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Research Article
Effects of Dietary Canola Seed with Rosemary Supplementation on Growth Performance, Lipid Oxidation and Meat Fatty Acid Composition of Broilers

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Abstract
Objective: This experiment was conducted to assess the effect of a diet containing full-fat canola seed and supplemented with rosemary leaves and vitamin C on broiler performance, carcass characteristics, meat fatty acid (FA) and lipid oxidation of broiler meat. **Methodology:** Two separate groups of 1 day old (d1) male broilers were fed from day 15-40 a diet containing 5% of canola seed and 27% of soybean meal as a control group. From days 40-56 chicks were allocated to three homogeneous groups. One group was fed a control diet without antioxidant, while the other two groups were fed with (canola seed supplemented with 10 g kg^{-1} of rosemary leaves) or 200 mg kg^{-1} of vitamin C. Birds were slaughtered at 56 days of age. After evisceration, thigh meat samples were separated, frozen at -20°C until to determine the fatty acid profile or stored at 4°C in the dark until to determine the lipid oxidation. **Results:** Results showed that the diet containing 5% of canola seed reduced (p<0.05) chicken growth by almost 4% at 35 and 40 days compared to the control diet. However, performance parameters of chicks were generally improved by the addition of rosemary leaves and vitamin C. The inclusion of canola seed increased (p<0.05) the concentration of omega-3 FA (2.14 and 1.79 against 0.85%) in meat, especially the proportion of α-linolenic acid (1.53 and 1.24 against 0.66%) and the polyunsaturated fatty acids: saturated fatty acids ratio and decreased widely the n-6: n-3 ratio. Dietary rosemary leaves were more effective (p<0.05) in inhibiting lipid oxidation of the thigh meat compared to vitamin C during storage at 4°C. **Conclusion:** These results indicate that the simultaneous use of rosemary and ground canola seed improves broilers performance and meat quality.

Key words: Broilers, canola seed, fatty acid, omega-3, antioxidants, rosemary leaves, vitamin C, lipid oxidation, meat quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The price of fat and oil is increasing due to increased demand from the biofuel industry and food sector and thus the costs of broiler feed have subsequently increased. Some broiler producers are including full-fat Canola Seed (CS) in broiler diets to reduce costs. Canola seed can contribute substantially more to the Metabolizable Energy (ME) than oil-extracted solvent or expeller canola meal. It contains approximately 400 g kg^{-1} oil and 210-230 g kg^{-1} protein[1] making it an attractive feed ingredient for broilers. Full-fat canola seed has a valuable amino acid composition, including high content of essential amino acids such as lysine, threonine, tryptophan and sulphur amino acids[2]. In addition, flaxseed (FS) and Canola Seed (CS) are the main sources of α-linolenic acid (ALA) of terrestrial origin. This fatty acid is the precursor for the synthesis of eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3), which play a major role in the control of cardiovascular diseases[3] and in neural and retinal development[4]. Consumption of n-3 PUFA has been shown to positively influence immune function, blood pressure, cholesterol and triglyceride levels, in addition to cardiovascular function in humans[5].

However, some reports indicated that the use of canola seed in high amount (175 g kg^{-1} of diet) decreased the growth performance of broiler chicks[6]. The lower energy digestibility of canola seed and subsequent poor growth performance depressing in broilers, is related to the oil covered by the cell wall polysaccharides in seed[7]. Also, increasing levels of polyunsaturated fatty acids in tissues by using CS lead to faster lipid oxidation, with a consequent quality loss and lower consumer acceptability.

So as to increase the growth performance of the birds various nutrients are to be incorporated in the diet. Studies have been conducted with various levels of vitamins like C and E. Several studies revealed a beneficial effect of vitamin C supplementation on live weight gains, meat protein[8,9] and on growth rate in stressed broilers[10-12]. Also, ascorbic acid (vitamin C) possesses antioxidant properties, although it can act as an antioxidant or as a prooxidant depending on the concentration, the presence of metal ions and the tocopherol content[13]. It decreases the lipid oxidation in meat of broiler chickens stored for some days[14].

