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Effects of dietary hemp seeds (*Cannabis sativa* L.) on breast and thigh meat quality attributes, fatty acid profiles, and health lipid indices in laying hens

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Abstract

This study evaluated the effects of graded dietary inclusion of Beldiya ecotype hempseed (HS) on the meat quality of laying hens. A total of 120 Lohmann Brown hens (22 weeks old) were randomly assigned to four dietary treatments containing 0%, 10%, 20%, and 30% HS, with six replicates of five birds each, over a 12-week feeding trial. Meat physicochemical traits (pH, water-holding capacity, and color), intramuscular fat content, mineral composition, fatty acid profiles, and lipid health indices were analyzed in the breast and thigh muscles. HS inclusion had no significant effect ($P>0.05$) on pH, water-holding capacity, or muscle lightness (L*). However, significant changes ($P\leq0.05$) were observed in muscle redness (a*) and yellowness (b*). Intramuscular fat decreased significantly ($P\leq0.05$) in the thigh muscle, whereas minor alterations were detected in certain trace minerals. More importantly, HS supplementation improved the fatty acid composition and lipid health indices. Saturated and monounsaturated fatty acids decreased ($P\leq0.05$), whereas polyunsaturated fatty acids (PUFAs), particularly the n-3 and n-6 series, increased ($P\leq0.05$). These changes were accompanied by beneficial shifts in health-related lipid indices, including reduced n-6/n-3 PUFA and LA/ALA ratios, as well as lower atherogenic (AI) and thrombogenic (TI) indices ($P\leq0.05$). Conversely, the PUFA/SFA and hypocholesterolemic/hypercholesterolemic (h/H) ratios were significantly elevated ($P\leq0.05$), indicating improved nutritional quality. In conclusion, dietary inclusion of HS enhanced the fatty acid profile and health indices of hen meat without compromising significant physicochemical properties, highlighting its potential as a functional feed ingredient.

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Introduction

Poultry diets generally consist of preparations based on maize and soya meal, which provide energy and protein. However, the need for substitute feed materials is increasingly of interest due to several factors: rising prices of conventional feed ingredients, insufficient availability, and the presence of antinutritional elements, which are likely to affect animal digestibility and health (1,2) adversely. Given this context, identifying profitable, nutritionally suitable, and locally accessible feed ingredients should be a key priority to promote the durability and resilience of the feed industry (3). Agricultural coproducts have attracted increasing interest as sustainable alternatives in animal nutrition, with the potential to reduce feed costs while improving the quality and environmental sustainability of animal products.

Coproducts, particularly plant-derived residues, can serve as valuable nutritional ingredients in animal feed (4). In recent years, emerging agro-industrial crops, such as hemp, have proven useful as feed raw materials due to their high nutritional and functional value (5,6). Hemp (*Cannabis sativa* L.), an annual, dioecious plant, is a sustainable and economically profitable crop with a wide range of food and feed applications. In addition, it is ideally suited to Mediterranean regions with low rainfall, making it an excellent alternative to oilseed crops such as soy and rapeseed (7). These features, together with its nutritious profile, make hemp a viable agro-industrial crop for sustainable poultry farming systems (5,6,8,9). Hemp seed, the edible fruit of the Cannabis plant, is considered a byproduct with the highest potential for use as animal feed based on its high crude protein content, well-balanced nutritional profile, and digestibility. On average, whole hemp seeds contain 20–30% crude protein, including all the essential amino acids in significant amounts. They are free from common antinutritional factors, such as trypsin inhibitors and oligosaccharides, which are often associated with soya (10,11). These seeds also provide approximately 30% oil, over 90% of which is polyunsaturated fatty acids (PUFAs), as well as approximately 25–34% carbohydrate, mainly in the form of dietary fiber (7,11,12). Other components include micronutrients such as phosphorus, potassium, magnesium, sulphur, calcium, iron, and zinc (7,10). Recent investigations into the incorporation of hemp seeds and their derivatives into animal diets have revealed promising outcomes, particularly with respect to lipid metabolism in poultry. In laying hens, the most frequently examined forms include whole hemp seeds and hemp oil, although some research has also assessed the use of hempseed cake or defatted meal (13). Overall, the literature suggests that the dietary inclusion of these hemp-based products does not markedly influence laying performance (14,15); however, slight improvements in parameters such as egg weight and eggshell thickness have occasionally been reported (16–19). In addition to production performance, lipid-related outcomes are auspicious. Supplementation with hemp seed products has been shown to enhance the fatty acid profile of eggs, particularly by increasing the levels of PUFAs, including those in the omega-3 and omega-6 series, which are known to offer significant health benefits (16,17,20). These improvements occur without adverse effects on sensory characteristics or the presence of undesirable residues (13). In fact, studies evaluating the potential transfer of cannabinoids into poultry products following hemp supplementation have consistently shown an absence of detectable residues in both eggs and tissues, confirming their safety for human consumption (21). Since the legalization of cannabis cultivation in Morocco under Laws 13-21 of March 2021, interest in the use of hemp-derived products in animal nutrition has increased considerably. However, knowledge remains limited regarding the optimal inclusion levels and effects of hemp seeds (HSs) in poultry diets, particularly for laying hens.

Most previous studies have focused on egg production and quality, whereas data on the effects of HS supplementation on meat characteristics remain limited. The present study aims to fill this gap by evaluating the effects of the gradual dietary incorporation of hemp seeds from the Moroccan Beldiya ecotype on meat quality characteristics and fatty acid composition in laying hens. We hypothesize that dietary HS supplementation will not hurt growth performance or the physicochemical parameters of meat but will improve the fatty acid profile and lipid indices related to poultry meat health**.**

Materials and methods

Ethics statement

This study was conducted in accordance with established ethical guidelines for animal research and was approved by the Royal Institute for Livestock Research in Kenitra, Morocco. The experimental procedures complied with Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and Council Directive 2007/43/EC, which outlines the minimum welfare standards for laying hens. Furthermore, the study adhered to the ARRIVE guidelines (22), ensuring transparency and reproducibility in animal research. All husbandry practices, including housing, feeding, and general animal care, were designed to minimize stress and discomfort throughout the experiment.

