USE OF FLAXSEED MEAL AND GRAPE SEED MEAL (2% AND 3%) AS NATURAL ANTIOXIDANT IN BROILER DIETS

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Abstract

This paper presents a 3-week „in vivo" feeding experiment conducted on 90, Cobb 500 broiler chicks, aged 14 days, assigned to 3 groups: a control group (C), and two experimental groups (E1, E2). Unlike the diet formulation for group C (based on corn, wheat, soybean meal and flax meal), the diet formulations for the experimental groups also included 2% (E1) and 3% (E2) grape seeds meal as natural antioxidant. The bioproductive parameters monitored throughout the feeding trial were not significantly (P>0.05) different between the groups. Six broilers per group were slaughtered in the end of the trial and six samples of breast meat and of thigh meat were formed for each group. the total amount of polyunsaturated fatty acids (PUFA) was significantly (P≤0.05) higher in the experimental groups, both in the breast meat: 31.50±1.59g (E1) and 33.74±0.29g (E2) vs. 29.29±0.96 g/100 g total fatty acids (C), and in the thigh meat: 30.28±1.09g (E1) and 34.08±1.80 (E2) vs 29.58±1.16 g/100gtotal fatty acids (C). The highest content of alfa linolenic acid (ALA), 1.12±0.07g, was recorded in the breast meat from group E1, which was significantly (P≤0.05) different from0.89±0.34g/100 g total fatty acids, in group C.

Key words: broiler, breast meat, thigh meat, flaxseed meal, grape seed meal, ALA

INTRODUCTION

Given the positive impact of including omega-3 fatty acids in human diets, there is a significant interest to enhance the omega-3 content of the meat and meat products. In monogastric animals, the fatty acids profile in the meat and fat is directly influenced by the dietary source of fat. Flaxseeds are an important category of oleaginous seeds because of their high concentration of α-linolenic acid. They contain 35-45% oil, of which ALA accounts for 45-52%. Therefore, feeding monogastric animals with diets including flaxseeds as rich source of omega-3 fatty acids, makes it possible to change the fatty acids profile of the poultry meat, particularly the omega-6/omega-3 ratio [15]. However, because of their high content of lipids, the diets enriched in polyunsaturated fatty acids are exposed to lipid peroxidation, which requires the use of antioxidants (synthetic or natural) which to slowdown lipid oxidation.

Winery by-products are one of the natural sources with a high level of polyphenols [8, 16] which bestows them with antioxidant properties [5, 11]. The literature [9,12] includes studies on the beneficial effects of the dietary winery by-products given to broiler chicks on broiler performance, protein and amino acids digestibility, intestinal microflora and broiler meat quality.

MATERIALS AND METHODS

The three-week in vivo experiment was conducted on 90, Cobb 500, fast growing broiler chicks, aged 14 days, housed in three-tier digestibility cages, assigned to 3 groups: a control group (C) and two experimental groups (E1 and E2). The initial average body weight was 409.96 ± 6.25 g/ chick with no significant differences between the experimental groups. Food and water was administered "ad-libitum". The light regimen

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was 23 hours / day, according to the Cobb 500 management guide.

The diets consisted of corn, wheat, soybean meal, with a 2% added flaxseed meal, which is rich in polyunsaturated fatty acids. The experimental diets differed from the control diet, and among them, by the addition of 2% and 3% grape seed meal as a natural antioxidant.

During the experimental period the following bioproductive parameters were monitored: the initial weight (g), the final weight (g), average daily compound feed intake (g/chick/day), average daily weight gain (g/chick/day), feed conversion ratio (g feed/g gain). At the end of the three weeks experimental period, respectively at the age of 35 days, 6 broilers/ group were slaughtered, from which 6 samples of breast meat/ group, respectively 6 samples of thigh / group were collected to determine their physical parameters, crude chemical composition and the fatty acid profile of fat. The following parameters were monitored throughout the experimental period: initial weight (g), final weight (g), average daily compound feed intake (g/chick/day), average daily weight gain (g/chick/day), feed conversion ratio (g feed/g gain).

The following standardized methods were used to determine the concentration of the main nutrients in the samples collected from feeds and meat: real dry matter (DM_r) was determined by the gravimetric method according to Regulation (EC) no. 152/2009 for combined feeds, respectively SR ISO 1442: 2010 for meat, using BMT drying oven model EcoCell Blueline Comfort model (Neuremberg, Germany); the crude protein (CP) was determined by the Kjeldahl method, in accordance with Regulation (EC) 152/2009 for combined feeds, respectively SR ISO 973: 2007 for meat, using a semiautomatic system KJELTEC auto 2300 - Tecator (Sweden); the ether extractives (EE) was determined by the organic solvent extraction method, in accordance with Regulation (EC) No. 152/2009 for feeds, respectively SR ISO 1443: 2008 for meat, using a SOXTEC-2055 FOSS-Tecator system (Sweden); the ash (Ash) was determined by gravimetric method, according to Regulation (EC) no. 152/2009 for compound feeds, and SR ISO 1442:2010 for meat, using 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.

