Letters in Animal Biology

Journal homepage: www.liabjournal.com

Growth pattern, lipid composition, oxidation status, and serum biochemical profile of broiler chicken fed flaxseed meal for different durations

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Article info

Received: 16 May 2021
Received in revised form: 06 June 2021
Accepted: 07 June 2021
Published online: 09 June 2021

Keyword:
Broiler chicken performance
Meat quality
Antioxidant enzymes
Serum biochemistry
Health indices

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Reviewed by: Dr. C. Sundharsan

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Abstract

This research studied the effects of flaxseed meal (FSM) feeding for different durations on growth performance, fatty acid profile, oxidative stability of meat, and serum biochemical profile of broiler chicken. In basal diet 100 g FSM replaced soybean meal. The FSM based diet was fed for different durations resulting in six dietary treatments *viz*. T1 (0-5 weeks), T2 (1-5 weeks), T3 (2-5 weeks), T4 (3-5 weeks), T5 (4-5 weeks), and T6 (without FSM). The results revealed significant negative effects of FSM feeding beyond 3 weeks duration (2-5 weeks) on the weight gain, feed efficiency, production efficiency factor, protein efficiency ratio, and energy efficiency ratio of broiler chicken. Significant improvement of fatty acid profile of chicken meat was observed by FSM feeding up to 3 weeks only. Increasing the FSM feeding beyond 1 week in case of thigh meat and 2 weeks in case of breast meat significantly decreased the antioxidant capacity of broiler chicken meat. The lipid oxidation of broiler chicken meat increased significantly by feeding FSM beyond 2 weeks. Increase in the duration of FSM feeding has significantly decreased the serum triglyceride, cholesterol, cardiac risk ratio, atherogenic coefficient, and atherogenic index of plasma, though, the decline was not significant beyond the 2 weeks (3-5 weeks) feeding duration. The progressive increase in serum HDL cholesterol, serum antioxidant enzyme activities, and MDA concentration was observed with increase of FSM feeding duration. Thus, the 100 g FSM feeding for 2 weeks duration (3-5 weeks) has no negative effect on the growth performance with better health indices and serum biochemical profile of broiler chicken. However, increasing the duration of feeding beyond 1 week (4-5 week) exerts negative effects on the oxidative stability of meat and serum antioxidant enzyme profile.

1. Introduction

Broiler chicken meat is characterized by low fat, sodium, and cholesterol content with relatively a higher concentration of polyunsaturated fatty acids (PUFA), thus considered healthier than other animal protein sources. The preferences of people, particularly in the western countries, for lean meat has increased and the demand for fatty tissues has decreased. Higher intake of ω-3 PUFA paralleled by reduced intake of trans-fatty acids, saturated fatty acids (SFA) and cholesterol reduce the incidence or prevent coronary heart diseases (del Gobbo 2016; Kumar et al. 2019). With intermediate levels of monounsaturated fatty acids (MUFA) and lower levels of saturated fatty acids (SFA) (Betti et al. 2009a), flaxseed feeding to broiler chicken can be a novel strategy for a healthy meat production.Flaxseed is a unique oilseed crop used to produce oil as well as to add to diet since it contains high levels of -linolenic acid (Chung et al. 2005). Flaxseed can be used to enrich poultry meat and eggs since it is a good source of protein, oil, and -linolenic acid (Leeson and Summers 2005).

However, because of the constituent anti-nutritional factors (ANF), non-starch polysaccharides (NSPs), mucilages, linatine dipeptide (a vitamin B6 antagonist), etc. flaxseed is known to exert adverse effects on broiler chicken performance (Alzueta et al. 2003; Hernandez 2013) and tissue oxidative stability to some extent because of increased

tissue lipid unsaturation (Mir et al. 2018a, 2018b). These ANF and NSPs are associated with increased intestinal viscosity, reduced litter quality, and poor growth performance in broiler birds (Hall et al. 2006). On the other hand, cost of flaxseed is much higher than that of conventional feed ingredients used in broiler chicken ration. So it is important to choose a proper inclusion level and duration of feeding flaxseed to contain the adverse effects and exploit the beneficial properties of flaxseed economically. Thus, current study was set to optimize the duration of feeding 100 g flaxseed meal (FSM)/kg diet in broiler chicken and it was hypothesised that decreasing the duration of FSM feeding exerts positive effects on the growth performance, meat quality, and serum biochemical profile of broiler chicken.

2. Materials and Methods

This study was carried out at the Division of Avian Nutrition & Feed Technology of ICAR-Central Avian Research Institute, Izatnagar, India.

2.1 Birds and management

The study was approved (IAEC No: 275/04/ab19/CPCSEA) by Institutional Animal Ethics Committee (IAEC) by adopting the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) 2012" which was established under the 'Prevention of Cruelty to Animals Act 1960' of Indian Penal Code. Crossbred commercial chicken (288), CARIBRO Vishal, with uniform body weight were procured from institutional hatchery and divided in to 36 groups/replicates at random with eight birds in each. The birds were raised in battery cages having separate troughs for feed and water for each battery cage. Eight birds were housed in each battery for a period of 42 days (1.25 ft^2/bird). Light was provided to birds for 24 h on first three days, thereafter it was reduced by 1 h each day till a light period of 18 h reached and continued till 42nd day.

