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Growth performance, tissue lipid composition and metabolism, health indices, and serum lipid chemistry of broiler chicken in response to dietary flaxseed and chromium

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Abstract

This study investigated growth performance, growth efficiency, lipid composition, lipid metabolism, health indices, and serum lipid chemistry of broiler chicken fed flaxseed meal (FSM) and Cr. Five dietary treatments were formulated – T1 (control), T2 (100 g FSM), T3 (100 g FSM + 0.5 ppm Cr), T4 (100 g FSM + 1.0 ppm Cr), and T5 (100 g FSM + 1.5 ppm Cr). Each treatment was assigned six replicate groups of day-old broiler chicks with eight birds in each. Feeding of 100 g FSM exerted negative effects on the growth performance during starter phase only (0-3 weeks) and overall growth efficiency parameters in broiler chicken, whereas, Cr supplementation reversed these negative effects. 100 g FSM reduced abdominal fat in chicken and Cr supplementation linearly decreased it with minimum at 1.5 ppm Cr. Feeding of 100 g FSM favourably improved the activities of lipid metabolism enzymes which resulted in improved fatty acid profile and health indices of chicken meat. No significant effect of Cr supplementation was observed on lipid metabolism, fatty acid profile, and health indices of chicken meat. 100 g FSM decreased serum total cholesterol, triglyceride, cardiac risk ratio, atherogenic coefficient, and atherogenic index of plasma, whereas, Cr supplementation decreased these parameters linearly with increasing levels. Antioxidant enzyme activities and lipid peroxidation were increased by FSM, whereas, Cr supplementation linearly decreased them with increasing levels; and inverse trend was observed in serum HDL cholesterol levels. This study concludes that feeding of 100 g FSM exert negative effects on growth performance of young chicken (0-3 weeks), favourably alter lipid metabolism which results in improved fatty acid profile and health indices of chicken meat. It improves the serum lipid profile and atherogenic indices in broiler chicken, but negatively affects the oxidative stability of lipids. However, Cr supplementation at 1.5 ppm level in diet successfully overcomes the negative effects of FSM feeding on growth performance and lipid oxidative stability.

1. Introduction

Chromium (Cr), an essential trace element required for normal carbohydrate and lipid metabolism in humans and animals (Kroliczewska et al. 2005), has generated considerable interest in its role in animal nutrition. In the field of animal nutrition, there are emerging evidences which suggest that dietary Cr requirement of poultry may be higher than the amount found in a typical corn soybean meal diet (Amata 2013). Poultry feeds of plant origin are poor in Cr and poultry birds may exhibit Cr deficiency (Debski et al. 2004) which may reflect badly on their growth performance. The Cr deficiency disrupts the normal carbohydrate, protein, and lipid metabolism and reduces the insulin sensitivity in peripheral tissues which results in impairment of growth rate in broiler chicken (Sahin et al. 2003). Improved growth performance (Toghyani et al. 2006), carcass yield, and reduced abdominal fat (Debski et al. 2004; Sahin et al. 2003; Toghyani et al. 2006) was reported in broiler chicken supplemented with dietary Cr. However, compared to ruminants very scanty literature pertaining to Cr supplementation in broiler chicken is available (Hossain 2007). Thus, because of this insufficient and inconclusive literature on Cr supplementation and its bioavailability there are no dietary Cr recommendations for poultry.

On the other hand, increase in incidences of cardiovascular diseases in past few decades have been

observed, because of newly evolved food and eating habits of people, which reflects badly on their health and involves high treatment costs (Mir et al. 2018a). Health professionals worldwide recommend the intake of ω-3 polyunsaturated fatty acids (PUFA) such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to help contain or prevent cardio and cerebro vascular diseases in humans (del Gobbo 2016). In the long run, use of marine sources for enrichment of human diets with EPA and DHA is an unsustainable solution which creates a negative impact on global fish stocks and aquaculture (Kumar et al. 2019). Under such circumstances flaxseed feeding in chicken, a richest terrestrial source of ω-3 fatty acids, is a most valuable nutritional strategy for developing the value-added poultry meat with improved health indices (Kumar et al. 2020). Because of direct absorption of dietary lipids without significant biohydrogenation broiler chicken are most suitable and an easy targets for ω-3 fatty acid fortification (Mir et al. 2018a, 2018b; Kumar et al. 2019; Kumar et al. 2020). The ω-3 fatty acid such as alpha-linolenic acid (ALA) abundantly present in flaxseed can be converted into long chain PUFA such as EPA, DPA, DHA by the desaturating enzymes (Kumar et al. 2020).

Though, flaxseed feeding in chicken increases the omega-3 fatty acid content of meat (Mir et al. 2018a; Kumar et al. 2019; Mir et al. 2018b) and reduces abdominal fat (Debski et al. 2004; Sahin et al. 2003; Toghyani et al. 2006) it exerts negative effects on the growth performance of broiler chicken (Zuidhof et al. 2009). Also, an increased UFA content in broiler chicken meat makes it more prone to lipid peroxidation. However, Cr has shown antioxidant properties which alleviate the negative effects of oxidative stress and confer protection against lipid peroxidation (Anderson 2000; Hoeck et al. 2020). Cr also influences the metabolism of cholesterol and triglycerides (Rajalekshmi et al. 2008) by which it exerts hypocholesterolemic and hypolidemic effects. Thus, this study was designed to analyse the effects of flaxseed meal (FSM) feeding and Cr supplementation on the growth performance, carcass characteristics, lipid composition, lipid metabolism, and health indices of meat along with the serum antioxidant and lipid chemistry in broiler chicken with a hypothesis that in chicken the lipid profile and health indices are improved by flaxseed feeding, and the Cr supplementation alleviates the consequent lipid peroxidation problem arising out of flaxseed meal feeding.

2. Materials and Methods

2.1 Experimental design, birds, husbandry conditions, and diets

The experimental trial was conducted on 300 day-old commercial broiler chicken, procured from the institutional hatchery, to investigate the effects of dietary FSM and Cr supplementation on their performance, lipid deposition, meat quality, and serum biochemistry. The birds were randomly assigned to five dietary treatments, having six replicates (cages) per treatment with 10 birds in each replicate. The birds were raised in battery cages for six weeks with ad libitum provision of mash feed and fresh water on daily basis. The birds were provided 23 h light first 3 days followed by a decrease of 1 h per day until it reached a 18 h light period which was continued until 42nd day. The initial cage temperature of 95 °F was reduced by 5 °F every week to provide thermo-comfort environment to the birds. The FSM and Cr were employed to formulate five iso-caloric and iso-nitrogenous dietary treatments – T1 (control), T2 (100 g FSM), T3 (100 g FSM + 0.5 ppm Cr), T4 (100 g FSM + 1.0 ppm Cr), and T5 (100 g FSM + 1.5 ppm Cr). The inclusion level of 100 g FSM was standardized by preliminary trials. The ingredients and nutrient composition of each dietary treatment is shown in Table 1. The organic Cr in the form of chromium picolinate was used for Cr supplementation.

