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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*

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The objective of the study was to investigate the effects of using increasing levels of hemp seed oil (HSO) instead of soybean oil (SO) in broiler diets during the first 21 d of the starting period on growth, meat and serum parameters and the fatty acid profile of abdominal fat. A total of 200 one-day-old broiler chicks (Ross 308) were allocated to 4 dietary groups having different levels of HSO as 5 replicates. Dietary groups included the control (basal diet and 100% SO), HOG1 (basal diet and 25% HSO+75% SO), HOG2 (basal diet and 50% HSO+50% SO), and HOG3 (basal diet and 100% HSO). Results showed that each level of HSO in the diet significantly suppressed growth when compared to the control group (P<0.05) with the worst performance observed in HOG3 (P<0.05). Dietary HSO did not affect meat quality and serum parameters. However, HSO prevented meat oxidation and thiobarbituric acid reactive substances (TBARS) concentration in the 1st d of storage was significantly low in all the HSO groups and in the 7th d of storage only in the HOG3 group (P<0.05). The abdominal fat profile was modulated by dietary HSO, with the highest α-linolenic acid (ALA) was detected in the HOG3 group (P<0.05). ΣMUFA (total monounsaturated fatty acid) and ΣPUFA (total polyunsaturated fatty acid) contents of abdominal fat changed depending on the level of HSO in the diet. Consequently, despite the advantageous effects of HSO on abdominal fatty acids and meat oxidation its levels added to the diet in the current study were not suitable for broiler chickens at an early age.

KEYWORDS: malondialdehyde concentration / meat colour/ fatty acid profile/ soy oil

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For thousands of years seeds of hemp, a plant from the Cannabaceae family (*Cannabis sativa L.*), have been an important source of nutrients in Old World cultures [Callaway 2004] with hempseed oil (HSO) being one of the major components that account for about 35% of the seed [Liang *et al.* 2015]. Depending on the variety HSO may contain up to 80% PUFA (polyunsaturated fatty acids), while linoleic acid and α-linolenic acid contents may reach up to 60% and 19%, respectively [Klir *et al.* 2019]. Also, HSO contains minor bioactive compounds including Vitamin E, polyphenols, carotenoids, phytosterols, other vitamins and dietary minerals that contribute to the nutritional values of the oil [Callaway *et al.* 2005, Liang *et al.* 2015, Klir *et al.* 2019]. Despite the confirmed nutritional value of HSO, it is rarely preferred as a food material by humans due to concerns related to its tetrahydrocannabinol (THC) and cannabinoid contents. However, cultivars of *Cannabis sativa* that can be used in Europe for seed production are those with a THC level below 0.2% [Citti *et al.* 2018]. As a result, THC contamination in hemp seed oil is generally extremely low and only exceptionally it exceeds the limit of 5 mg/kg (i.e. the maximum THC limit in food imposed by the German legislation in 2000) [Grotenhermen *et al.* 1998, Lachenmeier and Walch 2006, Sarmento *et al.* 2015]. Also, pigments and free fatty acids present in crude HSO act as pro-oxidants and can accelerate oil oxidation, thus deteriorating oil quality [Xu *et al.* 2021]. Despite the concerns related to cannabinoids and the pro-oxidant content of HSO, this by-product seems to have the potential to be a lipid source for livestock. The advantageous nutrient composition of HSO indicates that it may represent a potentially useful feed ingredient for poultry [Jing *et al.* 2017]. Previous studies reported that hempseed (HS) and its by-products including HSO and hempseed cake (HC) may be used in diets for laying poultry and may enrich the fatty acid profile of the egg yolk [Bazdidi *et al.* 2016, Klir *et al.* 2019]. On the other hand, HSO has rarely been investigated as a dietary lipid source for broiler chickens. Broiler chicks in the growing period need high metabolic energy and diets enriched with lipid sources. For this purpose, different types of oils and fats are used in broiler diets, including seed-based oils that have high PUFA contents such as Soybean oil (SO) [Baião and Lara 2005]. Moreover, dietary lipids can directly influence the lipid profile of chicken meat and therefore the fatty acid profile of broilers' diet may improve the nutritional quality of broiler meat [Rasool 2018]. Additionally, dietary antioxidants such as tocopherols and polyphenols may reduce thiobarbituric acid reactive substances (TBARS) in tissues and may improve functional properties of broiler meat [Mir *et al.* 2017]. It is reported that HSO contains higher phenolic contents than SO, with the total amount of tocopherols in HSO ranging from 80 to 150 mg/100 g oil and the phenolic content in HSO evaluated as gallic acid equivalents of 44-188 mg/100 g oil [Liang *et al.* 2015] All the knowledge regarding the micronutrient composition of HSO indicates that this valuable by-product may be an alternative to SO in broiler diets. However, due to the legal status of hemp in many countries [Silversides and Lefrancois 2005] it may not be evaluated as a feed additive. Nevertheless, several countries have recently changed their regulations to allow the cultivation of *Cannabis sativa* hemp varieties [Fabro *et*

