjeldahl method /*Regulation (CE) nr. 152/2009 and standard SR EN ISO 5983-2:2009*, with KJELTEC Auto 2300 system–Tecator;

-Ether extractives (EE) -by the extraction method /*Regulation (CE) nr. 152/2009 and standard SR ISO 6492:2001*, with SOXTEC-2055–Tecator;

-The fatty acids –by the chromatographic method /*standard SR CEN ISO/TS 17764-2:2008*, with Perkin Elmer-Clarus 500 chromatograph, fitted with a system for injection into the capillary column, high polarity stationary phase (60m x 0.25mm inner diameters and 0.25µm thick film);

-The crude fibre (CF)- by the method with filtering intermediary /*Regulation (CE) nr. 152/2009 and standard SR EN ISO 6865:2002*, with FIBERTEC 2010–Tecator;

-The ash (Ash)- by the gravimetric method /*Regulation (CE) nr. 152/2009 and standard SR EN ISO 2171:2010*, with Caloris CL 1206 furnace;

-The gross energy was determined by calculation from the gross chemical composition (dry matter, protein, fibre, fat, nitrogen-free extractives and ash) using the equations of [2];

-The polyphenol content of the methanol extracts, expressed in mg galic acid equivalents/g sample (mg GAE/g sample)-by the method described by [17], modified, with a UV-VIS Thermo Scientific spectrophotometer;

-The determination of the antioxidant capacity of the methanol extracts, expressed in mM Trolox equivalents/g sample (mM TE/g sample)-by the DPPH method proposed by [15], with a UV - VIS Analytik Jena Specord 250 Plus spectrophotometer.

The results of the experiment as presented as mean values with standard deviations, the statistic processing being done with StatView, the differences were considered statistically significant for P≤0.05.

RESULTS AND DISCUSSIONS

The flax meal used to enrich the experimental compound feeds E1 and E2, was characterized by 35.55% crude protein, 10.79% ether extractives, 9.60% fibre, 19.65 MJ/kg gross energy, a content of α linolenic acid (ALA) of 56.02 g /100 g total fatty acids methyl esters, total omega-3 fatty acids 56.41%, total omega-6 fatty acids 15.18%, and omega-3/omega-6 ratio of 0.27.

The grape meal, used as antioxidant, had 11.33 ± 0.52% crude protein, 0.69 ± 0.14% ether extractives, 39.51 ± 1.21% fibre and 16.08 ± 0.04 MJ/kg gross energy, similar to the results reported by [6], [18], [19].

The polyphenols content of the grape meal (Table 1) was, in average, of 3.865 mg EAG/g sample, while its antioxidant capacity was, in average, of 34.998 mM ET/g sample, these two parameters being rather well correlated, as shown by coefficient R^2 = 0.7360 (Figure 1).

Table 1 Oxidative status of the grape meal

Determination	MU	*Valori*
Polyphenols	mg GAE/g sample	3.865 ±0.040
Antioxidant capacity	mM ET / g sample	34.998 ±1.268

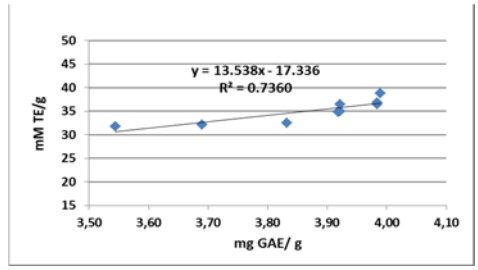

Fig. 1 Correlation between the polyphenols concentration and the antioxidant capacity of the grape meal

The nutrients from the samples of finished compound feeds showed that all three compound feeds (Table 2) were isoprotein (about 16.79% protein) and isocaloric (about 15.13 MJ/kg gross energy).