Whereas, consumers and health authorities increasingly dictate that the use of synthetic food additives should be phased out and where possible, only natural products should be used. Therefore, the incorporation of herbs and herbal products in livestock feeds instead of chemical products in order to stimulate or promote the effective use of feed nutrients which result in more rapid gain, higher production and better feed efficiency is a desirable method. Moreover, herbs contain active substances that can improve digestion and metabolism and possess bacterial and immunostimulant action of animals[15]. Biologically, rosemary extract improved feed conversion efficiency of broilers fed diet supplemented with such herb[16]. Rosemary is currently a widely used aromatic and medicinal plant which has high amounts of flavonoids and phenolic acids[17] that have antioxidant capacities. The use of essential oils of rosemary (*Rosmarinus officinalis*) has been thoroughly investigated and a significant decrease of lipid oxidation in chicken meat has been reported by Chang *et al.*[18] and Lopez-Bote *et al.*[19]. Also, supplementing ground rosemary to the feed has been successful in this respect in turkeys[20] and broilers[21].

The aim of this study is to investigate the effect of replacing soybean meal with full-fat canola seed and to compare the effective feed supplementation of vitamin C and rosemary leaves on the growth performances and lipid oxidation of broilers thigh meat.

MATERIALS AND METHODS

Animals and diets: This study was approved by a suitably constituted ethics committee of the university of Mostaganem. A total of 132, 1-day old Hubbard-ISA male broilers, obtained from a commercial hatchery were given for 2 weeks the same starter diet (3035 kcal kg^{-1}) and allowed free access to water and food. Chicks had an average initial Body Weight (BW) of 424.5±1.50 g at the age of 14 days.

From 15-39 days, birds were placed in 3 floor pens of 2×2.5 m with 44 birds per pen. Two diets were used, the first based on only corn (served as control diet), the second containing 5% of double zero full-fat canola seed (experimental diet). Diets were formulated to cover all nutrient requirements of broiler chicks for 56 days study period.

In the finisher period (from 40-56 days), the three dietary treatments consisted of: A control diet (C), diet containing 5% of ground canola seed+10 g kg^{-1} of rosemary leaves (CSRL) and diet containing 5% of ground canola seed+200 mg kg^{-1} of vitamin C (CSVC). Ingredients and nutrient composition of the diets are shown in Table 1. For this study, chickens were weighted on days 14, 28, 35, 40 and 56. Also, Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) were calculated throughout the experiment.

Canola seeds were purchased from Algiers institute of cultures, these seeds were finely ground using a grain mill before being mixed with the diet, 5% of soybean meal was substituted by 5% of ground canola seed.

Table 1: Composition of the experimental diets

Diet	C	CS
Ingredients (%)		
Corn	67.0	67.0
Canola seed	-	5
Soya bean meal	27.0	22.0
Wheat bran	4.0	4.0
*Vit-min premix	1.0	1.0
Calcium	0.5	0.5
Phosphorus	0.5	0.5
Calculated composition		
ME (kcal kg^{-1})	3035	3124.5
Crude protein (%)	21.0	19.7
Analyzed composition (%)		
Moisture	12.72	11
Lipids	4.62	3.24
Ash	3.9	4.1
FA analysis (Percentage of the identified FA)		
C14:0	0.06	0.08
C16:0	13.77	7.79
C16:1	0.13	0.18
C18:0	2.26	3.31
C18:1 (n-9)	24.45	44.07
Cl8:2 (n-6)	50.51	32.82
Cl8:3 (n-3)	3.21	6.72
C20:1 (n-9)	0.40	0.77
SFA	19.59	12.56
MUFA	25.85	47.39
PUFA	54.56	40.05
n-6	50.51	32.88
n-3	4.05	7.06
n-6:n-3	12.47	4.66

*Vit-min premix: Provided (in mg kg^{-1} of diet), Vitamin E: 6, Vitamin K3: 0.80, Vitamin B1: 1, Vitamin B2: 3, Pantothenate of Ca: 6, Vitamin B6: 1.5, Vitamin B12: 0.006, Folic acid: 0.2, Nicotinic acid: 12, Copper: 5, Cobalt: 0.65, Manganese: 65, Zinc: 65, Selenium: 0.25, Iron: 50, Iode: 0.8, Magnesium: 100, EM: Metabolisable energy, C: Control diet, CO: Canola seed diet, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