2.2 Experimental diets and design

The soybean meal in broiler chicken basal diet was replaced by 100 g FSM per kg diet and this level was standardized in preliminary trials carried out at our institute. Two diets were formulated – one with FSM and other without FSM. The FSM diet was offered to birds for one, two, three, four, and five weeks which resulted in five dietary treatments and the sixth one without FSM served as control. Six replicates of birds were assigned to each treatment (48 birds/treatment) at random. The FSM diet was fed for five weeks only and sixth week served as withdrawal period in which control diet was fed to all birds. The nutrient composition of diets and ingredients used for formulation of dietary treatments are given in Table 1.

2.3 Growth performance

The daily recording of feed intake and weekly body weight of birds was done throughout the experimental trial. The final body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of birds was calculated. The production efficiency factor (PEF), protein efficiency ratio (PEF), and energy efficiency ratio (EER), the measures of growth efficiency, were calculated as follows (Mir et al. 2019):

Table 1 Ingredients and nutrient composition of broiler chicken diets

Ingredients (g/kg)	Broiler starter		Broiler finisher	
	*T1 - T5	*T6	*T1 - T5	*T6
Maize	515.0	550.0	600.2	626.0
Flaxseed meal	100	0.0	100	0.0
De-oiled rice bran	8.20	4.55	0.0	3.55
Soybean	325.0	399.5	244.0	318.5
Vegetable oil	16.95	11.00	19.95	16.00
Limestone	8.0	8.0	11.5	12.0
Di-calcium Phosphate	18.0	18.0	15.5	15.0
Salt	3.0	3.0	3.0	3.0
DL-Methionine	1.3	1.3	1.3	1.3
Lysine	0.9	1.0	0.9	1.0
TM. Premix[1]	1.0	1.0	1.0	1.0
Vitamin Premix[2]	1.5	1.5	1.5	1.5
Vitamin B complex[3]	0.15	0.15	0.15	0.15
Choline Chloride	0.5	0.5	0.5	0.5
Toxin binder	0.5	0.5	0.5	0.5
Nutrient composition of diets (analysed)				
Crude Protein	229.7	229.9	200.0	200.2
M Energy (MJ/kg)	12.15	12.16	12.56	12.56
Calcium	10.0	10.0	10.0	10.0
Available P	5.0	5.0	4.0	4.0
Lysine	13.0	13.0	11.0	11.0

1 Trace mineral mixture (100 g): FeSO4.7H2O 8 g, ZnSO4.7H2O 10 g, MnSO4.H2O 10 g, CUSO4.5H2O 1 g, KI 30 g
2 Vitamin premix (1 g): Vitamin A 82.5 IU, Vitamin B2 50 mg, Vitamin D3 1200 unit, Vitamin K 10 mg
3 Vitamin B complex (1 g): Vitamin B1 8 mg, Vitamin B6 16 mg, Vitamin B12 80 mcg, Niacin 120 mg, Calcium panthotheonate 80 mg , Vitamin E 50% 160 mg, L-lysine 10 mg, and DL-Methionine 10 mg
* Diets fed for different time periods: T1 (0-5 weeks), T2 (1-5 weeks), T3 (2-5 weeks), T4 (3-5 weeks), T5 (4-5 weeks), T6 (control diet)

PEF = [Final body weight (kg) × Livability (%) × 100] /age in days × FCR

PEF = Weight gain /protein intake

EER = [Body weight gain (g) /total energy intake (ME Kcal)] × 100

2.4 Sample collection

After the completion of feeding trial at 42 days of age 12 birds were taken randomly from each treatment (2 bird/ replicate) and were sacrificed 12 hours after the complete withdrawal of feed with provision of *ad lib* clean drinking water. The blood samples were collected while sacrificing of birds and meat samples were collected after sacrificing. Meat sampling was done from both breast and thigh to evaluate the fatty acid profile, antioxidant capacity, and lipid oxidation status of broiler chicken meat. The serum was obtained from the blood samples collected without anticoagulant in sterile tubes for analysis of serum biochemical profile.

2.5 Fatty acid profile

The fatty acid methyl esters (FAMEs) were directly prepared from breast and thigh meat samples (O'Fallon et al. 2007). The standardised Gas chromatograph with CP-6173 60 m x 0.25 mm x 0.20 mm capillary column (Thermo Scientific Ceres 800 plus) was used for fatty acid profile analysis. The fatty acids were quantified by comparing their durations of retention to their corresponding fatty acid methyl ester standards under the standardised conditions of fatty acid profile analysis (Mir et al. 2018a). The results were presented as mg fatty acid per g meat. The fatty acid standard used in this study contained 37 different FAMEs and was purchased from Supelcon, Bellefonte- PA, USA.