Plant material

Hemp seeds (*Cannabis sativa* L.) from the local Moroccan ecotype known as “Beldiya” were sourced from smallholder cannabis cultivators in the Jebha region of northern Morocco (latitude: 35°05'N; longitude: 4°32'W; altitude: 520–650 m) during the 2023 harvest season. Following collection, the seeds were meticulously cleaned to remove dust, plant debris, and other impurities. To preserve nutritional integrity and prevent oxidation, the samples were stored until further analysis or incorporation into the experimental diets. The proximate composition and nutritional profile of the hemp seeds used in this study are detailed in Table 1.

Diet formulation

An isocaloric and isonitrogenous diet was formulated based on the recommendations outlined in the Nutrient Requirements of Poultry by the National Research Council (23) and in accordance with the guidelines for the Lohmann Brown strain. The basal diet consisted primarily of corn and soybean meal, with hemp seeds incorporated at 0%, 10%, 20%, and 30% of the total feed weight (Table 2). All experimental diets were prepared to ensure homogeneity and minimize component separation during feeding. The feed samples were collected directly from the feed mill during the manufacturing process for subsequent laboratory analysis. Proximate composition analyses were conducted to determine the contents of moisture, ash, crude protein, crude fiber, ether extract, calcium, and total and available phosphorus. These analyses followed the standard procedures recommended by the AOAC as described by Rbah *et al.* (24).

Slaughtering and Sampling

At 34 weeks of age, all the hens were fasted for 12 hours (overnight), during which time water remained available ad libitum. After the fasting period, three birds from each pen, representing the average body weight per replicate, were carefully selected and transferred to the slaughter facility.

Slaughter was performed according to Islamic halal procedures, as detailed in our previously published article (25). After bleeding, the birds were immersed in hot water to facilitate plumage removal and tissue access. Immediately thereafter, the breast and thigh muscles were carefully removed. The collected muscle samples were labeled and immediately stored at -20°C until further physical, chemical, and biochemical analyses were performed.

Table 1: Nutritional profile of nonindustrial Beldiya hemp seeds used in broiler diets, including proximate analysis, primary fatty acids, and some amino acids, used as feed in this study

		Hemp seed (g/kg)
Moisture		50.92±7.2
Crude protein		275.4±8.8
Crude fat		320.81±4.3
Crude fiber		248.4±9.0
Ash		42.9±0.3
Fatty acids (%)	Linoleic acid	51.12±0.02
	Linolenic acid	16.44±0.03
	γ- Linolenic acid	0.60±0.01
	n3 PUFAs	16.53±0.04
	n6 PUFAs/n3 PUFAs	3.09±0.02
Amino acid (g per kg)	L-Lysine	10.5±0.7
	DL-Methionine	6.4±0.2
	L-Threonine	7.6±0.4
Tocopherol (mg per kg of oil)	γ-Tocopherol	409.72±30.02
	α-Tocopherol	18.89±1.02

Table 2: Nutritional profile and chemical composition of diets formulated with and without hemp seeds (HS) for laying hens.

Item	Diet			
	Control	10HS	20HS	30HS
Ingredients (g. kg-1)				
Corn	575	545	470	334
Hempseed	0	100	200	300
Soybean meal	200	165	144	129
Sunflower meal	40	30	20	60
Soy oil	35	10	00	00
Limestone	90	90	98	99
Di calcium phosphate	19	19	22	24
Sodium chloride	4	4	6	7
DL-methionine	20	20	22	23
L-Lysine HCl	12	12	14	15
*Vitamin premix	5	5	5	5
Composition (Calculated unless noted)				
AME (Kcal/kg) **	2869	2868	2871	2875
Moisture (%)	12.4	12.3	12.7	12.9
Crude fat (%)	5.96	6.67	8.26	11.02
Crude protein (%)	18.55	18.57	19.03	19.24
Calcium (g/kg)	39	39	40	40
Phosphorus available (g/kg)	3.33	3.4	3.45	3.50
Sodium (g/kg)	1.55	1.57	1.65	1.7
α-Linolenic acid (%)	2.49	7.45	12.09	17.37
Lysine (g/kg)	8.35	8.64	8.91	8.94
Methionine (g/kg)	5.58	5.59	5.92	6.14

*Vitamin premix: The nutrient composition of the premix per gram includes 11,000 IU of retinol, 3,000 IU of cholecalciferol, 0.3 mg of menadione, 0.22 mg of thiamine, 0.65 mg of riboflavin, 0.45 mg of pyridoxine, two µg of cobalamin, 1 mg of pantothenic acid, 2.5 mg of niacin, 0.4 mg of folic acid, and 15 IU of tocopherol. **AME available metabolizable energy, calculated based on the NRC (23).

Physical analysis of meat quality

The physical traits of the breast and thigh muscles were evaluated postmortem to assess the effects of adding HSs to the diet of laying hens. The water-holding capacity (WHC) was determined gravimetrically according to the procedure described by Hashizawa *et al.* (26).

pH measurements were taken 24 hours after slaughter via a portable pH meter (HI99163, Hanna Instruments, Canada). The pH probe was calibrated with standard buffer solutions at pH 4.0 and 7.0 before analysis. Measurements were recorded at least three times per muscle sample to ensure accuracy. The color parameters brightness (L*), redness (a*), and yellowness (b*) were measured via a Minolta Konica CR-410 colorimeter. Measurements were taken on the surface of each sample at least three points after the meat had reached ambient temperature for 30 minutes.

In addition, hue angle (H)* and chroma (C)* values were calculated via the following standard formulas [I] and [II], as described by Rbah *et al.* (24): H*=tan-1 (b*/a*)×57.29 [I], and C*=√(a2+b2) [II].

Ash and mineral compositions

Mineral profiling of the ash content of both the muscle breast and thigh was performed as described by Rbah *et al.* (7) via a Shimadzu X-ray fluorescence energy dispersion spectroscopy instrument (EDX-7000). The instrument parameters were configured for the elements ranging from Al to U at 50 kV and 144 µA. The Na-to-Sc ratio was adjusted to 15 kV and 999 µA. A 5 mm collimator was used for all measurements to ensure consistency.

