

FEEDING QUALITY OF THE MEAT FROM BROILERS FED WITH DIETARY FOOD INDUSTRY BY-PRODUCTS (FLAXSEED, RAPESEEDS AND BUCKTHORN MEAL, GRAPE POMACE)

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Abstract

A feeding trial was conducted on 75, ROSS308 (0-42days) broilers, to evaluate the quality of the meat from broilers which received food industry by-products in their diets: rapeseeds meal and grape pomace, or flaxseeds meal and buckthorn meal. The broiler chicks were assigned to 3 groups (C, E1 and E2), and were housed in an experimental hall with controlled environmental conditions: average air temperature $27.07\pm2.75^{\circ}\text{C}$, humidity $64.80\pm9.57\%$. For 10 days, during the starter phase, all chicks received a conventional compound feed. In the other two stages (growing, finishing), compared to the conventional diet given to the C group, the diet formulations of the experimental groups included different proportions, depending on the phase of development, rapeseeds meal and grape pomace (E1), or flaxseeds and buckthorn meals (E2). The highest polyphenols concentration was determined in the finishing diet formulation for group E1 (with 8% rapeseeds meal and 4% grape pomace). The dietary concentration of $\omega 3$ polyunsaturated fatty acids ($\omega 3$ PUFA) in the diet formulations for group E2 increased with the level of dietary flaxseeds meal (2.5% during the grower phase and 8% in the finishing phase). Six broilers from each group were slaughtered in the end of the trial and meat (breast and thigh) and liver samples were collected and assayed for dry matter, protein, fat, ash, fatty acids and cholesterol. The highest concentration of $\omega 3$ PUFA, which are essential for human health, were determined in the breast and thigh of E2 broilers (flaxseeds meal and buckthorn meal). The cholesterol level in the breast meat and thigh samples was not significantly different between groups; however, it was lower in the experimental groups than in the control group. The fat level in the liver samples collected from C group broilers was significantly ($P\leq0.05$) higher than in the experimental groups.

Key words: broilers, by-products, feeding quality, breast meat, thigh, liver.

INTRODUCTION

The increasingly large amounts of vegetable by-products resulting from the food industry causes economic losses and bears an adverse impact on the environment. At the same time, the increasing cost of animal feeding increase the total production costs, in which the cost of feeding represents 60-70%. Most people are familiar with the three "R" concept (reduce, reuse, recycle). A fourth R, responsibility, might be the key to a sustainable society. The most efficient disposal of the by-products is to use them as animal feeds ingredient, but it is sometimes limited by the legislation and by

the nature of the particular by-product. Researchers have been increasingly concerned, lately, with finding new feeding solutions for poultry, which would allow achieving high performance at low costs (Lup, 2010).

The food industry by-products are rich in valuable nutrients: vitamins, minerals, polyphenols, polyunsaturated fatty acids, pigments, etc. Much of the food industry by-products, the meals, come from the oil extraction industry, being the wastes that remain after oil extraction from the oil seeds. Rapeseed meal is among the "classical" meals used in farm animal feeding. Rapeseed is

mainly an oil-source 40–45% DM, but the rapeseed meal obtained after extraction of oil is an interesting protein-source with protein content varying between 32% and 45% DM. While the advantage of rapeseed meal is the quality of its protein (its amino acid profile is more interesting than that of soybean), it contains a high proportion of fibre besides other anti-nutritional factors ANF such as tannins, sinapin and phytic acid (Burel et al., 2000). Several, authors warn against using a high level of rapeseeds meal in broiler diets (Karunajeewa, 1999; McNeill et al., 2004). On the other hand, Wetscherek et al. (1990) showed that up to 20% rapeseed oil meal can be included in broiler diets without affecting performance. Another oil industry by-product, the flaxseed meal, is increasingly used in poultry production due to its large content of fatty acids: 12.50% saturated FA, 24.21% monounsaturated FA, 43.23% ω :3 FA and 20.06% ω :6 FA (Aziza et al., 2013). Mridula et al. (2011) noticed that the alpha-linolenic acid, omega 3 acid, content in both breast and thigh meat was higher with an increasing level of flaxseed meal in the diets without affecting the sensory acceptability of meat. The same authors consider that ca up to 10% of flaxseed meal may be used in broiler diet to enhance the alpha-linolenic acid content in the broiler meat. As the industry of natural food supplements developed, new by-products appeared, such as the buckthorn meal. Buckthorn is a rich source of natural antioxidants such as ascorbic acid, tocopherols, carotenoids, flavonoids, while they contain proteins, vitamins (especially vitamin C), minerals, lipids (mainly unsaturated fatty acids), sugars, organic acids and phytosterols (Christaki, 2012). Kaushal and Sharma (2011) have shown that the buckthorn fruits are adequate for animal feeding too. In a study on the effect of flavones of sea Buckthorn on carcass characteristics and meat quality of Arbor Acres broilers, Li et al. (2008) have noticed that at the dose of 0.2% flavones of sea buckthorn, ether extract of thigh muscle and serum triglyceride were significantly decreased ($P \leq 0.05$).