Table 2 Main nutrients (%) and gross energy (MJ/kg) of the compound feeds

Item	DM	OM	CP	EE	CF	NFE	Ash	GE
C	90.10	77.50	16.65	3.87	4.23	52.75	12.61	15.37
E1	88.64	75.47	16.88	3.38	3.80	51.41	13.17	14.92
E2	89.50	76.87	16.85	3.07	4.89	52.05	12.64	15.12

DM – Dry matter; OM – Organic matter; CP – Crude protein; EE – Ether extractives; CF – Crude fibre; Ash - Ash; NFE – nitrogen-free extractives; GE – gross energy.

The addition of flax meal to the compound feeds of the experimental groupschanged the fatty acids profile compared to the control group compound feeds (Table 3). Thus, the level of α - linolenic acid, omega-3, polyunsaturated fatty acid, increased 11.17 times in E1 and 12.10 times in E2, compared to group C. On the other hand, the linolenic acid, omega-6 polyunsaturated fatty acid was slightly higher in group C compared to the experimental groups (E1 and E2).

Table 3 Fatty acids profile in the tested feeds and their ratio (g FAME*/100 g Total FAME)

Fatty acids	C	E1	E2
Lauric	0.06	0.05	0.03
Myristic	0.16	0.12	0.11
Palmitic	10.58	10.60	10.47
Palmitoleic	0.27	0.21	0.21
Stearic	3.11	3.10	3.06
Oleic cis	29.44	27.62	27.71
Linoleic cis	54.50	43.59	42.55
α-Linolenic	1.25	13.96	15.13
Heneicosenoic	0.22	0.21	0.21
Eicosadienoic	0.17	0.14	0.15
Eicosatrienoic	0.25	0.40	0.18
SFA, %	14.13	14.09	13.88
MUFA, %	29.71	27.83	27.91
UFA, %	85.87	85.91	85.92
PUFA, %	56.17	58.08	58.01
Ω3, %	1.25	13.96	15.13
Ω6, %	54.92	44.12	42.87
Ω6/Ω3	44.05	3.16	2.83

*FAME – Fatty acids methyl esters

These data explain the high omega-6/omega-3 ratio (44.05) in group C, while it was 3.16 for group E1 and 2.83 for group E2, which means that the flax meal improved the quality of the experimental groups compound feeds.

Layer performance (Table 4) shows that the lowest average daily feed intake was in group E1, 11.42% lower compared to group C. The use of 4% grape meal in group E2, however, increased significantly ($P \leq 0.05$) the average daily feed intake, by up to 10.30%, compared to group E1. Feed conversion ratio and the laying percentage displayed the same differences as the average daily feed intake, as the use of grape seeds meal (E2) improved significantly ($P \leq 0.05$) these parameters, feed conversion ratio being 13.01% better and the laying percentage being 13.33% higher than in group E1. However, no statistically significant difference was observed in the average egg weight between groups E1 and E2.

Table 4 Layer performance (average values ± std err /group)

Groups		
C	E1	E2
Average daily feed intake, (g CF /layer /day)		
113.536^{b} ± 0.884	100.571^{a,c} ± 1.235	110.929^{b} ± 1.259
Feed conversion ratio, (kg CF/ kg egg)		
1.755^{b} ± 0.047	1.568^{a,c} ± 0.064	1.772^{b} ± 0.036
Laying percentage, (%)		
87.664^{b} ± 2.026	75.435^{a,c} ± 2.217	85.493^{b} ± 1.308
Average egg weight, (g / egg)		
64.675^{c} ± 0.128	64.121^{c} ± 0.163	62.578^{a,b} ± 0.151

*a,b,c - significantly different ($P \leq 0.05$) from C; E1; E2.

As shown in Table 5, the physical and organoleptic parameters determined on eggs harvested at the end of the trial, there were no significant differences between groups in albumen and yolk weight, eggshell weight, breaking strength and yolk colour intensity.

The physical measurements showed that the Haugh unit was higher for the eggs from groups E1 and E2 than for the control group, but the differences were not significant ($P \leq 0.05$), although the compound feeds for these groups had a much higher concentration of polyunsaturated fatty acids.

