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THE EFFECT OF USING GRAPE SEEDS MEAL AS NATURAL ANTIOXIDANT IN BROILER DIETS ENRICHED IN FATTY ACIDS, ON MEAT QUALITY

Raluca Paula Turcu[1*], Margareta Olteanu[1], Rodica Diana Criste[1], Mariana Ropota[1], Tatiana Dumitra Panaite[1], Cristina Șoica[1], Dumitru Drăgotoiu[2]

[1]National Research-Development Institute for Animal Biology and Nutrition (IBNA), Calea Bucuresti no 1, Balotesti, 077015 Ilfov, Romania
[2]Faculty of Engineering and Animal Production Management, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Marasti Blvd. 59, District 1, 011464 Bucharest, Romania

[*]e-mail: raluca.socoliuc@yahoo.com

Abstract

Poultry meat is among the products liable to oxidative deterioration due to their rather high content of polyunsaturated fatty acids. Winery by-products are rich in polyphenols, which lends them the capacity to enhance meat stability to oxidation, hence its quality.

The 5-week feeding trial evaluated the effect of 2% grape seeds meal used as natural antioxidant in the diets for Ross 308 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 normal conditions of temperature, with 36% humidity, and 23 h light regimen. The basal ingredients of the conventional diet were: corn, wheat, gluten, soybean meal and 2% flaxseeds meal, which is rich in polyunsaturated fatty acids. The broilers had free access to the feed and water. Six broilers per group were slaughtered at the age of 49 days and 6 samples of breast/thigh meat per group were formed for subsequent analysis of the basic chemical composition and of the fatty acids profile by gas chromatography. Standardized methods were used to determine the main nutrients in the samples of raw feed ingredients, compound feeds and meat. The analytical data were compared by variance analysis (ANOVA and t test), using StatView for Windows (SAS, version 6.0). The results are expressed as mean values ± standard error.

The grape seeds meal had 13.38% crude protein, 7.57% ether extractives, 18.98 MJ/kg, gross energy, 29.45 mg equivalent gallic acid/g sample and an antioxidant capacity of 143.31 mM Trolox equivalents/g sample. The bioproductive parameters recorded for the entire experimental period (14 - 49 days) were not significantly ($P \geq 0.05$) different between groups. However, the total content of polyunsaturated fatty acids (PUFA) was 13.43% higher in the broiler breast meat and 15.49% higher in the broiler thigh, compared to the control group. Higher concentrations were recorded for omega-3 PUFAs, by 10.02% in the breast meat and by 18.70% in the broiler thigh, compared to the control group. These results show that the use of 2% grape seeds meal in broiler diets improved the quality of meat.

The main conclusion of this study is that the the higher concentration of omega-3 and omega-6 PUFAs in chicken meat from the experimental group is due to the dietary inclusion of 2% grape seeds meal, which inhibited the lipid degradation reactions, thus improving the meat quality.

***Key words**: Grape seeds meal, Natural antioxidant, Meat quality, Broiler, PUFA.*

1. Introduction

The literature presents a series of studies regarding the beneficial effects of polyunsaturated fatty acids (PUFA) on human health (Riediger *et al.*, [1]; Huang [2]; Pilkington *et al.*, [3]). This biochemical process results in toxic chemical reaction products, becoming a problem for both the food industry and consumers as they lead to the appearance of bad smells or unpleasant tastes in food (Bourré, [4]). Poultry meat is among the products

liable of lipid peroxidation because is relatively rich in polyunsaturated fatty acids and is, therefore, readily susceptible to oxidative deterioration (Kanner, [5]). Synthetic antioxidants have long been used to control lipid oxidation in stored meat and meat products. Nowadays, when the consumer is more and more interested in quality and not in the amount of food, there is a necessity to identify and use antioxidants from natural sources (Goni *et al.,* [6]).

Winery by-products (skin, seeds and grape meal) contain a wide range of bioactive compounds, polyphenols, flavonoids, that offer numerous opportunities to improve the quality of animal products. Using these by-products as natural antioxidants in diets could improve the meat stability to oxidation, hence its quality. *In vivo* and *in vitro* studies conducted in recent years have demonstrated the beneficial effects of administration of these bioactive compounds in monogastric diets due to their antioxidant and antimicrobial activity (Alonso *et al.,* [7]; Torres *et al.,* [8], Viveros *et al.,* [9]). Although the researchers have shown an increased interest in the properties of the by-products, these antioxidant sources have been relatively unexploited and studies are therefore limited (Brenes *et al.,* [10]).

