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Original Article

Grapeseed Meal Used as Natural Antioxidant in High Fatty Acid Diets for Hubbard Broilers

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ABSTRACT0

The 7-week feeding trial evaluated the effect of 2% grapeseed meal used as natural antioxidant in the diets for slow-growing Hubbard broiler chicks, aged 14 days. The chicks were weighed individually and assigned to two groups (C and E), with 40 chicks per group, housed under conditions of temperature, humidity and light regimen according to the hybrid management guide. The basal ingredients of the conventional diet were corn, wheat, gluten, soybean meal and 2% flaxseed meal, which is rich in polyunsaturated fatty acids. Compared to the control formulation, the experimental formulation included 2% grapeseed meal, both in the growing and finishing stages. The broilers had free access to feed and water. In the end of the feeding trial, blood samples were collected for serum biochemical determinations and six broilers per group were slaughtered and samples of breast and thigh meat were collected. The meat samples were assayed for the basic chemical composition, fatty acids profile and cholesterol content using standardized methods. The content of total polyunsaturated fatty acids (PUFAs) was significantly higher (p<0.05) in breast samples, while cholesterol content was significantly lower (p<0.05) in thigh samples from E group, compared to C. Blood glucose, cholesterol and triglyceride levels were significantly lower (p<0.05) in group E than in group C. The study showed that the grapeseed meal used as natural antioxidant in broiler diets enriched in polyunsaturated fatty acids given to Hubbard broilers had beneficial effects on broiler meat quality and on the metabolic profile of the blood plasma.

INTRODUCTION

Changes in human diets over the past 100 to 150 years, particularly in terms of dietary fat intake and its effect on human health has become a major concern in nutrition research (Ayerza *et al*., 2002). Chicken meat is low in fat and cholesterol and is usually considered healthier than other animal protein sources, especially red meats of mammalian origin (Ponte *et al*., 2004). The use of raw materials high in polyunsaturated fatty acids in the diets for farm animals with the view of obtaining foods with high nutritional value, with beneficial effects on consumer health, can be done rather easily by feeding. A good way to raise the omega-3 PUFA content in the diet, without making radical changes in the eating habits, seems to be the enrichment of frequently consumed food products. Flaxseed meal is a rich source of alpha-linolenic acid (ALA). ALA is an essential fatty acid in the human diet and is converted into two main long chain fatty acids, eicosapentaenoic acid and docosahexaenoic acid in a series of enzymatic reactions (Tapiero *et al.,* 2002). The fats, however, are likely to cause the feeds to get rancid (Ren *et al.,* 2013), which damages their quality, hence their acceptance by the animals.

Millions of tons of grapes are currently produced each year, and about 15% of the production is used by wineries. This socio-economic activity generates a large amount of solid waste, which represents up to 30% of the weight of the used material (Teixeira *et al.,* 2014). This calls for a higher use of these winery by-products, which contain active biocompounds with antioxidant properties (Radovanovic *et al.,* 2009; Granato *et al.,* 2010), as raw materials for pharmaceutics, cosmetics, food industry and feed additives. Grape seed flour, the residue from seed oil manufacture, has not received much attention but may be a potential rich source of natural antioxidants and other healthful bioactive compounds (Luther *et al.,* 2007). Grape seeds have about 15% of the solid waste produced in wine and are increasingly recognized as products that require a more accurate analysis of their value (Luque-Rodríguez *et al.,* 2005).

The literature has several studies reporting the use of winery by-products in broiler feeding and their effect on broiler performance, on protein and amino acids digestibility, on the gut microflora and on meat quality. Viveros *et al.* (2011) investigated the effects of dietary polyphenol-rich grape products on the intestinal microflora and gut morphology in broilers and concluded that winery by-products modify gut morphology and intestinal microflora and increase the biodiversity of the intestinal bacteria in broilers. Kasapidou *et al.* (2013) evaluated the effect of grape pomace supplementation on broiler meat quality characteristics. The inclusion of grape pomace in the diet did not affect lipid oxidation levels in breast and thigh muscle during storage. On the basis of microbiological data, there was neither a clear nor a consistent effect of grape pomace supplementation on poultry meat microbiological status. The inclusion of grape pomace in broiler diets did not affect the eating quality of the breast muscle. Fakhraddin & Shahria (2014) also evaluated the effect of red grape pomace on the performance, lipid peroxidation (MDA) and some serum biochemical parameters in broilers. They concluded that the supplementation of red grape pomace in broiler diets decreased broiler performance linearly after 2%. Grape pomace decreased blood glucose level, total antioxidant and MDA. Therefore, grape pomace can be used in broiler diets expecting to increase insolubility and decrease cholesterol and triglyceride in plasma. This is also important for the safety of meat consumers.