Grape (*Vitis vinifera*) is one of the world's largest fruit crops (FAO-STAT, 2007). Recent investigations have stressed the importance of by-products from wine processing as plant

materials that are particularly rich in polyphenols and have a wide range of biological activities. Grape pomace is the residue left after juice extraction by pressing grapes in the wine industry. This by-product (constituted by seeds, skin, and stem) is used every year either as animal feed (with low nutritional value) or for ethanol production by fermentation and distillation (Viveros et al., 2011). Grapes contain a large amount of polyphenols which include the phenolic acids, anthocyanins and proanthocyanidins (Lu and Foo, 1998). Goni et al. (2007) found out that the dietary grape pomace can delay lipid oxidation in breast and thigh chicken meats and reduce the potential risk induced by lipid oxidation products.

Within this context, we conducted a study to evaluate the quality of broiler chicken treated with pairs of food industry by-products: rapeseed meal and grape pomace, or flaxseed meal and buckthorn meal.

MATERIALS AND METHODS

A feeding trial was conducted on 75, ROSS 308 broilers, from 0 to 42 days of age. The day-old chicks were weighed individually and assigned to three homogenous groups: 42.39 ± 0.18 g (C); 42.16 ± 0.24 g (E1); 42.748 ± 0.21 g (E2). The broiler chicks were housed in an experimental hall with controlled environmental conditions, according to the management guide: average air temperature $27.07 \pm 2.75^\circ\text{C}$, humidity $64.80 \pm 9.57\%$, ventilation/broiler $0.50 \pm 0.24\%$; CO_2 level, 686.39 ± 104.38 (ppm). The broilers had free access to the feed and water.

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). For 10 days, during the starter phase, all chicks received a conventional compound feed, with the purpose of developing a good appetite and reaching the standard body weight at 7 days. In the other two stages (growing, finishing), compared to the conventional diet given to the control broilers, the diet formulations of the experimental groups included different proportions, depending on the phase of development (Table 1).

Table 1. Diet formulations

Ingredient	Phase II – growth (11 – 28 days)			Phase III – finishing (29 - 42 days)		
	C	E1	E2	C	E1	E2
	%					
Corn	51.32	46.1	50.22	60.23	51.98	59.61
Soybean meal	38.32	32.6	35.54	30.04	24.29	23.26
<i>Rapeseed meal</i>	-	8.00	-	-	8.00	-
<i>Grape pomace</i>	-	2.00	-	-	4.00	-
<i>Buckthorn meal</i>	-	-	2.00	-	-	2.00
<i>Flaxseed meal</i>	-	-	2.50	-	-	8.00
Plant oil	5.73	6.90	5.04	5.16	7.45	2.41
Lysine	0.02	0.05	0.13	0.11	0.14	0.36
Methionine	0.25	0.21	0.29	0.23	0.20	0.32
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Calcium carbonate	1.67	1.46	1.58	1.63	1.35	1.45
Monocalcium phosphate	1.23	1.22	1.24	1.17	1.15	1.15
Salt	0.41	0.41	0.41	0.38	0.39	0.39
Premix	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100

*1kg IBNA premix (A1)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; 6000 mg/kg antioxidant.