The purpose of this paper was to evaluate the meat quality as a result of the use of grape seeds meal as natural antioxidant in broiler diets enriched in fatty acids.

2. Materials and Methods

The trial was conducted within the experimental halls of the National Research - Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania), according to the provisions of the protocol approved by the ethics commission of the IBNA-Balotesti. The 5-week feeding trial was conducted on 80, Ross 308 broilers, aged 14 days, with an average weight of 311.79 g/chick. The chicks were weighed individually and assigned to two groups (C and E), with 40 chicks per group, housed under 27.02 ± 2.79 ^{0}C normal conditions of temperature, with 36% humidity, and 23 h light regimen. They had free access to the feed and water.

The basal ingredients of both conventional diets (Table 1), grower phase (14 - 28 days) and finisher phase (29 - 49 days), respectively, were: corn, wheat, gluten, soybean meal and 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% grape seeds meal, as a natural antioxidant. The grape seeds meal studied in this paper was purchased from 2E Prod SRL Alexandria, Teleorman County and linseed, raw feed material rich in polyunsaturated fatty acids.

Samples from grape seeds meal and flaxseed meal were collected to determine basic chemical composition. It was produced a single feed charge/group/experimental period from which were collected samples to determine their nutritional quality (crude protein, ether extractives, cellulose, ash, calcium, phosphorus, gross energy, polyphenols and antioxidant capacity). Throughout the experimental period (14 - 49 days) the following parameters were monitored: average daily feed intake (g feed/chick/day), average daily weight gain (g/chick/day), feed conversion ratio (g feed/g gain) and final weight (g). In the end of the 5-weeks feeding trial, at 49 days broilers, six broilers per group were slaughtered, according

Table 1. Diet formulation

Ingredients	Grower phase (14 - 28 days)		Finisher phase (29 - 49 days)	
	C	E	C	E
Corn, %	49.92	45.14	44.26	48.56
Wheat, %	15.00	15.00	16.00	10.00
Corn gluten, %	4.00	4.00	4.00	3.50
Soybean meal, %	19.85	22.00	24.00	24.00
Flaxseed meal, %	**2.00**	**2.00**	**2.00**	**2.00**
Grape seeds meal, %	**-**	**2.00**	**0.00**	**2.00**
Plant 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, %	1.00	1.00	1.00	1.00
Total	100	100	100	100

Legend:
*1kg premix vitamin-mineral contains: = 1.100.000 IU vit. A; 200.000 IU vit. D3; 2700 IU vit. E; 300 mg vit. K; 200 mg vit. B1; 400 mg vit. B2; 1485 mg pantothenic acid; 2700 mg nicotinic acid; 300 mg vit. B6; 4 mg vit. B7; 100 mg vit. B9; 1.8 mg vit. B12; 2000 mg vit. C; 8000 mg manganese; 8000 mg iron; 500 mg copper; 6000 mg zinc; 37 mg cobalt; 152 mg iodine; 18 mg/kg selenium.

to the Ethics commission of the IBNA-Balotesti. After slaughtering, six breast samples/group and six thigh samples/group were made in order to determine their basic chemical composition and fatty acid profile and fat degradation indices.

In order to determine the concentration of the main nutrients in the samples collected from raw materials, feeds and meat, standardized methods were used as follows: real dry matter (DM) was determined by the gravimetric method according to Regulation (EC) No. 152/2009 for combined feeds. For meat was used SR ISO 1442: 2010 and an analytical balance Sartorius (Gottingen, Germany) and BMT drying oven model Ecocell Blueline Comfort model (Nuremberg, Germany). The crude protein (CP) was determined by the Kjeldahl method, in accordance with Regulation (EC) 152/2009 for combined feeds. For meat we used SR ISO 973:2007 and a semiautomatic system Kjeltec auto 2300-Tecator (Sweden). Ether extractives (EE) were determined by the organic solvent extraction method, in accordance with Regulation (EC) No. 152/2009 for feeds. For meat analysis was employed SR ISO 1443:2008, using a Soxtec-2055 FOSS-Tecator system (Sweden). The ash was determined by gravimetric method, according to Regulation (EC) no. 152/2009 for compound feeds. For meat ash we used SR ISO 936:2009 and 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.

