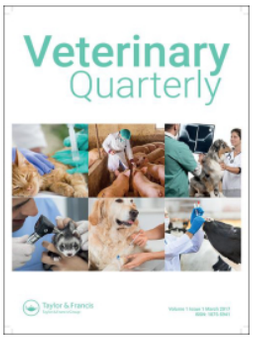

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Hemp seed (*Cannabis sativa* L.) cake as sustainable dietary additive in slow-growing broilers: effects on performance, meat quality, oxidative stability and gut health

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ABSTRACT

Hemp seed cake (HSC) (*Cannabis sativa* L.) is a rich source of polyunsaturated fatty acids, high-quality proteins and essential amino acids. The aim of this study was to evaluate the effects of dietary inclusion of HSC on growth performance, meat quality traits, fatty acids profile and oxidative status, and intestinal morphology in slow-growing broilers. A total of 180 male slow-growing broilers were randomly assigned to one of three dietary treatments containing different levels of HSC: 0 (HSC0), 5 (HSC5) or 10% (HSC10). Birds were slaughtered at 49 days of age: breast and thigh muscles were analysed and duodenum mucosa histomorphological features were evaluated. Regardless the level of HSC inclusion, no differences among groups were found for performance and meat quality traits. The thigh and breast fatty acid profile were significantly improved in both HSC groups, with an increase of the long chain fatty acids of n-3 series and decrease of n-6/n-3 ratio. The HSC diets lowered the MDA concentration and lipid hydroperoxides in breast meat. Histomorphometrical analysis revealed a significant increase in villus height, surface area and villus/crypt ratio, with a decrease of crypt depth, suggesting that dietary supplementation with HSC may boost intestinal health status in poultry. In conclusion, dietary HSC did not affect performance, carcass traits and meat quality, while it positively influenced the lipid profile of meat, and improved the oxidative status and gut health, thus representing a valuable and sustainable alternative ingredient in broiler diet.

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Broiler; growth; gut; hemp seed cake; meat; nutrition; sustainability

Introduction

Animal nutrition is one of the most important factor of determining farming efficiency and accounts for up to 70% on the total management costs (Mallick et al. 2020; Kasula et al. 2021). Moreover, the raising interest on sustainable production systems has focused on alternative feeding strategies based on the use of plants, herbs, vegetables and their by/co-products, which may be used to obtain final products that well fits the consumers' demand for healthy products, and on the other side represent an economically and environmentally advantageous solution for the livestock sector, increasing its profitability and sustainability (Kasapidou et al. 2015; Shehata et al. 2022; Tufarelli et al. 2022). Diet supplementation with natural extracts from plants and by/co-products may represent an alternative performance enhancer based on the use of plant-derived metabolites instead of synthetic additives without harmful residues in foods (Castanon 2007; Correddu et al. 2020).

Industrial hemp (*Cannabis sativa* L.) is a multi-purpose crop, providing food, fibre and nutraceuticals. In traditional medicine hemp has been used for lowering cholesterol in cardiovascular disease, for its immunomodulatory role and for the treatment of gastrointestinal disease (Prociuk et al. 2008; Cheng et al. 2011; Kaushal et al. 2020). Moreover, many studies reported that hempseed extracts possess strong antioxidant effects (Atalay et al. 2019).

Since 2011, the European Food Safety Authority (EFSA) panel on Additives and Products or Substances used in Animal Feed has introduced hempseed and hempseed cake (HSC) as feed ingredients for all animal species, including poultry, although with different dietary inclusion rates depending on animal species. Hemp seed and its processing by-products, i.e. cake and oil, are a valuable nutrition source with excellent fatty acid and amino acid profiles. Indeed,

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HSC obtained during the cold-pressed extraction of seeds is characterized by a high biological value of protein with an amino acid profile comparable to that of soybean meal (Callaway 2004; Wang and Xiong 2019). Thus, it may be included in poultry diets as a good source of crude protein and sulphur-containing amino acids, arginine and essential amino acids (Wang et al. 2008; Klir et al. 2019). Furthermore, HSC protein is free of trypsin inhibitors and oligosaccharides which are present in soybeans being responsible of gastrointestinal disorders (Eriksson and Wall 2012; Šťastník et al. 2019). The HSC also shows health-promoting properties due to its high amount of polyunsaturated fatty acids (PUFAs) (Da Porto et al. 2012; Della Rocca and Di Salvo 2020). Nowadays, it is well known that PUFAs, especially of the n-3 series, including alpha-linolenic acid (ALA), and derived long-chain (LC) n-3 FA eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), are essential nutrients for both humans and animals (Jing et al. 2017; Kasula et al. 2021). About 70% of total FAs in hemp are linoleic (LA) and alpha-linolenic (ALA) acid, and the n-6/n-3 ratio is about 2:1–3:1, which is considered ideal for human health (WHO 2003; Callaway 2004; Jurgoński et al. 2020; Rehman et al. 2021).

The use of HSC in meat production may also be advantageous as it contains a significant amount of antioxidants, phenolic compounds and tocopherols (Chen et al. 2012; Skřivan et al. 2020; Arango et al. 2022). Several researches showed that the bioactive compounds in hemp seed are mainly located in the hulls (Chen et al. 2012), thus remaining in the cake after oil cold-pressed extraction (Siano et al. 2018; Moccia et al. 2020). Recently, Antunović et al. (2021) found that hemp seeds and HSC contain γ-tocopherol at the rate of 60.85 and 33.72 mg/100 g DM, respectively. Oomah et al. (2002) have also reported that hempseed oil (HSO) contained about 800 mg/kg tocopherols, mostly in the form of γ-tocopherol (about 85%). These antioxidant components prevent oil oxidation (Rezvankhah et al. 2018); thus, their inclusion in poultry diets may have positive effects on oxidative stability of products and may enrich meat with some functional compounds (Konca et al. 2014; Mierlita 2019; Kanbur 2022). Another bioactive compound found in hempseed is the phytocannabinoid, cannabidiol (CBD). Cannabidiol (CBD) is a metabolite of tetrahydrocannabinol, with potential antioxidant, immunosuppressive and anti-inflammatory properties (Pubchem 2015). Hampson et al. (2000) reported that the antioxidant activity of cannabidiol found in hempseed was superior to α-tocopherol and ascorbate.

Therefore, the aim of the present study was to investigate the effects of different levels of dietary inclusion of hemp seed cake (5 and 10%) on growth performance, carcass and meat quality traits, fatty acid composition and oxidative stability in slow-growing broilers. Moreover, gut health was also assessed by studying broiler duodenal histomorphological architecture.

Materials and methods

The current trial was conducted at the Experimental Poultry Research Center of the University of Bari "Aldo Moro", Valenzano, Bari, Italy. Authors adhere that procedures imposed on the animals were carried out according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Authors also adhere to the EU regulations on feed legislation, such as regulation EC No 767/2009 of the European Parliament Council of July 13, 2009.

Birds, management and dietary treatments

A total of 180 male slow-growing broiler chickens (Hubbard) on day of hatch were purchased. On day 14, broiler chickens were individually weighed and divided among pens and randomly assigned to one of the three dietary treatment groups and grown up to 49 days of age. There were a total of 18 floor pens, six replicate pens (2.5 × 1.5 m) per treatment with ten broiler chickens in each pen. The pens were in a closed house with controlled environment. Each pen was equipped with a pan feeder and a manual drinker. Broiler chickens were raised on a concrete floor with straw as litter and stocking density was according to EU legislation. For the first two weeks of age, chickens were fed the same mashed diet without HSC. Then, broilers were fed a grower-finisher pelleted diet containing 0, 5 or 10% of HSC, respectively. Diets were formulated to meet NRC (1994) nutrient recommendations for broiler chickens. The ingredient and chemical composition of the diets are presented in Table 1. All broiler chickens were reared under similar conditions and feed and water were provided *ad libitum* throughout the whole feeding trial. The body weight (BW) and average daily feed intake (ADFI), from which average daily gain (ADG) and feed conversion ratio (FCR) were calculated, were measured. Mortality rate was considered when calculating the FCR.