The purpose of our study was to evaluate the effect of 2% grapeseed meal added to a broiler diet rich in polyunsaturated fatty acids on broiler performance, meat quality and on the metabolic profile of the blood plasma in slow-growing Hubbard broilers, at the end of the feeding trial.

MATERIAL AND METHODS

Birds and experimental design

The feeding trial was conducted in the experimental halls of the National Research-Development of Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to a protocol approved by the Commission of Ethics of our institute. The 7-week feeding trial was conducted on 80, Hubbard broiler chicks with slow growth, aged 14 days. They were weighed individually and assigned to two groups, C and E (40 chicks per group), with an initial weight of 233.38 ±6.14g (C), 233.25 ±6.53g (E), without significant differences ($p>0.05$) between the groups. The chicks were housed in an experimental hall raised to the ground under 22 - 24˚C normal conditions of temperature, with 60 – 70% humidity and 23 h light regimen throughout the experimental period, according to the_Hubbard CLASSIC Management Guide. They had free access to the feed and water. The following parameters were monitored throughout the experimental period (14 – 63 days): initial weight (g), final weight (g), average daily feed intake (g feed/broiler/day), average daily weight gain (g/broiler/day) and feed conversion ratio (g feed/g gain).

Diet formulation

The diet formulation was calculated using the results of the chemical analysis of the feed ingredients in agreement with the feeding requirements (NRC, 1994) and using a mathematical model for poultry diets formulation (Burlacu *et al.,* 1999). The basal ingredients of both conventional diets (Table 1), grower phase (14 - 28 days) and finisher phase (29 - 63 days) were: corn, wheat, gluten, soybean meal and 2% flaxseeds meal, which is rich in polyunsaturated fatty acids. The experimental diet (E) was differed from the control diet (C) by the inclusion of 2% grapeseed meal, as natural antioxidant, purchased from 2E Prod SRL Alexandria, Teleorman County.

Sampling and analysis

The grapeseed meal was analysed for the primary chemical composition, polyphenols content and antioxidant capacity. A single batch of compound feed was manufactured for each group and growth period

Table 1 – Diet formulation (%)

Specification	Grower (14 – 28 days)		Finisher (29 – 63 days)	
	C	E	C	E
Corn	49.92	45.14	44.26	48.56
Wheat	15.00	15.00	16.00	10.00
Corngluten	4.00	4.00	4.00	3.50
Soybean meal	19.85	22	24.00	24.00
Flax meal	*2.00*	*2.00*	*2.00*	*2.00*
Grapeseed meal	-	*2.00*	*0.00*	*2.00*
Sunflower oil	4.40	5.00	5.00	5.20
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
Vitamin-mineral premix (PVM)*	1.00	1.00	1.00	1.00
TOTAL	100	100	100	100
Analysed composition				
Gross energy, MJ/kg	16.83	17.04	17.61	18.05
Dry matter, %	90.18	90.72	89.81	90.04
Crude protein, %	20.19	20.30	19.84	20.29
Lysine, g/ 100 g DM	0.990	1.142	1.331	1.429
Methionine, g/ 100 g DM	0.433	0.363	0.376	0.406
Calcium, %	0.91	0.91	0.91	0.90
Phosphorus, %	0.81	0.75	1.27	0.88
Saturated fatty acids (SFA), %	11.04	11.14	14.74	14.75
Monounsaturated fatty acids (MUFA), %	23.62	21.94	24.11	24.23
Unsaturated fatty acids (UFA), %	88.96	88.86	84.96	84.98
Polyunsaturated fatty acids (UFA), %	65.33	66.92	60.86	60.75
SFA/UFA	0.12	0.13	0.17	0.17
PUFA/MUFA	2.77	3.05	2.52	2.51

*1 kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg vit. B1; 400 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; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.

(finishing); compound feed samples were collected and assayed for the primary chemical composition.