al. 2021]. The recent legalisation concerning hemp cultivation has led to an increase in research on the use of hemp products in animal nutrition. Studies have focused on improving the fatty acid profile of eggs using hemp products in diets for laying poultry [Goldberg *et al.* 2012,Göçmen *et al.* 2021] and researches on the use of hemp in the growth period of poultry have been scarce. The novelty of this study was to demonstrate the applicability of HSO as a substitute for SBO as an energy source in broiler diets and to investigate the effects on growth, biochemical blood components and meat characteristics at an early age. Therefore, the current study was designed to examine the effect of including three different levels of HSO in the diets of broiler chickens in the starting period on growth performance. The secondary objective of the study was to determine the influences of increasing levels of HSO instead of SO in broiler diets on meat oxidation, meat properties, serum biochemicals and the fatty acid profile of abdominal fat. To accomplish these objectives the experiment involving broiler chickens was conducted and the results from this study will provide data on safety and efficacy claims for HSO as a feed ingredient in broiler rations fed at an early age.

Material and methods

The animal experiment was conducted at the Poultry Unit of Prof. Dr. Orhan Düzgüneş Application and Research Farm Facility of the Selcuk University Agriculture Faculty. The animal experiment was carried out according to the local ethics committee directives of the Selcuk University that were prepared according to Directive 2010/63/EU is the European Union (EU) legislation. All the procedures in this study complied with the ethical principles of animal rights. In this study a total of 200 one-day-old broiler chicks (Ross 308) without sex discrimination were used and placed randomly in four dietary treatment groups with 5 replicates as to comprise 10 chicks per compartment. Animals were reared in cages with full control heating and lighting systems, the manger and tray nipple drinkers. In the 21 days of the starting-period experiment, a 23 h dark-1 h light illumination program was applied and feed and water were provided *ad libitum*. The cage ambient temperature was 32°C for the first three days, then it was gradually decreased by 0.5°C per day until the end of the trial and the final day temperature was 23°C**.** Diets were formulated to meet the nutrient requirements of broiler chickens for the first 21d starting period according to NRC [1994] as isocaloric and isonitrogenous. The feeds were prepared one week before the experiment was started and were conserved in a controlled dark storage area below room temperature (18-20°C) during the trial. The ingredients and nutrient composition of experimental diets are summarized in Table 1. Feed ingredients and crude soybean oil were purchased from a local commercial feed factory (Çöğenler Yem San. Tic. Ltd. Şti, Konya-Turkey). Hemp (*Cannabis sativa*) seeds were provided by a local supplier and crude oil was obtained by the cold pressing method (at about 45-50°C) from seeds of hemp using an expeller device (Karaerler Machine, NF 100 model, Ankara). Dietary groups were formulated according to oil type and level in total supplemental oil of the diet and named as the control group

that contained 100% crude soybean oil, hemp seed oil group 1 (HOG1) that contained 25% crude hempseed – 75% crude soybean oil, hemp seed oil group 2 (HOG2) that contained 50% crude hempseed – 50% crude soybean oil, and hempseed oil group

Table 1. The nutrient composition of experimental diets

Item	Experimental dietary groups			
	Control	HOG1	HOG2	HOG3
Ingredients				
Corn (%)	51.30	51.30	51.30	51.30
Soybean meal (47% CP)	38.80	38.80	38.80	38.80
Soybean oil (8800 kcal ME/kg)	6.10	4.88	3.05	0.00
Hempseed oil (8800 kcal ME/kg)	0.00	1.22	3.05	6.10
Limestone (%)	1.00	1.00	1.00	1.00
Dicalcium phosphate (%)	2.10	2.10	2.10	2.10
Salt (%)	0.30	0.30	0.30	0.30
Vitamin-mineral premix (%)[1]	0.25	0.25	0.25	0.25
L-Lysine (%)	0.02	0.02	0.02	0.02
DL-Methionine (%)	0.13	0.13	0.13	0.13
Nutrients				
Crude Protein (%)	22.00	22.00	22.00	22.00
Metabolizable energy (kcal/kg)	3100	3100	3100	3100
Calcium (%)	0.80	0.80	0.80	0.80
Available phosphorus (%)	0.30	0.30	0.30	0.30
Lysine (%)	1.30	1.30	1.30	1.30
Methionine (%)	0.50	0.50	0.50	0.50
Methionine + Cysteine (%)	0.80	0.80	0.80	0.80

