Impact of Dietary Oleaginous Seeds on Health Lipid Indices and Fatty Acids Profile on Broiler's Meat

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Abstract
Over the past few years, there has been a growing preference among consumers for broiler meat, primarily due to its rich protein content and its suitability in the obtained of functional foods. In this context, a 42-day feeding trial was conducted on 192, day-old Ross 308 chicks obtained from a local hatchery and kept in an experimental hall on permanent wood shaves litter (16 broilers/m2 capacity). The broilers were randomly divided into 4 groups (C, E1, E2, and E3), each group consisted of four replicates (12 chick/replicate). All the groups received the same basal diet for ten days. Broilers' were fed as follows: C group (basal diet), 6% flaxseed (E1), 10% camelina seeds (E2) and 15% hemp seeds (E3). At the end of the study, 32 broilers (8 chick/group) were slaughtered and thigh samples were collected for quality analyses (fatty acids profile, cholesterol content and health lipid indices). Prior to slaughter, the blood samples collected to determine the serum lipid parameters.The concentration of polyunsaturated fatty acids (PUFA), especially the n-3 fatty acid alpha-linolenic acid (ALA, g/100g total FAME) increased significantly (P=0.0001) in thigh samples for all experimental groups compared to C group, but the n3-PUFA concentration decreased inversely proportional to the rate inclusion of raw materials rich in PUFA. The healthy lipid indices, especially the atherogenicity (IA) and thrombogenicity (IT) indices, registered lower values ($P \leq 0.05$) in all experimental groups compared to C group. In conclusion, the supplementation of broilers' diet with oleaginous seeds positively influenced meat characteristics, including the health lipid indices and n-3 fatty acid ALA to promote an efficient conversion of ALA to eicosapentaenoic acid (EPA) and docosahexaenoic fatty acids (DHA), with beneficial implications for human health.

Keywords: broiler, meat, fatty acids, oleaginous seed, health lipid indices

1. Introduction

The dietary inclusion of oilseeds rich in polyunsaturated fatty acids in broiler chicken nutrition represents a functional feeding strategy aimed at improving the fatty acid profile of poultry meat and thereby enhancing its nutritional value for consumers. Flax seeds (*Linum usitatissimum* L.), hemp (*Cannabis sativa* L.), and camelina (*Camelina sativa* (L.) are valuable plant-based sources of lipids with a high content of essential omega-3 fatty acids, demonstrating that broiler diets' supplementation with these valuable ingredients leads to an increase in n-3 fatty acid levels in poultry meat and a reduction of the n-6/n-3 ratio, an important indicator of the nutritional quality of fat [1, 2]. There are numerous advantages associated with including flaxseed in broiler's feed: a substantial increase in the content of alpha-linolenic acid (ALA) and total n-3 polyunsaturated fatty acids (PUFA) in meat, a significantly improved n-6/n-

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3 ratio (⩽ 3:1 in some cases) [3]; AI and TI reduction in meat fat due to a decrease in the fraction of saturated fatty acids (SFA) and an increase in unsaturated fatty acids (UFA) [4]; lower cholesterol levels in poultry meat and a higher content of antioxidant compounds derived from flaxseed, such as lignans (e.g., secoisolariciresinol diglucoside, SDG) [5]. However, the presence of antinutritional factors and oxidative susceptibility in flaxseed requires controlled utilization, as they may affect broiler's performance parameters, oxidative stability of the meat, and carcass composition depending on the inclusion rate and the form of administration [6]. Hemp seeds, rich in high biological value proteins (25–30%), PUFA, and bioactive compounds (tocopherols, polyphenols), can enhance the lipid profile of poultry meat by reducing the n-6/n-3 ratio toward values close to 3:1 and by increasing oxidative stability, without significantly affecting growth performance at moderate inclusion levels (5–10%), although the enrichment in omega-3 fatty acids is more moderate compared to flaxseed supplementation [7, 8, 2]. In broiler nutrition, camelina seeds or camelina cake can partially substitute the conventional ingredients, improving the meat lipid profile without compromising productive performance, provided the inclusion level remains moderate [9]. Due to the intrinsic antioxidant content (tocopherols) of camelina seeds, the PUFA fats deposited in the meat are better protected against oxidation, and the risk of rancidity or oxidative spoilage of n-3 enriched meat is lower compared to flaxseed [1]. Camelina, due to its content of glucosinolates and secondary metabolites (sinapine and phytic acid), can reduce the palatability and digestibility of feed at high inclusion levels (>10%), negatively affecting broiler performance and requiring treatments such as toasting to mitigate antinutritional effects [10].