Similar results were reported by [14] who supplemented layer diets with 4% and 6% grape pomace and noticed that the Haugh unit was not significantly modified. The data on the Haugh unit, corroborated with the data on egg freshness (Table 5) prove that the used winery by -products acted as antioxidants thus preserving the organoleptic qualities of the eggs from the experimental groups.

Table 5 Physical and organoleptic parameters determined on eggs collected in the end of the trial (average values± std err /group)

Item		C	E1	E2
Albumen weight, g		38.28 ± 1.10	37.59 ±0.63	37.81 ± 0.44
Yolk weight, g		17.02 ± 0.34	16.55 ± 0.29	16.30 ± 0.31
Eggshell weight, g		8.51 ± 0.17	8.79 ± 0.30	8.69 ± 0.13
Eggshell thickness, mm		0.35^{b} ± 0.01	0.32^{a} ± 0.01	0.34 ± 0.01
Eggshell breaking strength, kf		3.44 ± 0.21	3.14 ± 0.24	3.43 ± 0.29
Colour intensity		4.11 ± 0.16	4.39 ± 0.12	3.94 ± 0.25
Haugh unit		84.54 ± 1.82	86.19 ± 2.68	87.58 ± 1.50
Freshness, %	AA	94.44	88.89	100
	A	0.00	5.56	0.00
	B	5.56	5.56	0.00

*a,b,c - significantly different ($P \leq 0.05$) from C; E1; E2

Analysing the fatty acids profile of the egg yolk at the end of the trial (Table 6), it can be noticed that de use of flax meal for the experimental groups increased significantly the level of omega-3 fatty acids: α-linolenic, docosapentaenoic and docosahexaenoic in the yolk of the eggs from these groups, their total amount increasing by 253.28% in group E1 and by 260.58% in group E2, compared to the control group. On the other hand, the level of omega-6 fatty acids decreased significantly ($P \leq 0.05$) in the yolk of the eggs from experimental groups: by 18.69% in group E1 and by 12.80 in group E2, compared to the control group. Thus, the ratio of Ω6/Ω3 polyunsaturated fatty acids decreased significantly ($P \leq 0.05$) in the yolk of the eggs from the experimental groups, by

75.89% in group E1 and by 75.72% in group E2, compared to the control group.

These results confirm the production of eggs enriched in omega-3 polyunsaturated fatty acids.

Table 6 Fatty acids profile in the egg yolk, in the end of the trial (g FAME*/100 g Total FAME)