Control – 100% soybean oil, HOG 1 – 75% soybean oil-25% hempseed oil, HOG2 – 50% soybean oil-50% hempseed oil, HOG3 – 100% hempseed oil.
[1]Vitamin-mineral premix (per kilogram of diet) – Vitamin A 15000 IU; Vitamin D3 1500 IU; Vitamin K 5 mg; Vitamin B1 3 mg; Vitamin B2 6 mg; Vitamin B6 5 mg; Vitamin B12 0.03 mg; Niacin 30 mg; Biotin 0.1 mg; calcium D-panthotenate 12.0 mg; folic acid 1.0 mg; coline chloride 400 mg; Manganese 80 mg; Iron 35 mg; Zinc 50 mg; Copper 5.0 mg; Iodine 2 mg; Cobalt 0.04 mg.

Table 2. Major fatty acids of dietary oils

Fatty acids (%)	Soybean oil	Hempseed oil
Palmitic 16:0	7.47	7.70
Stearic 18:0	3.00	3.55
Oleic 18:1	17.09	17.14
Linoleic 18:2	56.19	55.22
α-Linolenic 18:3	15.79	15.89
Arachidic 20:0	0.46	0.50
ΣSFA	10.92	12.12
ΣMUFA	17.10	16.80
ΣPUFA	71.98	71.11
Σn3	15.79	15.89
Σn6	56.19	55.22

ΣSFA – total saturated fatty acids, ΣMUFA – total unsaturated fatty acids, PUFA – total polyunsaturated fatty acids, Σn3 – total omega 3 fatty acids, Σn6 – total omega 6 fatty acids.

3 (HOG3) that contained 100 % crude hempseed oil. The fatty acid composition of dietary supplemental oils was determined and presented in Table 2.

Body weight (BW) of chicks in each replicate was determined at 1, 7, 14, and 21 days with group weighing. Weight gain (WG) was calculated as the difference between final and initial BW. Feed intake (FI) was calculated from the difference between supplied feed weight and remaining feed weight, which was then divided by the number of chicks in each replicate. The feed conversion ratio (FCR) was calculated for the starter period via [FI (g/bird)/weight gain (g/bird)] [Omar *et al.* 2021]. At the end of the experiment 15 chicks from each group, a total of 60 animals, were killed by cervical dislocation [Leary *et al.* 2013] and de-feathered, eviscerated and weighed, after which dressing percentages were determined, the carcasses and livers were weighed and the data were recorded. Carcass yield (CY) was calculated from a ratio of live body weights and carcass weight. After slaughter carcasses were stored for 24 hours at 4°C and then breast muscles were split for analysis. The pH value of breast meat was determined using a portable pH meter (WTW 2A20-1012 Waterproof pH-Meter) [Horwitz and Latimer 2000] and colour values (L*, a*, b*) were detected with the use of a Minolta Chroma Meter CR 400 (Minolta Co., Osaka, Japan) [Hunt *et al.* 1991]. The L*, a*, and b* parameters correspond to lightness (-100/+100, dark/white), redness (-100/+100, green/red) and yellowness (-100/+100, blue/yellow), respectively. A 2-g meat sample was centrifuged (4000 rpm-10 min) inside the tared filter paper to determine water holding capacity (WHC) and the amount of water which was retained in the filter [Gómez-Guillén *et al.* 2000].

To determine the MDA amount, 1g gram of an abdominal fat sample (W) was ground and placed in a 25mL test tube and supplemented with 5 mL solvent (100% glacial acetic acid), shaken for 1 h in a water bath shaker and centrifuged at 1000 rpm for 5 min and filtered. The extract (1 mL) was collected in a 10mL test tube and mixed with the thiobarbituric acid reagent (1mL). Afterwards the 2 mL mixture (V) was heated in a boiling water bath at 95°C for 60 min. The test tubes were cooled to room temperature and absorbance was measured (Ac) at 532nm using a UV-visible spectrophotometer and calculated as TBARS (μM/g) = (Ac × V)/W [Zeb and Ullah 2016].