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: 60m 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).

Gross energy (GE) was determined by calculation, using the gross chemical analysis data and the equations developed by Burlacu et al. (2002) [2].

The experimental results are expressed as mean values ± standard error; StatView software and the analysis of variance (ANOVA and t test) were used for statistical processing of the data, the differences being considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

Flaxseed meal, used to enrich the compound feeds in polyunsaturated fatty acids was characterized by 29.12% crude protein, 14.60% ether extractives, 8.80% cellulose, 20.03 Mj / kg gross energy, linolenic acid content (ALA) of 53.28 g, total omega-3 acids of 53.39 g, total omega-6 acids of 17.44 g / 100 g of fat and an omega-3 / omega-6 ratio of 0.33.

The defatted grape seed meal used as an antioxidant was characterized by a crude protein content of 14.12%, ether extractives content of 5.40%, 35.67% cellulose and 17.64 Mj / kg of gross energy, data similar to those reported by Mironeasa et al., (2010) and Elagamey et al., (2013) [4,13]. The fatty acid content of grape seed fat ranged from 66.20 g linoleic acid to 17.93 g oleic acid or 5.13 g stearic acid / 100 g fat, comparable to those presented by Tangolar et al., (2009), Kikalishvili et al., (2012) [10, 19]. Sabir et al., (2012) [17], who analysed grape samples of different varieties, reported that of the total weight of fatty acids found in the extracted fat, the linoleic acid accounts for 53.6-69.6%, followed by the oleic acid, 16.2-31.2%, palmitic acid, 6.9-12.9%, and stearic acid, 1.44- 4.69%.

The analytical results of the main dietary nutrients (Table 1) show that they were isoprotein (18.87% crude protein), the protein content being slightly lower in the control group but with insignificant differences (P≤0.05) between batches and isocaloric (17.11 MJ / kg of gross energy).

Table 1 Content of the main feed nutrients and gross energy

Specification	C	E1	E2
Dm_r ,%	89.06	89.27	89.11
OM, %	83.35	82.30	83.23
CD, %	17.36	19.69	19.56
EE, %	6.20	7.11	6.94
CELL, %	3.86	4.31	4.88
Ash, %	5.72	6.97	5.88
Ca, %	0.90	0.91	0.91
P, %	0.96	1.16	0.81
GE,MJ /kg	16.94	17.13	17.26

Chemical composition expressed on dry matter (DM); DMr-real dry matter; OM-organic matter; CP-crude protein; EE-ether extractives; CELL-cellulose; Ash-ash, Ca-calcium, P-phosphorus, GE-gross energy

The content of total polyunsaturated fatty acids, PUFA (Table 2), ranged from 53.77 to 56.43 g / 100g of fat, the omega-3 fatty acid content ranged between 2.75-2.67 g / 100g of fat and the omega-6 ratio / omega-3 recorded values between 18.55 - 20.47, with no significant differences between groups. The results obtained are comparable to those of Elagamey et al., (2013) who, following a study of six varieties of grapes, found that from the total fatty acids of the grape seed, UFA represents 86-88%, while SFA represents 12-14%; and with the data reported by Bardakçı and Canbay, (2011) [1] who, by performing gram-based fatty acid determinations, obtained a PUFA / SFA ratio of 3.17.

Table 2 Fatty acids content of the compound feeds, by level of saturation, (g/ 100 g fat)

Specification	C	E1	E2
SFA, %	14.50	13.97	13.25
UFA, %	85.06	85.72	86.47
MUFA, %	31.29	30.53	30.04
PUFA , %	53.77	55.19	56.43
PUFA/SFA	3.71	3.95	4.26
Ω3, %	2.75	2.57	2.67
Ω6, %	51.02	52.62	51.27
Ω6/Ω3	18.55	20.47	20.19

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 throughout the experimental period (Table 3) did not differ significantly (P> 0.05) between groups, but the highest final weight of 1973.5 ± 292.29 g, respectively average daily weight gain of 55.01 ± 12.92 g /chick/ day was obtained in experimental group E2 with 3% grape seed flour. The lowest feed conversion ratio, of 1.25 ± 0.07 g feed / g chick, was also obtained in group E2, with 3% grape seed flour. The results are similar to those obtained by Sossidou et al., (2013) [17] who found that the introduction of different levels of grape pomace 0.25 g; 0.5g and 1g / 100 feeds in dietary formulation for broiler over a period of 0-42 days had positive effects on bioproductive performance. Thus, the average daily weight gain recorded an average of 72.97 ± 0.85 g/day/ in the experimental group with 1 g of grape pomace/ 100 g feed, compared to 71.42 ± 0.85 g /chick/ day in group C; the weight of the broilers at the end of the experiment (42 days) recorded an average value of 2535 ± 25.92 g / chick, also in the group with 1 g grape pomace/ 100 feed, significantly higher (P≤0.05) versus 2484.55 ± 25,55 g / chick in group C.

