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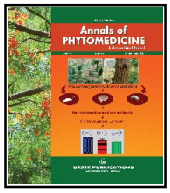

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Effect of dietary supplementation of linseed (*Linum usitatissimum* L.) on fatty acid profile and meat quality of broiler chickens

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Abstract

Broiler diets frequently include fats and oils to improve energy density, feed palatability, and fat deposition in broilers. Linseed (LS) is an oilseed crops comprising high level of n-3 FAs specifically ALA. The availability of n-3 FAs in broiler meat can be raised by appropriate feeding of LS. The main objective of this study was to examine the effect of various levels of LS on fatty acid profiles, metabolism and meat characteristics of broiler chicken. In this investigation, broiler chicken was fed with different levels of LS (2.5, 5, 7.5, and 10%, respectively) diet for five weeks. The results obtained, showed that feeding with different levels of LS to broiler chickens for five weeks, significantly (p<0.01) increases ALA, EPA, DHA, MUFAs, PUFAs, n-3 and n-6 PUFAs concentration in broiler's meat with regular decline in SFAs like stearic and palmitic acid concentration. Similarly, higher levels of LS showed increased Δ^9-desaturases enzyme (p<0.01), TI (p<0.01) and Δ^5 + Δ^6 DI activity (p<0.01), while feeding up to five weeks with no significant differences on EI. There were not any significant differences in meat attributes among the different levels of LS supplements. Present investigations suggest that, up to 10%, LS supplementation to broiler's diet enhances the n-3 FAs in chicken meat.

1. Introduction

In modern diets, animal meat plays an important role because of its fatty acids (FAs), protein, minerals, and vitamins composition. Broiler meat production and consumption has increased and has become popular across the globe over recent decades (Shah *et al.*, 2019). The modern poultry production industry aims to produce high-quality poultry meat by improving broiler health and performance as well as nutritious meat for consumers (Long *et al.*, 2020). Nowadays, dietary supplementation has contributed reduction in the ratio of omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs), which increases the risk of cardiovascular diseases (Mir *et al.*, 2018). According to health experts, n-3 PUFAs consumption may minimize the prevalence of coronary heart diseases (Bowen *et al.*, 2016). There has been an amazing increase in demands for wholesome and nutritious food in the past decade (Rai *et al.*, 2020). However, the average regular human diet is deficient in n-3 PUFA and the conversion of α-linolenic acid (ALA) to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in human body took place very slowly, resulting in lesser transformation of EPA and DHA (Abedi and Sahari, 2014). The appropriate enzymes being absent in the human body or unable to synthesize PUFA in the presence of an initial double binding on C3 and C6 from end of methyl (Orsavova *et al.*, 2015). This has led to carry out vast investigations, considering the possibility of producing poultry products that contain n-3 PUFAs required for human consumption.

The use of a mixture of fish oil, microalgae, LS oil, or a combination of these ingredients, in poultry diets has shown increased FAs profile (Lopez-Ferrer *et al.*, 2001). Previous findings showed that flaxseed meals supplementation in broiler chicken diet significantly improved the FAs profile of meat while retaining its quality (Kumar *et al.*, 2019). In addition, dietary factor performs desaturases activity, leading to PUFAs synthesis, for example EPA and DHA (Czumaj and Sledzinski, 2020).

Considering these facts, LS can serve as the richest terrestrial source of n-3 FAs, which is an excellent alternative for the poultry sector as it generates value addition in poultry meat. Approximately, 42-46 per cent of oil present in LS comprising, of which 45-71 per cent of ALA as sole FAs. It is a rich source of PUFAs, with a moderate amount of MUFAs, and a lower level of saturated fatty acids (SFAs) (Kumar *et al.*, 2019). In present investigation, the broiler chickens were fed different levels of LS for five weeks with a hypothesis that different levels of LS would enhance broiler chicken meat with n-3 FAs such as DHA and EPA.

2. Materials and Methods

This current investigation was performed at the poultry farm of Banaras Hindu University, Varanasi, India. The investigation was initiated after receiving the assent from the Central Animal Ethical

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Committee of the University (IAEC/3036). Two hundred one day old broiler male chicks (cobb-400) were procured from a commercial company (Pratap Hatchery, Varanasi). The chicks were kept in deep litter system and fed with a basal diet for one week just before starting of trial. After one week, body weights of the chicks were measured with measuring balance (model- BWT, K. Roy). The chicks were categorized in five different groups having 40 broilers each. In each group, five replicates of eight broilers were present. Fresh water and feed were provided *ad lib*. All chicks were kept under uniform management conditions throughout the 35 days of the experimental period.