Lipid extraction, fatty acid composition analysis, and calculation of lipid-related health indices

Lipid extraction from dietary samples and from thigh and breast muscle tissues was performed using the classical Folch method (27). Briefly, a 2:1:1 (v/v/v) mixture of chloroform, methanol, and sodium chloride solution was added to homogenized samples to extract total lipids. Following centrifugation, the lower lipid-rich phase was carefully collected, evaporated under nitrogen gas, and stored at -20°C until analysis. Fatty acid profiling of both diets and tissues was performed via gas chromatography with flame ionization detection (GC-FID). Lipid extracts were first methylated to fatty acid methyl esters (FAMEs) in accordance with the AOCS Ce 1k-09 method (28). The FAMEs were analyzed via an Agilent GC-6890 system equipped with a BPX70 capillary column (60 m * 0.32 mm i.d., 0.25 µm film thickness; SGE Europe). A 1 µL aliquot of each sample was injected in splitless mode, with helium serving as the carrier gas at a flow rate of 1 mL/min. The GC oven was programmed as follows: (i) initial temperature: 50°C, maintained for a short period; (ii) increased at a rate of 30°C/min to 170°C; (iii) then increased at a rate of 4°C/min to 220°C, then held for 10 minutes. Fatty acids were identified by comparing retention times to those of a standard FAME mixture comprising 37 components (Supelco 37 FAME Mix, Sigma–Aldrich, Bellefonte, PA, USA). The results are expressed as percentages of total identified fatty acids. Based on the fatty acid composition of the breast muscle, several lipid-related health indices were calculated: the linoleic acid/α-linolenic acid ratio (LA/ALA), the n-6/n-3 polyunsaturated fatty acid (PUFA) ratio, the PUFA/saturated fatty acid (SFA) ratio (P/S), the atherogenic index (AI), the thrombogenic index (TI), and the hypocholesterolemic-to-hypercholesterolemic fatty acid ratio (h/H). The equations and methodologies for calculating these indices were based on established references, including (29-32).

Statistical analyses

Statistical analyses were conducted using SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). One-way ANOVA followed by Tukey’s post hoc test was used to assess the effects of hemp seed inclusion on meat quality traits, intramuscular fat content, ash content, mineral composition, fatty acid profile, and lipid-related health indices, with significance set at $P < 0.05$. The results are presented as the means ± standard errors of the means (SEMs). Normality and homogeneity of variance were verified via the Shapiro–Wilk test and Levene’s test, respectively.

Results

Physical quality traits of meat

Table 3 shows the meat quality characteristics of laying hens fed hemp seed-enriched diets. No significant differences in pH values of the breast or thigh muscles were detected between the groups ($P = 0.480$; $P = 0.881$, respectively). Similarly, the dietary treatments did not significantly influence the WHC of either muscle type ($P = 0.158$ for the breast and $P = 0.296$ for the thigh). Furthermore, the brightness values (L*) of the breast and thigh meat did not significantly differ between the groups ($P > 0.05$).

With respect to the analysis of colorimetric parameters such as the red index (a*), a significant increase was observed in the breast muscle at the 10% and 20% hemp seed inclusion levels ($P = 0.003$). In contrast, a significant decrease was noted in the thigh muscle at the 20% inclusion level compared with the control group ($P = 0.019$). For the yellow index (b*), no significant variation was detected in the breast muscle ($P = 0.498$), whereas a marked reduction was recorded in the thigh muscle at the 20% and 30% levels ($P \leq 0.001$). With respect to hue angle, no significant difference was observed in the breast or thigh muscle ($P = 0.311$ and $P = 0.085$, respectively). Finally, chroma decreased significantly in the thigh muscles of all treated groups compared with the control group ($P \leq 0.001$).

Table 3: Effects of dietary HS supplementation on the breast and thigh muscle quality traits of laying hens

Attributes	Diet group				SEM	*p* Value
	Control	10HSs	20HSs	30HSs		
Breast muscle						
pH	5.91	5.93	5.93	5.95	0.02	0.480
Water holding capacity (%)	57.02	57.19	56.93	56.47	0.29	0.158
Color coordinates of the breast muscle						
*L**	54.83	54.05	55.11	54.77	0.63	0.430
*a**	1.17[a]	2.08[b]	2.28[b]	1.51[ab]	0.28	0.013
*b**	4.69	3.88	4.34	4.09	0.53	0.498
Hue angle	75.96	61.19	61.84	69.67	2.57	0.311
Chroma	4.83	4.41	4.91	4.36	0.48	0.571
Thigh muscle						
pH	6.09	6.10	6.10	6.11	0.02	0.881
Water holding capacity (%)	61.37	61.63	60.64	60.41	0.31	0.296
Color coordinates of the breast muscle						
*L**	48.85	50.90	50.05	51.27	0.87	0.092
*a**	8.49[b]	6.77[ab]	5.48[a]	6.39[ab]	0.72	0.019
*b**	3.91[b]	4.24[b]	2.62[a]	2.28[a]	0.29	≤.001
Hue angle	28.89	29.62	38.12	32.57	3.33	0.085
Chroma	9.70[b]	7.83[a]	7.02[a]	7.59[a]	0.29	≤.001

K: Potassium, S: Sulfur, P: Phosphorus, Zn: Zinc, Fe: Iron, Cu: Copper, SEM: Standard error of the mean, p significance. [abcd]Means marked with different superscript letters within each row are significantly different.

Intramuscular fat content, ash content, and mineral composition

Table 4 shows the results for the intramuscular lipid content, total ash content, and mineral composition of both muscles from laying hens fed hemp seed diets. For intramuscular fat, no significant difference was observed in the breast muscle between treatments (P = 0.252). In contrast, a significant reduction was observed in the thigh muscle at the 20% and 30% inclusion levels compared with the control ($P \leq 0.001$). With respect to total ash content, the results indicate no significant differences between the groups in either muscle ($P = 0.315$ for the breast and $P = 0.057$ for the thigh). Similarly, the macronutrients analyzed (potassium, sulfur, and phosphorus) showed no significant treatment-related variation across muscle types ($P > 0.05$). For trace elements, a substantial decrease in iron content was observed in the groups receiving 30% hemp seeds, both in the breast ($P = 0.036$) and in the thigh ($P = 0.042$). On the other hand, for copper, a significant decrease was noted only in the breast muscle at 30% inclusion ($P = 0.011$), whereas the thigh showed no difference ($P > 0.05$). Finally, zinc levels remained stable across treatments in both muscle types ($P > 0.05$).

Fatty acid profile and related health lipid indices in the breast and thigh muscles of laying hens fed hemp seed-based diets.