Preparation of hemp seed cake

A variety of industrial hemp authorized for cultivation in the European Union called "Earlina 8FC" was used. This variety, developed for the first time in France, offers a high seed yield (about 1.0–1.5 t/ha) in a Mediterranean climate and under optimal soil fertility and irrigation conditions. The oil extraction was achieved by the Komet Vegetable Oil Expeller system (IBG Monforts Oekotec GmbH & Co.KG, Germany) equipped with a special cold pressing system provided with a single transport auger to squeeze the oil from various oilseeds. The hemp seed oil was extracted using 7 mm nozzles, which led to 70% oil extraction. The HSC was grounded by using a hammer mill, then stored in polyethylene bags until used for feed formulations. The HSC was

Table 1. Ingredients and chemical composition of experimental diets fed to slow-growing broiler chickens.

Item[1]	HSC0	HSC5	HSC10
Ingredients (g/kg)			
Corn	565.0	585.0	600.0
Soybean meal (48% CP)	175.0	170.0	165.0
Sunflower meal (38% CP)	75.0	35.0	–
Wheat	75.0	120.0	95.0
Wheat middlings	60.0	–	–
Dicalcium phosphate	20.0	20.0	20.0
Soybean oil	10.0	–	–
Hemp seed cake	–	50.0	100.0
Calcium carbonate	10.0	10.0	10.0
L-Lysine HCl	2.0	2.0	2.0
Sodium chloride	2.0	2.0	2.0
Sodium bicarbonate	2.0	2.0	2.0
Vitamin-mineral premix[2]	2.0	2.0	2.0
L-Threonine	1.0	1.0	1.0
DL-Methionine	1.0	1.0	1.0
Chemical composition, % DM			
Crude protein	19.90	19.86	19.65
Crude fat	4.90	4.15	4.47
Ash	6.50	6.30	6.28
Calculated analysis			
ME (MJ/kg)[3]	13.80	13.85	13.90
Calcium	1.13	1.13	1.14
Available phosphorus	0.49	0.48	0.49
Lysine	1.09	1.09	1.10
Methionine	0.45	0.47	0.48
Methionine + Cysteine	0.83	0.84	0.86
Threonine	0.83	0.84	0.85

[1]HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake.
[2]Supplied per kilogram of diet: vitamin A 12,000 IU; vitamin E, 10 mg; vitamin D 2,200 IU; niacin 35.0 mg; d-pantothenic acid 12 mg; riboflavin 3.63 mg; pyridoxine 3.5 mg; thiamine 2.4 mg; folic acid 1.4 mg; biotin 0.15 mg; vitamin B 0.03 mg; Mn 60 mg; Zn 40 mg; Fe 1,280 mg; Cu 8 mg; I 0.3 mg; Se 0.2 mg.
[3]Metabolizable energy.

Table 2. Proximate chemical and fatty acids composition of hemp seed cake (HSC) included into broiler diets.

Item	
Dry matter, %	91.8
Crude protein, %	31.3
Crude lipids, %	8.42
Ash, %	5.36
Carbohydrates, %	15.2
Crude fiber, %	31.5
Metabolizable energy, MJ/kg DM[1]	12.0
Fatty acids, %	
C16:0 (palmitic)	8.6
C18:0 (stearic)	2.8
C18:1 n-9 *cis* (oleic)	13.1
C18:2 n-6 (linoleic)	53.2
C18:3 n-3 (α-linolenic)	12.3
C18:3 n-6 (γ-linolenic)	2.5
Other	7.5

[1]Estimated according to Van Der Klis and Fledderus (2007).

chemically analyzed to assess dry matter (DM), crude protein, crude lipids, crude fiber, and ash according to AOAC. (2005). The fatty acids profile of HSC was also assessed following the methods as described later for meat analysis. Metabolisable energy was estimated according to Van Der Klis and Fledderus (2007). The proximate chemical composition of the HSC included into broiler diet is illustrated in Table 2.

Sample collection

On day 49 of the feeding trial, five broilers of average BW were randomly selected from each pen following a 12-h fasting period, weighed individually, and slaughtered. The abdominal fat (consisting of fat surrounding the gizzard, proventriculus, and in the abdominal body cavity), breast and thigh muscle were removed and weighed immediately. Samples of meat muscles were immediately stored at −80 °C for assessing lipids content, and others were individually stored in plastic bags at 4 °C for meat quality analysis.

Meat quality parameters

At 24 h after slaughter, the breast muscle pH was measured at a depth of 2.0 cm below the surface. This was performed using a combined glass-penetrating electrode (Ingold, Mettler Toledo, Greifensee, Switzerland). Meat samples were analyzed for moisture, ash, and protein by oven, muffle furnace, and Kjeldahl methods, respectively, as described in AOAC. (2005). Total lipids were extracted according to the method of Folch et al. (1957). Meat color was assessed on samples excised from the *Pectoralis major* (breast) muscle. Meat color (L^* = lightness, a^* = redness, b^* = yellowness) was determined using a Hunter Lab MiniscanTM XE Spectrophotometer (Model 4500/L, 45/0 LAV, 3.20 cm diameter aperture, 10° standard observer, focusing at 25 mm, illuminant D65/10; Hunter Associates Laboratory Inc., Reston, VA, USA). Three readings were carried out for each sample by placing the instrument on different broiler meat areas. The instrument was normalized to a standard white tile before performing the analysis ($Y = 92.8$, $x = 0.3162$, and $y = 0.3322$). The reflectance measurements were performed after the samples were allowed to oxygenate in the air for at least 30 min, to take stable measurements.

Meat fatty acid profile

Chemical analysis and fatty acid profile were performed on raw meat from *Pectoralis major* and *Sartorius* muscles. Briefly, a 5 g homogenized (lyophilized) meat sample was blended with chloroform/methanol (2:1, v/v) overnight at 4 °C. Later, it was filtered, placed in separator funnels and mixed with saline solution (0.7% KCl). After separation into two phases, the methanol aqueous fraction was discarded, whereas the lipid chloroform fraction was evaporated using a rotary evaporator. Lipid extracts were transferred to test tubes for subsequent gas chromatographic analysis. Duplicate samples of chloroform extract, corresponding to 100 mg of lipid, were methylated by adding 1 ml of hexane/isopropanol 4:1 and 0.05 ml of 2 N methanolic KOH according to Christie (1982). Gas-chromatographic analysis of fatty acids was performed using a Shimadzu GC-17A instrument equipped with a capillary column BPX 70 (70% Cyanopropyl Polysilphenylene-siloxane Thermo Scientific, length 60 m, internal diameter 0.25 mm, film thickness 0.25 µm). Operating conditions were as follows: a helium flow rate of 1.2 ml/min.; a FID detector at 280 °C; a split–splitless injector at 245 °C.

The temperature programme of the column was: 7 min at 135 °C and a subsequent increase to 210° C at 4° C/min. Retention time and area of each peak were computed by using the CromatoPlus2008 software. The individual fatty acid peaks were identified by the comparison between retention times and those of known mixtures of standard fatty acids (FAME, Restek) run under the same operating conditions. Fatty acids were expressed as percent of total methylated fatty acids.

The atherogenic and thrombogenic indexes were calculated according to Ulbricht and Southgate (1991) as follows:

Atherogenic Index = (C12:0 + 4 × C14:0 + C16:0)/[ΣMUFA + Σ(n-6) + Σ(n-3)];

Thrombogenic Index = (C14:0 + C16:0 + C18:0)/[0.5 × ΣMUFA + 0.5 × Σ(n-6) + 3 × Σ(n-3) + Σ(n − 3)/Σ(n-6)]; where: MUFA = monounsaturated FA, PUFA = polyunsaturated FA.

Meat oxidation products

Thiobarbituric acid-reactive substances (TBARS) in meat samples were determined after 7 days of storage at 4 °C as described by Mcdonald and Hultin (1987). Tissue samples (2 g) were weighed into test tubes each with 18 ml of 3.86% perchloric acid; samples were homogenized with a Polytron (IKA Labortechnik T25-B, Selangor, Malaysia) 3 × 15 s at high speed. Fifty microliters of butylated hydroxyl anisole (BHA) (4.5% BHA in ethanol) was added to the sample prior to homogenization. The homogenate was filtered through a filter paper. The filtrate (2 ml) was mixed with 2 ml of 20 mM TBA in distilled water and incubated in a boiling water bath for 30 min. After cooling, the absorbance of filtrate was determined at 531 nm against a blank containing 2 ml of 3.86% perchloric acid and 2 ml of 20 mM thiobarbituric acid-reactive solution. The thiobarbituric acid-reactive substances values were expressed as milligrams of malonaldehyde per kg of meat.