2.1 Experimental diets

Table 1 shows five isocaloric and isonitrogenous experimental diets that were developed to reach the nutritional needs of broiler chicks as per (NRC, 1994). In addition to basal control diet without LS and four experimental diets were formulated comprising LS (2.5, 5, 7.5 and 10%, respectively). Besides the basal control diet, four experimental diets were formulated to contain linseed (2.5, 5, 7.5, and 10%), respectively. The diets were formulated for two-phases, first starter phase (7-21 d) and finisher phase (22-42 d).

2.2 Fatty acid profile determination

Total lipids from broiler meat samples (left side of breast and thigh muscle) were extracted by dispersion method using methanol to chloroform mixture (1:2 v/v) as per Folch method (Mubarak *et al.*, 2015). To enhance the volatility of analytes, lipids obtained after extraction of broiler meat samples were passed for trans-esterification with potassium hydroxide (KOH) to respective fatty acid methyl esters (FAMEs).

Table 1: Ingredient and nutrient composition of broilers starter and finisher ration

Ingredients (%)	Dietary groups of linseed									
	Starter phase (7-21 d)					Finisher phase (22-42 d)				
	0%	2.5%	5%	7.5%	10%	0%	2.5%	5%	7.5%	10%
Maize	51.05	49.15	47.06	45.63	44.1	59.23	57.91	56.65	54.43	53.12
Soybean meal	38.4	37.55	37.14	36.57	35.6	25.56	24.33	23.14	22.87	21.68
Wheat bran	5	5	5	5	5	5	5	5	5	5
Vegetable oil	2	2	2	2	2	7	7	7	7	7
Dicalcium phosphate	1.1	1.25	1.25	1.25	1.25	1	1	1	1	1
Shell grit	1	1	1	0.5	0.5	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Minerals premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamins premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.1	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0	0
DL-methionine	0.15	0.25	0.25	0.25	0.25	0.1	0.15	0.1	0.1	0.1
Vitamin E	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
Linseeds	0	2.5	5	7.5	10	0	2.5	5	7.5	10
Calculated composition										
Dry matter	88.92	89.38	89.67	89.89	90.04	89.84	90.05	90.12	90.1	90.22
Crude protein	22.2	22.21	22.24	22.25	22.27	19.18	19.28	19.88	19.36	19.32
Crude fibre	4.56	4.89	5.23	5.78	5.94	4.84	5.1	5.32	5.89	5.92
Lysine	1.27	1.3	1.38	1.35	1.34	1.08	1.1	1.08	1.12	1.15
Methionine	0.48	0.5	0.53	0.54	0.62	0.38	0.42	0.44	0.45	0.39
Calcium	1.1	1.12	1.15	1.17	1.19	1.08	1.12	1.31	1.3	1.07
Phosphorus	0.58	0.62	0.65	0.67	0.71	0.59	0.65	0.69	0.57	0.55
ME kcal/kg diet	2962.34	2983.54	2958.51	2970.74	2992.84	3185.65	3195.68	3210.04	3225.25	3227.85

[1]Supplied per kilogram: Vitamin A 8000 U; Vitamin D3 8560 U; Vitamin E 95 U; Vitamin B12 4.5 mg; Niacin 45 mg; Pyrodoxin 4 mg; Thiamin 5 m; Biotin 0.2 mg; Panthotenic acid 20 mg; Riboflavin 08 mg. [2]Supplied per kilogram: Iodine (Calcium iodate) 2 mg; Copper (Cupric sulfate pentahy-drate) 8 mg; Manganese (Manganese sulfate monohy-drate) 100 mg; Iron (Ferrous sulfate monohydrate) 55 mg; Zinc (Zinc sulfate monohydrate) 70 mg; Selenium (Sodium selenite) 0.15 mg.