Tables 5 and 6, alongside Figures 1 and 2, illustrate the impact of hemp seed supplementation on the fatty acid profile of thigh and breast muscles, as well as on lipid health indices. A significant reduction ($P < 0.001$) in total saturated fatty acid (SFA) content, particularly myristic, palmitic, and stearic acids, was observed in both the breast and thigh muscles, with the most pronounced decrease noted at 30% dietary hempseed inclusion. SFAs decreased from 31.29% to 23.93% in breast muscle and from 28.67% to 23.28% in thigh muscle. Similarly, monounsaturated fatty acids (MUFAs), including palmitoleic, vaccenic, and oleic acids, were significantly reduced in both muscle types ($P < 0.001$), with the most significant reduction again occurring at the 30% inclusion level. The MUFA content decreased from 30.49% to 24.66% in the breast muscle and from 33.42% to 25.34% in the thigh muscle.

Conversely, the total polyunsaturated fatty acid (PUFA) levels of both the n-3 and n-6 PUFA families significantly increased with increasing levels of hempseed in the diet. At 30% inclusion, total PUFAs reached 51.27% in the breast muscle (vs. 38.22% in the control) and 51.34% in the thigh muscle (vs. 37.90% in the control). Notably, omega-3 fatty acids increased markedly ($P < 0.001$), especially α-linolenic acid (α-LA), which rose from 1.93% to 6.35% in breast muscle and from 2.14% to 7.10% in thigh muscle. Docosahexaenoic acid (DHA) also increased in both muscles (from 0.78% to 1.21% in the breast and from 0.25% to 0.62% in the thigh), as did eicosapentaenoic acid (EPA), although to a lesser extent (from 0.01% to 0.26% in the breast and from 0.01% to 0.02% in the thigh). A significant increase ($P < 0.001$) in omega-6 fatty acids was also observed. The total omega-6 content increased from 35.45% to 43.42% in the breast muscle and from 35.47% to 43.55% in the thigh muscle, primarily due to the increase in linoleic acid (from

29.59% to 38.02% in the breast; from 33.19% to 40.38% in the thigh). Other n-6 fatty acids showed varied trends: arachidonic acid (ARA) decreased in breast muscle (from 5.14% to 4.35%) but increased in thigh muscle (from 1.84% to 2.59%); dihomoγ-linolenic acid (DGLA) slightly increased in the thigh (from 0.19% to 0.24%) but declined in the breast; and γ-linolenic acid (GLA) increased in both muscles (from 0.18% to 0.30% in the breast and from 0.22% to 0.37% in the thigh muscle).

Table 4: Effects of dietary HS supplementation on total intramuscular fat, Ash, and mineral composition of the breast muscle of laying hens

Item		Diet group				SEM	*P* Value
		Control	10HSs	20HSs	30HSs		
Breast muscle	Dry matter (DM)	26.29	26.13	26.06	26.21	0.22	0.061
	Intramuscular Fat (g/100 g DM)	4.75^{b}	4.41^{b}	4.56^{ba}	4.10^{a}	0.30	0.252
	Ash	4.55	4.57	4.50	4.35	0.11	0.315
	K	72.37^{ab}	73.57^{b}	70.64^{a}	72.07^{ab}	0.84	0.047
	S	16.18	15.39	16.83	16.42	0.61	0.193
	P	9.82	9.58	10.32	9.67	0.29	0.121
	Zn	0.44	0.38	0.35	0.36	0.03	0.152
	Fe	0.31^{ab}	0.34^{b}	0.30^{ab}	0.24^{a}	0.27	0.036
	Cu	0.55^{bc}	0.55^{c}	0.46^{ab}	0.45^{a}	0.03	0.011
Thigh muscle	Dry matter (DM)	25.24	25.49	24.70	25.39	0.27	0.074
	Intramuscular Fat (g/100 g DM)	12.38^{bc}	12.29^{bc}	10.79^{a}	11.30^{ab}	0.37	≤.001
	Ash	4.05	3.55	3.85	3.90	0.15	0.057
	K	65.09	68.58	67.91	68.38	1.18	0.058
	S	18.12	19.02	19.16	19.59	0.99	0.540
	P	8.43	8.61	8.68	8.60	0.32	0.880
	Zn	2.08	1.58	1.81	1.51	0.27	0.216
	Fe	0.87^{b}	0.63^{ab}	0.74^{ab}	0.59^{a}	0.08	0.042
	Cu	0.78	0.66	0.64	0.66	0.05	0.068

K: Potassium, S: Sulfur, P: Phosphorus, Zn: Zinc, Fe: Iron, Cu: Copper, SEM: Standard error of the mean, p significance. [abcd]Means marked with different superscript letters within each row are significantly different.

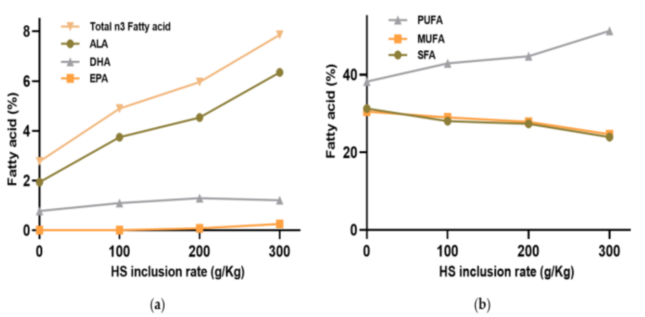

Figure 1: Effects of dietary hempseed on (a). The ω3 polyunsaturated fatty acid contents (ALA, EPA, DHA, and total n3-PUFAs, %) and (b)—the sum of PUFAs, MUFAs, and SFAs in breast muscle.

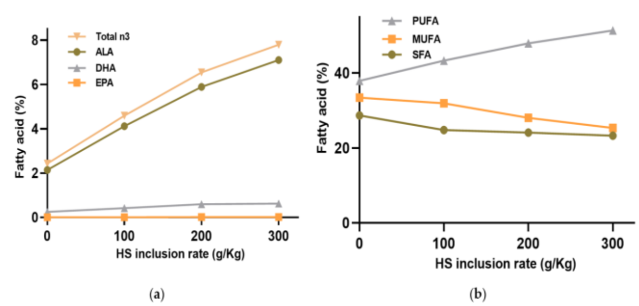

Figure 2: Effects of dietary hempseed on (a). The ω3 polyunsaturated fatty acid contents (ALA, EPA, DHA, and total n3-PUFAs, %) and (b)—the sum of PUFAs, MUFAs, and SFAs in thigh muscle.