For meat lipid hydroperoxides determination, about 1 g of meat muscle was homogenized in 5 ml of chloroform/methanol (1:1) for 30 s using a Polytron Type PT 10/35 (Brinkmann Instruments, Westbury, NY). Subsequently, the Polytron was washed for 30 s with 5 ml solvent. The homogenates and wash solutions were then combined. Three ml of 0.5% NaCl was added, and the mixture was vortexed for 30 s before centrifugation for 10 min to separate the mixture into two phases. Ice cold chloroform/methanol (1:1) (1.3 ml) was added to 2 ml of the lower phase and briefly vortexed. Twenty-five microliters of ammonium thiocyanate (4.38 M) and 25 µl ferrous chloride (18 mM) were added to assay for lipid hydroperoxides according to Shantha and Decker (1994). Samples were incubated for 20 min at room temperature before the absorbances at 500 nm were determined.

Meat protein oxidation, as measured by the total carbonyl content, was evaluated by derivatization with dinitrophenylhydrazine as described by Oliver et al. (1987) with slight modifications. Meat samples (1 g) were minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 6 M NaCl (pH 6.5) using an Ultraturrax homogenizer (IKA-Werke, Staufen, Germany) 2 × 30 s. Two equal aliquots of 0.2 ml were taken from the homogenates and dispensed in 2 ml Eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 ml) and subsequent centrifugation for 5 min at 4,200 g. One pellet was treated with 1 ml 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) dinitrophenylhydrazine in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 ml) and washed three times with 1 ml ethanol:ethyl acetate (1:1, v/v) to remove excess dinitrophenylhydrazine. The pellets were then dissolved in 1.5 ml of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 4,200 *g* to remove insoluble fragments. Protein concentration was calculated from the absorption at 280 nm using bovine serum albumin as the standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 $nM^{-1}cm^{-1}$ at 370 nm for protein hydrazones.

Histological examination

At day 49, on the same slaughtered broilers, intestinal segment samples (approximately 2 cm in length) of duodenum were excised and flushed with 0.9% saline to remove the contents. Segments were fixed in 10% neutral-buffered formalin for histological examinations. The segments collected were the loop of the duodenum (at 5 cm from the pylorus). Moreover, also liver samples were collected from the same subjects. Samples were dehydrated, cleared, and paraffin embedded. Intestinal segments from 12 birds per dietary treatment were sectioned at a 5–7 µm thickness, placed on glass slides, and processed in Masson's trichrome stain for examination by light microscopy according to Culling et al. (1985). Each sample section on slides were stained with haematoxylin and eosin (H&E) (Merck, Darmstadt, Germany) and Azan Mallory for morphological observations and morphometric measurements. The morphometric indices evaluated were: villus height from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, and the villus height to crypt depth ratio (Laudadio et al. 2012). The apparent villus surface area was calculated by the following formula: [(villus width at one-third + villus width at two-thirds of the height of the villus) × 2^{-1} × villus height], according to Iji et al. (2001). Morphometric investigations were performed on 20 intact villi and 30 crypts chosen from each duodenal segment of broiler chickens and evaluated at 10× and 25× magnification by using an image analysis system (X-Series, Alexasoft).

Statistical analysis

Data were analysed for variance (ANOVA) using the GLM procedure of SAS software version 9.2. All the studied traits were analyzed by using univariate linear model as the response variables and diet (0, 5, and 10% HSC, respectively) as the factor explanatory variables, assuming that the random residual variance follows a normal distribution. Means were separated and compared by Tukey's honestly significant difference (HSD) test. Results were reported as least squares mean and pooled standard error of the mean (SEM). Significance was set at $p < 0.05$.

Results and discussion

The results on growth performance parameters of slow-growing broilers are presented in Table 3. No significant differences in final BW, ADFI, ADG, and FCR were found among treated birds. Total mortality rate did not also differ among diets. It was reported by Kalmendal (2008) that HSC may be included in broiler diets up to 30% instead of soybean meal without adverse effects on performance parameters. Eriksson and Wall (2012) in broilers fed diets supplemented with 10 and 20% HSC found a significantly higher broiler BW only in the 20% HSC group at 21 and 35 days of age. On the other hand, Stastnik et al. (2015) reported that the inclusion of HSC up to 15% negatively affected broiler BW. So far, limited literature on dietary HSC in broilers is still available, while whole hemp seed and oil have been more investigated, although providing controversial results in terms of productive performance (Khan et al. 2010; Mahmoudi et al. 2015; Jing et al. 2017).

Results on broilers' carcass traits and meat quality characteristics are reported in Table 4. The carcass yield showed no significant differences ($p = 0.071$) among groups. Furthermore, dietary treatments did not influence abdominal fat ($p = 0.055$) and breast yield ($p = 0.095$). As for the chemical composition and pH values of breast meat, no significant differences among diets were observed; similarly, breast meat lightness (L*), redness (a*), and yellowness (b*) values did not significantly differ ($p > 0.05$).

Diet is one of the main factors that influence growth performance, nutritive profile and sensory traits of broiler meat (Šťastník et al. 2019). As indicated in our study, HSC can be included in broilers diet up to 10% without any adverse effects. Similar results were also reported by Eriksson and Wall (2012) and Vispute et al. (2019) who found that growth performance and carcass traits were not influenced by the inclusion of graded levels of HSC in broiler diet. Furthermore, Khan et al. (2009) reported that the inclusion of hemp seed powder at 20% of broiler diet had positive effects on the weight of breast, leg, thigh as well as liver, gizzard, intestines and abdominal fat. Šťastník et al. (2019) found that the dietary inclusion of hempseed expellers up to 15% affected the color of broilers' meat by increasing the red and yellow indices.

The fatty acid composition of thigh and breast meat of slow-growing broilers fed different levels of HSC are presented in Tables 5 and 6, respectively. Thigh meat (Table 5) from broilers fed the three dietary treatments showed similar amounts of total SFA, MUFA and PUFA ($p > 0.05$). On the other hand, significant differences were found for the individual and total n-3 FAs. The HSC5 diet provided the highest concentration of DHA, with significant differences as compared to the other HSC diet as well as to the control group ($p < 0.05$). Moreover, in both HSC groups, a significant increase of total n-3 FAs ($p < 0.05$), and a consequent reduction of the n-6/n-3 ratio ($p < 0.05$) was observed. Furthermore, the concentration of erucic acid (C22:1 n-9) was significantly higher in both the HSC groups as compared to control ($p < 0.05$). Significant differences among treatments were found neither for atherogenic nor for thrombogenic indices.

In breast muscle (Table 6), no significant differences among groups aroused for the total SFA, MUFA and PUFA. Dietary HSC inclusion led to a significant decrease of oleic acid ($p < 0.05$). The HSC5 diet led to significantly higher concentration of erucic acid (C22:1 n-9) as compared to the 10% HSC inclusion and control groups. The LA (C18:2n-6) concentration was significantly higher ($p < 0.05$) in broilers fed the control diet as compared to both the HSC groups, probably due to the presence of sunflower meal in control treatment. Despite the lack of significant differences among groups as for total PUFAs, the two hemp seed cake diets determined a significantly ($p < 0.05$) higher concentration of the C20:3 n-6 (DGLA) and C22:6 n-3 (DHA) long chain FAs. At the same time, the concentration of C20:5 n-3 (EPA) and C22:5 n-3 (DPA) was significantly ($p < 0.05$) higher in the two hemp seed cake groups as compared to control. This may be the result of elongation and desaturation of ALA to EPA and DPA, as previously reported also by other authors (Bou et al. 2009; Skiba

Table 3. Effect of dietary treatments on growth performance of slow-growing broiler chickens.

