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Article

Using grapeseed meal as natural antioxidant in slow-growing Hubbard broiler diets enriched in polyunsaturated fatty acids

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Abstract:

The purpose of the study was to assess the effect of the grapeseed meal, added to slow-growing Hubbard broilers diet high in polyunsaturated fatty acids (PUFA) due to the dietary flaxseed meal. The 7-wk feeding trial used 80 broiler chicks (14 d), assigned to two groups: control (C) and E, with 4 replicates of 10 chicks/group. The basal diet was similar for both groups during both feeding stages. The diet for group E was supplemented with 3% grapeseed meal. Six broilers from each group were slaughtered in the end of the feeding trial, and blood, breast and leg meat samples were collected. Serum cholesterol was significantly lower in group E (110.85 mg/dL), than in group C (146.82 mg/dL). The PUFA concentration was

significantly higher in group E, than in group C, both in the breast (31.34 %, compared to 27.73 % total fatty acid methyl ester - FAME) and in the leg (32.44 %, compared to 30.06 % total FAME). The cholesterol concentration was significantly lower in group E (42.52 mg), than in group C (60.91 mg/100 g fresh sample) in the leg. After 7 d of refrigeration, the peroxide value was significantly lower in group E (8.11 meq), than in group C (8.79 meq/kg fat) in the breast meat, while fat acidity was significantly lower in group E (40.82 mg KOH), than in group C (43.99 mg KOH / g fat) in the leg. The dietary 3 % grapeseed meal, used as natural antioxidant, in PUFA-enriched broiler diets, had positive effects on the blood parameters and meat quality.

Key words: Broiler, Hubbard, Flaxseed meal, Grapeseed meal, Fatty acids.

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Introduction

There are many factors influencing broiler meat quality, among which the genetic makeup, animal welfare, pre-slaughter factors and the post-mortem changes of the muscles[1]. Nowadays, the meat from slow-growing broiler hybrids became more known for its pleasant texture and flavour, less juicy, fitting the current consumer preferences[2,3]. In Europa these hybrids are adapting faster to the alternative production systems, several lines being available, even if the growth performance of the slow-growing hybrids are less efficient than that of the fast-growing hybrids[2,3]. Fatty acids contents were found to both hazards and beneficial effects on human health based on type of fats and meat consumption[4]. Furthermore, consumer concern for high omega – 3 (ω-3 PUFA) foods in their daily diet increased due to their beneficial effect on human health[5,6,7].

Animal foods can be enriched in PUFA by feeding the animals diets whose ingredients are high in PUFA. Some of the PUFA-high feed ingredients are the flaxseed[8,9] and flaxseed meal[10-13], camelina meal[14,15], and the rapeseed meal[16-19]. Flax is an oleaginous plant in which ω-6 / ω-3 ratio is lower than the unit (0.436%), hence, the flax, in all its forms (seeds, oil and meal) is a viable feed ingredient that can increase the PUFA level of the diets[8]. However, the increased levels of PUFA make the feeds prone to oxidation, which is why the diets have to be supplemented with antioxidants[20].

The winery by-products, grape pomace, grape seeds and grape peels, grapeseed cakes or grapeseed meals, have high levels of polyphenols, sources of natural antioxidants. The abundance of active polyphenols in these by-products is of real interest for animal nutrition, because they can be used as natural antioxidants, replacing the synthetic ones[21,22].

The grapeseed cakes or meals result from the cold extraction or chemical extraction of the oil. The concentration of polyphenols ranges between 0.642 mg gallic acid equivalents, in the cakes, 3.186 mg gallic acid equivalents, in the grape pomace and 90.41 mg gallic acid equivalents / g sample in the grape seed meal. The antioxidant capacity ranges between 8.554 mM Trolox equivalents, in the grape pomace, 6.241 mM Trolox equivalents, in cakes and 493.07 mM Trolox equivalents /g sample, in the grape seed meal[11,23,24]. The literature has several studies on the use of winery by-products, such as grape pomace[25,26,27] or grapeseed meal[28,29] in broiler feeding and on their beneficial effects on broiler performance, protein and amino acids digestibility, blood parameters and meat quality, due to the higher concentration of PUFA and lower lipid peroxidation.