Samples of the studied by-products (rapeseed, flaxseed and buckthorn meals, grape pomace) and of the compound feeds were collected and assayed for the basic chemical composition: dry matter (DM), crude protein (CP), ether extractives (EE), crude fibre (CF) and ash (Ash), using the chemical methods from Regulation (CE) no. 152/2009 (Methods of sampling and analysis for the official inspection of feeds). The fatty acids were determined by gas chromatography, according to SR CEN ISO/TS 17764 -2:2008. The content of total phenols in the methanol feeds extracts, egg white and yolk extracts was determined according to the method described by Mihailovic et al. (2013), while the flavonoids content of the methanol feeds extracts was determined according to the method described by Zhishen et al. (1999).

In the end of the feeding trial, according to the working protocol approved by the ethic commission of IBNA Balotesti (decision nr. 52/30.07.2014), 6 broilers/group were slaughtered and meat (breast and thigh) and liver samples were collected and assayed for their feeding quality (dry matter, protein, fat, ash, fatty acids and cholesterol). The crude protein of the meat was determined using a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 – Tecator (SR ISO 973,

2007). The meat fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 1444, 2008). The meat ash was determined by calcinations at 550°C (SR ISO 936, 2009). The meat fatty acids composition was determined by gas chromatography. After lipid extraction from the samples, the fatty acids were transformed into methyl esters by transmethylation, and the components were separated in the capillary chromatograph column. The fatty acids were identified by comparison with blank chromatograms and were subsequently determined quantitatively as percent for 100 g fat. The method used to determine the cholesterol was in agreement with AOAC International standard, 2002 (Cholesterol in multicomponent foods – Gas Chromatographic method. Assoc. of Anal. Chem. Arlington, VA). The working principle is the saponification of the sample followed by extraction in petrol ether, concentration and addition of chloroform. The sample is split in the GC, it is separated in the chromatographic column, and the results are compared with the standard chromatograms by measuring the peak area. It was used a Perkin Elmer-Clarus 500 chromatograph fitted with flame ionization

detector (FID) and capillary separation column HP-5, 30 in length, and 0.320 mm inner diameter, 0.10 µm thick film.

The analytical data were compared by variance analysis (ANOVA) using STATVIEW for Windows (SAS, version 6.0). The difference between the means was considered significant at P<0.05. The results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSIONS

The basic chemical analysis of the dietary by-products (Table 2) revealed a high protein level

in the rapeseed meal and flaxseed meal. The high fibre level from all used by-products show that they must be included in moderate level in poultry diets. The values from Table 2 are in agreement with the literature data, although the by-products have a lower level of chemical composition stability.

The rapeseed meal had 43.49% dry matter, 37.98% crude protein (Bell, 1990) and 12.8% crude fibre (Jensen, 1994). The grape pomace had 88.44% dry matter, 10.64% crude protein and 40.66% crude fibre (Olteanu et al., 2014). Panaite (2016) found that the flaxseed meal had 20.91% crude protein and 7.18 % crude fibre.

Table 2. Chemical composition of the studied by-products*

Item	Rapeseed meal	Flaxseed meal	Grape pomace	Buckthorn meal
DM%	90.03	90.46	89.92	88.94
CP%	31.15	29.97	12.33	11.66
EE%	1.02	15.69	5.95	12.46
CF%	12.40	11.16	35.17	15.10
Ash%	5.71	3.87	2.83	2.69
Σ SFA	14.88	13.67	30.06	10.13
Σ MUFA	41.53	19.51	42.63	19.55
Σ PUFA, (g/100g fatty acids) of which	43.19	70.33	66.60	27.33
- ω:3	5.68	43.42	1.12	4.88
- ω:6	37.51	26.91	65.48	22.45
- ω:6/ ω:3	6.60	0.62	58.40	4.60

where: DM=dry matter; CP=crude protein; EE= ether extractives; CF=crude fibre; Ash=ash; Σ= sum; SFA =saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.

*Chemical composition on dry matter (DM) basis.

The fatty acids profile (Table 2) showed that the flaxseed meal is particularly rich in omega 3 polyunsaturated fatty acids (omega 3 PUFA), as also shown by other authors (Aziza et al., 2013; Panaite et al., 2016).

The data shown in Table 3 reveal that the level of omega 3 polyunsaturated fatty acids (ω 3 PUFA) was higher in the diet formulation with flaxseed meal (E2), in both phases of development, than in groups C and E1. The level of ω 3 PUFA was higher in the finishing formulation for E2, with a higher flaxseed meal level, than in the grower formulation for the same group. In terms of the oxidative status of the

compound feeds (Table 3), the highest concentration of polyphenols (2.60 mg gallic acid equivalents/g) was determined in E1 formulation for the finishing phase (8% rapeseed meal and 4% grape pomace).