This study evaluates the effect of oleaginous seeds inclusion (flax, camelina and hemp) as protein alternative to soybean meal substitution in broiler diet enriched PUFA to improve the health lipid indices and fatty acids profile on broiler's meat quality.

2. Materials and methods

Experimental Design and Diets

The experiment complied with Directive 2010/63/EU on the protection of animals used for scientific purposes and the experimental procedures. The feeding trial was conducted in the experimental halls of The National Research-Development Institute of Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to an experimental protocol approved by the Ethics Commission of the institute. A 42-day feeding trial was conducted on 192 one-day-old broiler chicks Ross 308 obtained from a local hatchery. Prior to purchase, all birds were vaccinated at the hatchery, against Marek's disease and infectious bronchitis. The broilers were housed in an experimental hall, reared on a floor system with permanent wood shavings litter, under controlled environmental conditions (average temperature/total growth period 26.03±1.75°C; humidity 65.52±5.27%; ventilation/chick 0.50±0.14%; CO_2 level 639.40±115.38 ppm). The light regimen was adequate to chicks age (23h light/1h darkness). Feed and water were provided *ad libitum*. The broilers were randomly divided into 4 groups (C, E_1, E_2, and E_3), each group consisted of four replicates (12 chick/replicate). For ten days, all groups received the same basal diet. After 10 days, the experimental diet formulations were enriched in PUFA by including 6% flaxseed (E_1), 10% camelina seeds (E_2) and 15% hemp seeds (E_3) compared to C group. Flax, camelina, and hemp seeds were sourced from certified local producers in Romania, authorized for the cultivation of oilseed crops. Prior to their inclusion in the feed formulations, the seeds were ground using a universal hammer mill (MCU 7.5 kW) equipped with a 1 mm mesh screen to break the seed coats and enhance their digestibility. To optimize the nutritional parameters of the diet, the seeds were analyzed to determine their nutritional profile, previously reported by Iliescu et al. [11], and their fatty acid profile (Table 1). The diets were formulated using the Brill feed formulation software, based on the chemical composition of each feed ingredient, while considering the nutritional recommendations for the ROSS 308 hybrid and the maximum dry matter (DM) intake capacity.

Table 1. Fatty acids profile of oleaginous seeds (flax, camelina, hemp)

Fatty acids (g/100g total FAME)	Seeds		
	Flax	Camelina	Hemp
Ether extract*, %	32.17	26.92	30.52
∑SFA	10.06	11.47	14.35
∑MUFA	21.95	30.18	15.05
∑ PUFA, from which:	67.99	57.51	70.29
∑ n-3 PUFA	50.92	34.77	15.13
∑ n-6 PUFA	17.07	22.73	55.16
∑ n-6/∑ n-3	0.34	0.65	3.65

* Iliescu et al. [11]

∑SFA, sum of saturated fatty acids; ∑MUFA, sum of monounsaturated fatty acids; ∑PUFA, sum of polyunsaturated fatty acids; ∑n-3 PUFA omega, sum of omega 6 fatty acids, ∑n-3PUFA, sum of omega 3 fatty acids; ∑ n-6/∑ n-3, ratio of omega 6 fatty acids/omega 3 fatty acids

All diets (Table 2) were formulated to be iso-caloric and iso-nitrogenous. After the compound feed was produced, the bags were properly labeled according to each experimental group and stored in a temperature-controlled room (18–20 °C) throughout the experimental period. Approximately 500 g of feed were sampled from each bag containing the formulated and manufactured compound feed for further chemical analysis.