The studies conducted so far have shown that the inclusion of winery by-products in animal feeding, as natural antioxidants, is a good strategy for enhancing the oxidative stability of the animal products, meeting thus consumer requirement for high quality animal foods[30,31].

Within this context, the purpose of the present study was to assess the effect of 3 % grapeseed meal, winery by-product, added to Hubbard broilers diet high in polyunsaturated fatty acids due to the dietary 2 % flaxseed meal, on broiler performance, energy profile of the blood plasma, on fatty acids and cholesterol levels and on broiler meat degradation indices.

Material and methods

The feeding trial was conducted in the experimental facilities of the National Research Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania) on a protocol (no. 5122/03.08.2017) approved by the Ethics Commission of the institute in accordance with the EU Directive 2010/63/ EU and Romanian Law regarding Animal Protection.

Animals and experimental design

The 7-wk feeding trial was conducted on 80 Hubbard broiler chicks (14 d), reared on the floor, on wood shavings (10 cm thick). The chicks were weighed individually and assigned to two groups (40 chicks / group), having the average initial weight of 233.6 ± 5.53 g. Each group had four replicates of 10 chicks each. The chicks had free access to the feed and water. There was a 23 h light regimen, the temperature was 22 to 23 ºC, and the relative humidity was 60 to 70 % throughout the entire experimental period, according to the Management guide for Hubbard CLASSIC broilers[32].

The experimental design was monofactorial, completely randomized. It had two treatments, C (0%) and E (3%) with grapeseed meal applied for the two phases, growing (14-28 d) and finishing (28-63 d), according the feeding requirements of the slow-growing line Hubbard hybrid (Table 1). Both diets (C and E) were enriched in polyunsaturated fatty acids using 2% flaxseed meal. The flaxseed meal and the grapeseed meal were purchased from 2E Prod SRL, Romania. There was a single batch of diet / group for each period: grower and finisher. The growth performance of the broilers: average daily feed intake (g /broiler/d), initial weight (g), final weight (g) was monitored throughout the experimental period (14-63 d). It was calculated the average daily weight gain (g/broiler/d) and feed conversion ratio (g feed/g broiler), on basis of replicate of birds.

Table 1: Compound feeds formulation and chemical analysis

	Grower (14– 28 days)		**Finisher (29– 63 days)**	
	C	**E**	**C**	**E**
Corn	49.92	44.14	44.26	47.00
Wheat	15.00	15.00	16.00	10.00
Corn gluten	4.00	4.00	4.00	3.80
Soybean meal	19.85	22.00	24.00	24.00
Flax meal	2.00	2.00	2.00	2.00
Grapeseed meal	-	3.00	-	3.00
Sunflower oil	4.40	5.00	5.00	5.46
Monocalcium phosphate	1.32	1.50	1.30	1.40
Calcium carbonate	1.73	1.62	1.70	1.60
Salt	0.34	0.34	0.34	0.34
Methionine	0.15	0.15	0.15	0.15
Lysine	0.24	0.20	0.20	0.20
Choline	0.05	0.05	0.05	0.05
Premix*	1.00	1.00	1.00	1.00
Total	100	100	100	100

Chemical analysis results (% DM)				
Dry matter	90.18	90.16	90.00	90.11
Crude protein	21.80	21.87	19.12	19.20
Ether extractives	5.44	6.06	6.97	7.40
Crude fibre	4.59	4.93	4.64	4.61
Lysine	1.33	1.34	0.99	1.13
Methionine	0.43	0.41	0.38	0.39
Calcium	0.91	0.91	0.91	0.92
Total phosphorus	0.81	0.81	0.81	0.82
Concentration of total polyphenols (mg GAE / g)	1.47	1.68	1.36	1.56
Antioxidant capacity (mM TE / g)	2.15	3.09	2.01	2.82
Fatty acids (% of total FAME)				
Saturated fatty acids (SFA)	11.04	10.43	12.89	12.30
Monounsaturated fatty acids (MUFA)	23.62	21.06	23.87	22.16
Polyunsaturated fatty acids (PUFA)	65.33	68.51	63.10	65.47
α-linolenic acid (ALA)	4.35	4.90	5.76	6.34
Omega-3 polyunsaturated fatty acids (ω-3) Omega-6	4.46	5.01	5.89	6.47
polyunsaturated fatty acids (ω-6)	60.87	63.50	57.21	59.00
Calculated metabolizable energy (Mj / kg DM)**	13.18	13.20	13.39	13.40