	Diet[1]				
Item	HSC0	HSC5	HSC10	SEM	*p* value
Final BW,[2] g	2,119	2,148	2,155	38.4	0.197
ADG, g/d	53.1	53.3	53.5	0.75	0.212
ADFI, g/d	126.0	125.2	124.8	2.04	0.063
FCR, g/g	2.37	2.35	2.33	0.04	0.167
Mortality, %	1.31	1.29	1.33	0.221	0.598

[1]HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake.
[2]BW = final body weight at 49 days of age; ADG = average daily gain (14–49 d); ADFI = average daily feed intake (14–49 d); FCR = feed conversion ratio (14–49 d). SEM = standard error of the means.

Table 4. Effect of dietary treatments on carcass traits and breast muscle quality of slow-growing broiler chickens.

	Diet[1]				
Item	HSC0	HSC5	HSC10	SEM	*p* value
Carcass traits[2]					
Eviscerated carcass	73.5	73.8	74.0	0.40	0.071
Breast	20.4	20.5	20.7	0.23	0.095
Abdominal fat	1.8	1.9	2.1	0.04	0.055
Proximate composition					
Moisture, %	72.7	72.4	72.9	0.31	0.444
Protein, %	24.3	24.7	24.2	0.39	0.209
Fat, %	2.12	2.09	2.05	0.06	0.115
Ash, %	0.88	0.81	0.85	0.05	0.099
pH_{24}	5.70	5.77	5.79	0.101	0.088
Color at 24 h					
*L**	47.47	48.25	48.61	0.53	0.101
*a**	3.35	3.54	3.63	0.17	0.072
*b**	11.86	11.41	11.53	0.29	0.065

[1]HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake.
[2]Percentages of body weight at slaughter. SEM = standard error of the means.

Table 5. Fatty acid composition (% total FA methyl esters) of thigh muscle.

	Diet[1]				
Item	HSC0	HSC5	HSC10	SEM	*p* value
Total Fatty acids (g/100 g muscle)					
C4:0	0.04	0.05	0.05	0.005	0.224
C14:0 (myristic)	0.41	0.35	0.32	0.037	0.119
C15:0	0.08	0.06	0.05	0.011	0.128
C16:0 (palmitic)	21.43	21.04	20.05	0.580	0.152
C17:0	0.31	0.16	0.16	0.072	0.050
C18:0 (stearic)	9.96	12.04	13.81	1.575	0.069
C20:0	0.10	0.10	0.11	0.007	0.189
C21:0	0.24	0.26	0.25	0.009	0.113
C22:0	0.29	0.25	0.24	0.021	0.203
C23:0	0.02	0.03	0.04	0.006	0.132
∑ SFA	32.88	34.34	35.07	0.915	0.062
C14:1	0.05	0.04	0.03	0.005	0.177
C16:1 *trans*	0.27	0.27	0.29	0.009	0.095
C16:1 *cis*	2.51	2.01	2.03	0.231	0.055
C17:1	0.06	0.04	0.03	0.010	0.066
C18:1 n9 *trans* (elaidic)	0.00	0.00	0.07	0.031	0.056
C18:1 n9 *cis* (oleic)	34.91	32.42	31.88	1.322	0.051
C20:1 n-9	0.06	0.07	0.08	0.009	0.140
C22:1 n-9	2.96[b]	4.62[a]	4.67[a]	0.792	0.048
C24:1 n-9	0.11	0.12	0.11	0.004	0.111
∑ MUFA	40.94	39.59	39.19	0.747	0.069
C18:2 n6 (linoleic)	23.13	22.27	22.36	0.387	0.118
C18:3 n-6	0.94	1.06	0.94	0.056	0.135
C18:3 n3 (α-linolenic)	0.30	0.25	0.29	0.020	0.099
C20:2 n-6	0.03	0.03	0.04	0.007	0.117
C20:3 n-6	0.03	0.04	0.05	0.007	0.120
C20:5 n-3 (EPA)	0.71	1.08	0.99	0.156	0.051
C22:5 n-3 (DPA)	0.74	0.63	0.57	0.073	0.152
C22:6 n-3 (DHA)	0.30[c]	0.72[a]	0.51[b]	0.169	0.049
∑ PUFA	26.19	26.07	25.74	0.193	0.119
∑ UFA	67.13	65.66	64.93	0.915	0.112
n-6	24.13[a]	23.39[b]	23.38[b]	0.350	0.011
n-3	2.06[b]	2.68[a]	2.35[a]	0.253	0.048
n-6/n-3	11.71[a]	8.73[b]	9.94[b]	1.224	0.045
AI	0.36	0.35	0.34	0.008	0.123
TI	0.85	0.87	0.92	0.028	0.145

[1]HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake. SFA, saturated fatty acids (sum of C4:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0); MUFA, Monounsaturated fatty acids (sum of C14:1 + C16:1 + C17:1 + C18:1 + C20:1 + C22:1 + C24:1); Total n-6 (sum of C18:2 + C18:3 + C20:2 + C20:3); Total n-3 (sum of C18:3 + C20:5 + C22:5 + C22:6); PUFA, polyunsaturated fatty acids (sum of n-6 +n-3).
[a-c]Means within a row with no common superscript differ significantly ($p < 0.05$).

et al. 2015; Farinon et al. 2020). As found for the thigh muscle, the dietary HSC supplementation led to a significant increase of total n-3 FAs ($p < 0.01$) and to the consequent reduction of n6/n3 ratio ($p < 0.01$) in breast meat.

In both the muscles examined, dietary HSC significantly increased the proportion of n-3 PUFAs. Essential FAs are known to play an important role in human health and meat is a precious source of these nutrients (Arango et al. 2022), thus leading meat researchers to enhance the PUFA in fat deposits and muscle tissue in turn of a decrease of the SFA amount. In particular, the WHO guidelines recommended to decrease total FA intake, replace SFA with PUFA, especially the LC FA of the n-3 series, and to reduce the n-6/n-3 ratio to 5 as the maximum threshold value (WHO

Table 6. Fatty acid composition (% total FA methyl esters) of breast muscle.

Item	Diet[1]			SEM	*p* value
	HSC0	HSC5	HSC10		
Total Fatty acids (g/100 g muscle)					
C4:0	0.06	0.13	0.10	0.029	0.123
C14:0 (myristic)	0.35	0.21	0.23	0.062	0.102
C15:0	0.06	0.04	0.06	0.009	0.105
C16:0 (palmitic)	24.04	22.25	22.39	0.815	0.092
C17:0	0.14	0.11	0.14	0.014	0.151
C18:0 (stearic)	10.05	12.53	12.15	1.091	0.054
C20:0	0.06	0.04	0.08	0.016	0.125
C21:0	0.24	0.26	0.25	0.009	0.105
C22:0	0.28	0.60	0.47	0.131	0.125
C23:0	0.04	0.09	0.08	0.016	0.121
∑ SFA	35.31	36.24	35.94	0.385	0.059
C14:1	0.04	0.02	0.02	0.009	0.121
C16:1 *trans*	0.56	0.59	0.71	0.063	0.105
C16:1 *cis*	1.80[a]	0.93[b]	1.03[b]	0.389	0.049
C17:1	0.04	0.03	0.02	0.010	0.099
C18:1 n-9 *trans* (elaidic)	0.16	0.28	0.30	0.062	0.051
C18:1 n-9 *cis* (oleic)	33.91[a]	24.28[b]	26.67[b]	4.097	0.042
C20:1 n-9	0.05	0.05	0.07	0.007	0.122
C22:1 n-9	5.82[c]	14.28[a]	11.18[b]	3.494	0.042
C24:1 n-9	0.24	0.38	0.32	0.056	0.099
∑ MUFA	42.62	40.83	40.30	0.994	0.062
C18:2 n-6 (linoleic)	18.26[a]	15.14[b]	15.58[b]	1.378	0.049
C18:3 n-6	0.68	0.40	0.51	0.116	0.106
C18:3 n3 (α-linolenic)	0.22	0.20	0.20	0.009	0.104
C20:2 n-6	0.03	0.06	0.06	0.013	0.120
C20:3 n-6	0.10[b]	0.37[a]	0.32[a]	0.116	0.047
C20:5 n-3 (EPA)	1.13[c]	2.03[a]	1.58[b]	0.366	0.048
C22:5 n-3 (DPA)	0.87[c]	2.35[a]	1.83[b]	0.616	0.035
C22:6 n-3 (DHA)	0.75[b]	2.39[a]	2.18[a]	0.728	0.029
∑ PUFA	22.04	22.94	22.26	0.384	0.121
∑ UFA	64.66	63.77	62.56	0.861	0.112
n-6	19.07[a]	15.97[b]	16.47[b]	1.360	0.049
n-3	2.97[B]	6.97[A]	5.79[A]	1.679	0.009
n-6/n-3	6.43[A]	2.29[B]	2.85[B]	1.834	0.008
AI	0.41	0.37	0.38	0.16	0.119
TI	0.89	0.71	0.77	0.073	0.124