2.2 Growth performance

The body weight of individual birds were recorded on weekly basis and feed intake was calculated daily on replicate basis by taking the difference of feed offered and residual feed. The body weight gain (BWG), feed intake, and feed conversion ratio (FCR) were calculated for 0-3 week, 4-6 week, and 0-6 week durations. The mortality of the birds was recorded as and when it occurred. The growth efficiency parameters viz. production efficiency factor (PEF), protein efficiency ratio (PER), and energy efficiency ratio (EER) were calculated as follows (Mir et al. 2018c):

$$PEF = [\text{Final body weight (kg)} \times \text{Livability (\%)} \times 100] / \text{age in days} \times \text{FCR}$$

$$PEF = \text{Weight gain} / \text{protein intake}$$

$$EER = [\text{Body weight gain (g)} / \text{total energy intake (ME Kcal)}] \times 100$$

2.3 Carcass characteristics and cost economics

At the end of six weeks feeding trial 12 birds from each dietary treatment (1 male and 1 female per replicate) were selected randomly and sacrificed after 12 hours of fasting with ad libitum drinking water for evaluation of carcass characteristics. Cost of feed involved in the production of broiler chicken was calculated in terms of per kg live weight gain and per kg meat yield based on prevailing market price of the feed ingredients and expressed as percentage change in cost in each treatment group with respect to the control diet (T1).

2.4 Sample collection

The birds sacrificed for carcass trait study were used for collection of breast and thigh muscle samples without skin for the study of fatty acid profile, fatty acid metabolism indices, and health indices. Also, prior to sacrificing of birds

Table 1 Ingredients and nutrient composition (g/kg) of broiler chicken starter and finisher basal diets

Ingredients (g/kg)	Broiler starter					Broiler finisher				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Maize	537	535	535	535	535	625	622	622	622	622
Flaxseed meal	0.0	100	100	100	100	0.0	100	100	100	100
Soybean	390	290	290	290	290	300	200	200	200	200
Fish meal	30	30	30	30	30	30	30	30	30	30
vegetable oil	14	16	16	16	16	14	16	16	16	16
Limestone	9	9	9	9	9	11	11	11	11	11
DCP[1]	15.0	15.0	15.0	15.0	15.0	15.0	15.5	15.5	15.5	15.5
Salt	3	3	3	3	3	3	3	3	3	3
DL-Methionine	1	1	1	1	1	1	1	1	1	1
Lysine	0.0	0.3	0.3	0.3	0.3	0.0	0.3	0.3	0.3	0.3
Constant*	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765
Chromium (ppm)	0.0	0.0	0.5	1.0	1.5	0.0	0.0	0.5	1.0	1.5
Nutrient composition (Calculated based on the analyzed values of ingredients)										
Crude Protein	213	213	213	213	213	185	184	184	184	184
M Energy (MJ/ kg)	12.23	12.22	12.22	12.22	12.22	12.55	12.54	12.54	12.54	12.54
Calcium	10	10	10	10	10	11	11	11	11	11
Available P	5	5	5	5	5	4	4	4	4	4
Lysine	13	13	13	13	13	10	10	10	10	10
Fatty acid profile of diets (%)										
C14:0	1.70	0.12	0.12	0.12	0.12	1.81	0.12	0.12	0.12	0.12
C16:0	31.4	8.59	8.59	8.59	8.59	33.1	9.32	9.32	9.32	9.32
C16:1	9.80	3.59	3.59	3.59	3.59	11.0	4.23	4.23	4.23	4.23
C18:0	0.0	0.07	0.07	0.07	0.07	0.21	0.19	0.19	0.19	0.19
C18:1 ω-9	38.4	21.1	21.1	21.1	21.1	39.9	23.4	23.4	23.4	23.4
C18:2 ω-6	15.5	23.4	23.4	23.4	23.4	11.4	22.8	22.8	22.8	22.8
C18:3 ω-3	3.20	43.1	43.1	43.1	43.1	2.6	39.9	39.9	39.9	39.9
SFA	33.1	8.78	8.78	8.78	8.78	35.12	9.63	9.63	9.63	9.63
MUFA[2]	48.2	24.7	24.7	24.7	24.7	50.9	27.6	27.6	27.6	27.6
PUFA[3]	18.7	66.5	66.5	66.5	66.5	14.0	62.7	62.7	62.7	62.7

*Constant: (0.3% salt, 0.1% DL-Methionine, 0.1% Trace mineral premix, 0.15% Vitamin premix, 0.015% Vitamin B complex, 0.05% Choline chloride, 0.05% Toxin binder)

Trace mineral mixture (100 g): $FeSO_4.7H_2O$-8 g, $ZnSO_4.7H_2O$-10 g, $MnSO_4$. H_2O- 10 g, $CUSO_4.5H_2O$-1 g, KI- 30 g

Vitamin premix (1 g): Vitamin A-82.5 IU, Vitamin B2-50 mg, Vitamin D3-12000 unit, Vitamin K-10 mg

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

[1] DCP- Di-calcium Phosphate

[2] Monounsaturated fatty acids

[3] Polyunsaturated fatty acids

blood samples were collected in non-heparinised tubes, serum was harvested, and immediately stored at -20 C for the study of serum antioxidant enzyme activities and other biochemical parameters detailed ahead in this section.

2.5 Gas chromatography and fatty acid profile analysis

For fatty acid profile analysis of meat and feed samples a standardized protocol was followed at our research institute. Fatty acid methyl esters (FAMEs) were prepared directly from the feed, breast, and thigh meat samples by using C13:0 ME internal standard (0.5 mg C13:0/ml methanol) (O'Fallon et al. 2007). The FAMEs, prepared in hexane, were stored in Gas chromatograph (GC) vials and placed at -20 °C until analysis. The fatty acid composition of the FAME was determined by capillary Gas liquid chromatography on a CP-6173, 60 m × 0.25 mm × 0.20 mm capillary column (Varian) installed on a Thermo Scientific Ceres 800 plus gas chromatograph fitted with an Automatic sampler AI3000, integrator and flame ionization detector. The initial oven temperature was 120 °C, which was held for 5 min. Then,

subsequently the temperature was increased to 240 °C at a rate of 2 °C/min, and held for 60 min. Nitrogen was used as the carrier gas at a flow rate of 1 ml/min. The injector and the detector of GC were set at 260 °C. The split ratio was 30:1. Fatty acid standard purchased from Supelcon, Bellefonte- PA contained 37 different FAMEs and 0.5 µl was injected into GC to get the standard peaks. The fatty acids were identified by comparing retention time of their peaks with the respective fatty acid methyl ester standards and were expressed as percentage of total fatty acids in feed, breast, and thigh samples.