*Content per kg diet: vitamin A: 11000 IU; vitamin D_3: 2000 IU; vitamin E: 27 IU mg; vitamin K_3: 3 mg; vitamin B_1: 2 mg; vitamin B_2: 4 mg; pantothenic acid: 14.85 mg; nicotinic acid: 27 mg; vitamin B_6: 3 mg; vitamin B_7: 0.04 mg; vitamin B_9: 1 mg; vitamin B_{12}: 0.018 mg; vitamin C: 20 mg; manganese: 80 mg; iron: 80 mg; copper: 5 mg; zinc: 60 mg; cobalt: 0.37 mg; iodine: 1.52 mg; selenium: 0.18 mg.

**Metabolizable energy was calculated from the chemical composition

Sampling and chemical analysis

Samples of flaxseed meal and grapeseed meal were collected before feed manufacturing and their chemical composition determined. Grower and finisher compound feed samples were collected and assayed for the basic chemical composition, calcium, phosphorus, lysine, methionine, fatty acids profile, concentration of polyphenols and antioxidant capacity. At the end of the experimental period (63 d of broiler age), six broilers per group were selected randomly.

The blood samples (n= 6) were collected after which the broilers were slaughtered in 6 mL Vacutainer tubes, on anticoagulant heparin–lithium. The blood samples were centrifuged at 3,000 rpm, for 20 min, at +22 ºC. The plasma was stored at – 80 ºC until analysed. Six individual samples of breast meat and six individual samples of leg meat were formed. Each

sample was divided in three. One part was assayed for fatty acids and cholesterol, one sample was refrigerated (+4 °C) for 7 d and thereafter assayed for the fat degradation indices (peroxide value and fat acidity), and the third part was frozen (-18 °C) for one month and thereafter assayed for the fat degradation indices (peroxide value and fat acidity).

The basic chemical composition of the flaxseed meal, of the grapeseed meal and of the compound feeds was determined using methods standardized according to Regulation (EC) no. 152/2009. Dry matter (DM) was determined by the gravimetric method using a Sartorius (Gottingen, Germany) scale and BMT drying closet, ECOCELL Blueline Comfort (Nuremberg, Germany). Crude protein (CP) was determined by the Kjeldahl method using a semiautomatic KJELTEC auto 2300 system – Tecator (Sweden). Ether extractives (EE) were determined by the extraction in organic solvents using a SOXTEC-2055 FOSS system – Tecator (Sweden). Crude fibre (CF) was determined by the method with intermediary filtration using a FIBERTEC 2010 system – Tecator (Sweden). Ash (Ash) was determined by the gravimetric method using a Caloris CL 1206 furnace.

The calcium (Ca) was determined by the titrimetric method according to SR ISO 6491-1:2006. The phosphorus (P) was determined photometrically, according to Regulation (EC) no. 152/2009, using the Jasco V-530 spectrophotometer.

Lysine and methionine of the compound feeds were determined by the liquid chromatographic method, according to Regulation (EC) no. 152/2009, using a Finningan Surveyor Plus HPLC (Thermo-Electron Corporation, Waltham, USA), fitted with PDA detector (Photo Diode Array Detector). The amino acids are separated on a Hypersil BDS C18 column with silica gel (250 ×4.6 mm), particle size 5 µm, with reverse phase and a +45 ^{o}C temperature. The results were expressed in g amino acids / 100 g DM.

Concentration of polyphenols and antioxidant capacity of the grapeseed meal and compound feeds. The grapeseed meal samples and the compound feeds samples were first extracted in acidified methanol (methanol: hydrochloric acid = 80:20). The concentration of polyphenols in the methanol extracts was determined by spectrophotometer method[33], using a UV-VIS Thermo Scientific spectrophotometer. The results were expressed as mg gallic acid equivalents / g sample (mg GAE / g sample). The antioxidant capacity in the methanol extracts was determined by the DPPH method[34], 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).

Metabolic profiles of blood plasma. The blood biochemical parameters were determined from the plasma samples, using a biochemical analyser (Analyzer Chemistry Mindray BS - 130) with ACCENT - 200 kits, according the manufacturer's instructions.