At the end of the trial, a total of 60 with 3 chickens from each replicate were slaughtered and 5 mL of blood was drawn from the jugular vein into tubes. The serum was obtained by centrifugation of the blood at 3500 rpm for 5 min. Serum cholesterol, triglyceride, AST and ALT enzyme levels were determined by the photometric method using the Abbot C8000 device (USA).

Fatty acid methyl esters (FAMEs) of fats and oils were prepared according to the methods recommended by the European Union (EU) regulation 2568/91. FAMEs of soybean and hempseed oils were obtained using hexane-based methods [Ayyildiz *et al.* 2015]. Oils were weighed (0.10 g) into screw-cap glass tubes and dissolved in 10.0 mL hexane. Then, 100 μL 2N potassium hydroxide solutions in methanol were added to the tubes and manually shaken vigorously for 30 s. The tubes were centrifuged at 2500 × g for 5 min and the upper layer was removed to a small vial and stored at 0°C

until the date of analysis. FAMEs of abdominal fat were obtained using the BF_3 (Boron trifluoride) method [Metcalfe and Schmitz 1961]. For this purpose 0.15 g non-dried abdominal fat was ground and weighed in an Erlenmeyer flask, then 5 mL %2 NaOH: methanol solution were added, heated to the boiling point and boiled for 10 minutes. Afterwards 5mL BF_3 were added and manually shaken, then supplemented with 5 mL n-Hexane and cooled to room temperature. The obtained mixture was transferred to a 25 mL flask and completed with a saturated NaCl solution and manually shaken at least 10 times. The upper phase was collected to vials as methyl ester for gas chromatographic determination.

The fatty acid composition of FAMEs was detected by gas chromatography (GC; Shimadzu GC-2010 Plus 86, Japan) with an FID detector and an HP-88 column (100 m x 250 μm x 0.20 μm id). The temperatures of the GC-MS injection block and FID were 250°C and 280°C, respectively, and the set-point for the oven was 140°C. The heating programme was used for the fatty acid analysis. The initial temperature was 140°C for 5 min, then it was increased to 240°C at a rate of 4°C/min and held for 15 min and the final temperature was 40°C for 5 min. The carrier gas used was He (Helium) with a 1.3 mL/min flow rate. Fatty acids were identified by comparison of the retention time (min) and area (%) of determined peaks and classified with known FAME standards (Supelco® 37 Component FAME Mix, Merck- Germany) and presented as percentages. The experiment was designed as a complete randomized model. All the data from the experiment were examined using the homogeneity variance Bartlett test and then one-way analysis of variance was applied. Duncan's multiple range test was used to determine the differences between treatments that were found to be significantly different ($P<0.05$). The computations were performed using the Minitab software [Minitab 2000].

Results and discussion

The effects of increasing HSO levels in the diet instead of SBO on performance parameters in the first 21-d growth period of broiler chickens were summarized in Table 3. Except for SBW and CY, all the performance parameters were affected negatively by dietary HSO and the depressing impact of HSO on growth performance was increased depending on the additional levels of HSO in the diet ($P<0.05$). FBW, WG, FI, and CW significantly decreased with the increase in the HSO inclusion level in the diet, with the highest values found in the control group and the lowest results detected in the HOG3 group ($P<0.05$). The lowest FCR was found in HOG2 and HOG3 and even a 25% HSO inclusion in the diet caused lower FCR compared to the control group ($P<0.05$). LW was not affected by the dietary HSO up to the 50% addition level; however, when dietary oil was 100% HSO, LW was significantly reduced ($P<0.05$).

Kalmendal [2008] reported that HSC (hempseed cake), which is a by-product of HS (hempseed) and contains 11% fat, may be included in broiler diets up to 30%

Table 3. The effect of different levels of dietary HSO on performance parameters in broiler chickens