Blood and Meat Samples Collection and Analysis

Throughout the entire experimental period (up to 42 days of age), the broiler chickens were monitored for feed intake, body weight, and health status. At the end of the study, 32 broilers (8 chick from each group) were slaughtered and thigh samples were collected to determine the meat quality (fatty acids profile, cholesterol content and health lipid indices). Prior to slaughter, the blood samples were aseptically collected from the main brachial vein of birds into EDTA vacutainers tubes containing heparin anticoagulant to determine the serum lipid parameters. The cholesterol and fatty acids content in thigh muscles were determined by gas chromatography methods using a gas chromatograph Perkin Elmer Clarus-500 (Massachusetts, United States). Cholesterol content was determined according to AOAC International standard, 2002 (Cholesterol in multicomponent foods – Gas Chromatographic method. Assoc. Off. Anal. Chem. Arlington, VA). The fatty acids were determined using a standard method ISO 5508: 2002 (for preparation of the fatty acids methyl esters, FAME) and standard SR EN ISO 5509:2002 (for the analysis of the methyl esters). All fatty acids content in the samples were identified by matching the retention times with those of their certified reference material (wt. % (varied) F.A.M.E. Mix C4-C24; 18919-1 AMP SUPELCO).

Health Lipid Indices of Meat

Based on the results obtained for fatty acids, the meat quality indices for thigh muscles were calculated and classified according to the qualitative indices (SFA/UFA, saturated fatty acid/unsaturated fatty acid; PUFA/MUFA, polyunsaturated fatty acid/monounsaturated fatty acid; PUFA/SFA, polyunsaturated fatty acid/saturated fatty acid; n-6/n-3 ratio, omega 6 fatty acids/omega 3 fatty acids ratio [12]; LA/ALA, linoleic acid/alpha-linolenic acid ratio [13]; EPA+DHA, sum of eicosapentaenoic acid and docosahexaenoic acids [14, 15]; UI, unsaturation index [16]), and nutritional attributes (PI, peroxidability indices [17]; NVI, nutrition value index [18]; IA, index of atherogenicity [19]; IT, index of thrombogenicity [19]; HH ratio, hypocholesterolemic/hypercholesterolemic [20]; HPI, health-promoting index [21]).

Statistical Analysis

One-way analysis of variance (ANOVA), using the general linear model (GLM) procedures of SAS (Statistical Analysis System, Minitab version 17, SAS Institute Inc., Cary, NC, USA) was carried out to determine the effects of using oleaginous seeds enriched in PUFA to improve the meat quality (health lipid indices, fatty acids profile, cholesterol content) and serum lipid parameters. Significance between individual means was identified using the Tukey's multiple range test. Mean differences were considered significant at $P < 0.05$.

Table 2. Diet formulation and estimated chemical composition of experimental diet

Ingredients	*Starter (1-10 days)*	*Grower (11 – 22 days)*				*Finisher (23 - 42 days)*			
		C	E1	E2	E3	C	E1	E2	E3
Corn	33.86	27.00	40.00	40.00	38.00	52.37	50.89	47.36	43.73
Wheat	20.00	30.00	14.80	15.45	11.02	10.00	10.00	10.00	10.00
Soybean meal	37.98	33.71	31.86	26.65	27.96	28.30	24.70	23.59	22.77
L-lysine	0.25	0.20	0.26	0.40	0.38	0.22	0.33	0.36	0.40
DL-methionine	0.35	0.31	0.33	0.28	0.36	0.28	0.32	0.34	0.35
L-threonine	0.11	0.09	0.14	0.23	0.23	0.07	0.11	0.13	0.14
Calcium carbonate	1.28	1.24	0.72	0.73	0.54	1.28	1.29	1.29	1.29
Monocalcium phosphate	1.24	1.04	1.22	1.20	1.27	1.17	1.22	1.24	1.26
Salt	0.4	0.40	0.40	0.40	0.40	0.37	0.37	0.37	0.38
Soybean oil	3.47	4.95	3.03	3.42	3.60	4.88	3.53	4.08	3.44
Premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Colina	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase (2000 FYT)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Flaxseeed seeds	-	-	*6.00*	-	-	-	*6.00*	-	-
Camelina seeds	-	-	-	*10.00*	-	-	-	*10.00*	-
Hemp seeds	-	-	-	-	*15.00*	-	-	-	*15.00*
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Theoretical calculation									
Metabolizable energy, kcal/kg	3000.00	3124.11	3107.94	3100.45	3105.85	3200.79	3200.68	3200.66	3200.57
Crude protein, %	23.00	21.50	21.50	21.50	21.50	19.00	19.00	19.00	19.00
Crude fat, %	5.51	1.71	7.01	6.68	9.03	7.27	7.79	8.95	10.06
Crud fibre, %	3.49	3.38	3.85	4.14	4.70	3.10	3.99	4.22	7.12
Fatty acids profile									
∑SFA	13.07	11.91	11.74	12.07	12.67	11.17	10.98	11.16	12.49
∑MUFA	30.64	31.41	28.29	28.26	23.79	31.78	30.13	30.00	23.46
∑ PUFA	56.16	56.68	59.97	59.67	63.53	56.52	58.75	58.81	64.01
∑ n-3 PUFA	1.84	1.28	16.34	8.70	7.96	1.39	11.36	5.28	8.12
∑ n-6 PUFA	54.31	55.40	43.63	50.97	55.24	55.13	47.39	53.53	55.90
∑ n-6/∑ n-3	29.45	43.32	2.67	5.86	6.94	39.53	4.17	10.14	6.89