Table 2: Fatty acid profile in thigh meat of broiler chickens fed dietary supplementation of linseed

Fatty acid (%)	Dietary groups of linseed					SEM	*p*-value
	0%	2.5%	5%	7.5%	10%		
C14:0	0.60^{a}	0.56^{a}	0.47^{b}	0.40^{c}	0.32^{c}	0.01	<0.01
C16:0	34^{a}	31^{b}	29^{b}	26^{c}	25^{c}	0.70	<0.01
C18:0	5.90^{a}	5.61^{a}	4.80^{b}	4.21^{c}	4.12^{c}	0.07	<0.01
C20:0	0.28	0.29	0.27	0.28	0.29	0.04	NS
Σ SAF	41.22^{a}	37.73^{b}	34.85^{b}	31.22^{c}	30.01^{c}	0.74	<0.01
C16:1	3.85^{c}	4.20^{b}	4.36^{b}	5.65^{a}	5.81^{a}	0.07	<0.01
C18:1	31.50	31.90	32.10	32.84	33.12	0.70	NS
C20:1	0.47^{a}	0.42^{a}	0.37^{a}	0.22^{b}	0.17^{b}	0.01	<0.01
C24:1	0.20	0.19	0.19	0.18	0.17	0.01	NS
Σ MUFA	36.02^{c}	36.71^{bc}	37.02^{ab}	38.89^{a}	39.27^{a}	0.78	0.031
C18:2	16.50^{b}	16.90^{b}	19.50^{a}	19.70^{a}	20.10^{a}	0.32	<0.01
C20:2	0.32^{c}	0.38^{b}	0.42^{a}	0.45^{a}	0.48^{a}	0.01	<0.01
C20:3	0.72^{d}	1.05^{c}	1.24^{b}	1.29^{a}	1.31^{a}	0.01	<0.01
C20:4	0.22	0.22	0.23	0.24	0.25	0.01	NS
Σ n-6	17.76^{b}	18.55^{b}	21.39^{a}	21.66^{a}	22.14^{a}	0.32	<0.01
C18:3	1.72^{d}	2.67^{c}	3.90^{b}	4.31^{a}	4.45^{a}	0.02	<0.01
C20:5	1.97^{d}	2.20^{c}	2.70^{b}	2.86^{a}	2.91^{a}	0.01	<0.01
C22:6	1.17^{d}	1.62^{c}	2.70^{c}	2.87^{a}	2.95^{a}	0.01	<0.01
Σ n-3	4.86^{d}	5.58^{c}	9.30^{b}	9.89^{a}	10.31^{a}	0.03	<0.01
Σ PUFA	22.62^{d}	24.14^{c}	30.69^{b}	31.83^{a}	32.45^{a}	0.32	<0.01
Σ n-6:Σ n-3	3.65^{a}	3.32^{b}	2.30^{c}	2.19^{d}	2.14^{d}	0.03	<0.01

Mean bearing different superscript in a row differ significantly (p<0.05); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; n-6, omega-6 fatty acids; n-3, omega-3 fatty acids; PUFA, polyunsaturated fatty acids; Σ, total; SEM, standard error of the mean; NS, not significant.

2.2.1 Sample preparation for GC (Gas chromatography)

Approximately 60 mg of broiler meat sample was mixed with 4 milliliter (ml) of isooctane in a test tube, and then 200 ml of methanolic potassium hydroxide solution (2 mol/l) was added. The test solutions was kept for 30 seconds at room temperature with vigorous shaking. Further, 1 gram of sodium hydrogen sulfate monohydrate was added to neutralize the solution. After the salt was deposited, 1 milliliter (ml) from the upper phase was transferred to a 2 ml flask for further analysis (Leontopoulos *et al*., 2021). Gas chromatograph-mass-spectrometer (GC-MS-QP 2010 Plus, Shimadzu, Japan) with flame-ionization detector and silica capillary column (30 × 0.25 mm ID × 1µm df) was used for sample analysis and helium was used as a vector gas with a flow rate of 3.0 ml/min. The temperatures of injector and detector was set at 260°C and 280°C, respectively, while temperature of column was set at 120°C for 57 min with 3°C per minute increments. For identification, the peak retention times of sample mixtures were compared with those from FAME standard mixtures (Sigma-Aldrich, India) and the percentage of each individual fatty acid was calculated.