Item	Control	HOG1	HOG2	HOG3	P Value
SBW	43.21±0.91	44.44±0.39	43.89±0.66	44.53±0.65	0.064
FBW	847.2±32.73^{a}	710.0±23.65^{b}	658.0±7.30^{b}	541.2±34.25^{c}	0.000
WG	804.0±33.00^{a}	665.5±23.39^{b}	614.1±7.77^{b}	496.7±34.35^{c}	0.000
FI	1118±86.5^{a}	855.6±42.8^{b}	705.7±14.0^{c}	543.6±17.2^{d}	0.000
FCR	1.40±0.057^{a}	1.29±0.044^{b}	1.15±0.013^{c}	1.10±0.06^{c}	0.000
CW	530.7±22.02^{a}	443.0±16.53^{b}	404.5±6.45^{c}	336.5±19.67^{d}	0.000
LW	14.25±1.10^{a}	12.33±1.19^{a}	12.33±1.47^{a}	9.42±1.43^{b}	0.002
CY	62.64±0.51	62.40±1.11	61.48±1.27	62.19±0.85	0.410

^{abc}Differences between means in the same line are significant when marked with different letters ($P<0.05$).
Control – 100% soybean oil; HOG 1 – 75% soybean oil-25% hempseed oil; HOG2 – 50% soybean oil-50% hempseed oil; HOG3 – 100% hempseed oil; IBW – initial body weight; FBW – final body weight; WG – weight gain; FI – feed intake; FCR – feed conversion ratio; CW – carcass weight; LW – liver weight; CY – carcass yield.
Except for FCR, data for all the parameters are presented in grams.

replacement of soybean meal without adverse effects on performance parameters. Studies that employed HSO as a lipid source in the broiler diet are limited and contrarily to the current study, they showed no growth depressing effects. For example, Jing *et al.* [2017] stated that the introduction of HSO instead of corn oil at the level of 3 and 6 % to the diets for broiler chickens did not have any effect on the overall performance of the birds in the period of 0-21 days. Another comprehensive study [Rasool 2018] that used HS, HSC, and HSO in broiler diet showed that none of the hemp products caused detrimental effects on broiler performance. However, some literature sources presented results confirming that feeding broiler chickens with HS by-products containing different levels of HSO resulted in poor performance. Šťastník *et al.* [2019] studied the effects of two levels of HSE (hempseed expeller) addition (5 and 15%) in broiler diets and reported that dietary HSE at the level of 15% decreased live weight of broilers compared to the control diet (no HSE), while carcass weight was not significantly different. In a similar study [Stastnik *et al.* 2015], broiler chickens were fed with HSC added to the diets at 0, 5, and 15 % up to 37 days of age and results showed that both levels of HSC decreased the final live weight compared to the non-HSC diet, while carcass yield differed non-significantly between the groups. Mahmoudi *et al.* [2015] investigated the effects of different dietary levels (0, 25, 50, and 75 g/kg) of HS (containing 35% crude fat) on broiler performance and results indicated that diets containing 25 g/kg HS caused a significant decrease in average daily feed intake and average daily gain. In this study LW also decreased significantly with 100% HSO in diet, which was consistent with another study reporting a similar effect of HS, in which 5, 10 and 15 % whole HS addition to diet caused lower LW of quails compared to the non-HS group [Konca *et al.* 2014]. Similarly as in the current study, our former study which used HSO instead of SO in growing quail diets showed that dietary HSO in poultry fattening induced unsatisfactory growth performance of chicks [Göçmen and

Kanbur 2021]. In the current study, with the increased level of additional HSO in diet, FI decreased dramatically and this situation caused WG and BW to fall and consequently led to low carcass weight. On the other hand, low FCR in the HSO groups was also related to low FI and this should not be evaluated because of good feed utilization. The consequences of this study indicate that HSO may contain an antinutritional factor that reduces feed consumption and has a detrimental effect on the growth of broilers. However, concentrations of antinutritional components including phytic acid, tannins and trypsin inhibitors are relatively low even in the whole hemp seed [Wang and Xiong 2019, Xu *et al.* 2021]. The reduction in FI of the chickens may indicate THC presence in HSO, but it was reported that THC contamination in HSO is generally extremely low. It exceptionally exceeds the limit of 5 mg/kg [Citti *et al.* 2018] and also even *Cannabis* plants themselves have a low level of THC, insufficient to induce intoxication [Xu *et al.* 2021]. High-level PUFA in HSO is beneficial for health, but there is a considerable risk of peroxide and trans-fat formation and high-level unsaturation provides more opportunity for oxidation with atmospheric oxygen [Callaway 2010]. Also, it has been reported that high PUFA lipids in broiler diets may cause fatty acid β-oxidation and may decrease fat deposition [Fouad and El-Senousey 2014]. Eventually, our understanding is inadequate to explain the adverse effects of dietary HSO on growth performance of broiler chickens. Overall, the observed negative results on the performance parameters could be related to the physiological and developmental conditions of the birds in early age. The effects of incorporation of different HSO levels to the broiler diet in the starting period on meat oxidation, WHC, meat colour and pH values are presented in Table 4. The meat pH and WHC results were similar between the dietary groups. The HSO in the diet did not affect either L* or a* colour values regardless of the supplemental levels, whereas 100% HSO oil addition to the diet caused a lower b* value in meat ($P<0.05$). Dietary HSO significantly altered the MDA amount of meat both on the 1st and 7th day of storage ($P<0.05$). On the 1st day of meat storage MDA was significantly high in the control compared to the HOG groups and the presence of HSO in the diet decreased MDA of meat on the 1st day of the storage ($P<0.05$). The lowest MDA values in meat