Wild rosemary leaves (*Rosmarinus officinalis)* were collected during the period of March, 2014 from the region of the wilaya of Chlef (250 km to West Algiers) which is located at 86 m altitude, 36°10'26" Nord latitude and 1°20'12" East longitude. These leaves were first air-oven dried at 45°C for three consecutive days and then ground to pass a 2 mm screen and stored in clean labeled airtight bottles until the beginning of the animal study.

Ascorbic acid (vitamin C) was supplied by (Sigma-Aldrich Co., St., Louis, MO) with the concentration of 99%.

Measurements at slaughter: At the day 56th of age, 10 birds from each diet were selected, weighed, slaughtered and eviscerated in a local commercial slaughter house. After evisceration, the birds were apportioned by hand. Samples (100 g) from the left thigh were obtained. Some meat samples were placed in plastic bags and frozen at -20°C until analysis of total lipids and fatty acid profile. Whereas, the other samples were sliced at 0.5 cm thick, placed in open sterile polypropylene bags and stored at 4°C in the dark until to determine the oxidative stability. Liver and Abdominal Adipose Tissue (AAT) were also removed and weighed individually and the percentages of thigh muscle, liver and AAT (Percentage of eviscerated weight) for the individual bird were calculated.

Laboratory analysis

Analysis of diets: Samples of diets were dried and stored for subsequent analyses. Mineral content was determined by ashing at 600°C for 8 h[22]. Total phenol content was determined by the Folin-Ciocalteu method[23]. One mL of methanolic extract was mixed with 5 mL of Folin-Ciocalteu reagent (2 mol L^{-1}) diluted 10 times. The mixture was allowed to stand for 5 min, after which 4 mL of aqueous Na_2CO_3 solution at a concentration of 75 g L^{-1} was added. The absorbance of the mixture was measured at 765 nm after 60 min of incubation at room temperature using UV-vis spectrophotometer. All the experiments were carried out in triplicates and the amount of total phenols was expressed as Gallic Acid Equivalents (GAE g^{-1} herb), using a gallic acid calibration curve.

Analysis of meats: The Total Lipids (TL) of each sample (diet or meat) were extracted by chloroform:methanol (2:1) according to the method of Folch *et al.*[24]. The FA of lipids were freed by saponification (NaOH) and then methylated by methanol-BF3[25]. The methyl esters of FA were separated and quantified by gas chromatograph (Perkin-Elmer Auto System XL) equipped with flame ionization detector and a capillary column (30 m×0.25 mm internal diameter). The operating conditions of the gas chromatograph were as follows: Injector and detector temperature of 220 and 280°C, respectively, the oven temperature was programmed to increase from 45-240°C at 20-35°C min^{-1}, aliquots of 1 µL were injected with bicyanopropyl phenyl silicone as a stationary phase, hydrogen was used as conductor gas, FA peaks were identified by comparison with retention times of methyl fatty acid standards and quantification was made by reference to an internal standard (C17:0).

The lipid oxidation of meat samples was determined on day 1 and 5 post slaughter. Malondialdehyde (MDA), the compound used as an index of secondary lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method[26]. In brief, samples were homogenized in the presence of 8 mL of 5 g/100 mL aqueous trichloroacetic acid (Sigma Aldrich Co., St., Louis, MO) and 5 mL of 0.8 g/100 mL butylated hydroxytoluene (Sigma Aldrich) in hexane and the mixture was centrifuged. The top layer was discarded and a 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL of 0.8 g/100 mL aqueous 2-thiobarbituric acid (Sigma Aldrich Co., St., Louis, MO) and further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to conventional spectrophotometry (Shimadzu, Model UV-160A, Tokyo, Japan) in the range of 400-650 nm. Third-order derivative spectra were produced by digital differentiation of the normal spectra using a derivative wavelength difference setting of 21 nm. The concentration of MDA in the analyzed samples was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of standard calibration curve prepared, using 1,1,3,3-tetraethoxypropane (Sigma Aldrich Co., St., Louis, MO).