In both the breast and thigh muscles, the n-6/n-3 PUFA ratio exhibited a highly significant reduction ($P < 0.001$) in response to increasing dietary inclusion of hempseed at concentrations of 10%, 20%, and 30%. In the breast muscle, the ratio decreased from 12.81 in the control group to 5.52 at 30% inclusion. A similar trend was observed in the thigh muscle, where the ratio decreased from 14.59 to 5.59 across the same dietary treatments. Similarly, the LA-to-ALA ratio significantly reduced ($P < 0.001$) across all levels of hempseed supplementation. In the breast muscle, this ratio decreased from 15.29 in the control group to 5.99 at 30% inclusion, whereas in the thigh muscle, it decreased from 15.11 to 5.68 over the same dietary treatment.

Table 5: Effects of dietary HS supplementation on the fatty acid profile and related health lipid indices of the breast muscle of laying hens

Fatty acids	Diet group				SEM	*P* Value
	Control	10HS	20HS	30HS		
C14:0 (Myristic acid)	0.52[d]	0.42[c]	0.37[b]	0.25[a]	0.13	<.001
C15:0 (Pentadecanoic acid)	0.12[ab]	0.11[ab]	0.14[b]	0.09[a]	0.01	0.013
C16:0 (Palmitic acid)	21.77[d]	19.54[c]	18.52[b]	16.91[a]	0.08	<.001
C18:0 (Stearic acid)	8.78[b]	7.90[a]	8.23[ab]	7.61[a]	0.22	0.003
C20:0 (Arachidic acid)	0.03[a]	0.06[b]	0.07[c]	0.13[d]	0.01	<.001
C21:0 (Henicosanoic acid)	0.07[b]	0.04[a]	0.04[a]	0.04[a]	0.01	<.001
C24:0 (Lignoceric Acid)	0.03[bc]	0.02[a]	0.02[b]	0.03[c]	0.00	<.001
Σ SFA	31.29[c]	28.04[b]	27.33[b]	23.93[a]	0.26	<.001
C16:1n-7 (Palmitoleic acid)	1.94[c]	1.80[c]	1.38[b]	0.91[a]	0.05	<.001
C18:1n-9 (Oleic acid)	27.21[d]	26.42[c]	25.55[b]	23.06[a]	0.24	<.001
C18:1n-7 (Vaccenic acid)	1.34[c]	0.79[ab]	0.94[b]	0.69[a]	0.06	<.001
Σ MUFA	30.49[d]	29.01[c]	27.86[b]	24.66[a]	0.27	<.001
C18:2n-6 (Linoleic Acid; LA)	29.59[a]	33.14[b]	32.90[b]	38.02[c]	0.63	<.001
C18:3n-6 (γ- Linolenic acid GLA)	0.18[a]	0.21[ab]	0.25[bc]	0.30[c]	0.02	<.001
C20:3n-6 (Dihomoγ-Linolenic acid DGLA)	0.52[b]	0.32[a]	0.34[ab]	0.39[ab]	0.06	0.04
C20:4n-6 (Arachidonic acid ARA)	5.14[b]	4.35[a]	5.27[b]	4.73[ab]	0.24	0.017
Σ n6PUFA	35.45[a]	38.03[b]	38.77[b]	43.42[c]	0.42	<.001
C18:3n-3 (α- Linolenic acid ALA)	1.93[a]	3.74[b]	4.54[c]	6.35[d]	0.03	<.001
C20:5n-3 (Eicosapentaenoic acid EPA)	0.01[b]	0.01[a]	0.08[a]	0.26[b]	0.03	<.001
C22:6n-3 (Docosahexaenoic acid DHA)	0.78[a]	1.10[b]	1.30[c]	1.21[b]	0.05	<.001
Σ n3PUFA	2.77[a]	4.89[b]	5.96[c]	7.86[d]	0.04	<.001
Σ PUFA	38.22[a]	42.93[b]	44.74[c]	51.27[d]	0.41	<.001
Σ n6PUFA/Σ n3PUFA	12.81[d]	7.77[c]	6.51[b]	5.52[a]	0.15	<.001
PUFAs/SFAs	1.22[a]	1.53[b]	1.64[c]	2.14[d]	0.03	<.001
LA/ALA	15.29[d]	8.85[c]	7.25[b]	5.99[a]	0.18	<.001
h/H	2.96[a]	3.48[b]	3.73[c]	4.59[d]	0.02	<.001
AI	0.48[d]	0.41[c]	0.39[b]	0.32[a]	0.01	<.001
TI	0.76[d]	0.58[c]	0.53[b]	0.41[a]	0.01	<.001

Σ SFA: Sum of saturated fatty acids, Σ MUFA: Sum of monounsaturated fatty acids, Σ PUFA: Sum of polyunsaturated fatty acids, Σ n6PUFA/Σ n3PUFA: Omega 6 to Omega 3 ratio, LA/ALA: Linoleic to Linolenic ratio, h/H: Hypocholesterolemic to hypercholesterolemic fatty acid ratio, AI: Atherogenic index, TI: Thrombogenic index, SEM: Standard error of the mean, P significance. [abcd]Means marked with different superscript letters within each row are significantly different.

Concurrently, both the atherogenic index (AI) and the thrombogenic index (TI) significantly improved with increasing hempseed intake. Compared with the control group, all treatment groups showed notable reductions in these indices ($P < 0.001$). Specifically, the AI values in the breast muscle decreased from 0.48 in the control group to 0.32 at 30% inclusion, and those in the thigh muscle decreased from 0.42 to 0.31. Similarly, the TI values decreased from 0.76 to 0.41 in the breast muscle and from 0.69 to 0.40 in the thigh muscle. In contrast to the declining indices, the PUFA/SFA ratio and the hypocholesterolemic-to-hypercholesterolemic fatty acid ratio (h/H) both increased significantly ($P < 0.001$) with increasing hempseed inclusion relative to the control diet. Specifically, the PUFA/SFA ratio in the breast muscle rose from 1.22 in the control group to 2.14 at 30% inclusion and from 1.32 to 2.20 in the thigh muscle over the same dietary range. The h/H ratio improved markedly, increasing from 2.96 to 4.59 in the breast muscle and from 3.37 to 5.07 in the thigh muscle between the control and 30% inclusion groups, respectively.