The high antioxidant activity of the grape pomace has also been reported by other authors who used it in broiler diets (Goñi et al., 2007; Brenes et al., 2008). The concentration of polyphenols in E2 diet formulation for both phases, which contained 2% buckthorn meal, was also higher than in the control group (Table 3).

Table 3. Chemical composition of the compound feeds *

Item	Growth phase (11 – 28 days)			Finishing phase (29 - 42 days)		
	C	E1	E2	C	E1	E2
DM %	89.17	89.03	88.81	89.10	89.40	88.92
CP %	22.62	20.79	22.20	18.80	19.53	18.81
EE %	7.51	8.41	6.95	7.22	9.56	6.06
CF %	4.06	5.88	4.03	3.87	5.66	4.62
Ash %	5.60	5.74	6.03	6.02	5.41	5.29
g /100g total fatty acids:						
Σ SFA	12.15	11.66	12.94	13.17	12.81	13.91
Σ MUFA	28.24	28.73	28.25	29.32	29.25	28.24
Σ PUFA, of which:	59.09	59.20	58.37	57.07	57.62	57.54
- ω:3	1.03	0.92	4.13	1.48	1.02	9.71
- ω:6	58.06	58.27	54.24	55.59	56.60	47.83
- ω:6/ ω:3	56.36	63.31	13.13	37.69	55.25	4.92
Polyphenols (mg galic acid equivalents/g)	1.160	1.79	1.59	1.161	2.60	1.68

where: DM=dry matter; CP=crude protein; EE= ether extractives; CF=crude fibre; Ash=ash; Σ= sum; SFA =saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.

*Chemical composition on dry matter (DM) basis.

The total gains of the broilers, recorded in the end of the experiment, were comparable between groups: 2.39 kg (C); 2.32 kg (E1); 2.18 kg (E2). Therefore, the quality of broiler meat can be compared between the three groups. Table 4 data show that the best results were obtained for the breast meat samples from the broilers which received E2 formulations, rich in ω 3 PUFA. A significant ($P \leq 0.05$) decrease of the fat percentage was noticed in this group, compared to the broilers fed on the conventional formulation (group C). The concentration of ω 3 PUFA in the breast meat samples was higher ($P \leq 0.05$) in the experimental groups than in the control group. Betti et al., (2009) reported 300 mg ω 3 PUFA per 100 g breast meat, in 26.2 days, with 10% flaxseed meal. Kamboh and Zhu (2013) also reported a low proportion of saturated fatty

acids and an increasing proportion of polyunsaturated fatty acids in the broiler meat, by supplementation with bioflavonoids, such as groups E1 (grape pomace) and E2 (buckthorn) from this experiment.

The protein level didn't vary in the thigh samples collected from the 3 groups. However, unlike the breast meat samples, the proportion of fat was significantly ($P \leq 0.05$) higher in thigh from group E1 than in groups C and E2 (Table 5). The highest value ($P \leq 0.05$) of ω 3 PUFA concentration in the broiler thigh (Table 5) was noticed, same as for the breast meat samples, in group E2. However, an ω 6/ ω 3 PUFA ratio closer to the ideal value of 1 (Simopoulos, 2002) was determined in the thigh meat samples from group E2, the value of this ratio being 49.83% lower than in group C and 53.53% lower than in group E1 (Table 5).

Table 4. Chemical composition of the breast muscle

Item	C	E1	E2
Dry matter, %	24.07±1.23	24.74±0.58	23.34±2.16
Protein, %	22.09±1.72	22.17±0.51	21.71±2.82
Fat, % DM	1.12±0.07 c	1.25±0.13 c	0.83±0.18 a,b
Ash, % DM	1.19±0.10 c	1.17±0.05	1.09±0.09 a
Σ PUFA, (g/100g fatty acids) of which:	31.73±0.29 b	33.94±1.48 ac	32.24±1.93 b
- ω:3	1.865±0.116 bc	2.74±1.495 a	3.212±0.329 a
- ω:6	29.68±0.306	30.1±0.897	28.745±1.601
- ω:6 / ω:3	15.97±1.069b c	10.737±5.819 a	8.99±0.419 a

where: a,b,c, significant differences ($P \leq 0.05$) compared to C, E1, E2;