Table 4. The effect of different levels of dietary HSO on meat oxidation, pH, colour and WHC in broiler chickens

Item		Control	HOG1	HOG2	HOG3	P Value
Meat oxidation (μ	MDA 1st	0.18±0.01^{a}	0.12±0.01^{c}	0.13±0.01^{c}	0.15±0.01^{b}	0.000
	MDA 7th	0.18±0.01^{bc}	0.97±0.18^{a}	0.37±0.01^{b}	0.16±0.00^{c}	0.000
	WHC	16.99±1.32	17.42±3.81	18.80±1.62	14.12±1.16	0.069
Colour values	L*	49.80±9.76	52.19±1.85	49.59±0.48	48.90±2.43	0.816
	a*	1.20±0.64	1.23±0.82	0.99±0.52	1.05±0.37	0.931
	b*	4.63±1.56^{a}	3.53±0.27^{ab}	2.69±0.62^{ab}	1.68±1.00^{b}	0.008
	pH	5.67±0.14	5.61±0.07	5.73±0.13	5.69±0.10	0.518

^{abc}Differences between means in the same line are significant when marked with different letters ($P<0.05$). Control – 100% soybean oil; HOG 1 – 75% soybean oil-25% hempseed oil; HOG2 – 50% soybean oil-50% hempseed oil; HOG3 – 100% hempseed oil; MDA 1st – malondialdehyde concentration in the 1st day of storage; MDA 7th – malondialdehyde concentration in the 1st day of storage; WHC – water holding capacity.

were detected in HGO1 and HOG2 ($P<0.05$). However, on the 7th day of storage the effects of HSO levels in diet on MDA amount differed from those of the 1st day. The lowest MDA values were determined in HOG3 followed by the control and HOG2 on the 7th day of storage, while the greatest meat oxidation was detected in HOG1 ($P<0.05$).

The pH value and WHC are meat quality indicators and at 24 h post-mortem the ultimate pH of chicken meat falls to about 5.4-5.7 [Tougan *et al.* 2013]. The breast meat pH values in the current study were in this range (Table 4.) and were not affected by the HSO presence in the diet. The data on the effects of dietary HSO on chicken meat quality is inadequate; however, studies that used HS in quail diets or HSE in broiler diets reported no changes in the pH value of breast meat [Yalcin *et al.* 2018, Šťastník *et al.* 2019]. Results showed no effect of dietary HSO on WHC of breast meat and despite a slight decrease of WHC in HOG3 the differences were non-significant. The chicken meat colour may instantly affect consumers' preferences and the availability of lipid-soluble pigments such as carotenoids in the feedstuff determine the extent of skin pigmentation [Northcutt 2009, Mir *et al.* 2017]. HSO contains carotenoids and is also a rich source of chlorophylls that cause the colour change of the oil from dark green to yellow [Liang *et al.* 2015]. In this study with the introduction of HSO to the diet the b* value of breast meat fell significantly, while 100 % HSO in the diet changed meat colour from yellow to blue. The MDA is one of the most abundant aldehydes generated during secondary lipid oxidation and is used as an oxidation marker [Barriuso *et al.* 2013]. As seen in Table 4, the MDA amount of meat significantly fell with all levels of HSO in the diet on the 1st day of storage, while the lowest MDA value on the 7th day was found in HOG3. Previous studies reported that the use of whole HS in the diet decreased blood MDA concentration in quails [Konca *et al.* 2014] and HS usage in broiler diets caused a reduction of lipid peroxidation in meat [Vispute *et al.* 2021]. HS or its by-products such as HSO may display high antioxidant capacity, because they contain minor components such as tocopherols and polyphenols that have strong antioxidant properties [Liang *et al.* 2015]. These antioxidative effects of minor components in dietary HSO affect meat MDA concentration.