Statistical analyses: Data were statistically analyzed by analysis of variance using SAS Software[27]. Comparisons among means were conducted using Bonferroni's test.

RESULTS

Productive performance: The BW, BWG and FCR values are presented in Table 2 and 3. The CS diet reduced chicken growth by almost 4% (p<0.05) at day 35 and 40. There was enhancement in BWG of chicks after supplementation with rosemary leaves and vitamins C which explain why at day 56, birds on both diets reached similar final weights. During 14-35 days the control broilers chicken had better (p<0.05) FCR values compared to CS diet while no significant difference of this ratio was observed among experimental and control diets during 40-56 days of study.

Carcass parameters: The carcass parameters are shown in Table 4. There was no significant difference in carcass yield percentage of birds fed on CS supplemented with rosemary leaves (CSRL) or with vitamin C (CSVC) in comparison to the controls. A similar observation was made for AAT proportion, liver and thigh weights.

Table 2: Effect of experimental diet on growth performance of broilers from 14-35 days

Treatments	C	CS	SEM	Dietary effect
BW (g) (day 14)	424.5	424.5	1.50	NS
BW (g) (day 28)	1044	1040.5	1.75	NS
BW (g) (day 35)	1517[a]	1453.5[b]	6.75	p<0.05
BWG (g bird^{-1}) (14-28 days)	619,5	616	2.25	NS
BWG (g bird^{-1}) (28-35 days)	473[a]	413[b]	5.00	p<0.05
FCR (14-35 days)	2.07	2.12	0.03	NS

For each group n = 10, results are expressed as Mean and Standard Error of mean (SEM), means in the same line with different superscripts are significantly different (p<0.05), BW: Body weight, BWG: Body weight gain, FCR: Feed conversion ratio, C: Control diet, CO: Canola seed diet, NS: Not significant

Fatty acid composition: Total lipids and FA composition of the thigh muscle are shown in Table 5. No significant effect of the diet on TL level was detected (3.33 and 2.42 vs 2.83%).

Feeding the canola seed diet led to a decrease in the SFA level (p<0.05) and a lower palmitic acid content (p<0.05) compared to the control diet (25.29 and 24.57 vs 26.88%). In contrast, the amount of oleic acid was significantly higher (p<0.05) in the meat of broilers fed with the CS diet (38.16 vs 39.92 and 39.89%). However, no significant effect of diet on Linoleic Acid (LA) and total MUFA percentages was recorded. Incorporation of CS in the diet led to a higher PUFA level (p<0.05) (19.68 and 18.46 vs 16.49%) with a corresponding increase in the level of n-3 FA, especially Alpha Linolenic Acid (ALA) content (p<0.05) (1.53 and 1.24 vs 0.66% of the identified FA) and an increase in the PUFA:SFA ratio (p<0.05) (0.65 and 0.57 vs 0.45%).

For the content of other FA, no differences between the types of diet were detected. The n-6: n-3 ratio was lower in animals fed with CS than the controls (8.24 and 8.70 vs 18.22%). Vitamin C and rosemary leaves supplementation had no significant effect on FA composition of thigh meat.

Table 3: Effect of experimental diet on growth performance of broilers from 40-56 days

Treatments	C	CSRL	CSVC	SEM	Dietary effect
BW (g) (day 40)	1736[a]	1685[b]	1685[b]	7.3	p<0.05
BW (g) (day 56)	2233	2227	2209	12.04	NS
BWG (g bird^{-1}) (40-56 days)	497[a]	542[b]	524[b]	7,58	p<0.05
FCR (40-56 days)	1.91	1.89	1.91	0.03	NS

For each group n = 10, results are expressed as Mean and Standard Error of mean (SEM), means in the same line with different superscripts are significantly different (p<0.05), BW: Body weight, BWG: Body weight gain, FCR: Feed conversion ratio, CSRL: Diet containing 5% of canola seed+10 g kg^{-1} of rosemary leaves, CSVC: Diet containing 5% of canola seed+200 mg kg^{-1} of vitamin C, NS: Not significant