The collected samples were assayed for the dry matter, crude protein, ether extractives, fibre and ash using standardized methods according to Regulation (EC) no. 152/2009 for compound feeds. Dry matter (DM)was determined with the gravimetric method using Sartorius (Gottingen, Germany) scales and BMT ECOCELL Blueline Comfort (Nuremberg, Germany) drying oven; crude protein (CP) was determined using the Kjeldahl method, using a semiautomatic KJELTEC auto 2300 system – Tecator (Sweden); the ether extractives (EE) were determined by extraction in organic solvents with a SOXTEC-2055 system, FOSS –Tecator (Sweden); ash (Ash) was determined with the gravimetric method using Caloris CL 1206 furnace. Gross energy (GE) was determined by calculation, using the gross chemical composition, with the equations of Burlacu *et al.* (2002).

Polyphenols concentration and antioxidant capacity of the tested compound feeds. The polyphenols concentration was determined with the method described by Mihailovic *et al.* (2013). The phenol compounds were first extracted in acidified methanol (methanol:HCl=80:20), and then determined with a UV-VIS Thermo Scientific spectrophotometer. The results were expressed in mg gallic acid equivalents/ g sample (mg GAE / g sample). The determination of the antioxidant capacity of the methanol extracts has been done using the DPPH method, proposed by Marxen *et al.* (2007), using a UV-VIS Analytik Jena Specord 250 Plus spectrophotometer with thermostatic carousel. The results were expressed in Trolox equivalents/g sample (mM TE/ g sample).

Metabolic profile of the broilers blood plasma. The blood biochemical parameters were determined in the end of the experimental period on blood samples collected in 6 ml Vacutainer tubes on Heparin – Lithium

anticoagulant. The blood biochemical parameters were determined from the plasm obtained by blood samples centrifugation at 3,000 rpm, for 20 min at +22° C. The plasm was stored in a deep freezer at -80°C until analysed. The plasm energy (glycaemia, cholesterol, triglycerides), proteic (protein, albumin, creatinine, urea) and mineral (calcium, phosphor, magnesium, iron) profiles were determined from the plasm samples, using a biochemical analyser (Analyzer Chemisty Mindray BS -130) with ACCENT -200 kits, according to the manufacturer's instructions.

Six broilers per group were slaughtered at the end of the feeding trial (63 days, broiler age), according to the working protocol approved by the Ethics commission from IBNA Balotesti; six breast meat samples and six thigh meat samples per group were formed and used to determine the fatty acids profile, the cholesterol level and the fat degradation indicators.

Fatty acids content of the broiler meat samples. The fatty acids were determined by gas-chromatography, according to SR CEN ISO/TS 17764-2:2008 standard, using a Perkin Elmer-Clarus 500 chromatograph, with on-column injector, with high polarity stationary phase (BPX70: 60m x 0.25mm inner diameter and 0,25µm film thickness); or high polarity cyanopropyl phase with similar resolution for various geometric isomers (THERMO TR-Fame: 120m x 0.25mm ID x 0.25µm film). The results were expressed in g fatty acids/100 g fat.

Fat degradation indicators of meat samples. The peroxide value for the meat samples was determined with the iodometric method, according to SR EN ISO 3960:2017, while fat acidity was determined with the volumetric method, according to ISO 660:2009.

Cholesterol level in the broiler meat samples. The cholesterol level of the breast and thigh meat samples was determined by gas-chromatography, according to AOAC International 1996 AOAC Official Method 99410: Cholesterol in food, 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.

Statistical analysis

The effects of treatments were tested using ANOVA GLM procedure of Minitab software (version 17, Minitab® Statistical Software), with treatment as a fixed effect, according to the model Yi = Ti + ei, where Yi was the dependent variable, Ti is the treatment and ei is the error. When overall F-test was significant, differences between means were declared significant at $p<0.05$ using the Tuckey comparison test.

RESULTS

Chemical composition

The flaxseed meal (Table 2), raw material rich in fatty acids, had 34.44% crude protein, 10.97% ether extractives and 20.20 MJ/ kg gross energy. The fatty acids profile was as follows: 11.21 g SFA, 20.00 g MUFAs, 68.92 g PUFAs, of which 53.35 g omega-3 PUFAs, and 15.57 g omega-6 PUFAs / 100 g total fatty acids, and omega-6/ omega-3 of 0.29.

Table 2 – Content of the main nutrients of grapeseed meal and flaxseed meal.

Specification	Grapeseed meal	Flaxseed meal
Crude protein, %	12.90	34.44
Ether extractives, %	7.22	10.97
Gross energy, MJ/kg	18.55	20.20
SFA	12.30	11.21
MUFA	20.39	20.00
PUFA	67.14	68.92
Ω3, %	0.68	53.35
Ω6, %	66.45	15.57

SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA-polyunsaturated fatty acids; Ω3- omega 3 polyunsaturated fatty acids; Ω6- omega 6 polyunsaturated fatty acids.