The effects of different levels of dietary HSO on broilers' serum parameters are summarized in Table 5. None of the dietary HSO addition levels affected the serum parameters, and differences in serum cholesterol, triglyceride and liver enzymes AST and ALT concentrations were statistically non-significant.

Table 5. The effect of different levels of dietary HSO on serum parameters in broiler chickens

Item	Control	HOG1	HOG2	HOG3	P Value
Triglyceride (mg/dL)	43.59±6.18	36.16±3.32	37.50±4.04	41.75±4.57	0.133
Cholesterol (mg/dL)	144.0±22.2	127.2±6.29	147.7±13.45	167.5±26.15	0.066
AST (U/L)	204.7±15.59	237.0±26.80	253.2±51.36	219.7±23.47	0.219
ALT (U/L)	162.3±28.60	208.0±56.80	208.3±16.50	191.5±42.00	0.341

Control – 100% soybean oil; HOG 1 – 75% soybean oil-25% hempseed oil; HOG2 – 50% soybean oil-50% hempseed oil; HOG3 – 100% hempseed oil; AST – aspartate transaminase; ALT – alanine aminotransferase.

Numerous studies have demonstrated that intake of phytosterols lowers low-density lipoprotein (LDL) levels in human blood [Dunford 2020]. Previous knowledge regarding the high PUFA content and phytosterol capacity of HSO [Liang *et al.* 2015] has caused expectations regarding HSO to reduce serum total cholesterol and triglyceride levels of poultry. Moreover, it was stated that using PUFAs in poultry diets significantly reduces cholesterol and total lipid contents in the blood [Alagawany *et al.* 2019]. Similarly to the current study, Konca *et al.* [2014] stated that the whole HS in diets of laying quails (50, 100 and 200 g HS/kg feed) did not affect serum total cholesterol and triglyceride levels. Another study reported no changes in serum AST and cholesterol levels of laying hens that consumed 4.5 and 9% HSO oil with the diet [Neijat *et al.* 2014]. Any type of liver cell injury can reasonably increase ALT levels, while in toxin-induced liver damage the level of this enzyme rises greatly. AST levels are elevated in the case of tissue necrosis and also with chronic liver diseases [Gowda et al. 2009]. According to performance outcomes of the current study, it may be assumed that a toxic status occurred, which may affect liver tissue or its enzymes. However, ALT and AST enzyme levels between the dietary groups were similar and revealed that dietary HSO had no effect on liver secretions.

The effects of the inclusion of different HSO levels to the diet on the abdominal fat fatty acid profile in broiler chickens are presented in Table 6. Levels of myristic, palmitoleic and stearic acids in abdominal fat were not changed with the inclusion of HSO in the diet. On the other hand, palmitic acid content decreased in all the three HOG groups compared to the control ($P<0.05$). Concentrations of oleic and linoleic acids changed depending on the level of HSO in the diet, as seen in Table 6. Amounts of these two fatty acids were replaced with the inclusion of different HSO levels in the diet. The highest oleic acid content was found in the control and HOG2, while the highest linoleic acid content was determined in HOG1 and HOG3 ($P<0.05$). The amount of α-linolenic acid increased with the HSO inclusion level in the diet and HOG3 caused the richest ALA content in abdominal fat ($P<0.05$). ΣSFA content

Table 6. The effect of different levels of dietary HSO on abdominal fat fatty acid profile in broiler chickens

Fatty acids (%)	Control	HOG1	HOG2	HOG3	P Value
Myristic acid (14:0)	0.361±0.012	0.393± 0.071	0.397±0.063	0.421± 0.105	0.701
Palmitic acid (16:0)	24.10±1.14^{a}	19.96±0.71^{b}	23.37±2.07^{ab}	22.78±2.46^{ab}	0.028
Palmitoleic acid (16:1)	3.80±0.98	1.71±0.73	3.34±1.20	3.23±1.37	0.086
Stearic acid (18:0)	6.43±0.63	7.30±0.75	6.85±0.75	7.56±0.95	0.229
Oleic acid (18:1-n9)	37.56±1.08^{a}	29.84±0.48^{b}	34.36±2.64^{a}	29.28±1.06^{b}	0.000
Linoleic acid (18:2-n6)	26.33±2.00^{b}	37.39±1.49^{a}	29.59±2.48^{b}	31.88±4.28^{a}	0.00
α-Linolenic acid (18:3-n3)	1.42±0.10^{c}	3.42 ±0.37^{b}	2.81±0.51^{b}	4.85±0.47^{a}	0.00
ΣSFA	30.89±1.71	27.65±0.94	30.61±2.45	30.76±2.41	0.11
ΣMUFA	41.36±0.77^{a}	31.55±1.17^{b}	37.75±3.65^{a}	32.51±1.61^{b}	0.00
ΣPUFA	27.75±1.97^{c}	40.81±1.29^{a}	32.39±2.82^{bc}	36.73±3.95^{ab}	0.00