2.6 Fatty acid metabolism indices

The fatty acids present in body are acted upon by various enzymes of lipid metabolism to produce higher SFAs, monounsaturated fatty acids (MUFAs), and PUFAs. This fatty acid conversion occurs predominantly in liver and the products get deposited in muscle tissues. This conversion is measured in terms of certain indices by relating the percentage of products to the percentage of their corresponding precursors in chicken meat. The enzyme activities in the production of MUFAs was calculated as follows (Okada et al. 2005):

$$\Delta 9\text{-DI (18): } \Delta 9\text{-desaturase (18) index} = 100\,[C18:1/(C18:1 + C18:0)]$$

$$\Delta 9\text{-DI (16): } \Delta 9\text{-desaturase (16) index} = 100\,[C16:1/(C16:1 + C16:0)]$$

$$\text{Total } \Delta 9\text{-DI} = 100\,[(C16:1 + C18:1)/(C16:1 + C16:0 + C18:1 + C18:0)]$$

The enzyme activities in the conversion of myristic acid (C14:0) to palmitic acid (C16:0) and further to steric acid (C18:0) was measured in terms of thioesterase and elongase indices as follows (Zhang et al. 2007):

$$\text{Elongase index (EI)} = C18:0/C16:0$$

$$\text{Thioesterase index (TI)} = C16:0/C14:0$$

Similarly, the enzyme activities in the conversion of essential fatty acids (EFA), linoleic acid (LA) and ALA, into long chain PUFA was measured in terms of Δ5 + Δ6 desaturase index as follows (Kumar et al. 2019):

$$\Delta 5 + \Delta 6\text{-desaturase index} = 100\,[(C20:2\ \omega\text{-}6 + C20:4\ \omega\text{-}6 + EPA + C22:5\ \omega\text{-}3 + DHA) / (C18:2\ \omega\text{-}6\ (LA) + ALA + C20:2\ \omega\text{-}6 + C20:4\ \omega\text{-}6 + EPA + C22:5\ \omega\text{-}3 + DHA)]$$

2.7 Health indices of meat

The modification in the fatty acid profile of chicken meat alters its health profile. Different indicators or determinants of health profile are used to measure the health value of chicken meat. The ratios of ω-3 PUFA to ω-6 PUFA, PUFA to SFA, MUFA to SFA, and UFA to SFA along with saturation index (S/P), atherogenic index (AI), and thrombogenic index (TI) are considered as health indices of chicken meat and were calculated as follows (Kumar et al. 2019, 2020):

$$S/P = (C14:0 + C16:0 + C18:0)/ (MUFA + PUFA)$$

$$AI = (C12:0 + 4 \times C14:0 + C16:0)/(MUFA + PUFA)$$

$$TI = (C14:0 + C16:0 + C18:0)/[(MUFA + \omega\text{-}6\ PUFA)/2 + 3 \times \omega\text{-}3\ PUFA + \omega\text{-}3: \omega{-}6]$$

The other health indices of chicken meat viz. Desirable fatty acids (DFA), Hypercholesterolemic fatty acids (HFA), and ratio of hypocholesterolemic fatty acids to hypercholesterolemic fatty acids (h/H) were calculated as follows (Pilarczyk et al. 2015):

$$DFA = UFA + C18:0$$

$$HFA = (C12:0 + C14:0 + C16:0)$$

$$h/H = (C18:1 + PUFA)/(C14:0 + C16:0)$$

2.8 Serum lipid chemistry and health indices

The serum total cholesterol, HDL cholesterol, triglyceride, and glucose estimation was done by using the Cogent, SPAN diagnostics kits following the instructions of the manufacturer. The serum thiobarbituric acid reactive substances (TBARS) value measures the extent of lipid oxidation in the body and it was expressed in terms of µM malondialdehyde (MDA) (Yagi 1998). The antioxidant defence system of the body contains various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GR). These enzymes were assayed by using the Cayman diagnostic kits (Wheeler et al. 1990). All the samples and standards were measured in duplicate.

The atherogenic indices of serum were calculated as follows (Frolich et al. 2003):

$$\text{Cardiac risk ratio (CRR)} = \text{Total cholesterol/HDL cholesterol}$$

$$\text{Atherogenic Coefficient (AC)} = (\text{Total cholesterol - HDL cholesterol})/\text{HDL cholesterol}$$

$$\text{Atherogenic Index of Plasma (AIP)} = (\text{Total cholesterol - HDL cholesterol})/\text{HDL cholesterol}$$

2.9 Statistical analysis

For the analysis of data pertaining to feed intake, FCR, PEF, PER, EER, and cost economics replicate group was taken as an experimental unit, whereas, for all other parameters the sampled bird was taken as an experimental unit. All data were tested for normality and homogeneity of variances with the Shapiro-Wilk test and Levene's test respectively prior to analyses. The data were analysed by one way ANOVA using the General Linear Model procedure (IBM SPSS software-20). The significant mean differences were separated by Tukey post-hoc analysis with significance level defined at $P < 0.05$. Further, to validate the effects of Cr

supplementation the data were also subjected to polynomial orthogonal contrast.

3. Results

3.1 Growth performance

The BWG of birds during 0-3 weeks of age was lower (P = 0.009) in T2 group followed by statistically different T3 and T4 groups, whereas higher BWG was observed in T5 and T1 which did not differ significantly from each other (Table 2). Similarly, the FCR during 0-3 weeks of age was higher (P = 0.006) in T2 group compared to other treatments groups which did not differ significantly from each other. The feed intake and 4-6 week and 0-6 week BWG and FCR of birds were not affected by dietary treatments. The mortality pattern of birds also showed no significant dietary effects (data not shown, only 2 birds died from T3 and 1 bird each from T1, T2, & T4 treatment groups during whole experimental period).

3.2 Growth efficiency parameters

In line with the results of growth performance of birds given above, during 0-3 weeks of age lower PEF (P = 0.008), PER (P = 0.010), and EER (P = 0.007) of birds were observed in group T2 compared to other treatment groups which did not differ significantly from each other (Table 2). However, PEF, PER, and EER during 4-6 weeks and 0-6 weeks of age were not affected by dietary treatments.

3.3. Carcass characteristics and cost economics

The carcass characteristics revealed no significant dietary treatment effects except the abdominal fat (Table 3). Lower (P = 0.019) abdominal fat of birds was observed in T5 group followed by statistically similar T4 group. Higher abdominal fat was observed in T1 group followed by statistically different T2 and T3 groups which were not significantly different from T4 group. With respect to control diet (T1) all other treatment groups revealed an increase in cost per kg live weight (P = 0.021) and per kg meat yield (P = 0.018) with higher increase in group T2 and less increase in group T5. However, group T3, and T4 were statistically similar to both T2 and T5.

3.4 Fatty acid profile of chicken meat

The fatty acid profiles of broiler chicken thigh and breast meat are given in Table 4 & 5, respectively. Higher percentages of C16:0, C18:0, SFA; and lower percentages of C18:1, C18:2 (LA), C18:3 (ALA), C20:1, C20:3, C20:5 (EPA), C22:5 (DPA), C22:6 (DHA), MUFA, PUFA, ω-3 PUFA, and ω-6 PUFA were observed in group T1 compared to other treatment groups which did not differ significantly from each other.