[1]HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake. SFA, saturated fatty acids (sum of C4:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0); MUFA, Monounsaturated fatty acids (sum of C14:1 + C16:1 + C17:1 + C18:1 + C20:1 + C22:1 + C24:1); Total n-6 (sum of C18:2 + C18:3 + C20:2 + C20:3); Total n-3 (sum of C18:3 + C20:5 + C22:5 + C22:6); PUFA, polyunsaturated fatty acids (sum of n-6 +n-3).
[a-c] Means within a row with no common superscript differ significantly ($p < 0.05$).
[A,B] Means within a row with no common superscript differ significantly ($p < 0.01$).

2003; EFSA NDA Panel (EFSA Panel on Dietetic Products and Nutrition and Allergies)), 2009).

The concentration of n-3 FAs in animal products strongly depends on the fatty acid composition of the diet (Haug et al. 2007; Bou et al. 2009), and especially on the concentration of the precursors LA and ALA (Kahraman et al. 2004). The ability of ALA to be converted into LC FAs of the n-3 series is limited in humans who receive these FAs with the diet (Haug et al. 2007; Decker and Park 2010; Juodka et al. 2018; Farinon et al. 2020). As for chicken meat, Gregory et al. (2013) reported that broilers may be able to metabolize more docosapentaenoic acid (DPA) through tetracosapentaenoic acid, the precursor of DHA, than other species. Therefore, broiler meat could be a possible source of EPA and DHA for human nutrition, as long as these FAs could be synthesized from plant-derived ALA (Bou et al. 2009; Farinon et al. 2020; Skřivan et al. 2020).

Hemp seed and its by-products are well known for their high level of PUFA with a n-6/n-3 ratio of ~3.3:1 (Jing et al. 2017; Benkirane et al. 2022; Taaifi et al. 2023). It can be assumed that the effect of hemp seed on the higher production of LC n-3 PUFA was due to the high dietary supply of ALA as well as to the higher activity of Δ5 and Δ6 desaturases and elongases, as previously described by Garg et al. (1988). Similar results were found by Skřivan et al. (2020) on cockerels fed diets supplemented with HS or/and flaxseed in different combinations. Previous studies carried out in laying hens (Taaifi et al. 2023) and Japanese quails (Konca et al. 2014) showed that HS dietary inclusion from 5 to 30% raised the amount of n-3 fatty acids in the yolk as the level of dietary HS increased. Mierlita (2019) reported a significant increase in the individual n-3 FA (ALA, EPA and DHA) and in total n-3 FA concentration in egg yolks of hens fed with HS and HSC diets, probably due to the level of ALA in HS and HSC along with the high ability of hens to convert ALA to n-3 LC FAs (Howe et al. 2002).

In the present study, HSC supplementation led to different fatty acid composition in the thigh and breast, which are dark and white muscles, respectively. Our data showed that the inclusion of HSC in the diet provided a higher DHA concentration in thigh muscle, while higher EPA, DPA, and DHA contents in breast were also observed. According to Jing

Table 7. Effect of dietary treatment on breast meat oxidation products of slow-growing broiler chickens[1].

	Diet[2]				
Item	HSC0	HSC5	HSC10	SEM	*p* value
TBARS, mg MDA/kg of meat	0.53[b]	0.48[a]	0.47[a]	0.026	0.048
Lipid hydroperoxides, µmol/g of meat	0.47[B]	0.32[A]	0.30[A]	0.022	0.004
Protein carbonyls, nmol DNPH/mg protein	1.30	1.27	1.25	0.08	0.061

[1]Each value represents the mean of 12 birds per treatment.
[2]HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake. TBARS: Thiobarbituric acid reactive substances; MDA, malonaldehyde; DNPH, 2,4-dinitrophenyl hydrazine. SEM = standard error of the means.
[a,b] Means within a row with no common superscript differ significantly ($p < 0.05$).
[A,B] Means within a row with no common superscript differ significantly ($p < 0.01$).

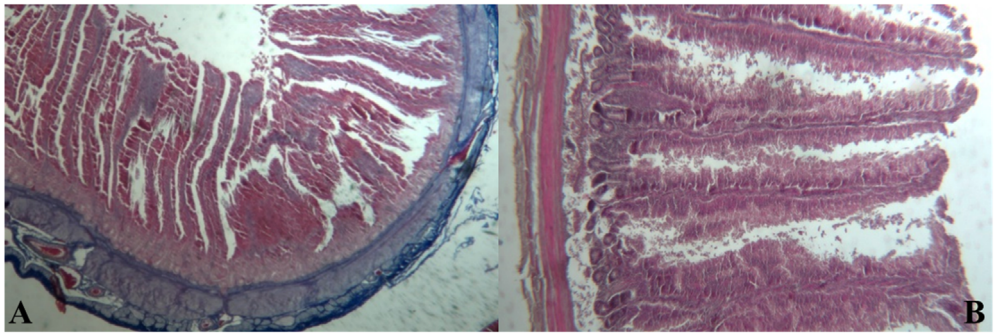

Figure 1. Cross section of small intestine of broiler chickens fed hemp seed cake (at 10% of diet). (A) Section of duodenum showing mucosa with several long villi and deep crypts (Azan Mallory, 10×). (B) Magnification of some villi with normal columnar epithelium (H&E, 250×).

Table 8. Effect of dietary treatments on duodenal mucosa morphometry of slow-growing broiler chickens[1].

	Diet[2]				
Item	HSC0	HSC5	HSC10	Pooled SEM	*p* value
Villus height, µm	1,017[B]	1,513[A]	1,531[A]	50.1	0.001
Crypt depth, µm	179[b]	148[a]	145[a]	11.2	0.017
Villus height/crypt depth	5.68[B]	10.22[AB]	10.56[A]	0.69	<0.001
Villus surface area, mm^2	0.129[C]	0.245[B]	0.267[A]	0.08	<0.001

[1]Each value represents the mean of 12 birds per treatment.
[2]HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake.
[a,b]Means within a row with no common superscript differ significantly ($p < 0.05$).
[A–C]Means within a row with no common superscript differ significantly ($p < 0.01$). SEM = standard error of the means.

et al. (2017) the extent of n-3 PUFA enrichments varied in relation to tissue type or animal species. The conversion of DPA to DHA takes place through the sequential reactions of elongation, desaturation, and β-oxidation (Sprecher 2000); the activity and the bioavailability of the enzymes involved in the biochemical pathway of LC n-3 PUFAs biosynthesis and their metabolism are different in red and white fiber muscles (Ng et al. 2012; Turner et al. 2014), in order to face the energy requirement of both muscles. Indeed, thigh muscle, which consist of type I red muscle fibres, has a greater oxidative capacity for FAs, while type II white muscle fibres, like breast muscle, contribute less to FA oxidation (Turner et al. 2014).