2.6 Tissue antioxidant and lipid oxidation status

The antioxidant status of broiler chicken meat was assayed by measuring its capacity to neutralize the long lived free radical cations of ABTS (2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (1, 1-diphenyl-2-picrylhydrazyl). The spectrophotometric (Perkin Elmer, Model: Lambda EZ 201) analysis of ABTS (Shirwaekar et al. 2006) and DPPH (Kato et al. 1988) free radical scavenging activity was done in fresh meat and after one month of refrigerated storage as well.

$$\text{ABTS (\% inhibition)} = \frac{(0.7 - At_{20})}{0.7} \times 100$$

$$\text{DPPH (\% inhibition)} = 100 - \left(\frac{At_{20}}{At_0} \times 100\right)$$

Where, At_0 is initial absorbency and At_{20} is absorbency taken after 20 minutes.

Further, the extent of lipid oxidation in meat under different treatment groups was measured by estimation of Thiobarbituric Acid reactive Substances (TBARS) value (Witte et al. 1970), free fatty acid value, and peroxide value (Koniecko 1979). The extent of lipid oxidation of meat samples was assessed in fresh meat and after one month of refrigerated storage as well.

2.7 Serum biochemistry, health indices, and antioxidant status

The SPAN diagnostics kits were used for estimation of serum glucose, triglyceride, total cholesterol, and HDL cholesterol by following the manufacturer's instructions. Also, Cardiac Risk Ratio (CRR), Atherogenic Coefficient (AC), and Atherogenic Index of Plasma (AIP) were calculated as follows (Frolich and Dobiasova 2003):

CRR = Total cholesterol /HDL cholesterol

$$\text{AC} = \frac{(\text{Total cholesterol} - \text{HDL cholesterol})}{\text{HDL cholesterol}}$$

AIP = log (Triglycerides /HDL cholesterol)

The liver function tests were done by estimation of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetate (SGOT) by using SPAN diagnostics kits following manufacturer's instructions. To measure lipid oxidation status under different dietary treatments the Cayman diagnostics kits were used for estimation of serum TBARS which was expressed in terms of µM malondialdehyde (MDA) (Yagi 1998). Similarly, the antioxidant status in broiler chicken under different treatment groups was done by measuring the activities of serum antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) by using Cayman diagnostic kits (Wheeler et al. 1990).

2.8 Statistical Analysis

The data obtained in this study were analysed by one way ANOVA following general linear model (GLM) procedure (IBM SPSS software-20). For analysis of growth performance data replicate served as experimental unit and for the data analysis of fatty acid profile, meat quality, and serum biochemistry sampled bird served as an experimental uint. The Tukey post-hoc analysis separated the group means having significant differences at a significance level $P<0.05$. The orthogonal polynomial contrast of the data was also presented to validate the effects of feeding FSM for different durations on the parameters studied.

3. Results

3.1 Growth performance

The results of growth performance (Table 2) revealed that the feeding of FSM up to 3 weeks (2-5 weeks) had no significant negative effect on the BWG ($P<0.01$), FCR ($P<0.01$), PEF

(P<0.05), PER (P<0.05), and EER (P<0.05) of birds. However, feeding of FSM beyond 3 weeks resulted in significant decline of growth performance. The FI of birds was not influenced by FSM feeding.

3.2 Fatty acid profile

The results revealed that the feeding of FSM up to 3 weeks improved (P<0.01) content of PUFA, ω-3 PUFA, and ω-6 PUFA of thigh and breast meat along with the decline of SFA and MUFA (only thigh meat) content (Table 3). Beyond 3 weeks (2–5 weeks), no discernible improvement in the fatty acid profile was seen, suggesting that 3 weeks of feeding 100 g FSM is best for improving the fatty acid profile in broiler chicken meat.

3.3 Antioxidant parameters

The DPPH and ABTS free radical inhibition (%) in fresh broiler chicken and after one month of storage at refrigeration (Table 4) revealed significantly (P<0.01, P<0.05) higher values in birds fed control diet followed by statistically similar values in the birds fed FSM for 1 week (4-5 weeks). The ABTS and DDPH values of thigh meat (fresh as well as stored) of birds fed FSM for 2 weeks (3-5 weeks) were significantly lower compared to control but statistically similar to the birds fed FSM for 1 week (4-5 weeks). However, the ABTS and DPPH values of breast meat of birds fed FSM for 2 weeks were statistically similar to control as well as birds fed FSM for 1 week only. Increasing the duration of FSM to 3, 4, and 5 weeks decreased the values of DPPH and ABTS in broiler chicken meat but generally did not differ significantly from each other.