where: [1]C – Control diet; E_1 – diet C supplemented with flaxseed (6 %); E_2 – diet C supplemented with camelina seed (10 %); E_3 – diet C supplemented with hemp seed (15 %);

*1kg premix IBNA (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

3. Results and discussion

The fatty acids profile evaluated for the three types of oleaginous seeds (flax, camelina, hemp) it is essential because these seeds are rich sources of essential fatty acids, particularly omega-3 and omega-6, and carry important nutritional benefits and considerations for both animal and human diets. Following the assessment of fatty acid profiles in our study (Table 1), it was observed that hemp seeds exhibited the highest saturated fatty acid (SFA) content at 14.35%, while camelina and flax seeds recorded lower levels at 11.47% and 10.06%, respectively. The omega-3 fatty acids content is more abundant in flax and camelina seeds compared to hemps seeds, while the flax and camelina seeds have lower Σ n-6/ Σ n-3 ratios compared to hemp seed value. The ratio Σ n-6/ Σ n-3 is considered a key indicator of the health benefits of these seeds. Similar with our results, other authors [22] noticed that camelina seeds, registered higher monounsaturated fatty acids (MUFA), which contribute to a better oxidative stability. Other studies showed that adding oleaginous seeds or cakes to poultry diets can triple the omega-3 levels in chicken meat and reduce the n-6/n-3 ratio in the muscle by 2-3-fold [23].

Table 3. Effects of oleaginous seeds dietary inclusion on serum lipid parameters

Parameter	C	E1	E2	E3	SEM	*p*-value
Glucose (mg/dL)	181.00^{a}	180.21^{a}	178.13^{ab}	152.29^{b}	6.85	0.021
Cholesterol (mg/dL)	88.61^{a}	85.51^{b}	82.75^{b}	79.89^{b}	8.68	0.047
Triglyceride (mg/dL)	62.72^{ab}	67.05^{a}	61.31^{ab}	56.32^{b}	3.40	0.022

where: C – Control diet; E1 – diet C supplemented with flaxseed (6 %); E2 – diet C supplemented with camelina seed (10 %); E3 – diet C supplemented with hemp seed (15 %); n = number of samples per group (8); SEM, standard error of the mean; $^{a-b}$Mean values within a row having different superscripts are significantly different at $p \leq 0.05$