3.5 Fatty acid metabolism of chicken meat

The fatty acid metabolism indices of thigh and breast meat revealed that DI (18), DI (16), total DI, and $\Delta^5 + \Delta^6$ desaturase index were lower (P < 0.05) and elongase and

Table 2 Effect of dietary flaxseed meal (FSM) and chromium (Cr) supplementation on growth performance and growth efficiency parameters of broiler chicken

Indices		T1	T2	T3	T4	T5	SEM	P value	LC
Body weight gain (BWG, g)	0-3 weeks	664^{c}	587^{a}	630^{b}	631^{b}	653^{c}	6.7	0.009	0.014
	4-6 weeks	1206	1179	1187	1206	1213	11.1	0.095	0.105
	0-6 weeks	1870	1766	1817	1837	1866	15.1	0.067	0.086
Feed intake (g)	0-3 weeks	861	827	815	809	847	7.5	0.058	0.079
	4-6 weeks	2540	2386	2449	2442	2504	22.7	0.076	0.096
	0-6 weeks	3401	3213	3264	3251	3351	26.8	0.091	0.112
Feed conversion ratio (FCR)	0-3 weeks	1.30^{a}	1.41^{b}	1.29^{a}	1.28^{a}	1.30^{a}	0.012	0.006	0.011
	4-6 weeks	2.11	2.02	2.06	2.06	2.06	0.015	0.078	0.095
	0-6 weeks	1.82	1.82	1.79	1.77	1.80	0.009	0.093	0.119
Production Efficiency Factor (PEF)	3rd week	243^{b}	198^{a}	233^{b}	231^{b}	239^{b}	4.1	0.008	0.146
	6th week	245	231	242	243	247	3.6	0.068	0.331
Protein Efficiency Ratio (PER)	0-3 weeks	3.62^{b}	3.33^{a}	3.63^{b}	3.66^{b}	3.62^{b}	0.03	0.010	0.163
	4-6 weeks	2.57	2.67	2.62	2.67	2.62	0.02	0.105	0.253
	0-6 weeks	2.76	2.76	2.80	2.84	2.80	0.02	0.096	0.278
Energy Efficiency Ratio (EER)	0-3 weeks	26.4^{b}	24.3^{a}	26.5^{b}	26.7^{b}	26.4^{b}	0.20	0.007	0.098
	4-6 weeks	15.8	16.5	16.2	16.5	16.2	0.11	0.071	0.268
	0-6 weeks	18.6	18.6	18.8	19.1	18.8	0.09	0.088	0.296

T1 = 0 g FSM + 0 ppm Cr, T2 = 100 g FSM + 0 ppm Cr, T3 = 100 g FSM + 0.5 ppm Cr, T4 = 100 g FSM + 1.0 ppm Cr, and T5 = 100 g FSM + 1.5 ppm Cr

Values bearing different superscripts within the row differ significantly; SEM: Standard error of mean; LC: Linear contrast of Cr levels

Table 3 Effect of dietary flaxseed meal (FSM) and chromium (Cr) supplementation on the carcass characteristics* and cost economics of broiler chicken

Indices	T1	T2	T3	T4	T5	SEM	P value	LC
Live weight (g)	1774	1692	1736	1754	1775	15.2	0.071	0.285
Eviscerated weight	66.7	66.0	66.0	66.4	66.4	0.18	0.103	0.354
Dressed yield	71.8	71.4	71.5	71.8	71.6	0.16	0.125	0.412
Abdominal fat	1.90^{c}	1.51^{b}	1.46^{b}	1.33^{ab}	1.11^{a}	0.051	0.019	0.021
Drumstick	9.59	9.71	9.35	9.64	9.68	0.074	0.095	0.314
Breast	16.5	16.5	16.8	16.6	16.9	0.13	0.125	0.407
Thigh	9.24	9.06	9.28	8.86	9.03	0.067	0.085	0.335
Cost change (live weight basis)[1]	0.0^{a}	+10.58^{c}	+8.51^{bc}	+8.76^{bc}	+7.72^{b}	0.82	0.025	0.021
Cost change (meat yield basis)[2]	0.0^{a}	+9.65^{c}	+7.47^{bc}	+7.67^{bc}	+6.68^{b}	0.96	0.027	0.018

T1 = 0 g FSM + 0 ppm Cr, T2 = 100 g FSM + 0 ppm Cr, T3 = 100 g FSM + 0.5 ppm Cr, T4 = 100 g FSM + 1.0 ppm Cr, and T5 = 100 g FSM + 1.5 ppm Cr

Values bearing different superscripts within the row differ significantly

SEM: Standard error of mean; LC: Linear contrast of Cr levels

* Based on percentage of live weight

[1] Percentage change in feed cost on live weight basis relative to control diet (T1)

[2] Percentage change in feed cost on meat yield basis relative to control diet (T1)

Table 4 Effect of flaxseed meal (FSM) and chromium (Cr) supplementation on fatty acid profile of broiler chicken thigh

Fatty acids (% of total fatty acids)	T1	T2	T3	T4	T5	SEM	P value	LC
C14:0	0.41	0.37	0.38	0.33	0.36	0.035	0.109	0.314
C16:0	25.1^{b}	19.8^{a}	20.0^{a}	18.9^{a}	18.9^{a}	0.97	0.031	0.262
C16:1	3.73	4.23	3.96	4.17	3.82	0.26	0.090	0.071
C18:0	13.2^{b}	5.0^{a}	6.5^{a}	4.7^{a}	5.9^{a}	1.56	0.008	0.141
C18:1 ω-9	34.9^{a}	38.8^{b}	38.4^{b}	38.5^{b}	37.8^{b}	0.71	0.024	0.263
C18:2 ω-6	17.3^{a}	21.0^{b}	20.3^{b}	22.3^{b}	22.0^{b}	0.96	0.019	0.098
C18:3 ω-3	1.99^{a}	3.53^{b}	3.19^{b}	3.55^{b}	3.46^{b}	0.296	0.013	0.476
C20:1 ω-9	1.43^{a}	3.49^{b}	3.46^{b}	3.78^{b}	4.07^{b}	0.468	0.008	0.224
C20:3 ω-3	0.58^{a}	1.42^{b}	1.35^{b}	1.33^{b}	1.28^{b}	0.154	0.025	0.096
C20:4 ω-6	1.40	1.41	1.39	1.43	1.40	0.017	0.089	0.368
C20:5 ω-3	0.18^{a}	0.56^{b}	0.57^{b}	0.62^{b}	0.55^{b}	0.036	0.007	0.263
C22:5 ω-3	0.26^{a}	0.65^{b}	0.66^{b}	0.64^{b}	0.67^{b}	0.041	0.012	0.367
C22:6 ω-3	0.19^{a}	0.41^{b}	0.43^{b}	0.40^{b}	0.44^{b}	0.029	0.016	0.174
SFA	38.7^{b}	25.1^{a}	27.0^{a}	23.9^{a}	25.2^{a}	2.52	0.004	0.086
MUFA	40.1^{a}	46.5^{b}	45.9^{b}	46.5^{b}	45.7^{b}	1.22	0.040	0.117
PUFA	21.9^{a}	29.0^{b}	27.8^{b}	30.3^{b}	29.8^{b}	1.07	0.017	0.158
ω-3 PUFA	3.20^{a}	6.57^{b}	6.20^{b}	6.54^{b}	6.40^{b}	0.447	0.011	0.273
ω-6 PUFA	18.7^{a}	22.4^{b}	21.6^{b}	23.7^{b}	23.4^{b}	0.98	0.019	0.088