Table 4: Effect of experimental diets on carcass characteristics of broilers

Treatments	C	CSRL	CSVC	SEM	Dietary effect
EW (g)	1590	1583	1588	2.08	NS
Carcass yield (%)	70.46	70.69	69.85	0.25	NS
AAT weight (g)	28	29	28	0.87	NS
AAT weight (Percentage of EW)	1.76	1.83	1.76	0.02	NS
Thigh weight (g)	338	336	330	1.70	NS
Thigh weight (Percentage of EW)	21.26	21.23	20.97	0.09	NS
Liver weight (g)	50	48	46	1.93	NS
Liver weight (Percentage of EW)	3.14	3.03	2.90	0.07	NS

For each group n = 10, results are expressed as Mean and Standard Error of the mean (SEM), EW: Eviscerated weight, AAT: Abdominal adipose tissue, CSRL: Diet containing 5% of canola seed+10 g kg^{-1} of rosemary leaves, CSVC: Diet containing 5% of canola seed+200 mg kg^{-1} of vitamin C, NS: Not significant

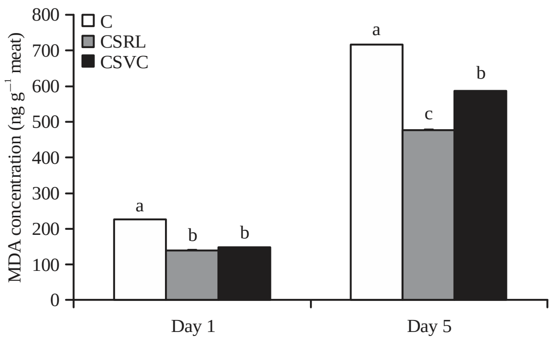

Fig. 1: Effect of rosemary and vitamin C supplementation on lipid oxidation of thigh meat (storage of samples at +4°C), C: Control diet, CSRL: Diet containing 5% of canola seed+10 g kg^{-1} of rosemary leaves, CSVC: Diet containing 5% of canola seed+200 mg kg^{-1} of vitamin C, MDA: Malondialdehyde

Table 5: Total lipid content (%) and fatty acid composition (Percentage of identified FA) of sartorius muscle of broilers fed control diet and canola seed diet supplemented with rosemary and vitamin C

Diet	C	CSRL	CSVC	SEM	Dietary effect
TL (%)	2.83	3.33	2.42	0.26	NS
C14:0	0.66	0.51	0.56	0.04	NS
C16:0	26.88^a	25.29^b	24.57^b	0.68	p<0.05
C16:1	0.53	0.52	0.60	0.03	NS
C18:0	7.53	6.30	6.15	0.44	NS
C18:1 (n-9)	38.16^a	39.92^b	39.89^b	0.58	p<0.05
Cl8:2 (n-6)	15.58	15.60	14.17	0.47	NS
Cl8:3 (n-3)	0.66^a	1.53^b	1.24^b	0.26	p<0.05
C20:1 (n-9)	0.44	0.49	0.61	0.05	NS
C20:4 (n-6)	0.69	0.72	0.72	0.01	NS
C20:5 (n-3)	0.01	0.09	0.10	0.03	NS
C22:4 (n-6)	0.17	0.25	0.18	0.03	NS
C22:5 (n-3)	0.09	0.23	0.17	0.04	NS
C22:6 (n-3)	0.07	0.16	0.11	0.03	NS
SFA	35.38^a	30.47^b	32.38^b	1.43	p<0.05
MUFA	48.13	50.17	49.85	0.63	NS
PUFA	16.49^a	19.68^b	18.46^b	0.93	p<0.05
n-6	15.47	17.38	15.54	0.63	NS
n-3	0.85^a	2.14^b	1.79^b	0.39	p<0.05
n-6:n-3	18.22^a	8.24^b	8.70^b	3.25	p<0.05
La/ala	20.91^a	10.55^b	11.44^b	3.31	p<0.05
PUFA:SFA	0.47^a	0.65^b	0.57^b	0.05	p<0.05