2.3 Fatty acid metabolism indices

Desaturase indices (DI) was estimated by corelating the product percentage to that to its precursor according to Okada *et al*. (2005) by using following equations:

$$\text{DI(18): } \Delta^{9} - \text{desaturase (18) index} = 100 \times \frac{(C18:1)}{(C18:0 + C18:1)}$$

$$\text{DI(16): } \Delta^{9} - \text{desaturase (16) index} = 100 \times \frac{(C16:1)}{(C16:0 + C16:1)}$$

$$\text{Total DI} = 100 \times \frac{(C16:1 + C18:1)}{(C16:0 + C16:1 + C18:0 + C18:1)}$$

Further, to examine the conversion efficiency, the activity of the enzymes that were involved in the conversion of essential fatty acids into long-chain PUFAs were determined in breast and thigh muscles. The thioesterase index (TI) and elongase index (EI) were calculated as per suggested by Zhang *et al*. (2007) by using following equations:

$$EI = \frac{C18:0}{C16:0}$$

$$TI = \frac{C16:0}{C14:0}$$

Evaluation of $\Delta^5 + \Delta^6$ DI activity was calculated according to Sirri *et al*. (2010) method.

2.4 Meat quality

The colour of broiler meat (L*, a*, b*) was monitored by Colour Flex EZ, (Model-CFEZ1048, Hunter Associates Laboratory Inc., Reston, VA, USA). For colour analysis, raw meat samples excised from the left thigh and breast portion of broilers were kept in petri plate, completely filled and then placed to the sensors of the colorimeter to analyze their color. Three color coordinate values were mean values of lightness (L*), redness (a*) and yellowness (b*). In order to measure the pH of meat after 24 h of slaughtering, 10 g of meat was taken from the left side of the thigh and breast and were homogenized with 100 ml distilled water. pH was recorded by using digital pH meter (Systrong μ pH system, type-361). The water-holding capacity (WHC) was estimated as per the protocol of Wilhelm *et al*. (2010).

2.5 Statistical analysis

This study involved a completely randomized design (CRD) with five different treatments and five replications. Data of this study were examined using one-way ANOVA and Duncan's multiple post-hoc range tests, which is a common statistical approach (Snedecor and Cochran, 1989). Statistical treatments were performed by using SPSS version 25. Significance levels were decided at (p<0.05).

Table 3: Fatty acid profile in breast meat of broiler chickens fed dietary supplementation of linseed

Fatty acid (%)	Dietary groups of linseed						
	0%	2.5%	5%	7.5%	10%	SEM	*p*-value
C14:0	0.62^{a}	0.55^{ab}	0.48^{b}	0.43^{c}	0.40^{c}	0.01	<0.01
C16:0	35.50^{a}	33.20^{ab}	28.50^{b}	25.10^{c}	24.30^{c}	0.07	<0.01
C18:0	7.93^{a}	6.32^{b}	5.85^{c}	5.55^{d}	5.43^{d}	0.01	<0.01
C20:0	0.28	0.29	0.28	0.28	0.29	0.01	NS
Σ SAF	44.33^{a}	40.36^{b}	35.11^{c}	31.36^{d}	30.42^{d}	0.08	<0.01
C16:1	3.02^{c}	3.78^{c}	4.42^{b}	4.58^{a}	4.65^{a}	0.01	<0.01
C18:1	30.40^{c}	31.34^{c}	32.78^{b}	34.08^{a}	34.20^{a}	0.12	<0.01
C20:1	0.64^{a}	0.51^{b}	0.44^{c}	0.31^{d}	0.30^{d}	0.01	<0.01
C24:1	0.20	0.19	0.20	0.19	0.19	0.01	NS
Σ MUFA	34.26^{c}	35.83^{c}	37.84^{b}	38.81^{a}	39.34^{a}	0.01	0.01
C18:2	14.50^{c}	15.50^{c}	16.20^{b}	17.82^{a}	18.20^{a}	0.07	<0.01
C20:2	0.42^{c}	0.47^{b}	0.50^{b}	0.56^{a}	0.58^{a}	0.01	<0.01
C20:3	0.75	0.74	0.74	0.73	0.74	0.01	NS
C20:4	0.22	0.23	0.23	0.22	0.21	0.01	NS
Σ n-6	15.89^{d}	16.94^{c}	17.67^{b}	19.20^{a}	19.73^{a}	0.07	<0.01
C18:3	0.38^{d}	0.47^{c}	0.54^{b}	0.61^{a}	0.64^{a}	0.01	<0.01
C20:5	1.60^{c}	2.13^{c}	2.67^{b}	2.76^{a}	2.80^{a}	0.01	<0.01
C22:6	0.27^{d}	0.37^{c}	0.48^{b}	0.59^{a}	0.62^{a}	0.01	<0.01
Σ n-3	2.25^{c}	2.97^{c}	3.69^{b}	3.94^{a}	4.06^{a}	0.01	<0.01
Σ PUFA	18.14^{c}	19.91^{c}	21.36^{b}	22.93^{a}	23.79^{a}	0.07	<0.01
Σ n-6:Σ n-3	7.06^{a}	5.70^{b}	4.79^{c}	4.82^{d}	4.86^{d}	0.03	<0.01