The natural antioxidant, the grapeseed meal (Table 2), had 12.90% crude protein, 7.22% ether extractives, 18.55 MJ/kg gross energy, 26.65 mg GAE/g polyphenols, and an antioxidant capacity of 148.35 mM TE/ g. The use of 2% grapeseed meal as natural antioxidant in the experimental diet (E) (Table 3) increased significantly ($p<0.05$), by 18.12%, the polyphenols concentration, and by 36.43% the antioxidant capacity, compared to the control diet (C). The fatty acids profile was: 12.30 g SFA, 20.39 g MUFAs, 67.14 g PUFAs, of which 0.68 g omega-3 PUFAs, and 66.45 g omega-6 PUFAs / 100 g total fatty acids.

Table 3 – Concentration of polyphenols and antioxidant capacity of the tested compound feeds*.

Specification	C	E	SEM	*p*-value
Concentration of polyphenols, mg gallic acid equivalents/ g	1.60^{a}	1.89^{b}	0.080	0.0493
Antioxidant capacity, mM Trolox equivalents/ g	2.47^{a}	3.37^{b}	0.241	0.0382

$^{a-b}$ Mean values within a row having different superscripts are significantly different by least significant difference test ($p<0.05$). SEM-standard error of the mean.

Broilers performance

Regarding broiler performance throughout the experimental period (14-63 days), Table 4 shows no significant (p>0.05) differences between the groups. E broilers had the highest average final body weight (2719.47±71.02 g), the highest average daily body weight gain (50.74 ±3.20 g /day/ broiler) and the best feed conversion ratio, 2.31 ±0.34 g feed/ g gain.

Table 4 – Broiler performance for the whole experimental period (14 – 63 days)*.

Specification	C	E	SEM	p-value
Average daily feed intake, g feed/broiler/day	114.21[a]	117.09[a]	3.912	0.7149
Initial weight, g	233.37[a]	233.25[a]	4.453	0.9889
Final weight, g	2545.00[a]	2719.47[a]	50.903	0.0866
Average daily weight gain, g/broiler/day	47.18[a]	50.74[a]	1.037	0.0900
Feed conversion ratio, g feed/g gain	2.42[a]	2.31[a]	0.265	0.8383

[a] = Not significant difference (p>0.05). SEM: standard error of the mean.

Metabolic profile of the blood plasma

Table 5 shows the metabolic profile of the blood plasma collected from the broilers slaughtered at 63 days of age. As it can be noticed, the values of the energy profile are significantly lower (p<0.05) in the experimental group, treated with grapeseed meal, compared to the control group. Glycaemia decreased by 18.58%, cholesterol by 30.24%, and the triglycerides by 25.57%, compared to group C. There were no significant (p>0.05) differences in the protein profile of the broiler meat, but the concentration of each parameter was higher in group E than in group C. The values of the mineral profile were significantly (p<0.05) different between the two groups. The calcium level was higher by 4.67%, the phosphorus level by 39.72%, and the iron level by 35.26% in group E than in group C.

Table 5 – Metabolic profile of the blood plasma for broilers aged 63 days.

Specification		C	E	SEM	p-value
Energy	Glycaemia, mg/dl	271.03[a]	220.67[b]	7.917	<0.0001
	Cholesterol, mg/dl	146.81[a]	102.41[b]	7.021	<0.0001
	Triglycerides, mg/dl	49.93[a]	37.16[b]	2.213	0.0002
Protein	Total protein, g/dl	2.27[a]	2.38[a]	0.052	0.3093
	Albumin, mg/dl	1.11[a]	1.16[a]	0.027	0.3939
	Creatinine, mg/dl	0.37[a]	0.41[a]	0.017	0.2936
	Urea, mg/dl	6.19[a]	6.17[a]	0.292	0.9725
Mineral	Calcium, mg/dl	5.78[a]	6.05[a]	0.160	0.6027
	Phosphorus, mg/dl	3.65[a]	5.10[b]	0.279	0.0027
	Magnesium, mg/dl	2.72[a]	2.88[a]	0.163	0.6400
	Iron, µg/dl	61.00[a]	82.51[b]	4.139	0.0026

[a-b] Mean values within a row having different superscripts are significantly different by least significant difference test (p<0.05). SEM-standard error of the mean.