Fatty acids profile of the feed ingredients, of the compound feeds and of the meat samples. The fat from the meat samples (breast and leg) were extracted, then saponified by reflux boiling in a solution of acidified methanol (2% H_2SO_4 in methanol), to obtain the fatty acids methyl esters (FAME). The fatty acids methyl esters were added to hexane and concentrated in rotavapor. The resulting samples were analysed by gas chromatographic method, according to the 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).

Cholesterol level in the meat samples. For the meat samples saponification, it was started with a method described by Dihn *et al*[35], adapted in our laboratory. The meat samples (breast and leg) were saponified by reflux boiling in a solution of methanol and potassium hydroxide (5% KOH in methanol), followed by extraction in petrol ether, concentration in rotavapor, and addition of chloroform. The resulting samples were analysed by gas chromatographic method, according to AOAC International[36], using a Perkin Elmer-Clarus 500 chromatograph, with on-column injector (splitting ratio, about 1:100), with programmable column heater; flame ionization detector (FID) and capillary separation column HP-5 (30m, 0.32mm ID, 0.1 µm film) AGILENT.

The peroxide value for the meat samples was determined with the iodometric method according to SR EN ISO 3960:2017. The results were expressed in milliequivalents / kg fat (meq/kg fat). The fat acidity for the meat samples was determined with the volumetric method according to ISO 660:2009. The results were expressed in mg potassium hydroxide / g fat (mg KOH / g fat).

Statistical analysis

The effects of treatments were tested by one-way analysis of variance using the GLM procedure of Minitab software[37] with treatment as a fixed effect, according to the model $Y_i = T_i + e_i$, where Y_i was the dependent variable, T_i is the treatment and e_i is the error. When overall F-test was significant, differences between means were declared significant at $P < 0.05$ using the Tukey comparison test.

Results

Characterisation of flaxseed meal and grapeseed meal

The flaxseed meal, used as feed ingredient high in polyunsaturated fatty acids (PUFA), had: 344.4 g/kg protein, 109.7 g/kg fat, and 20.20 MJ/kg gross energy. The fatty acids profile was: 11.21 g saturated fatty acids (SFA), 20.00 g monounsaturated fatty acids (MUFA), 68.73 g polyunsaturated fatty acids (PUFA), of which 53.35 g ω-3 PUFA, and 15.37 g ω-6 PUFA / 100 g FAME, with ω - 6 / ω – 3 ratio of 0.29. Table 2 shows the basic chemical composition, the fatty acids profile, the concentration of polyphenols and the antioxidant capacity of the grapeseed meal used as natural antioxidant in PUFA-high diets.

Table 2: Content of the main nutrients of grapeseed meal

Specification	**Grapeseed meal**
Dry matter, %	918.5
Crude protein, % DM	129.0
Ether extractives, % DM	72.2
Gross energy, MJ/kg	18.55
Concentration of total polyphenols, mg GAE/g	26.65
Antioxidant capacity, mM TE/g	148.35
Fatty acids (% of total FAME):	
SFA	12.30
MUFA	20.39
PUFA	67.14
ω -3	0.68
ω -6	66.45

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

Characterisation of compound feeds

The compound feeds were developed on the basis of the chemical composition of the feed ingredients, being isonitrogenous and isocaloric for each feeding phase, in agreement with the feeding requirements of the Hubbard hybrid (Table 1). Due to the inclusion of 3% grapeseed meal, the compound feed of group E had a higher concentration of polyphenols of 1.68 mg GAE (growing phase), respectively 1.56 mg GAE (finishing phase), compared to

the compound feed of group C 1.47 mg GAE (growing phase), respectively 1.36 mg GAE/g diet (finishing phase). Higher results were also determined regarding the antioxidant capacity of the E group compound feed of 3.09 mM TE (growing phase) and 2.82 mM TE (finishing phase), compared to C group which had 2.15 Mm TE (growing phase) and 2.01 mM TE/g diet (finishing phase).

Bird performance

Table 3 shows the effect of using a compound feed with 3% grapeseed meal on the growth performance of the broilers. The values for the entire experimental period (14- 63 d) were higher for group E, than for group C, for the final weight and feed conversion ratio. No mortalities were recorded.