T1 = 0 g FSM + 0 ppm Cr, T2 = 100 g FSM + 0 ppm Cr, T3 = 100 g FSM + 0.5 ppm Cr, T4 = 100 g FSM + 1.0 ppm Cr, and T5 = 100 g FSM + 1.5 ppm Cr

Values bearing different superscripts within the row differ significantly

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

SEM: Standard error of mean, LC: Linear contrast of Cr levels

thioesterase indices were higher ($P < 0.05$) in control group T1 compared to other treatments groups which did not differ significantly from each other (Table 6).

3.6 Health indices of broiler chicken meat

The health indices of chicken meat under the influence of FSM and Cr feeding revealed lower ($P < 0.01$) PUFA:SFA

Table 5 Effect of flaxseed meal (FSM) and chromium (Cr) supplementation on fatty acid profile of broiler chicken breast

Fatty acids (% of total fatty acids)	T1	T2	T3	T4	T5	SEM	P value	LC
C14:0	0.46	0.40	0.42	0.41	0.42	0.021	0.091	0.167
C16:0	26.9^{b}	21.3^{a}	21.3^{a}	20.8^{a}	20.9^{a}	1.17	0.018	0.281
C16:1	3.70	3.48	3.47	3.58	3.59	0.121	0.087	0.376
C18:0	17.7^{b}	10.9^{a}	10.7^{a}	10.2^{a}	11.0^{a}	1.41	0.005	0.273
C18:1 ω-9	30.9^{a}	36.4^{b}	36.8^{b}	36.4^{b}	35.9^{b}	1.09	0.027	0.138
C18:2 ω-6	15.3^{a}	18.5^{b}	18.5^{b}	19.1^{b}	19.3^{b}	0.73	0.018	0.097
C18:3 ω-3	0.86^{a}	1.82^{b}	1.82^{b}	1.87^{b}	1.94^{b}	0.133	0.010	0.127
C20:1 ω-9	1.35^{a}	3.93^{b}	3.93^{b}	4.61^{b}	3.66^{b}	0.451	0.007	0.371
C20:3 ω-3	0.37^{a}	1.14^{b}	1.14^{b}	1.02^{b}	1.20^{b}	0.154	0.037	0.185
C20:4 ω-6	0.83	0.81	0.79	0.83	0.80	0.008	0.078	0.247
C20:5 ω-3	0.33^{a}	0.99^{b}	1.00^{b}	0.98^{b}	1.01^{b}	0.042	0.006	0.069
C22:5 ω-3	0.48	0.42	0.43	0.36	0.41	0.039	0.073	0.161
C22:6 ω-3	0.29^{a}	0.71^{b}	0.78^{b}	0.89^{b}	1.07^{b}	0.057	0.011	0.105
SFA	0.26^{a}	0.59^{b}	0.61^{b}	0.69^{b}	0.86^{b}	0.042	0.018	0.162
MUFA	1.05	0.92	0.71	0.82	0.97	0.159	0.088	0.156
PUFA	46.6^{b}	31.9^{a}	31.6^{a}	30.5^{a}	31.7^{a}	2.65	0.002	0.274
ω-3 PUFA	36.0^{a}	43.8^{b}	44.2^{b}	44.6^{b}	43.2^{b}	1.10	0.006	0.095
ω-6 PUFA	17.4^{a}	23.4^{b}	23.8^{b}	24.6^{b}	25.4^{b}	1.08	0.017	0.137

T1 = 0 g FSM + 0 ppm Cr, T2 = 100 g FSM + 0 ppm Cr, T3 = 100 g FSM + 0.5 ppm Cr, T4 = 100 g FSM + 1.0 ppm Cr, and T5 = 100 g FSM + 1.5 ppm Cr
Values bearing different superscripts within the row differ significantly
SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids
SEM: Standard error of mean, LC: Linear contrast of Cr levels

ratio, MUFA:SFA ratio, UFA:SFA ratio, the DFA content, and h/H ratio in control group T1 compared to other treatment groups which did not differ significantly from each other (Table 7). However, higher ω-6: ω-3 fatty acid ratio, saturation index, atherogenic index (AI), thrombogenic index (TI), and hypercholesterolemic fatty acids (HFA) were

Table 6 Effect of flaxseed meal (FSM) and chromium (Cr) supplementation on fatty acid metabolism of broiler chicken meat

Indices	T1	T2	T3	T4	T5	SEM	P value	LC
				Thigh				
DI (18)	72.6^{a}	88.6^{b}	85.5^{b}	89.2^{b}	86.4^{b}	2.31	0.015	0.089
DI (16)	12.9^{a}	17.6^{b}	16.5^{b}	18.1^{b}	16.8^{b}	0.77	0.024	0.071
Total DI	50.3^{a}	63.4^{b}	61.5^{b}	64.4^{b}	62.6^{b}	2.21	0.017	0.084
Elongase index (EI)	0.53^{b}	0.25^{a}	0.33^{a}	0.25^{a}	0.31^{a}	0.053	0.037	0.275
Thioesterase index (TI)	61.6^{b}	54.7^{a}	53.4^{a}	55.2^{a}	52.6^{a}	2.80	0.073	0.078
Δ^{5} + Δ^{6}-desaturase	8.54^{a}	10.99^{b}	11.51^{b}	10.68^{b}	10.72^{b}	0.590^{b}	0.079	0.096
				Breast				
DI (18)	63.6^{a}	76.9^{b}	77.4^{b}	78.2^{b}	76.6^{b}	2.05	0.015	0.278
DI (16)	12.1^{a}	15.3^{b}	15.2^{b}	16.0^{b}	16.0^{b}	0.27	0.024	0.386
Total DI	43.7^{a}	56.9^{b}	57.3^{b}	58.0^{b}	57.0^{b}	1.40	0.019	0.178
Elongase index (EI)	0.66^{b}	0.57^{a}	0.56^{a}	0.54^{a}	0.58^{a}	0.021	0.035	0.067
Thioesterase index (TI)	58.9^{b}	47.8^{a}	45.6^{a}	45.5^{a}	44.5^{a}	2.746	0.064	0.095
Δ^{5} + Δ^{6}-desaturase	9.57^{a}	13.25^{b}	13.55^{b}	13.89^{b}	14.99^{b}	0.628	0.087	0.094

T1 = 0 g FSM + 0 ppm Cr, T2 = 100 g FSM + 0 ppm Cr, T3 = 100 g FSM + 0.5 ppm Cr, T4 = 100 g FSM + 1.0 ppm Cr, and T5 = 100 g FSM + 1.5 ppm Cr
Values bearing different superscripts within the row differ significantly
DI (18): Δ9-desaturase (18) index, DI (16): Δ9-desaturase (16) index
SEM: Standard error of mean, LC: Linear contrast of Cr levels

observed in control group T1 compared to other treatment groups which were statistically similar to each other.