Table 7 shows the effect of HSC inclusion on the occurrence of lipid oxidation in breast meat. The antioxidant status was evaluated by measuring the breast muscle concentration of MDA, lipid hydroperoxides, and protein carbonyls. The results revealed a significant decrease of the MDA concentration ($p < 0.05$) and lipid hydroperoxides ($p < 0.01$) in both HSC supplemented diets, while no significant effect was found for protein carbonyls values. Although the increase of PUFA muscle level is a key goal to obtain healthier meat products, high contents of PUFA in poultry meat could lead to a higher susceptibility to lipid oxidation during storage or cooking (Vercellotti et al. 1992; Engberg et al. 1996; Kanbur 2022). In this study, even if the HSC diets led to an increase of LC n-3 FAs (EPA, DPA, DHA), the MDA and lipid oxidation products decreased significantly. In accordance with Vispute et al. (2019), our results could be attributed to the bioactive compounds present in HS by-products, among which tochopherols, phenols and cannabidiol exert a strong antioxidant activity (Jiang et al. 2001; Stambouli et al. 2006; Konca et al. 2014; Liang et al. 2015).

The morphological observation of the duodenal mucosa of broilers fed HSC-diets displayed histological features similar to the control group, with normal columnar epithelium and mucous cells secreting a protective layer that lines the surface of the epithelium, and which are attached on a regular mucosal muscle layer, as shown in Figure 1. The effect of the HSC supplementation on the histomorphometric measures of the duodenum is shown in Table 8. The

duodenal villus height and the villus height/crypt depth ratio were significantly increased ($p = 0.001$ and $p < 0.001$, respectively) following the HSC diets, suggesting an increased epithelial cells turnover. The morphometric analysis showed a decrease of the crypt depth in the experimental groups compared to the control. Moreover, dietary supplementation with HSC resulted in a significant increase ($p < 0.001$) of the villus surface area; among the two HSC groups, the villus surface area was greatest at 10% level ($p < 0.001$).

Knowledge of the impact of the diet on the morphological features of intestinal tract is essential to evaluate digestive efficiency that depends on the enteric mucosal surface and the functional traits of the epithelium (Stillhart et al. 2020).Therefore, in the present study, we evaluated the gut morphology of slow-growing broilers to assess possible HSC influence on digestive and absorptive capacity of the intestinal tract. The histomorphological changes in the segments examined underlined that the enrichment of the diet with HSC, regardless the percentage of inclusion, improved the intestinal architecture, since a greater duodenal surface villus area and higher villus length/crypt depth ratio were observed.

Indeed, with regards to morphology, longer duodenal villi raise the villus surface area increasing the absorbitive efficiency, as well as shallow crypts are indicative of an increased villus mucosal cell turnover (Xu et al. 2003; Vispute et al. 2019). Additionally, villus length/crypt depth ratio is considered an indicative tool for evaluating the digestive capacity of the small intestine (Wu et al. 2004). Furthermore, healthy gut is a prerequisite for prevention of intestinal and inflammatory disorders that could negatively influence broiler performance (Thanabalan and Kiarie 2021). Dietary supplementation with HSC could also improve the homeostasis of the gut microbiota (Opyd et al. 2020) preventing dysbiosis and enhancing the protective barrier against pathogenic bacteria adhesion to the mucosa (Rubio 2019), as well as modulate the microbiome and the immune system (Cencic and Chingwaru 2010; Sugiharto 2016). Recently, Taubner et al. (2023) reported that HS contains biologically active compounds that may affect the digestive process due to the action of these substances on various receptors and enzymes. Also, Golzar Adabi et al. (2016) hypothesized that a high level of dietary PUFA positively influenced the morphological features of the villus. In our study, there is evidence that dietary supplementation with HSC improved the intestinal morphometric parameters sustaining the broiler production performance.

Conclusions

The results of this study demonstrated that dietary hemp seed cake supplementation up to 10% did not affect growth performance, carcass traits and meat physical and chemical traits in slow-growing broilers. Including HSC had positive influence on the fatty acid profile of meat, oxidative status and gut health. Thus, HSC may be considered a valuable and sustainable alternative feed ingredient in broiler diet. Further research is needed to thoroughly investigate the influence of functional hemp seed compounds in broiler production systems.

Authors' contributions

All the authors equally contributed and approved the final version of the manuscript.

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Data availability statement

Data are contained within the article.

References

Golzar Adabi S, Hajibabaei A, Casey NH, Bayraktaroglu AG. 2016. The effect of various dietary vegetable oil sources on villi morphology and liver aldehydes in young layers. SA J An Sci. 46(1):63–69. doi: 10.4314/sajas.v46i1.8.

Antunović Z, Šalavardić ŽK, Steiner Z, Đidara M, Ćavar S, Ronta M, Šabić AM, Pavić V, Novoselec J. 2021. The influence of hempseed cake on production traits, metabolic profile and antioxidant status of Merino landschaf lambs. Ann Anim Sci. 21(3):991–1006. doi: 10.2478/aoas-2020-0107.

AOAC. 2005. Official methods of analysis. 16th ed. Washington, DC: Association of Official Analytical Chemists.

Arango S, Guzzo N, Raffrenato E, Bailoni L. 2022. Effect of dietary hemp cake inclusion on the in vivo and post mortem performances of holstein veal calves. Animals. 12(21):2922. doi: 10.3390/ani12212922.

Atalay S, Jarocka-Karpowicz I, Skrzydlewska E. 2019. Antioxidative and anti-inflammatory properties of cannabidiol. Antioxidants. 9(1):21. doi: 10.3390/antiox9010021.

Benkirane C, Ben Moumen A, Fauconnier M-L, Belhaj K, Abid M, Caid HS, Elamrani A, Mansouri F. 2022. Bioactive compounds from hemp (Cannabis sativa L.) seeds: optimization of phenolic antioxidant extraction using simplex lattice mixture design and HPLC-DAD/ESI-MS analysis. RSC Adv. 12(39):25764–25777. doi: 10.1039/d2ra04081f.

Bou R, Codony R, Tres A, Decker EA, Guardiola F. 2009. Dietary strategies to improve nutritional value, oxidative stability, and sensory properties of poultry products. Crit Rev Food Sci Nutr. 49(9):800–822. doi: 10.1080/10408390902911108.

Callaway JC. 2004. Hempseed as a nutritional resource: an overview. Euphytica. 140(1–2):65–72. doi: 10.1007/s10681-004-4811-6.

Castanon JI. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult Sci. 86(11):2466–2471. doi: 10.3382/ps.2007-00249.

Cencic A, Chingwaru W. 2010. The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients. 2(6):611–625. doi: 10.3390/nu2060611.

Chen T, He J, Zhang J, Li X, Zhang H, Hao J, Li L. 2012. The isolation and identification of two compounds with predominant radical scavenging activity in hempseed (seed of Cannabis sativa L.). Food Chem. 134(2):1030–1037. doi: 10.1016/j.foodchem.2012.03.009.

Cheng CW, Bian ZX, Zhu LX, Wu JC, Sung JJ. 2011. Efficacy of a Chinese herbal proprietary medicine (Hemp seed pill) for functional constipation. Am J Gastroenterol. 106(1):120–129. doi: 10.1038/ajg.2010.305.

Christie WW. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. J Lipid Res. 23(7):1072–1075. doi: 10.1016/S0022-2275(20)38081-0.

Correddu F, Lunesu MF, Buffa G, Atzori AS, Nudda A, Battacone G, Pulina G. 2020. Can agro-industrial by-products rich in polyphenols be advantageously used in the feeding and nutrition of dairy small ruminants? Animals. 10(1):131. doi: 10.3390/ani10010131.

Culling CFA, Allison RT, Barr WT. 1985. Cellular pathology technique, Butterworth London.

Da Porto C, Decorti D, Tubaro F. 2012. Fatty acid composition and oxidation stability of hemp (*Cannabis sativa* L.) seed oil extracted by supercritical carbon dioxide. Ind Crops Prod. 36(1):401–404. doi: 10.1016/j.indcrop.2011.09.015.

Decker EA, Park Y. 2010. Healthier meat products as functional foods. Meat Sci. 86(1):49–55. doi: 10.1016/j.meatsci.2010.04.021.

Della Rocca G, Di Salvo A. 2020. Hemp in veterinary medicine: from feed to drug. Front Vet Sci. 7:387. doi: 10.3389/fvets.2020.00387.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). 2009. Scientific opinion of the panel on dietetic products, nutrition and allergies on a request from European Commission related to labeling reference intake values for n-3 and n-6 polyunsaturated fatty acids. EFSA J. 8:1461.