Table 3: Effect of the compound feed with grapeseed meal on the broiler performance: 14-63 d (mean values)

Items	C	E	SEM	*P*-value
Average daily feed intake, g/broiler/d	114.2[a]	119.5[a]	3.89	0.497
Initial weight, g	233.3[a]	233.7[a]	3.88	0.962
Final weight, g	2545[a]	2728.5[a]	48.03	0.056
Average daily weight gain, g/broiler/d	47.18[a]	50.70[a]	1.04	0.090
Feed conversion ratio, g feed/g gain	2.42[a]	2.35[a]	0.29	0.913
Death rate, %	0.00	0.00	-	-

C= Control; E= Control + 3% grapeseed meal; SEM= Standard error of the mean.
[a-b] Mean values within a row having different superscripts are different ($P<0.05$).

Metabolic profile of blood plasma

The metabolic profile of the blood plasma, collected at broiler age of 63 d (Table 4), shows significantly ($P<0.05$) lower energy profile in group E, than in group C, thus, glycaemia decreased by 16.88 %, cholesterol by 24.50 %, and the triglycerides by 34.90 %, in group E, compared to group C, the differences being statistically significant ($P<0.05$).

Table 4: Effect of compound feed with grapeseed meal on the metabolic profile of blood plasma in broilers of 63 d of age (mean values)

Items	C	E	SEM	*P*-value
Glycaemia, mg/dL	271.03[a]	225.28[b]	7.30	<0.0001
Cholesterol, mg/dL	146.82[a]	110.85[b]	5.99	<0.0001
Triglycerides, mg/dL	49.93[a]	32.50[b]	2.95	<0.0001

C= Control; E= Control + 3% grapeseed meal: SEM= Standard error of the mean.
[a-b] Mean values within a row having different superscripts are different (P<0.05).

Fatty acid concentration in the broiler meat

The PUFA concentration in the breast meat samples (Table 5) was significantly (P<0.05) higher in group E, than in group C, by 13.02 %. The concentration of ω - 3 PUFA, and of ω - 6 PUFA increased by 15.00 %, and by 12.84 %, in group E, compared to group C (P<0.05). The alfa linolenic acid (ALA) concentration in the breast meat was 1.89 g, in group E, and 1.82 g / 100 g total FAME, in group C (P>0.05). The PUFA concentration in the leg meat samples (Table 5) was significantly (P<0.05) higher in group E, than in group C, by 7.91 %. The concentration of ω –3 PUFA was also higher in group E, than in group C, by 9.66 %, but the difference was not statistically significant (P>0.05). The ALA concentration in the leg meat was 2.56 g in group E, significantly (P<0.05) higher than 1.97 g/ 100g total FAME, in group C.

Table 5: Effect of compound feed with grapeseed meal on the fatty acids concentration in the broiler meat samples (mean values)

Fatty acids (% of total FAME)	Breast meat				Leg meat			
	C	E	SEM	*P*-value	C	E	SEM	*P*-value
SFA	32.48[a]	32.33[a]	0.21	0.736	30.97[a]	29.29[b]	0.33	0.027
MUFA	39.09[a]	35.83[b]	0.54	< 0.001	38.40[a]	38.01[a]	0.29	0.517
PUFA, of which:	27.73[a]	31.34[b]	0.62	0.0002	30.06[a]	32.44[b]	0.62	0.049
ALA	1.82[a]	1.89[a]	0.03	0.276	1.97[a]	2.56[b]	0.11	0.002
ω -3	2.80[a]	3.22[b]	0.08	0.003	3.00[a]	3.29[a]	0.11	0.205
ω -6	24.84[a]	28.03[b]	0.55	0.0002	27.06[a]	29.14[b]	0.52	0.044
Ratio ω–6 / ω-3	8.92[a]	8.73[a]	0.13	0.475	9.02[a]	8.85[a]	0.19	0.656

FAME=fatty acid methyl esters; C=Control; E= Control + 3% grapeseed meal; SEM= Standard error of the mean.

SFA–saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; ALA -α-linolenic acid; ω-3 -omega-3 polyunsaturated fatty acids; ω-6 - omega-6 polyunsaturated fatty acids;
[a-b] Mean values within a row having different superscripts are different (P<0.05).