For each group n = 10, TL: Total lipid, results are expressed as Mean and Standard Error of the mean (SEM), means in the same line with different superscripts are significantly different (p<0.05), CSRL: Diet containing 5% of canola seed+10 g kg^{-1} of rosemary leaves, CSVC: Diet containing 5% of canola seed+200 mg kg^{-1} of vitamin C, NS: Not significant

Lipid stability of meat: The effect of dietary treatments on the lipid oxidation (MDA values) of thigh meat enriched with n-3 PUFAs at days 1 and 5 of storage is given in Fig. 1. The MDA concentrations increase in all thigh meat samples during storage at 4°C, but samples enriched with antioxidants had lower (p<0.05) MDA values than those of the controls. These treatments showed similar antioxidant activity at the 1st day of storage. However, rosemary leaves had higher antioxidant effect (p<0.05) than the vitamin C after 5 days of storage (480 vs 590 ng g^{-1} of meat).

DISCUSSION

The BW values obtained with diet containing canola seed especially at days 35 and 40 (Table 2, 3) agrees with observations of Roth-Maier *et al.*[28] who indicated that the use of 5, 10, 15, 20 and 25% of full-fat canola seed in the broiler ration has a negative effect on the chicken growth. This negative effects have been attributed to the lower availability of its fat fraction[7] and the presence of anti-nutritional factors[29,28] like lysine-arginine imbalance[30-33] also the existence of phytic acid that will reduce the calcium ability absorption and consequently, the proteolytic enzymes inhibition[34]. Our results of FCR obtained during (14-35) days are in agreement with those recorded by Roth-Maier *et al.*[28] and Talebali and Farzinpour[33]. At the end of the experiment, chicks fed with canola seed diet supplemented with either rosemary or vitamin C (CSRL or CSVC) had higher BWG than the C diet and the values of their BW and FCR (CSRL and CSVC) became similar to the controls. These results may be explained by the positive effect of aromatic herbs and their volatile oils in the digestive system, where they can improve the activity of enzymes that help in the digestion[35]. Also, Hernandez *et al.*[36] have demonstrated that adding 5,000 ppm of herbal mixture from different members of the Labiatae family such as rosemary can improve weight gain for 42 days. Thus, these findings agree with a previous researches conducted by Schildknecht *et al.*[37] and Kassim and Norziha[38] who found improved growth and feed efficiency in the broiler chicks supplemented with vitamin C. This vitamin is involved in growth by promoting collagen synthesis, calcium and vitamin D3 metabolism, carnitine synthesis for oxidation of fatty acids, oxidation of amino acids, electron transport in the cells and scavenging of free radicals[39]. No significant difference of performance parameters was observed among experimental diets (CSRL vs CSVC) in this study.

In agreement with Lee *et al.*[7] and Talebali and Farzinpour[33] studies, the present results demonstrated that diets containing 5% of canola seed resulted in similar carcass yield, AAT proportion and liver and thigh final weight as the control diet (Table 4).

A dearth of information exists in terms of carcass parameters to full-fat canola seed in poultry, therefore, direct comparisons cannot be made. In the present study, carcass composition seems not to be affected by supplementation of diet with ascorbic acid or with rosemary leaves which has been reported by others authors[40,41].