Breast and thigh meat fatty acid content

Table 6 shows the fatty acids content in the fat from the breast and thigh meat samples. The breast meat content of total PUFAs was significantly (p<0.05) higher in group E (by 5.48%) than in group C; at the same time, the content of omega-6 PUFAs was also significantly (p<0.05) higher in group E (by 5.27%) than in group C. The breast meat content of ALA was 1.82 g in group C and 1.97 g/ 100 g total fatty acids in group E. The thigh meat content of omega-3, omega-6 PUFAs and total PUFAs was higher in group E compared to group C, but the difference was not statistically different (p>0.05).

The evolution of the fat degradation indices at slaughter

Table 7 shows the evolution of the fat degradation indices at slaughter. The data show that, after 7 days of refrigeration at +4 ^{0}C, the peroxide value in the breast was significantly (p<0.05) lower, by 9.09%, in group E, than in group C. After one month of freezing, the decrease of the peroxide value in group E samples was not significant compared to group C samples. The same situation is with fat acidity, which was lower in group E, both after 7 days of refrigeration, and after one month of freezing; however, the difference was not statistically different (p>0.05). The peroxide value

Table 6 – Fatty acids content of the broiler meat samples according to the level of unsaturation (g acid/100 g total fatty acids).*

Fatty acids	Breast				Thigh			
	C	E	SEM	*p*-value	C	E	SEM	*p*-value
SFA	32.48[a]	32.47[a]	0.132	0.9719	30.97[a]	30.51[a]	0.218	0.3146
MUFAs	39.09[a]	37.76[b]	0.271	0.0052	38.40[a]	38.37[a]	0.285	0.9632
PUFAs, of which:	27.73[a]	29.25[b]	0.370	0.0310	30.06[a]	30.59[a]	0.519	0.6359
ω -3	2.80[a]	2.93[a]	0.067	0.3530	3.00[a]	3.19[a]	0.087	0.3000
ω -6	24.84[a]	26.15[b]	0.316	0.0292	27.03[a]	27.28[a]	0.447	0.7903
ω –6 / ω -3	8.90[a]	8.98[a]	0.162	0.8631	9.07[a]	8.56[a]	0.158	0.1094

*Where:SFA- saturated fatty acids; MUFAs- monounsaturated fatty acids; PUFAs- 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 significantly different by least significant difference test ($p<0.05$). SEM-standard error of the mean.

Table 7 – Evolution of the fat degradation indices in the broiler meat.

Specification	*Breast meat*				
	Period	C	E	SEM	*p*-value
Peroxide value (ml thiosulfate 0.1 N/g fat)	day 7- refrigeration	0.88[a]	0.80[b]	0.013	<0.0001
	1 month - freezing	0.50[a]	0.51[a]	0.002	0.0369
Fat acidity (mg KOH / g fat)	day 7- refrigeration	36.42[a]	36.19[a]	0.153	0.4827
	1 month - freezing	20.89[a]	20.09[a]	0.337	0.2524
	Thigh meat				
	Period	C	E	SEM	*p*-value
Peroxide index (ml thiosulfate 0.1 N/g fat)	day 7- refrigeration	1.12[a]	1.11[a]	0.010	0.5428
	1 month - freezing	0.63[a]	0.62[a]	0.005	0.2071
Fat acidity (mg KOH / g fat)	day 7- refrigeration	43.99[a]	41.05[b]	0.594	0.0053
	1 month - freezing	24.23[a]	20.77[b]	0.622	0.0007

[a-b] Mean values within a row having different superscripts are significantly different by least significant difference test ($p<0.05$). SEM-standard error of the mean.

in the thigh meat, after 7 days of refrigeration at +4^0 C, and after one month of freezing, was lower in group E than in group C, but the difference was not statistically different ($p>0.05$). However, fat acidity decreased significantly ($p<0.05$) in group E, both after 7 days of refrigeration at +4°C (by 6.68%), and after one month of freezing, by 14.27%, compared to group C

Cholesterol level

Table 8 shows that the cholesterol level in the fat from the breast meat samples was 8.13% lower in the experimental group compared to the control group, but the difference was not statistically significant ($p>0.05$). The thigh meat samples, however, showed a significantly ($p<0.05$) lower concentration of cholesterol in group E (by 23.85%), compared to group C.