Mean with bearing different superscript in a single row differ significantly (p<0.05); MUFA, monounsaturated fatty acids; n-6, omega-6 fatty acids; n-3, omega-3 fatty acids; PUFA, polyunsaturated fatty acids; Σ, total; SEM, standard error of the mean; SFA, saturated fatty acids;NS, not significant.

Table 4: Effect of dietary supplementation of linseed on the fatty acid metabolism of broiler chickens

Attributes	Dietary groups of linseed					SEM	*p*-value
	0%	2.5%	5%	7.5%	10%		
Thigh meat							
DI (18)	84.21^{b}	85.03^{b}	86.98^{ab}	88.64^{ab}	90.19^{a}	0.25	<0.01
DI (16)	10.17^{d}	11.93^{d}	13.07^{c}	17.89^{b}	20.19^{a}	0.27	<0.01
Total DI	46.97^{d}	49.64^{c}	51.89^{c}	56.04^{b}	59.43^{a}	0.28	<0.01
EI	0.17	0.18	0.16	0.16	0.17	0.01	NS
TI	56.70^{d}	56.45^{d}	61.66^{c}	65.15^{b}	71.89^{a}	1.8	<0.01
$\triangle^{5}$+ $\triangle^{6}$ DI	17.87^{c}	16.52^{d}	21.84^{b}	22.40^{a}	22.70^{a}	0.23	<0.01
Breast meat							
DI (18)	79.31^{c}	83.21^{b}	84.85^{ab}	85.89^{a}	86.29^{a}	0.04	<0.01
DI (16)	7.84^{d}	10.24^{c}	13.42^{b}	15.20^{a}	16.06^{a}	0.02	<0.01
Total DI	43.48^{d}	47.05^{c}	51.98^{b}	55.54^{a}	56.64^{a}	0.09	<0.01
EI	0.22	0.19	0.20	0.22	0.22	0.01	NS
TI	57.29^{b}	60.39^{a}	59.42^{b}	58.42^{b}	60.81^{a}	0.78	<0.01
$\triangle^{5}$+ $\triangle^{6}$ DI	15.88^{d}	18.11^{c}	20.18^{a}	19.76^{b}	19.60^{b}	0.09	<0.01

Mean with bearing different superscript in a single row differ significantly (p<0.05); DI (18),$\triangle^{9}$ desaturase (18) index; DI (16), $\triangle^{9}$ desaturase (16) index; EI, elongase index; TI, thioesterase index; SEM, standard error mean; NS, not significant.

Table5: Effect of dietary supplementation of linseed on meat quality of broiler chickens at 42 d

Attributes	Dietary groups of linseed					SEM	*p*-value
	0%	2.5%	5%	7.5%	10%		
Thigh meat							
L*	53.19	53.22	53.23	53.24	53.26	0.01	NS
a*	8.38	8.40	8.42	8.45	8.46	0.02	NS
b*	7.01	6.97	6.95	6.93	6.92	0.01	NS
pH	6.10	5.88	5.73	5.67	5.61	0.18	NS
WHC	63.52	63.55	63.54	63.50	63.48	0.04	NS
Breast meat							
L*	52.28	52.31	52.34	52.36	52.40	0.01	NS
a*	5.06	5.11	5.17	5.20	5.21	0.04	NS
b*	5.64	5.63	5.62	5.60	5.58	0.01	NS
pH	5.61	5.58	5.57	5.56	5.51	0.02	NS
WHC	64.45	64.43	62.44	64.42	64.45	0.01	NS

L*, lightness; a*, redness; b*, yellowness; WHC, water holding capacity; NS, not significant.