3.4 Lipid oxidation parameters

No significant effect of FSM feeding was observed on the free fatty acid and peroxide values of fresh chicken meat (Table 5). The free fatty acid and peroxide value of thigh and breast meat samples after one month of storage were significantly (P<0.01) lower in control diet fed birds followed by statistically similar values in birds fed FSM for 1 week (4-5 weeks) compared to other durations of FSM feeding which did not differ significantly from each other. However, the peroxide value of one month refrigerated stored breast and thigh meat in birds fed FSM for 1 week was statistically similar to control as well as to all other durations of FSM feeding in birds. In general the TBARS values of fresh as well as stored thigh, breast, and liver samples did not increase significantly by feeding FSM up to 2 weeks (3-5 weeks), whereas beyond 2 weeks feeding significant (P<0.01) increase was observed compared to control. However, the longer durations of feeding *viz*. 3, 4, and 5 weeks did not differ significantly from each other in terms of TBARS values of thigh, breast, and liver samples.

3.5 Serum biochemistry, health indices, and antioxidant parameters

The results of serum biochemistry (Table 6) have shown significant effects of FSM feeding except the serum glucose, SGOT, and SGPT concentrations. The serum triglyceride was significantly (P<0.01) lower in birds fed FSM for 5 weeks duration (0-5 weeks) which was statistically similar to 4 weeks (1-5 weeks) and 3 weeks (2-5 weeks) duration compared to birds fed control diet or FSM for 1 week (4-5 weeks). The serum triglyceride of birds fed FSM for 2 weeks (3-5 weeks) duration was statistically similar to 3 and 1 week duration and also to control diet without FSM. The FSM feeding for 5 weeks duration (0-5 weeks) resulted in significantly (P<0.01) lower serum cholesterol content of birds which was statistically similar to 4 weeks (1-5 weeks), 3 weeks (2-5 weeks), and 2 weeks (3-5 weeks) durations. The significantly higher concentration of cholesterol was observed in birds fed control diet which was statistically similar to birds fed FSM for 1 week duration (4-5 weeks). The serum HDL cholesterol concentration was lower (P<0.05) in control diet fed birds followed by statistically similar in values in birds fed FSM for 1 week duration (4-5 weeks) compared to birds fed FSM for 4 weeks (1-5 weeks) or 5 weeks (0-5 weeks) duration. The birds fed FSM for 2 weeks (3-5 weeks) or 3 weeks (2-5 weeks) durations resulted in intermediate serum HDL cholesterol.

The serum health indicators, CRR and AC, were significantly (P<0.05) lower in birds fed FSM for 5 weeks (0-5 weeks) or 4 weeks (1-5 weeks) durations which were statistically similar to 3 weeks (2-5 weeks) and 2 weeks (3-5 weeks) durations. The higher values of the indicators were found in birds fed control diet followed by birds fed FSM for 1 week (4-5 weeks) duration which was statistically similar to 2 and 3 weeks durations. The atherogenic index of plasma was significantly (P<0.01) higher in birds fed either control diet or FSM for 1 week duration (4-5 weeks) which were statistically similar to that of birds fed FSM for 2 weeks duration (3-5 weeks) compared to other durations which were statistically similar each other. Among the serum antioxidant enzymes significantly (P<0.01) lower SOD and GPx concentrations were observed in birds fed either control diet or FSM for 1 week (4-5 weeks) or 2 weeks (3-5 weeks) durations compared to other durations which did not differ statistically from each other. The serum catalase concentration was significantly (P<0.01) lower in birds fed control diet followed by statistically similar in birds fed FSM for 1 week duration (4-5 weeks) compared to 5 weeks (0-5 weeks), 4 weeks (1-5 weeks), and 3 weeks (2-5 weeks) durations which did not differ significantly from each other. Similarly, the serum GR concentration was significantly (P<0.01) lower in birds fed control diet followed by statistically similar in birds fed FSM for 1 week (4-5 weeks) compared to birds fed FSM for 5 weeks (0-5 weeks) duration. The 2 weeks, 3 weeks, and 4 weeks durations resulted in intermediate GR concentrations. The serum MDA

Table 2 Growth performance of broiler chicken fed flaxseed meal (FSM) for different durations (n = 48)

Performance parameters	Treatments (100 g FSM feeding durations)						SEM	P value	
	0-5 weeks	1-5 weeks	2-5 weeks	3-5 weeks	4-5 weeks	Control		Linear	Quadratic
Body weight gain (g)	1712^{a}	1739^{ab}	1802^{bc}	1813^{c}	1829^{c}	1846^{c}	12.1	0.006	0.098
Feed intake (g)	3165	3171	3110	3136	3104	3133	17.4	0.079	0.264
Feed conversion ratio	1.85^{c}	1.82^{bc}	1.73^{ab}	1.73^{ab}	1.70^{a}	1.70^{a}	0.021	0.007	0.087
Production efficiency factor	208^{a}	214^{a}	240^{b}	241^{b}	243^{b}	245^{b}	6.7	0.021	0.127
Protein efficiency ratio	2.51^{a}	2.60^{b}	2.67^{c}	2.72^{c}	2.72^{c}	2.71^{c}	0.034	0.018	0.246
Energy efficiency ratio	18.3^{a}	18.9^{b}	19.5^{bc}	19.8^{c}	19.8^{c}	19.8^{c}	0.25	0.027	0.196