Cholesterol level in the broiler meat

The cholesterol concentration in the breast meat samples (Table 6) were lower in group E, treated with 3 % grapeseed meal, than in group C, but the difference was not statistically significant (P>0.05). For the leg meat, the cholesterol concentration was 30.2 % lower in group E, compared to C (P<0.05).

Table 6: Effect of compound feed with grapeseed meal on the cholesterol level in the broiler meat samples (mean values), (mg / 100 g fresh meat)

Breast meat				**Leg meat**			
C	**E**	**SEM**	***P*-value**	**C**	**E**	**SEM**	***P*-value**
44.28[a]	36.49[a]	2.05	0.052	60.91[a]	42.52[b]	3.76	0.006

C= Control; E= Control + 3% grapeseed meal; SEM= Standard error of the mean.
[a-b] Mean values within a row having different superscripts are different (P<0.05).

Fat degradation indices in the broiler meat

Regarding the fat degradation indices (Table 7), the peroxide value of the breast meat samples, determined after 7 d of refrigeration at +4 ºC, was 8.11 meq/kg fat, in groups E, significantly (P<0.05) lower than 8.79 meq, in group C. However, after one month of freezing (-18 ºC), their values were similar (P>0.05). Fat acidity was lower in group E, than in group C, both after 7 d of refrigeration (+4 ºC), and after one month of freezing (-18 ºC), but the difference was not significant (P>0.05).

The peroxide value of the leg meat samples (Table 7) determined after 7 d of refrigeration (+4 ºC), and after one month of freezing (-18 ºC), was similar in both groups (P>0.05). However, fat acidity after 7 d of refrigeration (+4 ºC), 40.82 mg KOH / g fat, in group E, was significantly (P<0.05) lower than 43.99 mg KOH/g fat, in group C. After one month of freezing (-18 ºC), the decrease of fat acidity in group E was not statistically significant (P>0.05), compared to group C.

Table 7: Effect of compound feed with grapeseed meal on the fat degradation indices in the broiler meat samples (mean values)

Items	Breast meat				
	Period	**C**	**E**	**SEM**	***P*-value**
Peroxide value, meq/kg fat	day 7-	8.79[a]	8.11[b]	0.12	0.0002
Fat acidity, mg KOH/g fat	refrigeration	36.42[a]	36.44[a]	0.01	0.955
Peroxide value, meq kg fat	1 month-	5.08[a]	5.15[a]	0.02	0.161
Fat acidity, mg KOH/g fat	freezing	20.89[a]	20.30[a]	0.37	0.442
	Leg meat				
Peroxide value, meq/kg fat	day 7-	11.25[a]	11.10[a]	0.13	0.589
Fat acidity, mg KOH/g fat	refrigeration	43.99[a]	40.82[b]	0.52	<0.0001
Peroxide value, meq/kg fat	1 month-	6.39[a]	6.23[a]	0.04	0.043
Fat acidity, mg KOH/g fat	freezing	24.23[a]	23.72[a]	0.26	0.410

C= Control; E= Control + 3% grapeseed meal; SEM= Standard error of the mean.
[a-b] Mean values within a row having different superscripts are different ($P < 0.05$).

Discussion

The results on the flaxseed meal used as PUFA-high feed ingredient are in agreement with those of Panaite *et al*[11], who reported values of: 32.99 % protein, 9.42 % fat, 19.31 MJ / kg gross energy, 11.07 g SFA, 18.71 g MUFA, 70.23 g PUFA, of which 42.93 g ω-3 PUFA and 27.30 g ω-6 PUFA / 100 g FAME, and ω -6 / ω -3 ratio of 0.64.

The concentration of polyphenols and the antioxidant capacity of the grapeseed meal, used as natural antioxidant, are in agreement with those of Turcu *et al*[29], who studied the effect of 2 % grapeseed meal given to Ross 308 broilers and reported 28.08 mg GAE / g samples, and 145.83 mM Trolox equivalents / g sample, antioxidant capacity. The efficiency of the natural antioxidants depends on their chemical composition which, in turn, depends on the variety of grapes, soil type, agro-climatic factors and wine-making techniques[38].