DISCUSSION

Because the analysis of the primary chemical composition is the starting point in any experiment it is importance to characterize the by-products which will be used in the diet formulation (Table 2). The primary chemical composition of the winery by-products varies with the processing technology, cultivar, soil composition, climate, geographic origin, and cultivation practices or exposure to diseases, such as fungal infections (Bruno & Sparapano, 2007). The determined chemical composition of the grapeseed meal used in this experiment (12.90% crude protein, 7.22% ether extractives, 18.55 MJ/ kg gross energy) is similar with the results from other research which tested this by-product in broilers diet. Olteanu *et al.* (2018), in a study on the antioxidant role of the grapeseed meal

Table 8 – Cholesterol level in the broiler meat samples (mg/ 100 g).

Specification		C	E	SEM	p-value
Cholesterol	*Breast meat*	44.28[a]	40.68[a]	1.100	0.1026
	Thigh meat	60.91[a]	46.38[b]	3.278	0.0176

[a-b] Mean values within a row having different superscripts are significantly different by least significant difference test ($p<0.05$). SEM-standard error of the mean; means in the same row no common superscript significantly different ($p<0.05$).

in preventing the degradation of high-fatty acid broiler diets, used different levels of grapeseed meal inclusion (2% and 3) and 2% flax seed meal. The reported data for the basic chemical composition of grapeseed meal were 12.76% protein, 7.13% ether extractives and 35.30% crude fibre. In a research regarding the feeding value of some food industry by-products (Panaite *et al.,* 2016), grapeseed meals showed a content of 89.16% dry matter, 11.91% crude protein, 5.96% ether extractives and 35.68% fibre.

Among the bioactive compounds of the grapes, phenols represent the third most abundant constituent in grapes after carbohydrates and fruit acids (Singleton, 1980), they accumulate in solid parts of grapes, namely seeds, skins and clusters (Mironeasa, 2017). The grapeseed meal used in this study showed similar polyphenol concentration (26.65 mg GAE/ g) and antioxidant capacity (148.35 mM TE/ g) with those from the literature. The investigations regarding the biological activities of polyphenols reported values from 36.6 mg GAE/g to 49.7 mg GAE/g DW in the grape seed extract (Ky *et al.,* 2014), to 2178.8, 374.6, 23.8, and 351.6 mg/g GAE in seed, skin, flesh, and leaf (Pastrana-Bonilla *et al*., 2003). Being most notable the bioactivity of phenolic compounds from grapes, the antioxidative characteristics have been widely studied (Meyer *et al*., 1997). The antioxidant capacity data from literature show 16.8 - 92 mM Trolox equivalents (TE) /g sample for the grape seeds, 15.7 – 113.3 mM TE/ g sample for the grape peel (En-Qin Xian *et al.,* 2010), 3.186 mg in the grape pomace (Poudel *et al.,* 2008) and 493.07 mMTrolox/g sample in the grapeseed meal (Panaite *et al.,* 2016).

The work presented in this paper showed that grapeseed meal incorporation (2%) into Hubbard broilers diet was able to increase the polyphenol concentration and antioxidant capacity (Table 3) in the experimental group (E), compared to the control group (C). It should be mentioned that broiler performance (Table 4) for the entire experimental period (14-63 days) was not affected by the dietary grapeseed meal, and that the final weight of E broilers was higher than that of C broilers. The literature data regarding the influence of the dietary winery by-products on the bioproductive performance is varied. Viveros *et al*., (2011) studied the effect of the inclusion of grape pomace concentrate and grape seed extract in the diet of broilers. Dietary treatments included an antibiotic-free diet, a positive control (50 mg/kg of avoparcin), and antibiotic-free diets containing grape pomace concentrate (60 g/ kg) or grapeseed extract (7.2 g/kg). The reported data showed that broiler performance was not affected by dietary treatment, except for the grapeseed extract diet (7.2 g/kg), which showed a decreased weight gain and feed efficiency. Growth performance was depressed in chicks fed the grapeseed extract diet compared with those fed the grape pomace concentrate diet (60 g/ kg). Wang *et al.,* 2008 concluded in a study that the incorporation of grapeseed extract as low as 10 to 20 mg/kg in broiler diets is able to enhance the growth performance of broilers and significantly reduce the mortality of chicks after the *E. tenella* infection. On the other hand, Abu *et al*. (2018) reported that the inclusion of 10, 20 and 40 g/kg grapeseed powder in the broilers diet improved broiler performance.