Fat degradation indices by feed: the peroxide value and fat acidity were determined by the volumetric method and STAS 12266:84. For the meat samples, the peroxide value was determined by iodometric method, according to official method AOCS Cd 8b-90, and fat acidity was determined by the volumetric method according to ISO 660:200. The Kreiss reaction is a qualitative method and was performed according to SR 9065-10:2007. Gross energy (GE) was determined by calculation, using the gross chemical analysis data and the equations developed by Burlacu *et al.,* [11].

The fatty acids were determined by gas chromatography, according to standard SR CEN ISO/TS 17764-2: 2008, using Perkin Elmer-Clarus 500 gas chromatograph, with capillary column injection system, high polarity stationary phase (BPX70: 60 m x 0.25 mm inner diameter and 0.25 µm thick film), and high polarity cyanopril phase, which give similar resolution for different geometric isomers (THERMO TR-Fame: 120 m x 0.25 mm ID x 0.25 µm film).

The grape seed samples concentration of polyphenols was determined according to the method described by Mihailovic *et al.,* [12] using a thermo scientific UV-VIS spectrophotometer. The results are expressed in mg equivalent gallic acid/g sample (mg EAG / g sample). The antioxidant capacity of the grape seed samples was determined according to the method proposed by Marxen *et al.,* [13] using a UV-VIS analytik Jena specord 250 plus spectrophotometer. The results are expressed in mM Trolox equivalents/g sample (mM ET/g sample).

The analytical data were compared by variance analysis (ANOVA and t test), using StatView for Windows (SAS, version 6.0). The experimental results were expressed as mean values, the differences being considered statistically significant for $P < 0.05$.

3. Results and Discussion

The regarding data on the main nutrients of the tested grape seed meal are shown in Table 2. As can be seen, the data recorded are consistent with those in the literature. Concerning the polyphenol content, the grape seeds meal used as a natural antioxidant in the Ross 308 broiler diets was characterized by 29.45 mg equivalent gallic acid/g sample and an antioxidant capacity of 143.31 mM Trolox equivalents/g sample.

The flaxseed meal added to the control and experimental diets, due to its rich content of polyunsaturated fatty acids, was characterized by: 33.6% crude protein, 12.45% ether extractives, 11.88% cellulose, 5.39% ash, 0.39% Ca, 1.14% P and 20.68 Mj/kg of gross energy. The α-linolenic acid PUFA concentration of meal was

Table 2. Content of the main nutrients of grape seeds meal and gross energy

Specification	CP (%)	EE (%)	CELL (%)	Ash (%)	Ca (%)	P (%)	GE (MJ/kg)
Determined content	13.38	7.57	31.35	3.05	0.58	0.49	18.98
Brenes *et al.,* [14]	13.85	9.87	15.18	2.41	-	-	-
Llobera and Canellas, [15]; Molina-Alcaide *et al.,* [16]; Spanghero *et al.,* [17]; FEDNA Tables [18]; Deng *et al.,* [19]	11.2 - 13.8	5.6 - 11.7	32.5 - 56.3	2.4 - 5	0.5 - 0.7	0.2 - 0.3	-
Zhao *et al.,* [20]	11.5	6.5	-	8.1	1.2	0.05	-

56.84%. The obtained data in this study are in agreement with those reported by Aziza *et al.,* [21] in a study on the flaxseed meal use in monogastric diets. The recorded concentrations were 34.3% crude protein, 4.12% ether extractives, 0.4% Ca and 0.9% P.

The regarding data on the main nutrients of feed tested in this experiment (Table 3) reveals that they had a balanced primary chemical composition. No remarkable differences were between groups, with an average concentration of 20.4% crude protein and 17.84 Mj/kg gross energy.

Table 3. Content of the main feed nutrients and gross energy

Specification	Grower phase (14 - 28 days)		Finisher phase (29 - 49 days)	
	C	E	C	E
Dry matter, %	90.55	90.18	89.92	89.94
Organic matter, %	84.00	84.85	89.92	89.94
Crude protein, %	20.60	20.41	20.50	20.10
Ether extractives, %	5.73	6.03	6.50	6.76
Cellulose, %	4.93	5.06	4.37	4.22
Ash, %	6.55	5.33	6.86	5.42
Calcium, %	0.93	0.91	0.90	0.91
Phosphorus, %	0.99	0.88	0.84	0.98
Gross energy, Mj/kg	17.19	17.39	18.41	18.39

As it can be seen in Table 4, in the growth phase (14 - 28 days), at the experimental lot (E) was recorded a higher value, with 2.21% for the total content of polyunsaturated fatty acids (PUFA), with 11.61% for omega-3 and 1.54% for omega-6, compared to the control group (C). No significant differences were found ($P > 0.05$) during the finisher phase (29 - 49 days), but there was an increase with 2.72% in the total saturation fatty acids content (SFA) for the experimental group (E), compared to the control group (C).