The effects of oleaginous seeds dietary inclusion on serum lipid parameters are presented within table 3. There was a statistically significant difference for all parameters. The lowest level (p = 0.021) of glycemia was observed in the E3 group compared to C and E1 groups, with no significant differences compared to E2 group. Total cholesterol levels showed a significantly (p = 0.047) decreasing for all experimental groups compared to C group. A similar decreasing trend was noted for triglycerides, but only the diferences (p = 0.022) were registered between E3 and E1 groups. Many studies recorded an increased glycemia under high levels of flaxseed (up to 26%) dietary inclusion compared to a control diet, when replacing soybean meal in broilers [24]. On the other hand, a recent study [25] using 5–10% camelina seed or meal in broilers found no effect on plasma glucose at 42 days, consistent with our flaxseed and camelina groups. In our study the significant decreasing of glucose concentration in E3 serum can be attributed to hemp seed rich content in omega-6 rather than omega-3. Omega-6 fatty acids have been reported to support insulin function and glucose uptake in some contexts, potentially preventing hyperglycemia [26]. Regarding serum cholesterol concentration, similar results to ours were obtained by other researchers [27] who reported a significant decrease in total serum cholesterol in broilers concomitantly with increased dietary supplementation with flaxseed oil or replacement of sunflower oil with flaxseed oil in broiler diets [28]. A similar effect is also exerted by soluble fiber from flaxseeds, which can reduce cholesterol absorption, thus lowering serum cholesterol levels [29]. Other studies show that dietary camelina seeds or meal (5–10%) significantly lower total cholesterol and LDL cholesterol in broilers, as a rapid response to dietary PUFAs [25]. Also, hemp seed, rich in omega-6 and gamma-linolenic acids, has been found to reduce cholesterol levels in poultry. Mahmoudi et al. [30] found that broilers fed 0-7.5% hemp seed reduced total cholesterol and beneficial HDL cholesterol, with the highest hemp seed group having the lowest serum cholesterol. Vispute et al. [31] adding hemp seeds (25, 50, 75 g/kg) to broiler diets obtained 75% total serum cholesterol reduction and lipoprotein density. On other hand, other authors [32] found that broilers fed 6% hemp seed oil showed lower total cholesterol.

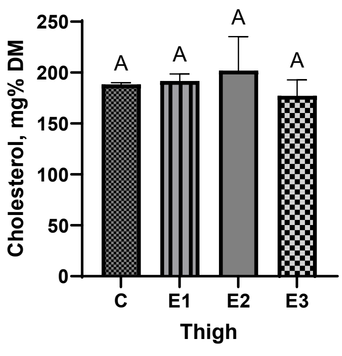

Figure 1. Effects of oleaginous seeds dietary inclusion on cholesterol content in thigh

The effects of including oleaginous seeds in the broiler diet on cholesterol concentration in the thigh samples are illustrated in figure 1. There are no significant differences (p > 0.05) noticed between all groups for thigh samples.

Table 4 shows the effects of including oleaginous seeds in the broiler diet on the profile of fatty acids in meat. In the thigh meat, the C and E3 groups exhibited the highest ΣSFA content, significantly exceeding the levels observed in E1 and E2 groups. Significant differences were noted for individual SFA (C14:0, C16:0, and C18:0), where C and E3 groups were significantly higher (p = 0.016) compared to E1 (C12:0); E3 group was significantly higher (p = 0.002) compared to E1 and E2 groups (C14:0); E1, E2, and E3 groups were significantly lower (p = 0.001) compared to C group (C16:0); E1 and E3 groups were significantly higher (p = 0.029) compared to E2 (C18:0). The ΣMUFA content was also significantly higher (P = 0.001) in the C group compared to all experimental groups, with significant effects observed for C14:1 (p = 0.0001), C16:1 (p = 0.001), and C18:1 (p = 0.002), where the C group had the highest values. Conversely, ΣPUFA values were significantly higher in E1 and E2 groups compared to C and E3. Among the n-6 PUFA, C18:2n6 was significantly higher in E2 group, while C20:4n6 levels were higher in E1 and E2 compared to C and E3 groups. The omega-3 fatty acids C18:3n3, C20:5n3 and C22:6n3 were significantly increased in E1 and E3 groups, indicating an improved beneficial fatty acid profile in these diets. Similar results with our study were obtained by Mridula et al., [33] when using 0%, 2.5%, 5.0%, 7.5% and 10% flaxseed dietary inclusion in broiler diets and noticed a significantly increased the ALA content in meat and Zineb et al. [34] when fed 5% ground flaxseed and obtained significant higher levels of LA in thigh meat compared to C (6% vs. 2.68%). Feeding with 10% flaxseed meal for at least 3 weeks significantly improved the meat fatty acid profile and reduced the fat and cholesterol [35].