Discussion

Chicken meat remains a highly popular and inexpensive source of animal protein worldwide and is suitable for all age groups and cultural and religious backgrounds (33). The poultry industry plays a vital role in meeting global nutritional requirements by providing meat and eggs that are rich in protein and key micronutrients (34). The increasing interest in hemp seeds as functional feed ingredients is primarily attributed to their rich content of bioactive compounds, particularly essential fatty acids, tocopherols, and carotenoids (7,35). Several studies have documented the generally beneficial effects of hemp seed supplementation on poultry growth performance and product quality (14,15,35–37). However, the outcomes of these studies vary considerably due to differences in inclusion levels, which often exceed 20%, despite hemp seeds containing up to 30% fiber. While excessive dietary fiber is typically associated with reduced nutrient digestibility, existing evidence suggests that this does not pose a significant limitation to the effective use of hemp seeds in poultry nutrition (18). This study investigated the effects of incorporating varying levels of hempseed into the diets of laying hens on several meat quality parameters, including physical traits, intramuscular fat content, ash and mineral composition, fatty acid profiles, and lipid indices related to health in both thigh and breast muscles.

Table 6: Effects of dietary HS supplementation on the fatty acid profile and related health lipid indices of the thigh muscle of laying hens

Fatty acids	Diet group				SEM	*P* Value
	Control	10HS	20HS	30HS		
C14:0 (Myristic acid)	0.61[c]	0.47[b]	0.41[ba]	0.35[a]	0.29	<.001
C15:0 (Pentadecanoic acid)	0.12[b]	0.11[b]	0.10[ba]	0.08[a]	0.01	0.005
C16:0 (Palmitic acid)	19.60[c]	17.24[b]	17.49[b]	14.32[a]	0.14	<.001
C18:0 (Stearic acid)	8.26[c]	6.86[b]	5.98[a]	8.42[c]	0.23	<.001
C20:0 (Arachidic acid)	0.04[a]	0.09[b]	0.08[b]	0.08[b]	0.01	0.012
C21:0 (Henicosanoic acid)	0.03[b]	0.03[a]	0.04[b]	0.03[a]	0.00	<.001
C24:0 (Lignoceric Acid)	0.01[b]	0.01[a]	0.01[ba]	0.01[a]	0.00	0.001
Σ SFA	28.67[c]	24.80[b]	24.11[bc]	23.28[c]	0.37	<.001
C16:1n-7 (Palmitoleic acid)	2.68[d]	2.46[c]	1.86[b]	1.68[a]	0.05	<.001
C18:1n-9 (Oleic acid)	29.90[d]	28.74[c]	25.50[b]	23.26[a]	0.30	<.001
C18:1n-7 (Vaccenic acid)	0.84[d]	0.73[c]	0.66[b]	0.41[a]	0.02	<.001
Σ MUFA	33.42[d]	31.93[c]	28.02[b]	25.34[a]	0.33	<.001
C18:2n-6 (Linoleic Acid; LA)	33.19[a]	36.45b	38.20c	40.38d	0.09	<.001
C18:3n-6 (γ- Linolenic acid GLA)	0.22[a]	0.28b	0.31b	0.37c	0.01	<.001
C20:3n-6 (Dihomoγ-Linolenic acid DGLA)	0.19[a]	0.22ab	0.24b	0.21ab	0.01	0.01
C20:4n-6 (Arachidonic acid ARA)	1.84[b]	1.72[a]	2.56[c]	2.59[c]	0.03	<.001
Σ n6PUFA	35.47[a]	38.68[b]	41.33[c]	43.55[d]	0.07	<.001
C18:3n-3 (α- Linolenic acid ALA)	2.14[a]	4.12[b]	5.89[c]	7.10[d]	0.03	<.001
C20:5n-3 (Eicosapentaenoic acid EPA)	0.01[a]	0.01[a]	0.02[b]	0.01[a]	0.00	<.001
C22:6n-3 (Docosahexaenoic acid DHA)	0.25[a]	0.42[b]	0.59[c]	0.62[c]	0.02	<.001
Σ n3PUFA	2.43[a]	4.59[b]	6.55[c]	7.79[d]	0.03	<.001
Σ PUFA	37.90[a]	43.27[b]	47.88[c]	51.34[d]	0.08	<.001
Σ n6PUFA/Σ n3PUFA	14.59[d]	8.42[c]	6.31[b]	5.59[a]	0.15	<.001
PUFAs/SFAs	1.32[a]	1.74[b]	1.99[c]	2.20[d]	0.03	<.001
LA/ALA	15.51[d]	8.84[c]	6.48[b]	5.68[a]	0.13	<.001
h/H	3.37[a]	4.07[b]	4.10[b]	5.07[c]	0.06	<.001
AI	0.42[c]	0.34[b]	0.33[b]	0.31[a]	0.01	<.001
TI	0.69[d]	0.50[c]	0.44[b]	0.40[a]	0.01	<.001

Σ SFA: Sum of saturated fatty acids, Σ MUFA: Sum of monounsaturated fatty acids, Σ PUFA: Sum of polyunsaturated fatty acids, Σ n6PUFA/Σ n3PUFA: Omega 6 to Omega 3 ratio, LA/ALA: Linoleic to Linolenic ratio, h/H: Hypocholesterolemic to hypercholesterolemic fatty acid ratio, AI: Atherogenic index, TI: Thrombogenic index, SEM: Standard error of the mean, P significance. [abcd]Means marked with different superscript letters within each row are significantly different.

pH, WHC, and color parameters

Physicochemical attributes such as pH, water-holding capacity (WHC), and color are essential indicators of poultry meat quality (38). Among these factors, color remains one of the most influential factors affecting consumer perception and purchasing behavior (39). In chickens, particularly in breast and thigh cuts, meat color is influenced by genetic, nutritional, and physiological factors (40). In this study, hemp seed inclusion had no statistically significant effect on pH or WHC in either the breast or thigh muscle ($P > 0.05$). These findings are consistent with earlier studies by Kaić et al. (41) and Kanbur (42), which showed that incorporating hemp leaves or hemp oil into poultry diets did not alter these parameters. Similar conclusions were drawn by Tufarelli *et al.* (43) and Šťastník *et al.* (44), who noted no significant pH variation following the use of hempseed cake or expellers in broiler diets. The consistency in pH values across treatment groups may reflect the absence of preslaughter stress and stable postmortem glycolysis. A relatively high pH generally correlates with improved WHC and meat coloration, although these traits also depend on factors such as age, breed, and slaughter conditions (45,46). Our WHC data reinforce the findings of Kanbur (42) and Kaić et al. (41), supporting the conclusion that moderate hemp inclusion does not adversely affect this parameter. Nevertheless, Bień *et al*. (47) reported reduced drip loss in hemp extract-fed broilers, suggesting that the bioactive compound composition and form of hemp used may play a role in modulating moisture retention.