Khodayari and Shahria, (2014) [9] analysing the effect of introducing different levels of 0, 2, 4 and 6% grape pomace in broiler diet on the bioproductive performances found that only the inclusion of 2% grape pomace in broilers ration influenced positively feed consumption and body weight at 42 days, the difference being significant, compared to group C.

Table 3 Bioproductive parameters throughout the experimental period of 14 - 35 days (average values / group)

Groups		
C	E1	E2
Average daily feed intake, g/broiler/day		
75.81±6.96	73.97±6.65	73.92±6.91
Initial weight, g		
47.62±3.49	49.21±3.58	48.19±3.29
Final weight, g		
1825.00 ±199.22	1863.33 ±243.68	1973.54 ±292.29
Average daily weight gain, g/broiler/day		
50,78±10.99	51,83±11.47	55,01±12.92
Feed conversion ratio, g feed/g broiler		
1,37±0.11	1,33±0.09	1,25±0.07

Cross et al., (2011) [3] also evaluated the effect of inclusion in broiler (females) fed from 0-42 days on grape pomace, along with other natural antioxidants, and found that the performance and digestibility of the broilers that have been fed with grape pomace were similar to those of the control group, and this supplement seems suitable for dietary inclusion. At the same time, Goñi et al., (2007) [6] investigated the effect of the fermented grape pomace added at different levels (5, 15 and 30 g / kg feed) and found that a rate of inclusion of up to 30 g / kg of grape pomace did not affect growth performance nor protein digestibility.

The data regarding the content of the main nutrients in the breast and thigh meat from broiler did not differ significantly between groups (Table 4).

Table 4 Level of the main nutrients in broiler meat (average values/group)

Specificaion	C	E1	E2
Breast meat			
DMr, %	24.69±1.43	25.89±0.87	25.53±1.35
OM, %	22.55±1.23	23.67±1.41	23.18±1.35
CP, %	21.09^{a}±1.20	22.40^{b}±0.39	21.84^{c}±0.42
CF, %	1.28^{d}±0.11	1.24^{e}±0.12	1.28^{f}±0.17
Ash, %	1.14±0.10	1.23±0.11	1.19±0.10
RE, Mj/kg	5.56±0.35	5.83±0.28	5.72±0.36
Thigh meat			
DMr, %	33.97±1.91	31.29±2.09	28.94±2.04
OM, %	31.47±1.32	28.80±1.96	26.59±1.57
CP, %	15.71^{a}±0.64	19.15^{b}±0.92	18.53^{c}±1.04
EE, %	9.38^{d}±0.84	8.31^{e}±1.06.	7.61^{f}±0.92
Ash, %	1.03±0.16	1.01±0.12	1.06±0.09
GE, Mj/kg	8.56±0.72	8.09±0.86	7.51±1.02
DM_r-real dry matter; OM-organic matter; CP-crude protein;EE- ether extractives;Ash- ash, GE-gross energy; a,b,c–significant differences (P≤0.05) of the protein level between the breast and thigh meat within the same group: C, E1, E2; d,e,f – significant differences (P≤0.05) of the fat level between the breast and thigh meat within the same group: C, E1, E2;			

However, there were differences (P≤0.05) within the same groups, in terms of protein content, which was higher in broiler breast meat than in broiler thigh meat, by 34.25% (group C), by 16.97% (lot E1), and by 17.86% (groupE2); the fat level was lower in the breast meat than in the thigh meat by85.05% in group C, by 85.07% in group E1, and by 83.18% in group E2.

The obtained results are similar to those reported by Mridula et al., (2015) [14] where, with an addition of 2.5% flaxseeds, they obtained a protein content of 20.17 ± 1.02% (breast), respectively 19.10 ± 0.77% (thigh); with a fat content of 2.55 ± 0.21% (breast), respectively 5.81 ± 0.72% (thigh) and an ash content of 1.21 ± 0.18% (breast), respectively 1.20 ± 0.20% (thigh).

The values of total polyunsaturated fatty acids (PUFA) in the broiler breast fat (Table 5) were significantly higher (P≤0.05) in the experimental groups than in group C, the highest increase (15.19%) being recorded in groupE2, with 3% grape seed meal.