The metabolic profile of the blood plasma (Table 5) showed significantly (p<0.05) lower values of the energy profile parameters in the meat from group E (glycaemia, cholesterol and triglycerides) compared to group C. The literature (Hibbeln & Salem, 1995) shows that the quantity and distribution of dietary omega-6 and omega-3 polyunsaturated essential fatty acids influence serum. Another aspect to be considered is that polyunsaturated fatty acids are thought to lower the serum cholesterol level more effectively than monounsaturated fatty acids (Mensink *et al*., 1989). In this study too, the grapeseed and flaxseed meal had a rich content of PUFA, higher than MUFA content. Researchers (Keys *et al*., 1965; Hegsted *et al*., 1965) found that replacing the saturated fat by (omega-6) polyunsaturated fat in the form of linoleic acid caused a larger decline in total serum cholesterol levels than monounsaturated fatty acids, carbohydrates, or protein (Mensink *et al*., 1989). Omega–3 fatty acids are essential for the normal growth and development and displayed serum lipid lowering, antithrombotic, antiarrhythmic, and anti-inflammatory effects (Hu *et al.,* 1999). The obtained results show that grapeseed meal played a strong antioxidant role, being consistent with the statement that feeding high-polyphenols increase the oxidative stability and provide a source of compounds that are useful for human nutrition and health (Laudadio *et al.,* 2015). The fact that blood cholesterol and triglyceride levels decreased in this study means an improvement in the health status of broilers over the experimental period due to the applied treatment, namely the inclusion of grapeseed meal in high omega-3 fatty acids diets.

Through the parameters of the mineral profile, the calcium and iron concentrations were significantly (p<0.05) higher in samples from the experimental group (Table 5). The literature results are varied.

Chamorro *et al*. (2013) investigated the use of grapeseed extract in various proportions (0.025; 0.25; 2.5 and 5.0 g/ kg compound feed) in the diets of broilers aged 21 days. The addition of increasing levels of grapeseed extract in the chicken diets did not change the analysed plasma parameters, but caused a significant decrease in the concentrations of plasma iron (up to 27%) and zinc (up to 12.3%) compared to the animals fed the control diet. The blood plasma results (Table 5) are in agreement with those reported by Fakhraddin & Habib (2014), who studied the effect of various grape pomace levels (0, 2, 4 and 6%) in broiler diets on performance, lipid peroxidation and on some biochemical parameters. The results showed a significant decrease of the blood triglycerides (from 52.00 mg/dl to 35.33 mg/dl), and of the cholesterol (from 163.33 mg/dl to 129.33 mg/dl). The protein profile was not significantly different between the groups (p>0.05). The blood mineral content varied as follows: 0.063 – 0.066 µg/ ml copper, 0.65 – 0.89 µg/ ml iron, and 1.83 – 2.04 µg/ ml zinc.

The fatty acids composition of the meat is considered an important index for meat quality. The grapeseed meal improved the nutritional quality of the broiler meat (breast and thigh) in group E (Table 6). Significantly (p<0.05) higher values of the total PUFAs and of omega-6 fatty acids were noticed in the breast meat from the experimental group compared to the control group. PUFAs concentration was also higher in the thigh meat from group E compared to group C, but the difference was not statistically significant (p>0.05). The results for breast and thigh meat fatty acids content are similar with those reported by Olteanu *et al.* (2017) who used 2% flaxseed meal and 3% grapeseed meal as natural antioxidant in Cobb 500 broiler diets. In the breast meat, PUFA content was 32.46 g/ 100 g and for thigh meat was37.68 g/ 100 g total fatty acids. Chamorro *et al.* (2015), who studied the effect of including different levels (0, 5, and 10%) of grape pomace phenolic compounds and the addition (individually or combined) of hydrolysing enzymes (carbohydrase enzyme complex and tannase at 500 ppm) in broiler diets reported increased PUFA concentration and reduced MUFA content of the thigh meat.

The fat degradation indices (Table 7) showed a significantly (p<0.05) lower peroxide value, after 7 days of refrigeration, in the breast meat samples from the group treated with grapeseed meal. Fat acidity was also significantly (p<0.05) lower in the thigh meat samples from group E, both after 7 days of refrigeration, and after one month of freezing, which means that grapeseed meal had a strong antioxidant character, since the concentration of PUFA from thigh meat was higher in E group compared to C group. The determined peroxide value of meat samples (breast and thigh) in this study are in agreement with the literature data. Thus, Goni *et al.* (2007) investigated the effect of fermented grape pomace on broiler performance and antioxidant activity. The study showed that, in the meat samples refrigerated for 1, 4 and 7 days, lipid oxidation decreased linearly with the increase of the dietary grape pomace (0.5, 1.5 and 3%). Olteanu *et al.* (2017) also reported lower fat acidity values in the meat samples from broilers treated with grape marc, while Brenes *et al.* (2008) concluded that the dietary grape pomace enhances the antioxidant activity in the broiler breast meat. Investigations by Kasapidou *et al.* (2016) on the inclusion of grape meal in broilers diet showed that the tested treatments did not affect the lipid oxidation of meat samples (breast and thigh) during refrigeration storage (4 ° C) for 2 and 5 days.