Table 4. Effects of oleaginous seeds dietary inclusion on tight fatty acids profile

Thigh fatty acids profile	C	E1	E2	E3	SEM	*p*-value
ΣSFA	27.70^{a}	24.09^{b}	23.59^{b}	27.57^{a}	0.710	0.0001
C12:0	0.16^{a}	0.07^{b}	0.10^{ab}	0.16^{a}	0.020	0.016
C14:0	0.52^{ab}	0.44^{b}	0.48^{b}	0.61^{a}	0.027	0.002
C16:0	18.47^{a}	15.53^{b}	15.85^{b}	16.32^{b}	0.478	0.001
C18:0	6.41^{ab}	6.60^{a}	5.90^{b}	6.60^{a}	0.170	0.029
ΣMUFA	37.21^{a}	33.48^{b}	33.60^{b}	32.08^{b}	0.741	0.001
C14:1	0.08^{a}	0.05^{b}	0.05^{b}	0.03^{b}	0.004	0.0001
C16:1	2.93^{a}	2.13^{b}	2.44^{ab}	2.00^{b}	0.148	0.001
C18:1	33.26^{a}	30.65^{b}	30.58^{b}	29.09^{b}	0.652	0.002
ΣPUFA	34.26^{b}	41.74^{a}	42.10^{a}	39.63^{ab}	1.380	0.002
Σn-6	32.09	31.91	34.43	34.27	1.130	0.260
C18:2n6	29.17	29.10	29.95	31.82	0.933	0.173
C20:2n6	0.31^{c}	0.27^{c}	1.82^{a}	0.65^{b}	0.078	0.0001
C20:4n6	1.60^{a}	1.46^{a}	1.63^{a}	0.77^{b}	0.133	0.0001
Σn-3	1.94^{d}	9.64^{a}	7.48^{b}	5.00^{c}	0.251	0.0001
C18:3n3	0.65^{d}	7.57^{a}	6.16^{b}	2.99^{c}	0.216	0.0001
C20:5n3	0.27	0.25	0.22	0.24	0.035	0.800
C22:5n3	0.06^{c}	0.59^{a}	0.36^{b}	0.56^{a}	0.047	0.0001
C22:6n3	0.18^{b}	0.48^{a}	0.28^{ab}	0.40^{ab}	0.061	0.013
n-6/n-3	16.58^{a}	3.31^{d}	4.61^{c}	6.96^{b}	0.218	0.0001
SFA / UFA	0.39^{a}	0.32^{b}	0.31^{b}	0.39^{a}	0.014	0.001
PUFA/MUFA	0.92^{b}	1.25^{a}	1.26^{a}	1.26^{a}	0.069	0.005
PUFA/ SFA	1.24^{b}	1.74^{a}	1.79^{a}	1.47^{ab}	0.089	0.001

where: C – Control diet; E1 – diet C supplemented with flaxseed (6 %); E2 – diet C supplemented with camelina seed (10 %); E3 – diet C supplemented with hemp seed (15 %); n = number of samples per group (8); SEM, standard error of the mean; $^{a-c}$Mean values within a rown having different superscripts are significantly different at p ≤ 0.05; Abbreviations: ΣSFA, sum of saturated fatty acids; ∑MUFA, sum of monounsaturated fatty acids; ΣPUFA, sum of polyunsaturated fatty acids; n-6/n-3, ratio of omega 6 fatty acids/omega 3 fatty acids; SFA/UFA, saturated fatty acid/unsaturated fatty acid; PUFA/MUFA, polyunsaturated fatty acid/monounsaturated fatty acid; PUFA/SFA, polyunsaturated fatty acid/saturated fatty acid;