Σ= sum; PUFA= polyunsaturated fatty acids

Table 5. Chemical composition of the thigh samples

Item	C	E1	E2
Dry matter, %	25.51±1.06	25.72±1.32	26.49±0.89
Protein, %	18.96±0.62	19.21±1.21	19.22±0.70
Fat, % DM	4.08±0.37 ^b	5.01±0.34 ^{a,c}	4.28±0.33 ^b
Ash, % DM	0.99±0.06	0.91±0.07	0.88±0.04
Σ PUFA, (g/100g fatty acids) of which:	33.11±0.57 ^{b,c}	39.53±0.68 ^{a,c}	41.40±0.19 ^{a,b}
- ω:3	1.75±0.08 ^c	1.95±0.15 ^c	4.18±0.10 ^{a,b}
- ω:6	31.00±0.57 ^{b,c}	37.16±0.55 ^a	37.07±0.22 ^a
- ω:6 / ω:3	17.70±0.92 ^{b,c}	19.11±1.55 ^{a,c}	8.88±0.22 ^{a,b}

where: a,b,c, significant differences ($P\leq 0.05$) compared to C, E1, E2;
 Σ = sum; PUFA= polyunsaturated fatty acids

The diet formulations enriched in ω 3 PUFA determine a higher content of these fatty acids in the meat and eggs, resulting thus foods that are a natural source of these essential nutrients for the consumers (Leskanich and Noble, 1997).

Figure 1 shows the concentrations of ω 3 PUFA, which are essential acids for human health (Simopoulos et al., 2000), in the breast and thigh meat samples.

The concentration of α -linolenic acid (C 18:3n3) was 68.80% and 68.52 % lower in the breast from group C broilers, than in the breast samples from groups E1 and E2, respectively.

The concentration of docosapentaenoic acid (C 22:5n3) too, was 43.3 % and 76.09% lower in the breast samples from group C broilers, than in the breast samples from groups E1 and E2, respectively.

The docosahexaenoic acid (C 22:6n3) was 13.79% (E1) and 44.44% (E2) higher in the breast samples of these groups, than in group C. The concentration of α -linolenic acid in the thigh samples from group E2 was 7 times higher than in the thigh samples from group C, while the concentration of docosahexaenoic acid was over 4 times higher.

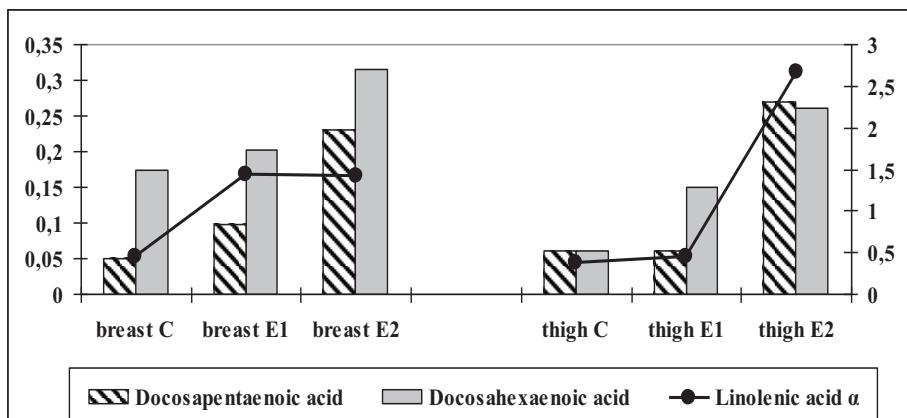


Figure 1. Concentration of omega 3 polyunsaturated fatty acids (g/ 100g total acids) in the breast and thigh meat sample

Figure 2 shows that the cholesterol concentration in the thigh samples from groups C and E2 was higher than in the breast meat but the difference was not statistically significant. The lowest cholesterol concentration in the thigh samples was recorded in group E1 (Figure 2). The breast samples from group C had the highest concentration of cholesterol,

but also the difference was not statistically significant.