^{abc}Differences between means in the same line are significant when marked with different letters ($P<0.05$)., Control – 100% soybean oil; HOG 1 – 75% soybean oil-25% hempseed oil; HOG2 – 50% soybean oil-50% hempseed oil; HOG3 – 100% hempseed oil; ΣSFA – total saturated fatty acids; ΣMUFA – total unsaturated fatty acids; ΣPUFA – total polyunsaturated fatty acids.

of abdominal fat was not affected by dietary HSO levels and results were similar between the dietary groups. However, in connection with oleic acid, ΣMUFA content showed differences between the treatment groups and 25% and 100% HSO addition to the diet instead of SO caused lower ΣMUFA contents in abdominal fat ($P<0.05$). ΣPUFA content of abdominal fat also increased with the HSO content in the diet and the highest values were found in HOG1 and HOG3, respectively, while 100% SO in the diet decreased ΣPUFA amount in abdominal fat ($P<0.05$).

Today, in view of knowledge on the relationship of dietary fatty acids with cardiovascular diseases in humans, studies on the modulation of the fatty acid profile in poultry meat have continued. It has been revealed that n-6 PUFA is predominant in the meat of poultry, whereas n-3 PUFA content is low, which is desirable for human health. To modify poultry meat toward high levels of PUFAs and n-3 fatty acids numerous studies have been conducted and conventional plant-based oils such as SO have been used in poultry fattening to provide high PUFA content in the diet. The abdominal fat tissue grows faster compared with other fat tissues in poultry and is considered to be a reliable parameter for judging total body fat content [Białek *et al.* 2021] It is well known that dietary fatty acid profiles are reflected in tissue fatty acid [Mir *et al.* 2017]. This study was conducted to investigate the effects of HSO in the diet of broiler chickens at an early age. At this age, even in normal growth, meat fat content is quite low. In this study the BW of animals was reduced and the fat content of meat was not adequate to properly determine the fatty acid composition of meat. Therefore, it was decided to determine the fatty acid composition of abdominal fat to investigate the effects of dietary HSO on fat tissue fatty acids. Seed-based oils such as HSO that have high PUFA contents may be a promising alternative to improve the fatty acid profile of poultry meat. In this study, both SO and HSO dietary oils contained a high PUFA content (Tab. 2.). However, HSO was more effective in the enrichment of the linoleic acid (n6) and ALA (n3) content of abdominal fat (Tab. 6). Consistently with our results, Jing *et al*. [2017] reported that with the 3 or 6% addition of HSO to the broiler diet the chicken tissue PUFA amount increased, while MUFA content decreased. Rasool [2018] stated that a 3, 6 and 9% HSO addition in broiler diets caused higher linoleic, α-linolenic, total n-3 and n-6 fatty acid concentrations in chicken tissues. Also, the outcomes of the current study showed that the supplemental level of HSO may have an effect on the fatty acid composition of abdominal fat, because 75% SO -25% HSO mixed dietary oil caused higher PUFA content in abdominal fat compared to the other groups. The results confirmed that dietary HSO had a greater effect than SO on the abdominal fat FA composition in broiler meat and it has a high potential to modulate the FA profile of poultry tissues.

Conclusion

Theoretically, HSO appears a remarkable lipid source for broiler feeding due to its superior fatty acid profile, antioxidant and micronutrient capacity. In this study, HSO use instead of SO in the diet prevented meat oxidation during 7-d storage and

improved the fatty acid profile of abdominal fat. However, performance results were unacceptable for broiler production and the results revealed that all the three levels of HSO (1.22, 3.05 and 6.10 %) in the diet to replace SO were not suitable for the first 21 d period of broiler chicken fattening. When considering the rich nutritional capacity of HSO, further research is required to determine the effects of HSO in broiler diets in the growing or finishing periods. Moreover, the antinutritional factors in HSO that negatively affect broiler performance at an early age are needed to be clarified with biochemical and physiological analyses.

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