Values bearing different superscripts within the row differ significantly
SEM: Standard error of mean

Table 3 Lipid composition of broiler chicken meat fed flaxseed meal (FSM) for different durations (n = 12)

Lipid composition (mg/g meat)	Treatments (100 g FSM feeding durations)						SEM	P value	
	0-5 weeks	1-5 weeks	2-5 weeks	3-5 weeks	4-5 weeks	Control		Linear	Quadratic
Thigh									
SFA	24.8^{a}	26.5^{a}	27.8^{a}	39.8^{b}	43.4^{bc}	45.8^{c}	0.85	0.008	0.196
MUFA	35.9^{a}	35.8^{a}	35.9^{a}	37.2^{b}	38.2^{b}	38.1^{b}	0.61	0.016	0.108
PUFA	30.4^{b}	29.7^{b}	29.3^{b}	26.1^{a}	25.4^{a}	24.1^{a}	0.94	0.007	0.069
ω-3 PUFA	9.96^{c}	9.63^{c}	9.22^{c}	7.32^{b}	5.82^{ab}	4.68^{a}	0.412	0.032	0.274
ω-6 PUFA	20.4^{b}	20.1^{b}	20.1^{b}	18.7^{a}	19.6^{a}	19.3^{a}	0.49	0.024	0.202
Breast									
SFA	16.7^{a}	17.3^{a}	17.9^{a}	24.7^{b}	26.8^{bc}	28.3^{c}	0.71	0.009	0.178
MUFA	20.8	20.8	20.6	20.8	21.1	21.1	0.38	0.086	0.238
PUFA	16.0^{c}	15.8^{c}	15.5^{c}	13.6^{b}	13.1^{ab}	12.4^{a}	0.47	0.013	0.137
ω-3 PUFA	4.44^{c}	4.38^{c}	4.26^{c}	3.23^{b}	2.78^{ab}	2.49^{a}	0.318	0.016	0.289
ω-6 PUFA	11.6^{b}	11.4^{b}	11.2^{b}	10.3^{a}	10.4^{a}	9.9^{a}	0.42	0.018	0.194

Values bearing different superscripts within the row differ significantly
SEM: Standard error of mean
SFA: Saturated fatty acids; MUFA: Mono unsaturated fatty acids; PUFA: Poly unsaturated fatty acids

concentration was significantly (P<0.01) lower in control diet fed birds or the birds fed FSM for 1 week (4-5 weeks) compared to birds fed FSM for 5 weeks (0-5 weeks) or 4 weeks (1-5 weeks) duration. The FSM feeding for 3 weeks (2-5 weeks) and 2 weeks (3-5 weeks) resulted in intermediate serum MDA concentration of birds.

4. Discussion

According to the findings of the current study, FSM has a negative impact on the growth performance of broiler chicken because of. insufficient energy availability, anti-nutritional elements present, and low flaxseed digestibility (Mir et al.

Table 4 Antioxidant parameters of broiler chicken meat under the influence of flaxseed meal (FSM) feeding for different durations (n = 12)

Antioxidant parameters		Treatments (100 g FSM feeding durations)						SEM	P value	
		0-5 weeks	1-5 weeks	2-5 weeks	3-5 weeks	4-5 weeks	Control		Linear	Quadratic
DPPH (% inhibition)										
Fresh meat	Thigh	16.2^{a}	16.5^{a}	17.1^{a}	17.2^{ab}	17.8^{bc}	18.8^{c}	0.35	0.008	0.172
	Breast	21.2^{a}	21.4^{a}	22.0^{a}	23.8^{b}	24.0^{b}	24.9^{b}	0.32	0.007	0.181
After 1 month	Thigh	9.1^{a}	9.9^{ab}	10.8^{abc}	11.1^{bc}	12.4^{cd}	13.3^{d}	0.35	0.003	0.098
	Breast	11.8^{a}	12.2^{a}	14.6^{b}	16.2^{bc}	15.8^{bc}	17.1^{c}	0.43	0.006	0.221
ABTS (% inhibition)										
Fresh meat	Thigh	83.9^{a}	83.9^{a}	84.1^{a}	84.3^{a}	85.6^{ab}	86.1^{b}	0.27	0.026	0.286
	Breast	87.7^{a}	88.1^{ab}	88.3^{ab}	89.7^{bc}	89.5^{bc}	90.1^{c}	0.27	0.021	0.216
After 1 month	Thigh	69.2^{a}	69.8^{ab}	70.7^{b}	70.4^{b}	72.9^{c}	73.8^{c}	0.33	0.010	0.201
	Breast	84.4^{a}	85.0^{a}	84.9^{a}	86.3^{b}	86.5^{b}	87.3^{b}	0.23	0.005	0.148

Values bearing different superscripts within the row differ significantly
SEM: Standard error of mean
DPPH: 1, 1-diphenyl-2-picrylhydrazyl
ABTS: 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid

2017a). However, the growth performance of birds fed FSM up to three weeks duration did not differ significantly from the control birds. It clearly indicates that the negative effects could be overcome by reducing the duration of FSM feeding from five weeks to three weeks. The previous studies reported negative effects of full time flaxseed feeding on growth performance of broiler chicken (Mir et al. 2017a, 2018a, 2018b; Azcona et al. 2008; Mridula et al. 2011). The decline in growth performance of broiler chicken with increasing duration of flaxseed feeding has been reported (Zuidhof et al. 2009), however, the decline of growth performance was noticed in birds after 12-16 days of feeding compared to 21 days (3 weeks) in the present study. However, in contrast to the present study there are the reports showing no significant effects of supplementing flaxseed/flaxseed oil for different durations on growth performance of broiler chicken (Kanakri et al. 2017; Mirshekar et al. 2015; Stanacev et al. 2014; Tarek et al. 2014).

In the present study chicken diets were made isocaloric and isonitrogenous to avoid the confounding effects of energy and nitrogen imbalance on the deposition of fatty acid in chicken meat. In monogastrics such as poultry, dietary unsaturated fatty acids are absorbed rapidly without much biohydrogenation which may be the reason for higher PUFA and lower SFA content in meat of birds fed ω-3 PUFA rich flaxseed meal (Mir et al. 2017a; Mirshekar et al. 2015). Increasing the feeding duration of FSM/flaxseed oil in broiler chicken reduces the SFA content of chicken meat (Mirshekar et al. 2015; Kumar et al. 2019). However, increasing FSM feeding beyond 3 weeks duration did not produce any significant effect on fatty acid composition in this study.

The DPPH and ABTS assays of meat reveal its free radical neutralizing ability. And, the TBARS (mg MDA/kg sample) value, free fatty acid value, and peroxide value are the markers of lipid oxidation in chicken meat (Mir et al. 2018a, 2018b; Kumar et al. 2020). The present study establishes that increasing the duration of FSM feeding in broiler chicken negatively affects the DPPH and ABTS values of broiler chicken meat. The feeding of FSM beyond 1 week in case of thigh meat and 2 weeks in case of breast meat decreased the DPPH and ABTS values. This negative effect of reduced free radical scavenging ability of meat due to FSM feeding has been linked to increased unsaturated fatty acid content of chicken meat which fosters more free radical generation (Mir et al. 2018a, 2018b; Kumar et al. 2020). It has been reported that inclusion of FSM in broiler chicken ration caused significant decline in the values of DPPH and ABTS in broiler chicken meat (Mir et al. 2017b, 2018a, 2018b). The free fatty acid value and peroxide value of broiler chicken meat on refrigerated storage and TBARS value of fresh as well as stored meat increased significantly in current study with increasing FSM feeding duration. In general an increase of lipid oxidation was observed by feeding FSM beyond 2 weeks. It suggests that increasing the duration of FSM feeding increases the deposition of unsaturated fatty acids in broiler chicken meat which are

Table 5 Antioxidant parameters of broiler chicken meat under the influence of flaxseed meal (FSM) feeding for different durations (n = 12)

Oxidation parameters		Treatments (100 g FSM feeding durations)						SEM	P value	
		0-5 weeks	1-5 weeks	2-5 weeks	3-5 weeks	4-5 weeks	Control		Linear	Quadratic
Free fatty acid (%)										
Fresh meat	Thigh	0.08	0.08	0.09	0.08	0.09	0.09	0.004	0.078	0.478
	Breast	0.09	0.09	0.10	0.10	0.10	0.11	0.003	0.085	0.563
After 1 month	Thigh	0.21^{b}	0.20^{b}	0.20^{b}	0.19^{b}	0.17^{a}	0.16^{a}	0.004	0.008	0.271
	Breast	0.21^{b}	0.20^{b}	0.19^{b}	0.18^{b}	0.16^{a}	0.15^{a}	0.006	0.005	0.187
Peroxide value (meq/kg)										
Fresh meat	Thigh	1.42	1.40	1.38	1.36	1.34	1.31	0.025	0.112	0.442
	Breast	1.33	1.31	1.28	1.27	1.22	1.15	0.027	0.131	0.743
After 1 month	Thigh	1.85^{b}	1.79^{b}	1.77^{b}	1.75^{b}	1.69^{ab}	1.52^{a}	0.032	0.031	0.225
	Breast	1.54^{b}	1.52^{b}	1.49^{b}	1.44^{b}	1.36^{ab}	1.25^{a}	0.029	0.018	0.086
TBARS value (mg MDA/kg meat)										
Fresh meat	Thigh	1.40^{c}	1.37^{c}	1.32^{bc}	1.16^{ab}	1.11^{a}	1.03^{a}	0.035	0.005	0.162
	Breast	1.03^{c}	0.97^{c}	0.91^{bc}	0.78^{ab}	0.72^{a}	0.68^{a}	0.033	0.010	0.278
	Liver	0.93^{b}	0.89^{b}	0.80^{b}	0.60^{a}	0.56^{a}	0.46^{a}	0.04	0.007	0.074
After 1 month	Thigh	3.10^{c}	3.09^{c}	2.90^{c}	2.68^{bc}	2.24^{ab}	1.85^{a}	0.104	0.004	0.081
	Breast	2.34^{c}	1.97^{bc}	1.86^{bc}	1.50^{ab}	1.33^{a}	1.23^{a}	0.095	0.006	0.153
	Liver	1.72^{c}	1.60^{c}	1.39^{bc}	1.21^{abc}	0.97^{ab}	0.81^{a}	0.086	0.008	0.079