The use of 2 % flaxseed meal, high in PUFA, in the compound feeds for Hubbard broilers, produced close values of the fatty acids profile both in the growing and in the finishing phases. As expected, the use of 3 % grapeseed meal as natural antioxidant, in the formulation for group E, increased the concentration of polyphenols and the antioxidant capacity compared to the feed formulation for group C, in both stages. Thus, the concentration of polyphenols was 14.28 % higher and the antioxidant capacity was 43.72% higher for the growth stage, and 14.70 % and 40.29 % higher, respectively, for the finishing stage. These results are in agreement with those reported by Vlaicu *et al*[28], who studied the effect of 2 %

grapeseed meal given to Ross 308 broilers (growth stage) and reported 54.31% higher concentration of polyphenols. Broiler performance for the entire experimental period, show that the use of grapeseed meal in the compound feed formulation for group E did not have adverse effects on the broilers, the differences from the control group not being statistically significant ($P>0.05$).

The values of the blood plasma energy profile (glycaemia, cholesterol, triglycerides) (Table 4) were significantly ($P<0.05$) lower in group E, than in the control group. The lower blood cholesterol might be due to the flavonoid content of the grapeseed meal, which inhibits the formation of mycelia within the small intestine, thus reducing the absorption of the intestinal cholesterol[39]. The results in this study on the blood profile are in agreement with those reported by Abu Hafsa and Ibrahim[40] who evaluated the effect of the dietary grapeseed powder (0; 10; 20 and 40 g/kg) given to Cobb 500 broilers. They reported decreases in the following blood parameters: glucose, from 188.42 mg/dL in group C, to 151.39 mg/dL, in the group with 40 g grapeseed powder; cholesterol, from 122.46 mg/dL, in group C, to 95.88 mg/dL, in the group with 40 g grapeseed powder; triglycerides from 67.46 mg/dL, in group C, to 60.75 mg/dL, in the group with 40 g grapeseed powder. Khodayari and Habib[26] studied the effect of various levels of dietary grape pomace (0, 2, 4 and 6 %) given to Ross 308 broilers, on broiler performance, lipid peroxidation and blood biochemical parameters, and reported the decrease of blood triglycerides from 52.00 mg/dL, in group C, to 35.33 mg/dL, in the group with 6 % dietary grape pomace. The blood cholesterol decreased from 163.33 mg/dL, in group C, to 129.33 mg/dL, in the group with 6 % dietary grape pomace.

The fact that this study revealed lower values for blood glycaemia, cholesterol and triglycerides in group E, shows a better health state of those broilers, compared to the control group, due to the grapeseed meal added, as antioxidant, to the diet high in polyunsaturated fatty acids.

Broiler meat enhanced in PUFA, particularly in ω -3 PUFA, has established beneficial effects for consumer health[4]. Kamboh and Zhu[41] shows changes in the proportion of fatty acids (decrease in SFA and increase in PUFA), when the broiler diets were treated with bioflavonoids.

The significantly ($P<0.05$) difference of the breast meat PUFA concentration in group E, can be explained by the fact that the dietary grapeseed meal, due to its antioxidant properties given by the content of polyphenols and flavonoids it slowed down the lipid degradation reactions[42,43]. Other work[44] showed the positive effects of the winery by-products in preventing the oxidation of broiler meat PUFA. They studied the use of an extract of grape seeds and onion, alone or in combination with vitamin E, in the diet formulations for Ross broilers exposed to heat, and reported higher PUFA values in the broiler breast meat, compared to the control group. The significantly ($P<0.05$) higher concentration of ω –3

PUFA in the breast meat from group E, reported in this study, was also reported[29]. By the addition of 2 % flaxseed meal and 2 % grapeseed meal to Ross 308 broiler diet, the concentration of ω –3 PUFA was 11.14 % higher in the experimental group than in the control group.