In terms of brightness (L*), our results revealed no significant differences between the treatments. This observation is consistent with studies by Serrapica *et al*. (48) and Tufarelli *et al*. (43), who also reported no effect of hempseed inclusion on meat lightness. However, some authors, such as Šťastník *et al*. (44) and He *et al.* (49), have reported reductions in L* values following hemp supplementation, potentially enhancing meat appearance given that consumers tend to prefer slightly darker poultry meat (50).

Our findings revealed a significant increase in the redness index (a*) of the breast muscle at 10% and 20% hempseed, whereas the redness index (a*) of the thigh muscle decreased at 20% hempseed. These results are partially in agreement with those of He *et al*. (49) and Kasula *et al*. (51), who also reported increased a* values in response to hemp-based diets. Comparable outcomes were noted by Yalcin *et al*. (52) in quail meat following phytobiotic supplementation. For the yellowness index (b*), no significant alterations were detected in the breast muscle. However, the thigh muscle markedly decreased in response to 20% and 30% hempseed. This aligns with Kanbur (42), who reported reduced b* values when soybean oil was substituted entirely with hemp oil in broiler diets. Conversely, Kaić *et al*. (41) noted an increase in b* values with low levels of hemp leaf supplementation, possibly due to differences in plant fractions and carotenoid concentrations.

Intramuscular fat content, ash content, and mineral composition of laying hen meat fed hemp seed-based diets

The findings show that intramuscular lipid content in breast muscle was not significantly affected by the treatments. In contrast, a significant reduction in thigh muscle was observed in the 20% and 30% hemp seed inclusion groups compared with the control group. These results concur with those of Baeza *et al*. (53), who reported that the energetic nature of the diet (carbohydrates or fats) does not significantly affect the intramuscular fat content in chickens. Furthermore, Keeton *et al*. (54) noted that the relationships among moisture, protein, and ash are inversely proportional to fat content, which could explain the low lipid levels observed.

The intramuscular lipid content is also influenced by physiological factors such as age, genotype, and production system (55), which may explain the lack of significant response in some cases despite variation in diet. Furthermore, the limited lipogenic capacity in poultry, with hepatic lipogenesis dominant (90%), means that the response to lipid supplementation of exogenous origin (such as hemp seeds) could be modulated at the hepatic level before translation into muscle tissue (56,57). With respect to total ash content, no significant differences were observed between groups in either the breast or thigh muscles. This finding corroborates the observations of Baéza *et al*. (40), who reported that the mineral composition of poultry meat (mainly calcium, phosphorus, and potassium) is generally not strongly affected by feeding, provided intake meets the animal's needs.

In terms of mineral composition, the macronutrients analyzed (potassium, phosphorus, and sulfur) presented no significant treatment-related variations. This is consistent with the results of Farinon et al. (11), who reported that hemp meal is rich in phosphorus, magnesium, and calcium. Nevertheless, as suggested by Neijat *et al*. (57), mineral concentrations in tissues often reflect the animal's physiological needs more than gross dietary intake. In laying hens, these minerals can also be mobilized for other physiological functions, such as eggshell formation (58).

For trace elements, significant reductions in iron were observed in both muscles at 30% inclusion. A decrease in copper was also observed in the pectoral muscle at the same dose, but not in the thigh. In contrast, the zinc levels remained stable. This result contrasts with that of Skřivan *et al.* (18), who reported a negative correlation between increasing hemp seed inclusion and mineral concentrations, which could reflect different metabolic distributions across tissues or competition for intestinal absorption. Minerals such as calcium and phosphorus also play a central role in bone health and the maintenance of acid–base balance (58), and their presence in hemp seeds could therefore contribute indirectly to these functions without their muscle concentration directly reflecting their effects. Certain bioactive compounds in hemp, such as cannabidiol and α-tocopherol, have been associated with beneficial effects on bone quality via improved bone healing and increased osteogenic bone mass (18). However, these effects were not directly measured in this study.

Fatty acid profile in laying hen meat fed hemp seed-based diets

The gradual enrichment of laying hens fed hemp seeds led to substantial changes in the lipid profile of the pectoralis major and thigh muscles. One of the main findings of this study concerns the significant reduction in saturated fatty acids (SFAs), particularly myristic, palmitic, and stearic acids. This decrease, which was particularly pronounced at the 30% inclusion level, is consistent with several previous studies conducted on both eggs and meat (5). Indeed, Silversides and Lefrançois (59) as well as Cufadar *et al*. (60) reported a similar decrease in SFAs in the eggs and muscle tissue of poultry fed hemp byproducts. In parallel, a decrease in monounsaturated fatty acids (MUFAs), particularly oleic acid, was also observed. This instance could be explained by the inhibition of the enzyme stearoyl-CoA desaturase-1 (SCD-1), which is responsible for the conversion of SFAs into MUFAs, under the effect of omega-3, as suggested by Bellenger *et al*. (61). This result is consistent with the results of Konca *et al*. (62). Still, it contrasts with those of Cufadar *et al*. (60), who reported an increase in MUFAs at higher levels of hemp meal inclusion.

Conversely, the polyunsaturated fatty acid (PUFA) profile was significantly improved, particularly the omega-3 fatty acid profile. Alpha-linolenic acid (ALA) increased substantially in both muscle types, consistent with results from several studies, notably Jing et al. (15), Shahid et al. (63), and Neijat et al. (64), which have reported similar enrichments in eggs. The metabolic conversion of ALA to long-chain fatty acids, such as EPA and DHA, although limited in poultry, nevertheless allows for a significant improvement in the lipid profile, as demonstrated by Gakhar *et al*. (16) and Simopoulos (65). These conversions are catalyzed by the Δ-5 and Δ-6 desaturase enzymes, whose expression is well documented in the avian liver, the primary organ of lipogenesis (28,56). With respect to omega-6 fatty acids, the results revealed a significant increase in linoleic acid (LA) and γ-linolenic acid (GLA), consistent with the intrinsic composition of hemp seeds, which are rich in these acids (7). These increases were also observed by Park *et al.* (66) and Kasula *et al.* (21) in eggs and muscle tissue. In contrast, the change in arachidonic acid (ARA) differed between muscles: a decrease in the breast muscle but an increase in the thigh muscle. This variability can be explained by differences in tissue metabolism or by desaturation rates that vary with muscle type, as suggested by Baéza *et al.* (40) and Skřivan *et al.* (18).