Recent recommendations by health professionals call for foods which are low in cholesterol and high in polyunsaturated fatty acids (PUFA) and therefore are healthier (Yalçyn *et al*., 2007). In this study, the recorded cholesterol concentration in the meat (Table 8) highlighted a significant (p<0.05) decrease in the thigh meat from the group with 2% grapeseed meal inclusion, in contrast to the group C. The fatty acid profile of the meat and fat is directly affected by the source of fat in the poultry diet. Therefore, it is possible to change the fatty acid profile, especially the ratio of omega-3 fatty acids and to reduce the cholesterol level (Mridula *et al*., 2015). Cholesterol is necessary to maintain cellular integrity, having an important role in regulating the viscosity of the cell fluids (blood). For this reason, a low cholesterol content contributes to the good circulation of the blood in the body. Also, cholesterol plays a role in the metabolism of liposoluble vitamins: A, D, K, E, being a major precursor of vitamin D and interfering with the immune system. Decreasing cholesterol and triglyceride levels through a healthy diet contributes to the optimal functioning of the entire body (Holick, 1996). Therefore, the cholesterol level should be balanced, recommended by doctors.

Panaite *et al*. (2016) investigated the effect of layer diets enriched in omega-3 fatty acids supplemented with 5% flaxseed meal and 2% camelina meal. The use of the experimental diets produced eggs with properties of functional foods because the yolk concentration of α linolenic acid was higher than in group C, while the cholesterol concentration was

significantly lower than in group C. Abu *et al*. (2018) have reported that the inclusion of 10, 20 and 40 g/kg grapeseed powder in the broilers diet reduced blood lipids, enhanced antioxidant capacity, and decreased the detrimental bacteria in the ileum. Jiao *et al.* (2010) investigated the effect of grapeseed proanthocyanidins inclusion on blood cholesterol level and gene expression of cholesterol-regulating enzymes in hamsters. The researchers concluded that the inclusion of 0.5% and 1% grapeseed proanthocyanidins in diets can regulate the blood lipids metabolism of animal bodies, with the function of lowering cholesterol. Other studies (Farahat *et al.*, 2017) on the effect of supplementation of broiler diets with grapeseed extract as a natural antioxidant at levels of 125, 250, 500, 1000 and 2000 ppm revealed a decrease in total cholesterol and low density lipoprotein cholesterol compared to those which received a synthetic antioxidant, BHT. Also, the inclusion of the viticultural by-product in diets has significantly decreased ($p < 0.05$) the level of malondialdehyde in the meat tissue. The recorded results did not reveal any significant differences in the performance of the chickens.

The positive effects of cholesterol-lowering in meat from broiler (breast and thigh) are important for human nutrition and health, reducing in particular the cardiovascular disease problems. Studies have shown that lowering cholesterol may reduce the risk of heart attack, so whether or not they suffer from heart disease, the adult population must necessarily keep their cholesterol levels under constant surveillance (Pavlovic *et al*., 2018). Within this context, we can consider that the inclusion of grapeseed meal in broiler diets acted as a feed additive with qualities of natural antioxidant due to the rich content of bioactive compounds with antioxidant properties.

CONCLUSIONS

In summary, from the nutritional perspective the results obtained in this study showed that the inclusion of 2% of grapeseed meal in the diet of broilers did not affect their performance (weight gain or feed consumption). From the nutritional and health perspective, our results are in agreement with other research suggesting the antioxidant properties of grapeseed meal. The inclusion of this by-product as natural antioxidant in high fatty acids diets for Hubbard broilers had beneficial effects both on the quality of the broiler meat, and on the metabolic profile of the blood plasma. Grapeseed meal led to a significantly ($p>0.05$) lower cholesterol content in the thigh meat and also decreased all the energy parameters profile from the blood plasma, compared to group C. Reducing the level of cholesterol in the meat, as well as the values of the parameters of the energy profile, reveals the improvement of the broiler health status, as well as the positive effects of consuming low cholesterol-broiler meat on human health.

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