Engberg RM, Lauridsen C, Jensen SK, Jakobsen K. 1996. Inclusion of oxidized vegetable oil in broiler diets. Its influence on nutrient balance and on the antioxidative status of broilers. Poult Sci. 75(8):1003–1011. doi: 10.3382/ps.0751003.

Eriksson M, Wall H. 2012. Hemp seed cake in organic broiler diets. Anim Feed Sci Technol. 171(2–4):205–213. doi: 10.1016/j.anifeedsci.2011.10.007.

Farinon B, Molinari R, Costantini L, Merendino N. 2020. 2020. The seed of industrial hemp (Cannabis sativa L.): nutritional quality and potential functionality for human health and nutrition. Nutrients. 12(7):1935. doi: 10.3390/nu12071935.

Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226(1):497–509. doi: 10.1016/S0021-9258(18)64849-5.

Garg ML, Sebokova F, Thomson BR, Clandinin MT. 1988. 6-Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or W-3 fatty acids. Biochem J. 249(2):351–356. doi: 10.1042/bj2490351.

Gregory MK, Geier MS, Gibson RA, James MJ. 2013. Functional characterization of the chicken fatty acid elongases. J Nutr. 143(1):12–16. doi: 10.3945/jn.112.170290.

Hampson AJ, Grimaldi M, Lolic M, Wink D, Rosenthal R, Axelrod J. 2000. Neuroprotective antioxidants from marijuana. Ann New York Acad Sci. 899(1):274–282. doi: 10.1111/j.1749-6632.2000.tb06193.x.

Haug A, Eich-Greatorex S, Bernhoft A, Wold JP, Hetland H, Christophersen OA, Sogn T. 2007. Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. Lipids Health Dis. 6(1):29. doi: 10.1186/1476-511X-6-29.

Howe PRC, Downing JA, Grenyer BFS, Grigonis-Deane EM, Bryden WL. 2002. Tuna fishmeal as a source of DHA for n-3 PUFA enrichment of pork, chicken, and eggs. Lipids. 37(11):1067–1076. doi: 10.1007/s11745-002-1002-3.

Iji PA, Saki A, Tivey DR. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br Poult Sci. 42(4):505–513. doi: 10.1080/00071660120073151.

Jiang Q, Christen S, Shigenaga MK, Ames BN. 2001. γ-Tocopherol, the major form of vitamin E in the US diet, deserves more attention. Am J Clin Nutr. 74(6):714–722. doi: 10.1093/ajcn/74.6.714.

Jing M, Zhao S, House JD. 2017. Performance and tissue fatty acid profile of broiler chickens and laying hens fed hemp oil and HempOmega TM. Poult Sci. 96(6):1809–1819. doi: 10.3382/ps/pew476.

Juodka R, Juska R, Juskiene V, Leikus R, Stankeviciene D, Nainiene R. 2018. The effect of feeding with hemp and Camelina cakes on the fatty acid profile of duck muscles. Arch Anim Breed. 61(3):293–303. doi: 10.5194/aab-61-293-2018.

Jurgoński A, Opyd PM, Fotschki B. 2020. Effects of native or partially defatted hemp seeds on hindgut function, antioxidant status and lipid metabolism in diet-induced obese rats. J Funct Foods. 72:104071. doi: 10.1016/j.jff.2020.104071.

Kahraman R, Özpınar H, Abas İ, Kutay HC, Eseceli H, Grashorn M. 2004. Effects of different dietary oil sources on fatty acid composition and malondialdehyde levels of thigh meat in broiler chickens.

Kalmendal R. 2008. Hemp seed cake fed to broilers [master's thesis]. Swedish University of Agricultural Sciences. Department of Animal Nutrition and Management.

Kanbur G. 2022. Growth-depressing effect of dietary hempseed oil on broiler performance in the starting period and alterations in meat oxidation, serum parameters and abdominal fatty acids. Animal Sci Papers Rep. 40(2):203–216.

Kasapidou E, Sossidou E, Mitlianga P. 2015. Fruit and vegetable co-products as functional feed ingredients in farm animal nutrition for improved product quality. Agriculture. 5(4):1020–1034. doi: 10.3390/agriculture5041020.

Kasula R, Solis F, Shaffer B, Connett F, Barrett C, Cocker R, Willinghan E. 2021. Characterization of the nutritional and safety properties of hemp seed cake as animal feed ingredient. Int J Livestock Product. 12(2):53–63.

Kaushal N, Dhadwal S, Kaur P. 2020. Ameliorative effects of hempseed (Cannabis sativa) against hypercholesterolemia associated cardiovascular changes. Nutr Metab Cardiovasc Dis. 30(2):330–338. doi: 10.1016/j.numecd.2019.09.006.

Khan RU, Durrani FR, Chand N, Anwar H, Naz S, Farooqi MF, Manzoor MN. 2009. Effect of Cannabis sativa fortified feed on muscle growth and visceral organs of broiler chicks. Inter J Biol Biotech. 4(1):79–81.

Khan RU, Durrani FR, Chand N, Anwar H. 2010. Influence of feed supplementation with Cannabis sativa on quality of broilers carcass. Pakistan Veterinary J. 30:34–38.

Klir Ž, Novoselec J, Antunović Z. 2019. An overview on the use of hemp (Cannabis sativa L.) in animal nutrition. Poljoprivreda (Online). 25(2):52–61. doi: 10.18047/poljo.25.2.8.

Konca Y, Yalcin H, Karabacak M, Kaliber M, Durmuscelebi FZ. 2014. Effect of hempseed (Cannabis sativa L.) on performance, egg traits and blood biochemical parameters and antioxidant activity in laying Japanese Quail (Coturnix coturnix japonica). Br Poult Sci. 55(6):785–794. doi: 10.1080/00071668.2014.978264.

Laudadio V, Passantino L, Perillo A, Lopresti G, Passantino A, Khan RU, Tufarelli V. 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poult Sci. 91(1):265–270. doi: 10.3382/ps.2011-01675.

Liang J, Appukuttan AA, Chary A, Thiyam-Holländer U. 2015. Hemp seed oil: minor components and oil quality. Lipid Tech. 27(10):231–233. – doi: 10.1002/lite.201500050.

Mahmoudi M, Farhoomand P, Nourmohammadi R. 2015. Effects of different levels of hemp seed (Cannabis sativa L.) and dextran oligosaccharide on growth performance and antibody titer response of broiler chickens. Ital J Anim Sci. 14:114–119.

Mallick P, Muduli K, Biswal JN, Pumwa J. 2020. Broiler poultry feed cost optimization using linear programming technique. J Operations Strategic Plan. 3(1):31–57. doi: 10.1177/2516600X19896910.

Mcdonald RE, Hultin HO. 1987. Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. J Food Sci. 52(1):15–21. doi: 10.1111/j.1365-2621.1987.tb13964.x.

Mierlita D. 2019. Fatty acid profile and oxidative stability of eggs from laying hens fed diets containing hemp seed and hempseed cake. South African J Animal Sci. 49(2):310–321.

Moccia S, Siano F, Russo GL, Volpe MG, La Cara F, Pacifico S, Piccolella S, Picariello G.,. 2020. Antiproliferative and antioxidant effect of polar hemp extracts (Cannabis sativa L., Fedora cv.) in human colorectal cell lines. Int J Food Sci Nutr. 71(4):410–423. doi: 10.1080/09637486.2019.1666804.

Ng JM, Azuma K, Kelley C, Pencek R, Radikova Z, Laymon C, Price J, Goodpaster BH, Kelley DE. 2012. PET imaging reveals distinctive roles for different regional adipose tissue depots in systemic glucose metabolism in non-obese humans. Am J Physiol Endocrinol Metabol. 303:1134–1141.

NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. Washington, DC: Natl. Acad. Press.

Oliver CN, Ahn BW, Moerman EJ, Goldstein S, Stadtman ER. 1987. Age-related changes in oxidized proteins. J Biol Chem. 262(12):5488–5491.

Oomah BD, Busson M, Godfrey DV, Drover JC. 2002. Characteristics of hemp (Cannabis sativa L.) seed oil. Food Chem. 76(1):33–43. doi: 10.1016/S0308-8146(01)00245-X.