Table 4. Fatty acids content of the compound feeds, by level of saturation, (g/ 100 g fat)

Specification	Grower phase (14 - 28 days)	Finisher phase (29 - 49 days)		
	C	E	C	E
SFA	13.07	12.99	15.73	16.17
MUFA	25.22	23.90	24.53	23.99
PUFA	61.71	63.11	59.75	59.69
Ω3, %	3.73	4.22	7.45	7.39
Ω6, %	57.98	58.89	52.29	52.30
Ω6/Ω3	4.92	64.62	7.02	7.07

Legend: 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 bioproductive parameters recorded over the entire experimental period (14 - 49 days) presented in Table 5 did not differ significantly ($P > 0.05$) between groups. The obtained results are similar to those presented in literature. Some researchers (Brenes *et al.,* [14]; Viveros *et al.,* [9]; Chamorro *et al.,* [22]; Zhao *et al.,* [20]) conducted studies on the inclusion of different levels of grape meal in poultry and other animal diets without significant differences between groups ($P > 0.05$) at the end of the experimental period in terms of bioproductive performance. In Figure 1 there can be observed a collage of images captured during the experiment while the broilers were weighed.

Table 5. Bioproductive parameters throughout the experimental period of 14 - 49 days (average values/group)

Specification	C	E	SEM	P
Initial weight, g	311.75^a	311.75^a	4.215	> 0.9999
Final weight, g	2847.17^a	2835.12^a	35.592	0.0179
Average daily feed intake, g/broiler/day	133.81^a	130.18^a	3.880	0.5796
Average daily weight gain, g/broiler/day	72.45^a	71.71^a	1.031	3.932
Feed conversion ratio, g feed/g broiler	1.85^a	1.85^a	0.063	0.9383

Legend: 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 ($P < 0.05$).

Figure 1. Weighing the broilers

Figure 2 shows the content of the main nutrients in the breast meat samples, collected from the control and experimental groups. As we can see, the recorded values did not differ significantly ($P > 0.05$) between the

two groups, but the ether extractives of the E group was lower by 2.56% and the gross energy by 0.86% compared to the breast meat from C group.

The content of the main nutrients in thigh meat samples is shown in Figure 3. Although there were no significant differences (P > 0.05) between the two groups (C and E), in E group the ether extractives concentration was lower with 7.82% compared to C group, and gross energy by 6.39%.

The fatty acids content of broiler meat fat is shown in table 5. Regarding the fatty acid content of the breast meat samples from the experimental group, it can be noticed that the grape seeds meal introduction into the experimental diet determined the obtaining of higher concentrations, compared to those recorded in the control group. Thus, the inclusion of 2% grape seeds meal in the experimental diet resulted in significant levels (P < 0.05) in breast meat samples from the experimental group, with 13.43% of the total content of polyunsaturated fatty acids (PUFA), respectively 10.02% for omega-3 and 14.22% for omega-6, as opposed to the control group samples. As in the case of breast meat, the obtained results for thigh meat from the experimental group reveal significant values (P < 0.05) for the polyunsaturated fatty acid profile (PUFA) higher by 15.49%, by 18.7% for omega-3 and by 15.34% for omega-6 compared to the control group. The obtained results are comparable to those obtained from Chamorro *et al.,* [22] which evaluated the grape meal in poultry diets and recorded a high content of polyunsaturated fatty acids in the thigh meat, but also an increase in the oxidative stability of the meat.

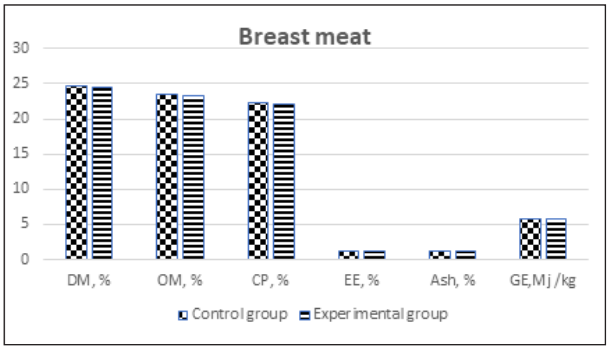

Figure 2. Level of the main nutrients in breast meat (average values/group)