3.7 Serum lipid chemistry and health indices

The serum lipid chemistry, antioxidant enzyme activities, and associated health indices are given in Table 8. No significant dietary effects were observed on the serum glucose concentration. However, higher serum triglyceride (P = 0.011) and total cholesterol (P = 0.021) concentrations were observed in control group T1 compared to group T2 and linear decrease (P = 0.021 & 0.016, respectively) was observed in groups supplemented with increasing Cr levels. Serum HDL cholesterol was lower (P = 0.011) in control group T1 compared to group T2 and it increased linearly (P = 0.014) in treatment groups supplemented with increasing Cr levels. Serum antioxidant enzyme activities and MDA concentrations have shown a unique trend. Higher activities of SOD (P = 0.007), CAT (P = 0.001), GSH-Px (P = 0.002), GR (P < 0.001), and TBARS value (P = 0.009) were observed in group T2 compared to control group T1 and these serum parameters decreased linearly (P = 0.004, < 0.001, 0.001, < 0.001, & 0.006, respectively) with increasing Cr levels in the diets, such that group T5 depicted lower values compared to control group T1. Higher values of health indices such as CRR (P = 0.033), AC (P 0.022), and AIP (P = 0.007) were observed in control group T1 compared group T2 and linear reduction of CRR (P < 0.001), AC (P = 0.003), and AIP (P = 0.012) was observed with increasing Cr levels in the chicken diets.

4. Discussion

A number of researchers consistently reported poor growth performance of birds with increasing levels of flaxseed in broiler chicken diets (Pekel et al. 2009; Kumar et al. 2021) due to poor energy availability, presence of anti-nutritional factors, low digestibility of flaxseed and high viscosity of jej-

Table 7 Effect of flaxseed meal (FSM) and chromium (Cr) supplementation on health indices of broiler chicken meat

Indices	T1	T2	T3	T4	T5	SEM	P value	LC
				Thigh				
ω-6: ω-3 Fatty acid ratio	5.38^{b}	3.41^{a}	3.49^{a}	3.63^{a}	3.66^{a}	0.302	0.001	0.253
PUFA:SFA Ratio	0.57^{a}	1.15^{b}	1.03^{b}	1.26^{b}	1.18^{b}	0.098	0.010	0.078
MUFA:SFA Ratio	1.04^{a}	1.85^{b}	1.70^{b}	1.94^{b}	1.81^{b}	0.108	0.009	0.067
UFA:SFA Ratio	1.60^{a}	3.00^{b}	2.73^{b}	3.21^{b}	3.00^{b}	0.144	0.002	0.089
Saturation index (S/P)	0.62^{b}	0.33^{a}	0.37^{a}	0.31^{a}	0.33^{a}	0.054	0.021	0.276
Atherogenic Index (AI)	0.43^{b}	0.28^{a}	0.29^{a}	0.26^{a}	0.27^{a}	0.017	0.017	0.351
Thrombogenic Index (TI)	0.86^{b}	0.44^{a}	0.48^{a}	0.41^{a}	0.44^{a}	0.016	0.001	0.098
DFA (%)	75.1^{a}	80.5^{b}	80.2^{b}	81.4^{b}	81.4^{b}	0.60	0.018	0.157
HFA (%)	25.5^{b}	20.1^{a}	20.4^{a}	19.2^{a}	19.2^{a}	0.58	0.007	0.201
h/H ratio	2.23^{a}	3.37^{b}	3.25^{b}	3.58^{b}	3.51^{b}	0.107	0.006	0.064
				Breast				
ω-6: ω-3 Fatty acid ratio	7.66^{b}	3.67^{a}	3.60^{a}	3.66^{a}	3.30^{a}	0.74	<0.001	0.441
PUFA:SFA Ratio	0.37^{a}	0.74^{b}	0.75^{b}	0.81^{b}	0.80^{b}	0.060	0.010	0.162
MUFA:SFA Ratio	0.77^{a}	1.37^{b}	1.40^{b}	1.46^{b}	1.36^{b}	0.101	0.010	0.085
UFA:SFA Ratio	1.15^{a}	2.21^{b}	2.15^{b}	2.27^{b}	2.16^{b}	0.079	0.010	0.096
Saturation index (S/P)	0.84^{b}	0.45^{a}	0.45^{a}	0.42^{a}	0.44^{a}	0.044	0.029	0.264
Atherogenic Index (AI)	0.54^{b}	0.31^{a}	0.31^{a}	0.30^{a}	0.30^{a}	0.034	0.031	0.451
Thrombogenic Index (TI)	1.13^{a}	0.60^{b}	0.59^{b}	0.56^{b}	0.57^{b}	0.038	0.001	0.275
DFA (%)	71.2^{a}	78.5^{b}	78.7^{b}	79.3^{b}	79.5^{b}	1.02	0.013	0.313
HFA (%)	27.4^{b}	19.7^{a}	19.7^{a}	19.2^{a}	19.3^{a}	0. 40	0.009	0.467
h/H ratio	1.77^{a}	3.06^{b}	3.07^{b}	3.18^{b}	3.17^{b}	0.174	0.015	0.166

T1 = 0 g FSM + 0 ppm Cr, T2 = 100 g FSM + 0 ppm Cr, T3 = 100 g FSM + 0.5 ppm Cr, T4 = 100 g FSM + 1.0 ppm Cr, and T5 = 100 g FSM + 1.5 ppm Cr
Values bearing different superscripts within the row differ significantly
SFA: Saturated fatty acids, UFA: Unsaturated fatty acids, MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids,
DFA: Desirable fatty acids, HFA: Hypercholesterolaemic fatty acids,
h/H: hypocholesterolemic/hypercholesterolemic ratio
SEM: Standard error of mean, LC: Linear contrast of Cr levels

Table 8 Effect of dietary flaxseed meal (FSM) and chromium (Cr) supplementation on serum biochemistry and health indices of broiler chicken