The chemical analysis of the liver samples (Table 6) collected in the end of the experiment showed that the fat level of these samples was significantly ($P\leq 0.05$) higher in group C than in the experimental groups (E1, E2).

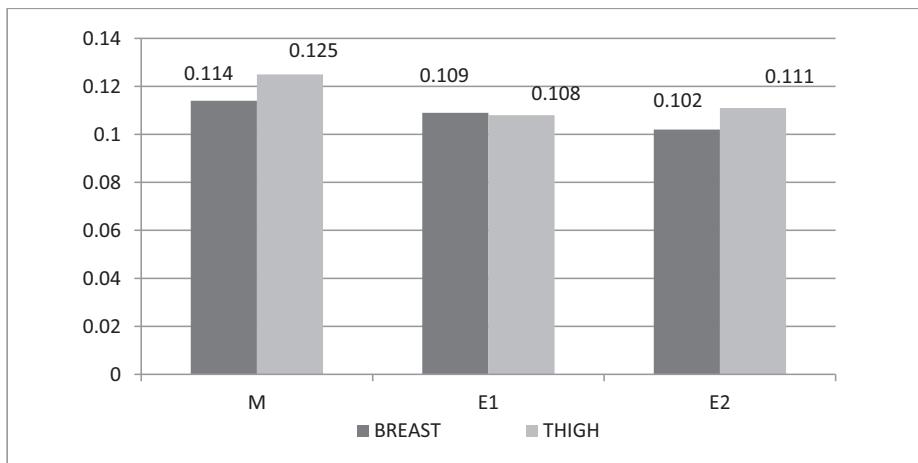


Figure 2. Cholesterol (g %) concentration in the breast and thigh meat samples

Table 6. Chemical composition of the liver samples

Item	C	E1	E2
Dry matter, %	26.73±2.57	25.96±0.82	25.71±0.74
Protein, %	18.11±1.10	17.85±0.95	18.12±0.79
Fat, % DM	3.92±1.15 ^{b,c}	2.95±0.34 ^a	2.60±0.10 ^a
Ash, % DM	1.21±0.15	1.30±0.08	1.30±0.08
Σ PUFA, (g/100g fatty acids) of which:	36.85±3.92 ^{b,c}	43.68±1.89 ^a	42.32±1.07 ^a
ω:3	1.90±0.31 ^c	1.60±0.15 ^c	5.46±0.60 ^{a,b}
ω:6	34.84±3.73 ^b	41.89±1.79 ^{a,c}	36.71±1.10 ^b
ω:6 / ω:3	18.58±2.48 ^{b,c}	26.31±2.20 ^{a,c}	6.80±0.75 ^{a,b}

where: a,b,c, significant differences ($P\leq 0.05$) compared to C, E1, E2; Σ= sum; PUFA= polyunsaturated fatty acids.

The concentration of omega 3 fatty acids determined in the liver samples from group E2 was significantly ($P\leq 0.05$) higher than in the samples from groups C and E1 (Table 6). All the omega 3 acids essential to human health, α-linolenic, docosapentaenoic and docosahexaenoic, were significantly ($P\leq 0.05$) higher in the liver samples from group E2 (flaxseed meal) than in the other groups. Also in this group, ω 6/ ω 3 PUFA ratio was significantly ($P\leq 0.05$) lower than in the other groups.

CONCLUSIONS

The high fibre level from all the used by-products shows that they have to be included in moderate levels in the diet formulations for poultry. The highest polyphenols concentration was determined in the diet formulation for group E1, the finishing phase (8%

rapeseed meal and 4% grape pomace), which is due to the high antioxidant activity of the grape pomace. The dietary grape pomace also increased the level of ω 3 PUFA in this group. As the dietary level of flaxseed meal increased in the formulations for group E2 (2.5% in the growing phase and 8% in the finishing phase), the dietary concentration of ω 3 PUFA also increased.

The feeding trial has shown that the highest concentration of ω 3 polyunsaturated fatty acids, essential to human health, was determined in the breast and thigh meat samples from E2 broilers (flaxseed and buckthorn meals). The cholesterol concentration in the breast meat samples was not significantly different between groups, although they were lower in the experimental groups than in the control group. In group C, the fat level from the liver samples was significantly ($P\leq 0.05$)

higher than in the liver samples from the experimental groups.

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