3. Results

3.1 Fatty acid profile

The fatty acids profile in thigh muscle of broiler chicken is shown in Table 2. It depicts that, the SFAs content gradually declined in all the treatments ranging from 2.5 to 10% LS groups. Monounsaturated fatty acid content showed significant increment (p<0.05) in 2.5 to 10% LS groups. Similarly, increasing trends were observed in palmitoleic acid (C16:1) content. However, eicosenoic acid (C20:1) was significantly (p<0.05) decreased. There were no significant difference was observed in oleic (C18:1) and nervonic acid (C24:1) content in all the LS groups. The overall PUFAs concentration was significantly (p<0.05) higher in 10% LS group comparatively to other groups. The n-3 PUFAs and n-6 PUFAs content increased (p<0.01) progressively from 2.5 to 10% LS groups while the ratio of n-6: n-3 decreased significantly (p<0.01) progressively from 2.5 to 10% LS groups. However, there were no significant differences recorded in FAs profile between 7.5 and 10% LS groups.

The fatty acid profile in breast muscle of broiler chicken is shown in Table 3. A continuous decline of total SFAs ($p<0.01$) was observed while varying the 2.5 to 10% LS groups, which reflects the trend of myristic (C14:0), palmitic (C16:0), and stearic acid (C18:0) content. The amount of arachidic acid (C20:0) in the muscles did not differ significantly across the groups. The palmitoleic (C16:1) and oleic acid (C18:1) content of breast muscle increased significantly, while the eicosenoic acid (C20:1) content dropped ($p<0.01$), resulting in a net remarkable change in the content of MUFA of breast muscle from 2.5 to 10% LS groups. The content of n-3 and n-6 PUFAs levels gradually increased ($p<0.01$), which reflected the trend of linoleic (C18:2), ALA (C18:3), eicosadienoic acid (C20:2), dihomo-γ-linolenic (C20:3), EPA (C20:5) and DHA (C22:6) content. The content of C20:3 (n-6) and C20:4 (n-6) levels in muscle did not differ significantly across all groups. Furthermore, the FAs profile did not differ significantly between the 7.5% and 10% LS groups.

3.2 Fatty acid metabolism

Fatty acid metabolism in breast and thigh muscles of broiler chicken were shown in Table 4. The current study shows that various levels of LS fed up to 5 weeks, significantly increased the DI (18) ($p<0.01$), DI (16) ($p<0.01$), total DI ($p<0.01$), TI ($p<0.01$) and $\Delta^5 + \Delta^6$ DI ($p<0.01$). Neither the thigh muscle nor the breast muscles showed significant differences in the activity of EI between treatments and controls. However, there were no significant difference observed in fatty acid metabolism index in breast and thigh muscles in between 7.5% and 10% of LS groups.

3.3 Meat quality

The effect of various levels of LS on the attributes of chicken meat is shown in Table 5. There were no significant difference in the colour value of the meat (L*, a*, b*) in the breast and thigh throughout the experimental period. As the dietary levels of LS rises, the pH value of the thighs and breasts decreases compared to control diet. pH value was decreased from 6.10 to 5.61 in thigh meat and 5.61 to 5.51 in breast meat from 7 to 42 days. As a result of feeding various levels of LS for five weeks, the results of WHC of thigh and breast meat were not significantly different for the control and the treatments.

4. Discussion

The energy and nitrogen balance in mono-gastric animals such as chickens show significant impact the lipid composition of the tissues. In order to maintain the balance for desired outcome, iso nitrogenous and isocaloric diets were created in this investigation. In the present investigation, it was clearly deduced that feeding of different levels of LS for five weeks increased the percentage of MUFAs and PUFAs significantly (Tables 2 and 3). On the other hand, SFAs decreased significantly. This experiment agrees with previous results of Mridula *et al.* (2015) who showed that increase in the consumption of different levels of flaxseed increases the ALA content in breast and thigh meat of broiler chickens. The results from this study support an earlier report that indicated longer fish oil, flaxseed, and rapeseed consumption enhanced the PUFA content of chicken meat, possibly due to a greater amount of *de novo* fatty acid synthesis (Konieczka *et al*., 2017). According to Mirshekar *et al*. (2015), as the duration of flaxseed oil feeding was increased, there was significant decreases in SFAs such as eicosanoic acid, palmitic acid and stearic acid were observed in poultry meat. In this investigation, reduction in n-6/n-3 PUFA in breast and thigh muscles may be due to low dietary n-6/n-3 PUFA. The supplementation of dietary LS oil, fish oil and microalgae can significantly enhance the n-3 PUFAs deposition in chicken muscle while decreasing n-6 PUFAs deposition (Morales-Barrera *et al*., 2013). Similarly, dietary supplementation of 2% LS oil and antioxidants in broiler enhanced the n-3 PUFAs, particularly EPA and DHA in meat, and decreased n-6: n-3 PUFA ratio significantly (El-Samee *et al*., 2019). This is in conformity with previous findings which showed that increased dietary flaxseed levels have raised the n-3 FAs content, especially ALA content in broiler chicken muscles (Kumar *et al*., 2019). Although, the higher levels of PUFAs in flaxseed, especially n-3 FAs, which pass through the gut more rapidly without undergoing significant bio-hydrogenation, may be responsible for meat containing higher PUFAs and low SFAs (Mir *et al*., 2017).