Also, a high content of PUFA was obtained by Turcu et al., [36] using a level of 4% flax meal combined with 3% grape oil, but other researchers [37, 38] when using up to 20% flaxseeds dietary inclusion in broiler noticed a PUFA n-6 significant increase. Also, using hemp seeds inclusion in broiler diets, Skřivan et al., [39] obtained the lowest n-6/n-3 PUFA ratio ($P < 0.001$) when testing 40 g/kg hempseed and 60 g/kg extruded flaxseed dietary combination in cockerel's broiler diets. Tufarelli et al. [2] incorporated 0, 5, or 10% hemp seed dietary cakes, resulting in increased n-3 content, and a reduced n-6/n-3 ratio in thigh and breast samples. Similarly, He et al. [40] found that 20% hemp seed cake dietary inclusion in yellow chickens (50 days old) over 9 weeks significantly enhanced n-3 PUFA concentration. Sopian et al. [41] following a meta-analysis study on hempseed products in broiler production found that hemp product increased ω-3 FA and ω-6 FA but reduced body weight and feed intake, without negatively influencing feed conversion ratio. Concerning camelina studies, Ciurescu et al., [42] obtained significant increases in omega n-3 fatty acids profile in the breast samples when using oil (2.5%) or seeds (5 % and 10%). Orczewska-Dudek et al., [1] included 40 and 100 g/kg of camelina oil and cakes, and noted that MUFA content decreased, while PUFA content increased significantly ($P < 0.05$), especially the α-linolenic acid. Juodka et al. [43] found that feeding broilers camelina products (cake, seeds, oil) increased total n-3 PUFA content in muscles and liver samples by 1.5 to 3.9 times while lowering the n-6/n-3 PUFA ratio, resulting in a healthier product for human nutrition. Dalle Zotte et al. [44] observed similar results using 15% camelina cake of different varieties in breast samples.

Table 5. Effects of oleaginous seeds dietary inclusion on health indices

Indices	C	E1	E2	E3	SEM	*p*-value
LA/ ALA	45.09^{a}	3.85^{c}	4.87^{c}	11.23^{b}	0.764	0.0001
EPA + DHA	1.51^{a}	0.60^{b}	0.87^{ab}	0.77^{ab}	0.198	0.022
UI	113.62^{c}	133.82^{a}	131.45^{ab}	122.31^{bc}	2.64	0.0001
PI	46.48^{c}	64.54^{a}	60.46^{ab}	55.75^{b}	2.20	0.0001
NVI	2.15^{c}	2.40^{a}	2.31^{ab}	2.19^{bc}	0.038	0.001
IA	0.29^{a}	0.23^{b}	0.24^{b}	0.27^{ab}	0.010	0.002
IT	0.63^{a}	0.37^{c}	0.39^{c}	0.49^{b}	0.020	0.0001
HH ratio	3.54^{b}	4.52^{a}	4.44^{a}	4.08^{ab}	0.164	0.001
HPI	3.46^{b}	4.34^{a}	4.26^{a}	3.85^{ab}	0.153	0.002

where: C – Control diet; E1 – diet C supplemented with flaxseed (6 %); E2 – diet C supplemented with camelina seed (10 %); E3 – diet C supplemented with hemp seed (15 %); n = number of samples per group (8); SEM, standard error of the mean; $^{a-c}$Mean values within a row having different superscripts are significantly different at $p \leq 0.05$; *abbreviations*: LA/ALA, linoleic acid/α-linolenic acid ratio; EPA+DHA, sum of eicosapentaenoic acid and docosahexaenoic acid; UI, unsaturation index; PI, peroxidability indices; NVI, nutrition value index; IA, index of atherogenicity; IT, index of thrombogenicity; HH ratio, hypocholesterolemic/hypercholesterolemic; HPI, health-promoting index)