The concentration of PUFA in the leg meat was significantly (P<0.05) different in group E than in group C. Just like for the breast meat samples, for the leg meat sample too, this can be explained by the fact that the dietary grapeseed meal, due to its antioxidant properties given by the content of polyphenols and flavonoids it slowed down the lipid degradation reactions[42,43]. Significantly (P<0.05) higher PUFA concentrations were also reported by 19.38 %, and 20.02 %, compared to the control group, in the groups of Ross 308 broilers treated with 2 %, and 4 %, respectively, grapeseed meal[28]. Other teams of researchers[27], studied the effect of another winery by-product, the grape pomace, given to broilers in amounts of: 0 %, 5 % and 10 %, reporting increases of PUFA concentration in the broiler leg meat, from 37.0 g, in group C, to 47.4 g, in the group treated with 5 % grape pomace, and to 53.1 g / 100 g total fatty acids, in the group treated with 10 % grape pomace.

The alfa linolenic acid (ALA) is an essential fatty acid because it cannot be synthesized by the organism, the main source being the food. In this study, the ALA concentration in the broiler leg meat samples were significantly (P<0.05) higher in group E than in group C, as also reported by Chamorro *et al*[27], who used grape pomace in the diet formulations for Cobb broilers.

As it is known, cholesterol is a component of fats, playing an important role in the formation of hormones and in vitamin metabolism. Cholesterol synthesis in the liver can be modified by the addition of derivatives of animal fats. Recently, the education and research in food safety increased consumer awareness regarding the health effects of the food cholesterol[4] Nutrition can be used to modify the profile of the fatty acids, to decrease ω - 6 / ω – 3 ratio, and the cholesterol level[10].

The cholesterol level in the broiler breast and leg meat samples was lower in group E, treated with grapeseed meal. However, only for the leg meat, the decrease was statistically significant: 30.19 %, may be due to high lipid content in leg meat. These results are in contrast with those reported by Yong *et al*[45] who studied the effect of adding 0; 0.25 and 0.5 % grape meal to broiler diets on the cholesterol level in broiler meat. They observed that the cholesterol content of thigh and breast meat was not significantly affected by grape meal supplementation. The antioxidant effect of the grapeseed extract was investigated by Farahat *et al*[46], who used it in concentrations of 125 ppm to 2,000 ppm, in broiler diets, and reported lower values of the total cholesterol than in the broilers treated with synthetic antioxidant, BHT.

The lipid degradation indices showed lower values in the breast and leg meat from group E, both after 7 d of refrigeration and after one month of freezing. However, the decrease was significant only for the 7 d of refrigeration: the peroxide value of the breast meat sample was 7.73 % lower, compared to group C, and fat acidity of the leg meat was 7.21 % lower, compared to group C. Olteanu *et al*[47] reported similar effects, i.e., lower values of fat acidity after 7 d of refrigeration of the meat samples from Cobb 500, treated with 2 % grapeseed added to PUFA-high diet formulations, but the differences from the control group were not statistically significant. The available data from the literature include various information on the ability of grape meal to slow down the lipid peroxidation processes of broiler meat. Chamorro *et al*[27] studied the effect of the dietary grape pomace, and reported increasing oxidative stability of the broiler leg meat after 1 and 4 d of refrigeration, with the increasing dietary grape pomace by MDA decreasing levels. The obtained results showed significant lower differences ($P<0.05$) from 0.217 µg (C) to 0.112 µg (5 % GP) and 0.114 µg/g meat (10% GP) after 4 d of refrigeration. Kasapidou *et al*[48] concluded that the dietary grape pomace (2.5 % and 10 %) did not influence lipid oxidation of the broiler meat (breast and thigh) samples during 2 - 5 d of refrigeration. Also, it was found that antioxidants improved meat quality of Japanese quail and delayed oxidative rancidity[49].

The results obtained in this study show that the grapeseed meal, a winery by-product with a lower price, can be used as natural and non-toxic antioxidant in broiler diets, replacing the synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)[45].

Conclusions and implications

The inclusion of 3% grapeseed meal, as natural antioxidant, in Hubbard broiler diets high in polyunsaturated fatty acids did not affect broiler performance. The feeding quality of the broiler meat (breast and leg) was improved by the higher levels of total PUFA and omega – 3 PUFA, and by the lower cholesterol level. The health state of the broilers was also enhanced by the lower concentrations of cholesterol and triglyceride in the blood. These results highlight the antioxidant properties of the plant additive from the winery industry. The grapeseed meal added to broiler diets high in polyunsaturated fatty acids allowed the production of safe food of beneficial impact on human health.

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*** Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (Text with EEA relevance).

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