Opyd PM, Jurgoński A, Fotschki B, Juśkiewicz J. 2020. Dietary hemp seeds more effectively attenuate disorders in genetically obese rats than their lipid fraction. J Nutr. 150(6):1425–1433. doi: 10.1093/jn/nxaa081.

Prociuk MA, Edel AL, Richard MN, Gavel NT, Ander BP, Dupasquier CM, Pierce GN. 2008. Cholesterol-induced stimulation of platelet aggregation is prevented by a hempseed-enriched diet. Can J Physiol Pharmacol. 86(4):153–159. doi: 10.1139/Y08-011.

Pubchem. 2015. National center for biotechnology information. [online]. Publisher Chemical Compound Database. https://pubchem.ncbi.nlm.nih.gov/compound/2543.

Rehman M, Fahad S, Du G, Cheng X, Yang Y, Tang K, Liu L, Liu F-H, Deng G.,. 2021. Evaluation of hemp (Cannabis sativa L.) as an industrial crop: a review. Environ Sci Pollut Res Int. 28(38):52832–52843. doi: 10.1007/s11356-021-16264-5.

Rezvankhah A, Emam-Djomeh Z, Safari M, Askari G, Salami M. 2018. Investigation on the extraction yield, quality, and thermal properties of hempseed oil during ultrasound-assisted extraction: a comparative study. J Food Process Preserv. 42(10):e13766. doi: 10.1111/jfpp.13766.

Rubio LA. 2019. Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. Poult Sci. 98(2):695–706. doi: 10.3382/ps/pey416.

Shantha NC, Decker EA. 1994. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J AOAC Int. 77(2):421–424. doi: 10.1093/jaoac/77.2.421.

Shehata AA, Attia Y, Khafaga AF, Farooq MZ, El-Seedi HR, Eisenreich W, Tellez-Isaias G. 2022. Restoring healthy gut microbiome in poultry using alternative feed additives with particular attention to phytogenic substances: challenges and prospects. Ger J Vet Res. 2(3):32–42. doi: 10.51585/gjvr.2022.3.0047.

Siano F, Moccia S, Picariello G, Russo GL, Sorrentino G, Di Stasio M, La Cara F, Volpe MG. 2018. Comparative study of chemical, biochemical characteristic and ATR-FTIR analysis of seeds, oil and flour of the edible Fedora cultivar hemp (Cannabis sativa L.). Molecules. 24(1):83. doi: 10.3390/molecules24010083.

Skiba G, Poławska E, Sobol M, Raj S, Weremko D. 2015. Omega-6 and omega-3 fatty acids metabolism pathways in the body of pigs fed diets with different sources of fatty acids. Arch Anim Nutr. 69(1):1–16. doi: 10.1080/1745039X.2014.992173.

Skřivan M, Englmaierová M, Taubner T, Skřivanová E. 2020. Effects of dietary hemp seed and flaxseed on growth performance, meat fatty acid compositions, liver tocopherol concentration and bone strength of cockerels. Animals. 10(3):458. doi: 10.3390/ani10030458.

Sprecher H. 2000. Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochim Biophys Acta. 1486(2–3):219–231. doi: 10.1016/s1388-1981(00)00077-9.

Stambouli H, El Bouri A, Bouayoun T, Bellimam A. 2006. Seed oil characterization of Cannabis sativa L. cultivated in Northern Morocco. Ann Toxicol Anal. 18(2):119–125. doi: 10.1051/ata:2006004.

Šťastník O, Jůzl M, Karásek F, Fernandová D, Mrkvicová E, Pavlata L, Nedomová Š, Vyhnánek T, Trojan V, Doležal P. 2019. The effect of hempseed expellers on selected quality indicators of broiler chicken's meat. Acta Vet Brno. 88(1):121–128. doi: 10.2754/avb201988010121.

Stastnik O, Karasek F, Stenclová H, Trojan V, Vyhnanek T, Pavlata L, Mrkvicova E. 2015. The effect of hempseed cakes on broiler chickens performance parameters. MendelNet 2015: Proceedings of International PhD Students Conference. Brno. p. 157–160.

Stillhart C, Vučićević K, Augustijns P, Basit AW, Batchelor H, Flanagan TR, Gesquiere I, Greupink R, Keszthelyi D, Koskinen M. 2020. Impact of gastrointestinal physiology on drug absorption in special populations–an UNGAP review. Eur J Pharm Sci. 147:10528.

Sugiharto S. 2016. Role of nutraceuticals in gut health and growth performance of poultry. J Saudi Soc Agric Sci. 15(2):99–111. doi: 10.1016/j.jssas.2014.06.001.

Taaifi Y, Belhaj K, Mansouri F, Rbah Y, Melhaoui R, Houmy N, Ben Moumen A, Azeroual E, Addi M, Elamrani A, et al.

2023. The effect of feeding laying hens with nonindustrial hemp seed on the fatty acid profile, cholesterol level, and tocopherol composition of egg yolk. Int J Food Sci. 2023:1360276–1360215. doi: 10.1155/2023/1360276.

Taubner T, Skřivan M, Englmaierová M, Malá L. 2023. Effects of hemp seed and flaxseed on enzyme activity in the broiler chicken digestive tract. Animal. 17(4):100765. doi: 10.1016/j.animal.2023.100765.

Thanabalan A, Kiarie EG. 2021. Influence of feeding omega-3 polyunsaturated fatty acids to broiler breeders on indices of immunocompetence, gastrointestinal, and skeletal development in broiler chickens. Front Vet Sci. 8:653152. doi: 10.3389/fvets.2021.653152.

Tufarelli V, Passantino L, Zupa R, Crupi P, Laudadio V. 2022. Suitability of dried olive pulp in slow-growing broilers: performance, meat quality, oxidation products, and intestinal mucosa features. Poult Sci. 101(12):102230. doi: 10.1016/j.psj.2022.102230.

Turner N, Cooney GJ, Kraegen EW, Bruce CR. 2014. Fatty acid metabolism, energy expenditure and insulin resistance in muscle. J Endocrinol. 220(2):T61–T79. doi: 10.1530/JOE-13-0397.

Ulbricht TLV, Southgate DAT. 1991. Coronary heart disease: seven dietary factors. Lancet. 338(8773):985–992. doi: 10.1016/0140-6736(91)91846-m.

Van Der Klis JD, Fledderus J. 2007. Evaluation of raw materials for poultry: what's up? Proceedings of the 16th European Symposium on Poultry Nutrition. Strasbourg, France: World Poultry Science Association.

Vercellotti JR, St. Angelo AJ, Spanier AM. 1992. Lipid oxidation in foods. In: Lipid Oxidation in Food. Vol. 500. Washington, DC: American Chemical Society; p. 1–11.

Vispute MM, Sharma D, Mandal AB, Rokade JJ, Tyagi PK, Yadav AS. 2019. Effect of dietary supplementation of hemp (*Cannabis sativa*) and dill seed (*Anethum graveolens*) on performance, serum biochemicals and gut health of broiler chickens. J Anim Physiol Anim Nutr (Berl). 103(2):525–533. doi: 10.1111/jpn.13052.

Wang Q, Xiong YK. 2019. Processing, nutrition, and functionality of hempseed protein: a review. Compr Rev Food Sci Food Saf. 18(4):936–952. doi: 10.1111/1541-4337.12450.

Wang XS, Tang CH, Yang XQ, Gao WR. 2008. Characterization, amino acid composition and in vitro digestibility of hemp (Cannabis sativa L.) proteins. Food Chem. 107(1):11–18. doi: 10.1016/j.foodchem.2007.06.064.

WHO. 2003. Diet, nutrition and the prevention of chronic diseases: Report of the joint WHO/FAO expert consultation. WHO Technical Report Series, No. 916 (TRS 916). http://www.who.int/dietphysicalactivity/publication.

Wu YB, Ravindran V, Thomas DG, Birtles MJ, Hendriks WH. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Br Poult Sci. 45(1):76–84. doi: 10.1080/00071660410001668897.

Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora, and morphology of male broilers. Poult Sci. 82(6):1030–1036. doi: 10.1093/ps/82.6.1030.