Indices	T1	T2	T3	T4	T5	SEM	P value	LC
Glucose (g/L)	2.14	2.04	1.99	1.93	1.94	0.039	0.811	0.367
Triglyceride (g/L)	1.11^{c}	1.03^{b}	0.99^{b}	0.97^{ab}	0.94^{a}	0.030	0.011	0.021
Total cholesterol (g/L)	1.35^{c}	1.21^{b}	1.16^{ab}	1.13^{ab}	1.07^{a}	0.047	0.021	0.016
HDL cholesterol (g/L)	0.73^{a}	0.82^{b}	0.84^{b}	0.89^{bc}	0.93^{c}	0.033	0.011	0.014
SOD (U/mL)	112.1^{b}	141.4^{e}	136.6^{d}	123.9^{c}	101.2^{a}	2.82	0.007	0.004
Catalase (nmol/min/mL)	51.2^{b}	68.1^{d}	70.6^{d}	60.1^{c}	41.9^{a}	2.02	0.001	<0.001
GPx (nmol/min/mL)	10.7^{b}	14.4^{e}	14.1^{d}	12.4^{c}	9.1^{a}	0.38	0.002	0.001
GR (nmol/min/mL)	13.1^{b}	15.9^{d}	15.8^{d}	14.3^{c}	11.4^{a}	0.32	<0.001	<0.001
TBARS Value (MDA µM)	4.28^{b}	6.94^{e}	6.78^{d}	5.55^{c}	3.18^{a}	0.270	0.009	0.006
Cardiac Risk Ratio (CRR)	1.84^{d}	1.48^{c}	1.41^{c}	1.27^{b}	1.16^{a}	0.053	0.003	<0.001
Atherogenic Coefficient (AC)	0.84^{d}	0.48^{c}	0.41^{c}	0.27^{b}	0.16^{a}	0.033	0.022	0.003
Atherogenic Index of Plasma (AIP)	0.18^{c}	0.10^{b}	0.07^{b}	0.02^{a}	0.02^{a}	0.011	0.007	0.012

T1 = 0 g FSM + 0 ppm Cr, T2 = 100 g FSM + 0 ppm Cr, T3 = 100 g FSM + 0.5 ppm Cr, T4 = 100 g FSM + 1.0 ppm Cr, and T5 = 100 g FSM + 1.5 ppm Cr
Values bearing different superscripts within the row differ significantly
SOD: Super Oxide Dismutase; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; TBARS: Thiobarbituric acid reactive substances; MDA: Malondialdehyde
SEM: Standard error of mean, LC: Linear contrast of Cr levels

-unal digesta (Mir et al. 2017b). However, results of the present were peculiar in that negative effects of 100 g FSM on BWG and FCR of birds were observed only up to 3 weeks of age, indicating the age dependence of negative effects of FSM feeding in broiler chicken. Similar age dependence of negative effects of FSM feeding was observed on the PEF, PER, and EEF of birds. Poor growth performance and efficiency of birds fed FSM indicates their poor efficiency of feed, protein, and energy utilization (Mridula et al. 2015). Even some old studies maintain that feeding ground flaxseed beyond 7.5% level in broiler chicken reduced the growth and conversion efficiency, explaining the reduction in PER and net protein ratio, which could be due to lower nitrogen and amino acid retention because of the presence of mucilage (Zuidhof et al. 2009). However, in present study the negative effects of FSM feeding during starter phase seem to be overcome by Cr supplementation. But Cr levels did not differ significantly from each other except that the BWG of birds supplemented with only 1.5 ppm Cr could match the BWG of control diet fed birds. Similarly, Cr supplementation in broiler chicken was reported to improve BWG and efficiency of feed utilization (Kroliczewska et al. 2005). Contrary to this no significant effects of Cr supplementation were observed in other studies (Jackson et al. 2008; Ramarao et al. 2012). The mortality of birds, well within permissible limits, was found to have no impact of different dietary treatments in the present study. On the similar lines in earlier studies flaxseed feeding (Pekel et al. 2009) and Cr supplementation (Jackson et al. 2008) were found to have no significant effect on broiler chicken mortality.

The decline of abdominal fat in broiler chicken fed FSM can be attributed to enhanced UFA content in meat at the cost of SFA and former undergo rapid oxidation compared to their saturated counter parts. The FSM also exerts its effect by increasing gut viscosity which hinders the micelle formation and thus diminish lipid uptake, thereby reducing its deposition in the body (Mir et al. 2018a). Further, the increased leptin protein levels were observed in rabbits fed diet supplemented with 100 g flaxseed (McCullough et al. 2011) which can also be responsible for reduction of abdominal fat deposition in animals. On the other hand linear reduction in abdominal fat was observed with increasing Cr levels in the present study. This can be attributed to the fact that Cr supplementation improves insulin sensitivity of tissues which causes enhanced deposition of dietary protein and carbohydrate in muscle cells compared to fat (Mir et al. 2017a). In line with the results of present study carcass characteristics in broiler chicken were not affected by 100 g flaxseed feeding (Pekel et al. 2009) or Cr supplementation (Ramarao et al. 2012). The feed cost per kg live weight and per kg meat yield increased by 10.50% and 9.65%, respectively, due to the inclusion of 100 g FSM in chicken diets. However, Cr supplementation could linearly decrease this rise of feed cost by just 2.86% and 2.97%, respectively,

by improving the feed efficiency of birds. This increase of feed cost can be justified based on the fact that FSM results in ω-3 PUFA enrichment of meat whose consumption has well documented health benefits in humans. Previous studies have also reported increase in feed cost by inclusion of flaxseed in broiler chicken ration (Zuidhof et al. 2009). However, no such studies are available which show the effect of Cr supplementation on feed cost of broiler chicken.

The dietary inclusion of FSM as a source of PUFA, especially ω-3 fatty acids, in broiler chicken diets has been carried out to alter the fatty acid composition of chicken meat in earlier studies (Mir et al. 2018a, 2018b, 2017a; Kumar et al. 2019, 2020, 2021). Significant improvement in fatty acid profile of chicken meat was observed in present study by inclusion of 100g FSM/kg diet of broiler chicken. Flaxseed is the richest terrestrial source of ω-3 fatty acids (particularly ALA), which undergo faster absorption in the gut of birds without any significant bio-hydrogenation and can be the reason for higher UFA content in chicken meat at the expense of SFA content (Mir et al. 2018a, 2018b, 2017a). Increase in long chain PUFA such as EPA, DPA, and DHA content of chicken meat could be because of enhancement of de novo synthesis of long chain PUFA from LA and ALA supplied by dietary FSM (Smink et al. 2010). On similar lines increased ω-3 fatty acids, particularly ALA, EPA, DPA, and DHA content in broiler chicken meat was observed by inclusion of flaxseed in broiler chicken ration (Mir et al. 2018a, 2018b, 2017a; Kumar et al. 2019, 2020) which indicates that EPA and DHA can be made available from chicken meat conveniently other than the conventional marine sources. There are no such reports available on chicken showing the effects of Cr on fatty acid profile of chicken meat.