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

Specification	C		E1		E2		SEM	P
SFA, %	29.94^{b,c}	±0.57	28.62^{a,c}	±0.86	27.69^{a,b}	±0.19	0.287	0.0003
MUFA, %	40.18^{b,c}	±0.52	39.45^{a,c}	±0.68	38.20^{a,b}	±0.34	0.253	0.0002
UFA, %	69.47^{b,c}	±0.61	70.94^{a,c}	±0.97	71.93^{a,b}	±0.21	0.315	0.0003
PUFA, % of which:	29.29^{b,c}	±0.96	31.50^{a,c}	±1.59	33.74^{a,b}	±0.29	0.550	0.0001
ALA, %	0.89^{b,c}	±0.34	1.12^{a}	±0.07	1.09^{a}	±0.02	0.037	0.0105
Ω3, %	2,14^{b}	±0.05	2.33^{a}	±0.25	2.29	±0.07	1.485	0.043
Ω6, %	27,00^{b,c}	±0.99	28.91^{a,c}	±1.39	31.21^{a,b}	±0.25	0.518	<0.0001
Ω6/Ω3	12,64^{c}	±0.58	12.47^{c}	±0.90	13.62^{a,b}	±0.37	0.208	0.0348

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,c- significant differences (P≤0.05) compared to C, E1, E2

Also, the content of omega 3 polyunsaturated fatty acids recorded significantly higher values (P≤0.05) for the experimental groups compared to group C, the highest increase, of 8.87%, being in groupE1, with 2% grapes seed meal. The highest value of ALA in broiler breast was 1.12 ± 0.07 g / 100g of fat in group E1, lower value compared to that obtained by Mridula et al. (2015), where an inclusion of 2.5% flaxseed produced an ALA concentration of 3.21 ± 1.29% in the broiler breast.

Table 6 Fatty acids content of the thigh meat fat, depending on the level of saturation (g acid/100 g total fatty acids)

Specification	C	E1	E2	SEM	P
SFA, %	27.81±1.53	27.91^{c}±0.45	26.57^{b}±0.50	0.282	0.0903
MUFA, %	42.29^{c}±0.48	41.38^{c}±0.77	39.07^{a,b}±1.30	0.424	0.0004
UFA, %	71.87^{c}±1.37	71.66^{c}±0.36	73.16^{a,b}±0.53	0.214	0.0001
PUFA, % of which:	29.58^{c}±1.16	30.28±1.09	34.08^{a}±1.80	0.624	0.0005
ALA, %	0.90^{c}±0.06	0.81^{c}±0.03	1.03^{a,b}±0.02	0.032	0.0062
Ω3, %	1.39^{c}±0.40	1.48±0.19	1.74^{a}±0.08	0.067	0.0030
Ω6, %	28.12^{c}±0.81	28.72±0.95	32.20^{a}±1.80	0.568	0.0050
Ω6/Ω3	22.38^{c}±9.41	19.64±2.44	18.54^{a}±1.38	1.423	0.5596

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,c- significant differences (P≤0.05) compared to C, E1, E2

Also, similar studies on the enrichment of the broiler carcass in fatty acids by addition of ration of flaxseed meal were also conducted by researchers Gonzalez-Esquerra and Leeson (2010) [7], whose results revealed significant meat enrichment in omega-3, depending on the concentration of flaxseed meal introduced in ration, the sensory quality of the broiler breast is being unaffected. Analysing the data on total polyunsaturated fatty acids (PUFA) in the case of breast meat fat (Table 6), it can be seen that their values are significantly higher ($P \leq 0.05$) in the experimental groups, compared to group C, the highest increase of 15.21% being registered in group E2.

Significantly higher values ($P \leq 0.05$) were also found for the omega 3 fatty acids (Ω3) in the experimental groups compared to the C group, the highest increase of 25.18% being recorded also for the E2 group.

The highest value of ALA in the thigh meat was 1.03 ± 0.02g / 100g total fatty acids, in the case of broilers from group E2, whose diet included 2% grape seed meal, but lower than the value of 3.01 ± 0.80% reported by Mridula et al. (2015) [14] in the case of an addition of 2.5% flaxseed in broilers diet.

CONCLUSIONS

Flaxseed meal, a raw material rich in polyunsaturated fatty acids, introduced in a proportion of 2% in broiler diets has increased the content of polyunsaturated fatty acids both in feed and broiler meat.

Grape seed meal used as an antioxidant in experimental diets (groups E1 and E2) determined the improvement of the nutritional qualities of broiler meat by inhibiting lipid degradation reactions, thus increasing the content of total polyunsaturated fatty acids (PUFA) respectively PUFA omega-3 fatty acids in the breast meat of the experimental group (E1), respectively in the thigh meat of the experimental group E2, compared to the control group (C).

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