On the basis of this study, in chicken meat, different fatty acid profiles were observed, which might affect FAs accumulation and fat metabolism in broilers (Dal Bosco *et al*., 2012). Different levels of LS feeding increased the total DI ($p<0.01$), DI (18) ($p<0.01$), DI (16) ($p<0.01$), TI ($p<0.01$), and $\Delta^5 + \Delta^6$-DI activity ($p<0.01$) in the present investigation (Table 4). However, stearic acid and endogenous or dietary palmitic acid were converted into oleic acid and palmitoleic by the Δ^9 desaturase enzyme. Therefore, these enzymes play an important role in converting long-chain SFAs and specific medium into corresponding MUFAs (Reh *et al.*, 2004). In the present study, the broiler chickens of same age group were used, so it could become easier because of the different levels of linseed may increase Δ^9 desaturase activities. In addition, the enhanced Δ^9 desaturase activity also occurs due to increase in oleic and palmitoleic acids, as well as decreases in stearic and palmitic acids in the meat of broiler chickens. Essential fatty acids like ALA and LA from acetyl-CoA cannot be synthesized by broiler chickens, but can be converted to unsaturated long-chain fatty acids, if a diet contains these essential fatty acids. The conversion is catalyzed by enzymes that terminate, elongate, and desaturate. The $\Delta^5 + \Delta^6 -$ DI, which is used to measures the capability of chickens to synthesis long-chain FAs from the ALA and linolenic acid, is a rate-limiting enzyme (Dal Bosco *et al*., 2012). Chickens can convert ALA into DHA, which is an intermediary product of EPA, using desaturase. The elongase enzymes, which mean EPA and DHA, are available from the meat of chicken as well as fish and other marine products (Kumar *et al*., 2020). Increasing the TI as well as $\Delta^5 + \Delta^6$-desaturase activity corresponds to increasing the EPA and DHA corresponding to different LS levels fed for five weeks in the present study.

Considering consumer acceptance, meat color is the most important aspect of meat production. In this investigation, the WHC of the breast and thigh meat does not show any significant difference among different levels of LS. The reason for this is that LS feed increases the unsaturation within muscle tissues, making it more susceptible towards lipid peroxidation, which produces free radicals. Protein degradation is caused by free radicals in broiler chicken meat, which negatively impacts WHC (Mir *et al*., 2017). Traits associated with meat attributes, such as water holding capacity and shearing forces are closely related to pH, since pH is a good indicator of meat color (Liu *et al*., 2019). In accord with the following findings, in various reports, it is mentioned that there is no significant effect on pH value (Anjum *et al*., 2013) and chicken meat color (Mir *et al*.,

2018) supplemented with flaxseed diet. Similarly, there was (p>0.05) effect on color and pH value of pork fed with fish oil, flaxseed oil, flaxseed (Martinez-Ramirez *et al*., 2014). Myoglobin and pH affect meat color, and meat pH is favorably connected to redness, but negatively to yellowness and lightness (Han *et al*., 2012).

5. Conclusion

The present findings clearly depict that feeding broiler chickens with 10% linseed diet for at least five weeks continuously will improve the FAs profile of the chicken meat of broiler. Further, it increases the desaturase enzyme system activity, facilitating the conversion of SFAs into MUFA and catalyzing long-chain PUFAs synthesis. As a result of these effects, broiler chicken meat is better for the consumer's health, as evidenced by the enhanced health indices of the meat. This may be scale up and prototype can be developed for its commercialization with better consumer acceptability.

Conflict of interest

The authors declare no conflicts of interest related to this article.

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