Related health lipid indices of laying hen meat fed hemp seed-based diets.

The inclusion of hempseed in laying hen diets led to significant improvements in health-related lipid indices, underscoring the nutritional potential of this feed additive. A progressive and substantial reduction in the n-6/n-3 PUFA and LA/ALA ratios was observed in both the breast and thigh muscles with increasing levels of dietary hempseed. These results are consistent with several reports demonstrating that hempseed and its derivatives are rich in alpha-linolenic acid (ALA), which contributes to lowering the n-6/n-3 ratio in animal products (15,16,20,52). This shift is critical from a nutritional perspective, as modern diets often display an excessive n-6/n-3 ratio, exceeding 15:1. In contrast, a ratio below 4:1 is recommended to reduce the risk of inflammatory and cardiovascular diseases (65,67). Concurrently, a significant improvement in the PUFA/SFA ratio was recorded in both muscle types, with values well above the minimum threshold of 0.45 considered beneficial for human health (68,69). This trend reflects the substitution of SFAs, particularly palmitic and stearic acids, with unsaturated fatty acids, a phenomenon previously documented by Neijat *et al*. (64) and Cufadar *et al*. (60) in poultry products supplemented with hempseed or oil.

Our findings also revealed a consistent and marked increase in the hypocholesterolemic/hypercholesterolemic (h/H) ratio, accompanied by a corresponding decrease in both the atherogenic (AI) and thrombogenic (TI) indices. These indices are critical predictors of lipid-associated health risks in human diets. As previously reported by Ulbricht and Southgate (29) and reiterated in recent studies (30,70), lower AI and TI values, ideally below 0.5 and 1.0, respectively, are associated with a reduced risk of atherosclerosis and thrombosis. In our study, values as low as 0.31 for the TI and 0.32 for the AI were achieved at 30% hempseed, which are comparable to or better than those reported for quail and broiler meat by Kasula *et al*. (21), Skřivan *et al*. (71), and Kaić *et al*. (41).

Conclusion

The gradual incorporation of hemp seeds into the diets of laying hens did not significantly alter the main physical characteristics of the meat, including pH, water-holding capacity, or lightness. However, notable improvements were observed in the lipid profile and associated health indices. Hemp seed inclusion reduced saturated and monounsaturated fatty acids while increasing polyunsaturated fatty acids, particularly omega-3 fatty acids (ALA, DHA, and EPA). These changes resulted in favorable shifts in lipid-related health metrics, including reduced n-6/n-3 and LA/ALA ratios, lower atherogenic and thrombogenic indices, and higher PUFA/SFA and h/H ratios. Overall, these findings highlight the potential of hemp seeds as a functional feed component to increase the nutritional value of poultry meat. Nevertheless, the study was limited to a single local hemp seed ecotype and a specific feeding period, which may restrict the generalizability of the results. Future studies should consider larger populations, diverse production systems, and long-term feeding trials to validate and extend these observations.

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Conflict of interest

There is no conflict of interest.

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آثار تغذية الدجاج البياض ببذور القنب على خصائص جودة لحم الصدر والفخذ، ومكونات الأحماض الدهنية، ومؤشرات الدهون الصحية

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الخلاصة

تهدف هذه الدراسة إلى تقييم آثار إدراج بذور القنب من الصنف المحلي "بلدية" (HS) بنسب متدرجة في النظام الغذائي للدجاج البياض على معايير جودة اللحم. تم توزيع ١٢٠ دجاجة من سلالة Lohmann Brown بعمر ٢٢ أسبوعًا، عشوائيًا على أربعة أنظمة غذائية تحتوي على ٠٪، ١٠٪، ٢٠٪، و٣٠٪ من بذور القنب، حيث تضمن كل نظام ستة تكرارات من خمس طيور، استمرت التجربة لمدة ١٢ أسبوعًا. وتضمنت المعايير التي تم تقييمها الخصائص الفيزيائية والكيميائية للحوم (درجة الحموضة، القدرة على الاحتفاظ بالماء، واللون)، محتوى الدهن العضلي، التركيب المعدني، ملامح الأحماض الدهنية، ومؤشرات الصحة المتعلقة بالدهون في عضلات الصدر والفخذ. لم تؤثر إضافة بذور القنب ($P>0.05$) على درجة الحموضة، قدرة احتباس الماء، أو درجة السطوع (L*) في اللحم. ومع ذلك، لوحظت تغيرات كبيرة ($P\leq0.05$) في قيم احمرار (a*) واصفرار (b*) لون العضلات. كما انخفض محتوى الدهن العضلي في عضلة الفخذ بشكل ملحوظ ($P\leq0.05$)، مع بعض التغيرات الطفيفة في بعض المعادن. والأهم من ذلك، أن بذور القنب حسّنت بشكل ملحوظ تركيب الأحماض الدهنية ومؤشرات صحة الدهون، حيث انخفضت نسب الأحماض الدهنية المشبعة والأحادية ($P\leq0.05$)، بينما ارتفعت نسبة الأحماض الدهنية المتعددة غير المشبعة (PUFAs)، خصوصًا سلسلة أحماض n-3 وn-6 ($P\leq0.05$). كما سُجلت تعديلات إيجابية في مؤشرات الصحة المتعلقة بالدهون، تمثلت في انخفاض نسب n-6/n-3 وLA/ALA، وانخفاض مؤشري التصلب والتخثر (AI وTI) ($P\leq0.05$)، في حين ارتفعت نسبة PUFA/SFA ونسبة h/H ($P\leq0.05$)، مما يعكس تحسن القيمة الغذائية للحوم. تُظهر النتائج أن إدراج بذور القنب في علائق الدجاج البياض يعزز الخصائص الغذائية للحوم دون الإضرار بصفاتها الفيزيائية الأساسية، مما يبرز إمكاناتها كمكوّن وظيفي في التغذية الحيوانية.