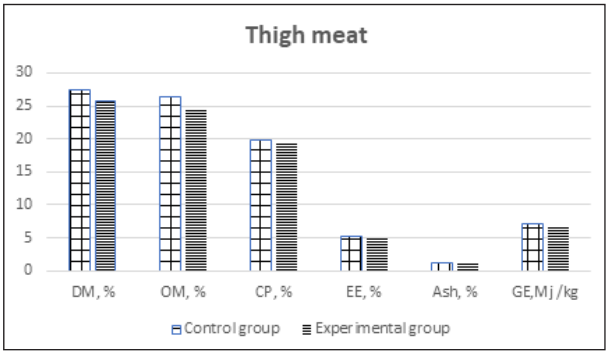

Figure 3. Level of the main nutrients in thigh meat (average values/group)

Table 5. Fatty acids content of broiler meat fat, depending on the level of saturation (g acid/100 g total fatty acids)

Specification	C	E	SEM	P
	Breast meat			
SFA, %	32.40^{a}	30.78^{b}	0.241	<0.0001
MUFA, %	37.83^{a}	34.96^{b}	0.364	<0.0001
UFA, %	67.10^{a}	68.77^{b}	0.255	<0.0001
PUFA, % of which:	29.26^{a}	33.80^{b}	0.598	<0.0001
Ω3, %	3.23^{a}	3.59^{b}	0.063	0.0024
Ω6, %	25.81^{a}	30.08^{b}	0.561	<0.0001
Ω6/Ω3, %	7.99^{a}	8.37^{a}	0.086	0.1674
	Thigh meat			
SFA, %	34.29^{a}	32.46^{a}	0.467	0.0001
MUFA, %	41.33^{a}	38.58^{b}	0.554	<0.0001
UFA, %	65.22^{a}	66.84^{b}	0.467	<0.0001
PUFA, % of which:	23.88^{a}	28.26^{b}	1.010	<0.0001
Ω3, %	2.39^{a}	2.94^{b}	0.091	0.0032
Ω6, %	21.18^{a}	25.02^{b}	0.960	<0.0001
Ω6/Ω3, %	8.95^{a}	8.50^{a}	0.269	0.0047

Legend:

SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; Ω3 - omega 3 polyunsaturated fatty acids; Ω6 - 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; means in the same row no common superscript significantly different (P < 0.05).

The evolution of the fat degradation indices in breast meat samples highlights the antioxidant character of the grape seeds meal added to the experimental diet. While the values of the peroxide indices on day 0 were identical for both groups (C and E), after 7 days of freezing of the samples, there was a significant decrease ($P < 0.05$) of the peroxide value, by 6.97%, compared to the samples in the control group. Regarding the acidity of the samples from the experimental group, the values obtained were significantly reduced ($P < 0.05$) both on day 0, by 5.75%, and after 7 days of freezing by 5.28%, compared to the acidity of the sample fat in the control group. Brenes *et al.,* [14] in a study on the grape seeds meal introduction into poultry diets concluded that its use intensifies the antioxidant activity in breast meat. Specialty studies have reported that supplementing of poultry diets with grape seeds meal as an antioxidant increased the vitamin E concentration in muscle tissues, improving oxidative stability of the meat during storage (Carreras *et al.,* [23]).

In the case of thigh meat, the evolution of fat degradation indices was decreasing. Peroxide indices were significantly different ($P < 0.05$) at both measurement moments, day 0 and day 7. The reduction was by 15.18% on day 0 and by 12.84% on day 7 for the experimental group compared to the control group. As for the acidity of thigh meat fat, there were no significant differences ($P>0.05$) between groups, there was a decrease of 3.97% on day 0 and 0.23% on the last analysis, on day 7.

4. Conclusions

- The dietary flaxseed meal, raw material rich in polyunsaturated fatty acids, given to Ross 308 hybrid broilers had a significant contribution to achieving high levels of fatty acids in broiler meat samples.

- The inclusion of 2% grape seeds meal in broiler diets of the experimental group as a natural antioxidant inhibited the lipid degradation reactions of the meat from this group, thus recording significantly ($P < 0.05$) higher concentrations of total polyunsaturated fatty acids (PUFA), omega-3 fatty acids and omega-6 fatty acids compared to meat samples from the control group. At the same time, the evolution of fat degradation indices has been declining both for the breast meat, and especially for the thigh meat.

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