Values bearing different superscripts within the row differ significantly

SEM: Standard error of mean

TBARS: Thio-barbituric acid reactive substances; MDA: Malondialdehyde

highly prone to rapid oxidation than the saturated fatty acids. Comparatively lower free radical scavenging ability and higher lipid oxidation was observed in thigh meat than the breast meat because of greater fat deposition rate in chicken thighs (Kumar et al. 2020). The increase of free fatty acid and peroxide values of broiler chicken meat due to the feeding of FSM were observed in previous studies (Mir et al. 2017b, 2018a, 2018b). An increase in TBARS values of thigh and breast meat was observed in earlier study by increasing the duration of flaxseed feeding in broiler chicken (Betti et al. 2009b). In swine, fed 6% crushed linseed diet, Kouba et al (2003) reported increased pork TBARS values with increasing duration of feeding. Increase in the TBARS values of broiler chicken meat due to the feeding of flaxseed in broiler chicken has also been reported by various researchers (Mir et al. 2017b, 2018a, 2018b; Anjum et al. 2013; Abdulla et al. 2015). However, notwithstanding the knowledge of authors of this manuscript there is no report suggesting the effects of FSM feeding for different durations on the DPPH, ABTS, free fatty acid, and peroxide values of broiler chicken meat.

In the present study, an increase in duration of FSM feeding significantly decreased the serum triglyceride, cholesterol, cardiac risk ratio, atherogenic coefficient, and atherogenic index of plasma, though, the decline was not significant beyond 2 weeks (3-5 weeks) feeding duration. However, in general an increase of FSM feeding duration beyond 2 weeks (3-5 weeks) progressively increased the serum HDL cholesterol, serum antioxidant enzyme (SOD, catalase, GPx, GR) activities, and MDA concentration. The FSM produces hypocholesterolemic effects by preventing the recycling of bile acids from intestines due to which more and more cholesterol is converted to bile acids to maintain the supply resulting in lower serum cholesterol levels (Mir et al. 2018b; Saxena and Katare 2014). On the hand low serum triglyceride levels have been associated with increase of ω-3 fatty acids in chicken due to FSM feeding (Mir et al. 2017a). Flaxseed consumption had hypocholesterolemic effects in animal models like rats, mice, and rabbits, usually as a result of increased faecal excretion of lipids (Kristensen et al., 2012). The results of present study are corroborated by Mir et al (2017a) and Saxena and Katare (2014) who observed significant reduction in serum cholesterol and triglyceride levels due to flaxseed consumption, whereas, significant increase in serum HDL cholesterol was observed (Saxena and

Table 6 Effect of feeding flaxseed meal (FSM) for different durations on serum biochemical parameters and health indices of broiler chicken (n = 48)

Serum biochemical parameters	Treatments (100 g FSM feeding durations)						SEM	P value	
	0-5 weeks	1-5 weeks	2-5 weeks	3-5 weeks	4-5 weeks	Control		Linear	Quadratic
Glucose (mg/dl)	188	191	196	203	201	208	8.2	0.065	0.386
Triglyceride (mg/dl)	78.7^{a}	80.3^{a}	81.4^{ab}	86.2^{bc}	86.9^{c}	87.4^{c}	1.91	0.009	0.147
Cholesterol (mg/dl)	121^{a}	126^{ab}	127^{ab}	128^{ab}	130^{bc}	136^{c}	2.2	0.006	0.098
HDL cholesterol (mg/dl)	81.4^{c}	79.7^{c}	77.2^{b}	75.6^{b}	72.5^{ab}	69.8^{a}	1.8	0.027	0.228
Cardiac risk ratio	1.49^{a}	1.58^{a}	1.65^{ab}	1.69^{ab}	1.79^{b}	1.95^{c}	0.067	0.018	0.187
Atherogenic coefficient	0.49^{a}	0.58^{a}	0.65^{ab}	0.69^{ab}	0.79^{b}	0.95^{c}	0.067	0.023	0.219
Atherogenic index of plasma	-0.01^{a}	0.00^{a}	0.02^{a}	0.06^{ab}	0.08^{b}	0.10^{b}	0.008	0.019	0.201
SGOT (U/ml)	104	106	108	111	112	111	4.1	0.131	0.473
SGPT (U/ml)	21.7	22.6	23.8	23.5	24.3	24.4	1.56	0.096	0.363
SOD (U/ml)	119^{b}	117^{b}	116^{b}	111^{a}	109^{a}	108^{a}	1.96	0.005	0.061
Catalase (nmol/min/ml)	59.3^{c}	58.1^{c}	56.2^{c}	51.5^{b}	49.7^{ab}	47.4^{a}	0.96	0.007	0.079
GPx (nmol/min/ml)	13.8^{b}	13.1^{b}	12.7^{b}	11.0^{a}	11.2^{a}	10.7^{a}	0.29	0.006	0.56
GR (nmol/min/ml)	16.5^{d}	16.1^{cd}	15.9^{c}	14.4^{b}	14.0^{ab}	13.6^{a}	0.22	0.010	0.194
TBARS Value (MDA µM)	7.7^{d}	7.4^{d}	6.9^{c}	6.0^{b}	5.5^{a}	5.2^{a}	0.18	0.002	0.069