Fatty acids		C		E 1		E 2	
Myristic	C 14:0	0.27	±0.02	0.24	±0.02	0.23	±0.04
Mristoleic	C 14:1	0.00	±0.02	0.00	±0.02	0.00	±0.01
Pentadecanoic	C 15:0	0.07	±0.01	0.20	±0.30	0.08	±0.01
Pentadecenoic	C 15:1	0.12	±0.02	0.15	±0.03	0.09	±0.02
Palmitic	C 16:0	24.22	±0.59	24.17	±0.62	24.29	±0.65
Palmitoleic	C 16:1	2.77	±0.22	2.94	±0.40	2.82	±0.57
Heptadecanoic	C 17:0	0.14	±0.02	0.15	±0.02	0.16	±0.01
Heptadecenoic	C 17:1	0.09	±0.02	0.14	±0.02	0.10	±0.03
Stearic	C 18:0	11.25	±0.62	11.42	±0.63	11.31	±1.32
Oleic	C 18:1n9	34.57	±1.78	34.49	±0.86	34.20	±1.85
Linoleic	C 18:2n6	18.35	±0.90	16.30	±0.74	17.08	±0.74
Linolenic γ	C 18:3n6	0.10	±0.01	0.11	±0.02	0.11	±0.01
Linolenic α	C18:3n3	$0.22^{b,c}$	±0.03	$1.19^{a,c}$	±0.13	$1.29^{a,b}$	±0.24
Eicosadienoic	C20 (2n6)	0.17	±0.01	0.15	±0.03	0.13	±0.02
Eicosatrienoic	C20 (3n6)	0.28	±0.04	0.16	±0.08	0.20	±0.02
Erucic	C22 (1n9)	0.11	±0.02	0.07	±0.05	0.06	±0.01
Eicosatrienoic	C20 (3n3)	0.22	0.03	0.27	±0.05	0.24	±0.03
Arachidonic	C20 (4n6)	4.20	±0.42	3.89	±0.35	3.65	±0.69
Nervonic	C24 (1n9)	0.36	±0.02	0.22	±0.03	0.21	±0.04
Docosatetraenoic	C22 (4n6)	1.50	±0.24	0.30	±0.05	0.29	±0.08
Docosapentaenoic	C22 (5n3)	$0.08^{b,c}$	±0.01	0.23^{a}	±0.06	0.25^{a}	±0.05
Docosahexaenoic	C22 (6n3)	$0.85^{b,c}$	±0.07	3.15^{a}	±0.25	3.16^{a}	±0.64
SFA, %		35.96	±0.69	36.18	±0.47	36.07	±1.77
MUFA, %		38.02	±1.86	38.02	±1.02	37.47	±2.28
UFA, %		64.00	±0.69	63.78	±0.47	63.86	±1.73
PUFA, % of which:		25.98	±1.43	25.76	±1.02	26.39	±0.60
Ω3, %		$1.37^{b,c}$	±0.09	4.84^{a}	±0.32	4.94^{a}	±0.48
Ω6, %		$24.61^{b,c}$	±1.38	$20.01^{a,c}$	±1.08	$21.46^{a,b}$	±0.29
Ω6/Ω3		$17.92^{b,c}$	±1.05	4.32^{a}	±0.40	4.35^{a}	±0.47

*FAME – Fatty acids methyl esters

The introduction of grape meal in the diet for group E2 had beneficial effects on egg quality, the cholesterol level decreasing significantly ($P \leq 0.05$), from 197.49 mg in the control group, to 189.90 mg in group E1 an ti 150,70 mg/100 g egg in group E2. Similar results have been reported by [10], [25] who used winery by-products rich in polyphenols and who noticed beneficial effects on layer performance and on egg quality, by decreasing the cholesterol level.

Figure 2 shows that the antioxidant capacity of the eggs from groups E1 and E2 was higher by 9.07% in the methanolic yolk extracts and by 12.56% in the methanolic albumen extracts compared to the control group.

These results prove that although the eggs from the experimental groups were enriched in polyunsaturated fatty acids, the use of grape meal had beneficial effects on egg quality.

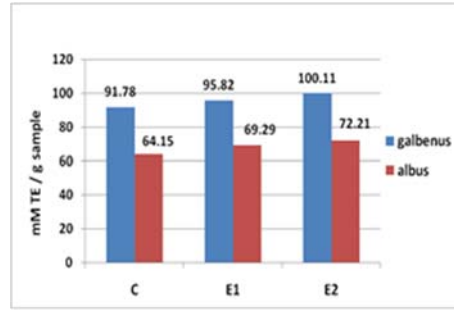

Fig. 2 Antioxidant capacity in the methanolic egg extracts

CONCLUSIONS

The use of flax meal in the compound feeds for the experimental groups increased the concentration of alfa-linolenic acid (ALA), omega-3 acid, respectively decreased significantly the ratio of omega-6/omega-3 polyunsaturated fatty acids, both feed and eggs, show that the eggs from the experimental groups have a high feeding value.

Although the concentration of polyunsaturated fatty acids was higher in the compound feeds for the experimental groups, the oxidative status was balanced both in the feeds and in the eggs by the presence of the natural feed additive, the grape meal, by-product of the winery industry.

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