The total lipid content (Table 5) of thigh meat was not affected by treatment ($p>0.05$) which can be related to the moderate enrichment of muscles. Some researchers have shown that the dietary polyunsaturation level of fat does not influence intramuscular lipid content of breast[42,43]. Oleic acid was the predominant FA in thigh muscle of both groups, whereas, its proportion was higher in the CS group. In agreement with these results with Ajuyah *et al.*[44] and Crespo and Esteve-Garcia[45] who reported that oleic acid was the major FA found in carcass and muscle fats. Also, linoleic acid content was not affected by the type of diet. Rahimi *et al.*[46] reported that canola seed is rich in oleic acid and contains more Linoleic Acid (LA) than flaxseed. In the present study, fatty acid composition of the chick's tissues generally reflected the FA profile of the diets, dietary supplementation with n-3 PUFA (7.06% of n-3 PUFA in CS diet vs 4.05% in C diet) increases the content of these FA in poultry meat[47-50] especially the proportion of alpha linolenic FA ($p<0.05$)[46]. In this study, eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3) were not affected by the type of diet, which may be explained by the limited ability of chicks to desaturate and elongate ALA and the very low deposition rate of these metabolites in muscle tissues[51,52]. Feeding broilers canola seed caused a large decrease in the n-6/n-3 ratio in the thigh muscle ($p<0.05$), this ratio was more favourable with the inclusion of CS in broilers diet, but higher than that found by Kamran Azad *et al.*[53]. Also, Salamatdoustnobar *et al.*[50] observed that when canola oil level increased this ratio decreased and the quality of fatty acid composition is improved.

However, our results showed that birds fed CS had enriched their muscle with a higher PUFA proportion (19.68 and 18.46 vs 16.49%) while, the content of SFA was significantly greater in the controls (35.38 vs 30.47 and 32.38%). These observations were consistent with an increase in the PUFA:SFA ratio on CS compared to the control diet (0.65 and 0.57 vs 0.47%), which has been also obtained by Kamran Azad *et al.*[53]. In broiler chickens, it had been described that dietary PUFA, as compared to more saturated or mono-unsaturated FA sources, diminishes fat deposition which may be attributed to increased lipolysis or diminished lipogenesis or both[54].

Thiobarbituric acid (TBA) analysis is an efficient way to measure antioxidant activity in meat products. This analysis is an indicator of MDA a product of oxidation, thus the MDA value increases during storage period. Lopez-Ferrer *et al.*[55] reported that n-3 PUFAs are deposited in higher concentration in thigh meat than in breast meat when the n-3 PUFA content in the feed is increased. Moreover, some researchers have suggested that the lower oxidative stability of thigh meat is related to a higher absolute content of PUFAs with more than two double bonds in the fat[56,57]. In the present study, sartorius meat samples from all treatments enriched with antioxidant had lower MDA values than those of the control. However, rosemary leaves treatment had higher antioxidant effect than the vitamin C treatment after 5 days of storage. Our results are in agreement with those of Loetscher *et al.*[41] who reported that when supplementing rosemary at 25 g kg^{-1}, there was a clear effect on the oxidation stability of breast meat and it was similar than that of the supranutritional vitamin E treatment. In current experiment, analyses showed that the total phenolic content of rosemary was 38.40 mg of gallic acid equivalents g^{-1} which is lower than the total phenolic content obtained by Yesilbag *et al.*[21] (62.5 mg GAE g^{-1}), who found that rosemary, added as ground plant material either at 11.5 g kg^{-1} or at the corresponding amount of rosemary essential oil (200 mg kg^{-1}) was even more effective than 200 IU of α-tocopheryl acetate kg^{-1} of feed in preventing oxidation in broiler breast meat. Also, Govaris *et al.*[58] reported that rosemary incorporation was more effective in delaying lipid oxidation of turkey breasts compared to α-tocopheryl acetate at 150 mg kg^{-1} during refrigerated storage, in their study, the total phenol content of rosemary had been lower than in this study (29.4 mg GAE g^{-1}). According to our results and also to other authors results[21] even low concentration of rosemary can induce a higher antioxidative protection which is based on its ability to inactivate free radicals produced during the auto-oxidation process[59]. The rosemary plant is rich in polyphenols that might inhibit free radical formation and lipid oxidation[21].

CONCLUSION

In conclusion, broilers fed diet containing CS enriched their meat with omega-3 FA, while their performance was negatively affected by oil seed supplementation. The results of this experiment suggest that rosemary may be considered as a natural growth promoter for broilers, moreover, it showed good antioxidant properties in meat when added to the diet of animals. We suggest the simultaneous use of rosemary and ground canola seed to enrich broilers meat by n-3 FA and reduce the lipid oxidation during storage.

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