Values bearing different superscripts within the row differ significantly
SEM: Standard error of mean
SOD: Super oxide dismutase; GPx: Glutathione peroxidase; GR: Glutathione reductase; TBARS: Thio-barbituric acid reactive substances; MDA: Malondialdehyde
SGOT: Serum glutamic oxalacetic and SGPT: Serum glutamic pyruvic transaminase

Katare 2014). A major risk factor for the development of cardiovascular diseases is decreased plasma concentrations of HDL cholesterol (Martirosyan et al. 2007). And, researchers have shown that increase in plasma HDL cholesterol concentration reduces the cardiovascular risks (Assmann and Gotto 2004) which supports the decline of health indices like cardiac risk ratio, atherogenic coefficient, and atherogenic index of plasma observed in the present study. The faster growth rates in modern day broiler chicken increase the workload on cardiovascular system which predisposes them to disorders such as right ventricular failure, ascites syndrome, cardiac arrhythmias, cardiopulmonary disorders, and sudden death (Julian 2005). The increase of dietary long-chain ω-3 fatty acids, such as flaxseed, have been reported to decrease the incidence of sudden death in broiler chicken due to cardiomyopathy (Cherian et al. 2005).

Similar to the observations of present study Mir et al (2017a) observed a significant increase of antioxidant enzyme activities (SOD, CAT, GSH-Px, GR) and serum TBARS values due to the incorporation of FSM in broiler chicken ration. This may be due to higher lipid unsaturation in chicken meat which induces greater lipid peroxidation and in turn greater free radicals. This enhanced lipid peroxidation is supported by the decline of values of ABTS and DPPH in chicken meat and the increased antioxidant defence enzymes is considered as a regulatory response. Anjum et al (2013) has also reported an increased serum SOD, catalase, GSH-Px and GR activities due to higher inclusion rate of flaxseed in the broiler chicken diet. In rats it has been reported that feeding of diet supplemented with ω-3 fatty acids from flaxseed oil increased the activity of the serum antioxidant enzymes like catalase and glutathione peroxidase (Ramaprasad et al. 2005). On the other hand when type 2 diabetic patients were fed defatted flaxseed for 2 months, significant improvement in plasma lipid profile and reduction of lipid peroxidation was observed (Mohamed et al. 2012). Similar to the results of present study Beheshti Moghadam et al (2017) found that the bird fed 100 g flaxseed revealed no effects on serum SGOT and SGPT levels indicating no negative effects of dietary FSM on liver functioning. Further, in laying hens it has been shown that feeding moderate level of flaxseed (50-100 g) exerts no significant effect on serum SGPT (Yassein et al. 2015). Further, the nonsignificant effect of supplementing FSM in broiler chicken (Mir et al. 2017a) and linseed oil in turkey (Jankowski et al. 2012) on serum glucose have been reported. However, contrary to the current study observations

the serum cholesterol and triglyceride concentration were not affected by feeding 1% and 2% linseed oil in broiler starter and finisher ration (Mandal et al. 2014). Also, Jankowski et al (2012) found no significant differences in values of blood biochemical parameters (triglycerides, total cholesterol, and HDL cholesterol) after 15 weeks feeding of soybean, rapeseed, and linseed oil in turkey.

5. Conclusions

Based on the results of present study it has been concluded that in pursuit of using flaxseed meal for omega-3 fatty acid enrichment, the feeding of 100 g flaxseed meal for 2 weeks duration (3-5 weeks) has no negative effect on the growth performance with improved fatty acid composition, health indices, and serum biochemical profile of broiler chicken. However, to increase the duration of 100 g flaxseed meal feeding beyond 1 week (4-5 week) the addition of some suitable feed antioxidant is necessary for better oxidative stability of meat and serum antioxidant enzyme profile.

Declarations

Funding: None

Conflict of interests: The authors declare no conflict of interest

Ethics approval: The study was approved (IAEC No: 275/04/ab19/CPCSEA) by the Institutional Animal Ethics Committee (IAEC)

Acknowledgement: None to acknowledge

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Citation

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