Table 5 presents the effects of oleaginous seeds dietary inclusion on health indices with direct (PUFA/SFA, EPA + DHA%, NVI, HH ratio, HPI) and indirect (n-6/n-3 ratio, LA/ALA, IA, IT) impact on human health. SFA and PUFA register an important part in human nutrition as prevention of atherosclerosis and coronary thrombosis, due to their effects on serum cholesterol and low-density lipoprotein-cholesterol levels, including low values for AI and IT indices in a healthy diet [45]. The lowest statistically significant ratio value n-6/n-3 for thigh was observed on E1 group compared to all other experimental groups. In thigh meat samples, the diet E1 group, significantly improved the fatty acid profile. The n-6/n-3 ratio decreased significantly ($P \leq 0.0001$), alongside with a decreasing of SFA/UFA and LA/ALA ratios, indicating a healthier balance of fatty acids. The E1 and E2 groups also enhanced PUFA/MUFA and PUFA/SFA ratios and increased omega-3s (EPA+DHA). Both, UI and PI increased significantly, reflecting a higher degree of unsaturation. Importantly, the IA and IT indices decreased, while HPI, and HH ratio improved. Similar results to our study were presented by Albergamo et al. [4] tested 3–4% extruded flax meal + antioxidants, obtaining a lower n-6/n-3 ratio, decreased AI and TI indices, reflecting an increased MUFA/PUFA and decreased SFA. On the other hand, high values of peroxidability index (PI), ratio of hypocholesterolemic and hypercholesterolemic (HH) and HPI, health-promoting index (FPI) indices are recommended as well as beneficial healthy promoters for a healthy diet [45]. In our study, the 10% camelina group showed a significant ALA concentration increases (EPA+DHA) and a decreasing of n-6/n-3 ratio which determine a higher h/H and a lower AI/TI ratio. It is important to take into account the dietary inclusion of camelina seed level and form: whole camelina seed has more antinutrients than oil or meal, possibly tempering the effect [9]. On the other hand, Tufarelli et al. [2] reported that feeding slow-growing broilers with 5–10% hemp seed cake reduced the n-6/n-3 ratio, increased EPA concentration increased and DHA, therefore total EPA+DHA almost doubled. The AI and TI indices significantly declined using 5% hemp cake. Our 15% hemp seed dietary inclusion registered similar results: the n-6/n-3 ratio decreased significantly, and EPA+DHA and total PUFA increased significantly in thigh samples.

Table 6. Person correlation between the fatty acids profile and healthy indices in broiler thigh

Item	LA/ALA	EPA + DHA	UI	PI	NVI	IA	IT	HH ratio	HPI
ΣSFA	.585**		-.924**	-.841**	-.759**	.943**	.871**	-.899**	-.928**
ΣMUFA	.705**		-.758**	-.808**	-.440*	.740**	.712**	-.801**	-.756**
ΣPUFA	-.741**		.962**	.945**	.687**	-.960**	-.906**	.972**	.962**
Σn-6	-.472*	-.693**		.504*	.528**				
C18:2n6			.583**	.547**		-.664**	-.433*	.676**	.663**
C20:2n6			.462*	.455*		-.548**		.573**	.548**
C20:4n6	-.416*								
Σn-3	-.887**	-.470*	.885**	.892**	.772**	-.804**	-.948**	.810**	.808**
C18:3n3	-.872**	-.418*	.873**	.855**	.755**	-.796**	-.943**	.792**	.796**
C20:5n3		.775**							
C22:6n3	-.761**	-.742**	.481*	.600**	.434*		-.574**	.464*	.411*
n-6/n-3	.986**	.562**	-.775**	-.798**	-.618**	.685**	.896**	-.724**	-.693**
SFA / UFA	.572**		-.920**	-.837**	-.756**	.941**	.866**	-.895**	-.924**
PUFA/MUFA	-.707**		.885**	.897**	.573**	-.881**	-.818**	.916**	.891**
PUFA/ SFA	-.709**		.978**	.932**	.748**	-.981**	-.923**	.974**	.980**

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

Table 6 presents the correlation between the fatty acids profile and healthy indices in broiler thigh samples. Broiler thigh meat samples from experimental groups showed an increase in PUFA concentration as a result of the dietary inclusion of oleaginous seeds. C18:3n3 was found to have a statistically significant ($P < 0.01$) positive correlation with the health indices UI ($r = 0.873$), PI ($r = 0.855$). NVI ($r = 0.755$), HH ratio ($r = 0.792$), and ($r = 0.796$). Additionally, a statistically significant ($P < 0.01$) positive correlation was found, between the health indices LA/ALA ($r = 0.986$), IA ($r = 0.685$), and IT ($r = 0.896$) and n-6/n-3.

4. Conclusions

Oleaginous seed supplementation (flax, camelina, hemp) in broilers′ diet improved fatty acid ratios, n-3 fatty acid ALA to facilitate an effective conversion of ALA to EPA and DHA, and improved meat characteristics, such as the health lipid indices, with positive implications for human health.

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