The changes in the lipid composition of broiler chicken meat observed in this study due to FSM feeding could be the results of alteration in lipid metabolism and the consequent lipid deposition in chicken tissues (Dal Bosco et al. 2012). Feeding of 100 g FSM increased Δ9-DI (16), Δ9-DI (18), total Δ9-DI, and Δ5 + Δ6 desaturase activities in broiler chicken and decreased elongase and thioesterase indices. Diet of broiler chicken is an important determinant of Δ9 desaturase activity and this FSM induced increase in Δ9 desaturase activity increases the conversion of endogenous and dietary specific medium- and long-chain SFA (C16:0 and C18:0) to their corresponding MUFA (C16:1 and C18:1) (Kumar et al. 2019). The decreased elongase and thioesterase indices indicate lower conversion of myristic acid (C14:0) to palmitic acid (C16:0) and further to stearic acid (C18:0) which reflects the trend of steric acid in the fatty acid profile of chicken meat. On the other hand, animals do not have the ability to synthesize essential fatty acids (EFA) such as LA and ALA from acetyl-CoA, but they can convert them to more unsaturated long chain FA in liver when supplied in diet. This process is catalysed by Δ5 and Δ6 desaturases which are rate limiting enzymes in this process of conversion. In present study 100 g FSM feeding induced higher Δ5 + Δ6 desaturase index which reflects the trend of EPA, DPA, and DHA content of the chicken meat and this relationship between long chain PUFA and Δ5 + Δ6 desaturase activities in chicken tissues has also been established in earlier studies (Kumar et al. 2019). However, Cr supplementation did not show any significant effect on the fatty acid metabolism indices which corroborates the trend of fatty acid profile of chicken meat observed in this study. Notwithstanding the knowledge of authors, there is no literature available pertaining to the effects of Cr supplementation on fatty acid metabolism in broiler chicken meat.

The level of unsaturation has a direct influence on health value of chicken meat. The greater the degree of UFA content, particularly ω-3 fatty acids, greater are the health benefits of chicken meat as observed by decreasing S/P in present study (Kumar et al. 2019). The ratio of ω-6 to ω-3 is an important index of meat quality of animals/birds. The metabolism of EFA, LA (ω-6 fatty acid family) and ALA (ω-3 fatty acid family), to their corresponding long chain PUFAs share common pathways and pool of metabolic enzymes due to which an excess of substrate of one family hinders the metabolism of other and reduces its incorporation into the tissues (Kumar et al. 2019). In this regard, flaxseed has been reported containing 32 - 45% oil, of which 51 - 55% is ALA and 15 - 18% is LA (Prasad 2009) and presenting an ω-6: ω-3 ratio of 0.3:1 which makes it most suitable for ω-3 enrichment of chicken meat. Thus, the present study provides intuitions that feeding of FSM in broiler chicken offers a unique strategy of increasing the long chain ω-3 PUFAs such as EPA, DPA, and DHA in chicken meat at the expense of ω-6 PUFAs. As a result of enhanced unsaturation of chicken meat due to dietary FSM PUFA:SFA ratio, MUFA:SFA ratio, UFA:SFA ratio, the DFA content, and h/H ratio of chicken meat increased, whereas, ω-6: ω-3 fatty acid ratio, saturation index, atherogenic index (AI), thrombogenic index (TI), and hypercholesterolemic fatty acids (HFA) decreased. The ω-6: ω-3 fatty acid ratio improved from 5.4:1 to 3.4:1 in thigh meat and from 7.7:1 to 3.3:1 in breast meat which indicates differential deposition rate of different fatty acids in breast and thigh meat. Similarly, in recent studies on broiler chicken dietary flaxseed improved tissue ω-6: ω-3 fatty acid ratio and PUFA:SFA ratio (Kumar et al. 2019, 2020; Usoro et al. 2006). Lower AI and TI, observed in present study, have been reported to exert protective action against coronary heart diseases (Usoro et al. 2006). Furthermore, 100 g flaxseed feeding elevated the leptin protein levels in rabbits, which has a strong positive correlation with adipose ALA levels (McCullough et al. 2011) and hence inversely correlated with the risk of cardiovascular diseases (Goyal et al. 2014). The diets rich in saturated fatty acids, such as lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0), have shown

higher correlation with increased risk of atherosclerosis, obesity, and coronary heart diseases (Kumar et al. 2019). But the higher Δ9 desaturation activity observed in the current study is supposed to counter these unwanted outcomes to a certain extent by converting palmitic acid to palmitoleic acid. However, Cr supplementation in present did not affect the health indices of broiler chicken meat because of nonsignificant effect on the metabolism and composition of fatty acids of chicken tissues. Notwithstanding the knowledge of authors, no literature deciphering the influence of Cr supplementation on the health indices of broiler chicken/ animal/humans is available.

In present study feeding of 100 g FSM resulted in lower serum cholesterol, triglyceride, CRR, AC, and AIP; and linear decrease was observed in all these parameters due to Cr supplementation. Flaxseed interferes in bile acid metabolism by inhibiting the re-uptake of bile acids, which diverts cholesterol towards the hepatic synthesis of bile acids and thus reduces its serum concentration in broiler chicken/ humans (Mir et al. 2018b; Saxena and Katare 2014). The decrease in serum triglyceride levels may be because of increased tissue ω-3 fatty acid content due to FSM feeding. In line with the results of present study decline of serum cholesterol and triglyceride levels occurred as a result of flaxseed feeding in broiler chicken (Mir et al. 2018c, 2017b) and humans (Saxena and Katare 2014). Similarly, flaxseed feeding increased serum HDL cholesterol in humans (Saxena and Katare 2014). On the other hand, Cr seems to exerts hypocholesterolemic and hypolipidemic effects by activating 5-AMP-activated kinase which negatively regulates sterol regulatory element binding protein-1 required for synthesis and uptake of cholesterol and triglycerides (Rajalekshmi et al. 2008). The Cr supplementation reduced the muscle cholesterol by 40% and serum cholesterol by 30% in broiler chicken supplemented by organic Cr (Debski et al. 2004). Researchers have shown that an increase in plasma HDL-C concentration reduces cardiovascular risk (Ramaprasad et al. 2005) which supports the decline of CRR, AC, and AIP in the present study. The serum lipid profile has a direct relationship with the risk factors of cardiovascular diseases (Goyal et al. 2014) and improved lipid profile results in lower atherogenic indices which provide protection against coronary heart diseases (Usoro et al. 2006). Similar to the results of present study, a significant improvement in atherogenic indices in humans (Saxena and Katare 2014) and chicken (Kumar et al. 2019) were observed due to dietary flaxseed. The increase in serum antioxidant enzyme activities and TBARS value of broiler chicken in present study has been reported in recent studies (Mir et al. 2018c, 2017b). This can be attributed to increased lipid unsaturation in chicken meat, supported by increased TBARS values, which induces the enhanced antioxidant enzyme activities (Ramaprasad et al. 2005). Linear decrease in antioxidant enzyme activities and TBARS value were observed with increasing Cr levels in diet of chicken. Cr serves as an antioxidant which provides protection against lipid peroxidation (Anderson 2000) and hence reverses the antioxidant enzyme induction property of FSM. Cr supplementation in cows significantly lower plasma SOD with no effect on GPx or GR (Kumar et al. 2015) and reduced lipid peroxidation due to Cr supplementation with no significant effect on serum antioxidant enzymes was observed in birds (Ramarao et al. 2012).

5. Conclusions

The study concludes that 100 g FSM feeding exerts negative effects on the growth performance during starter phase only (0-3 weeks) and overall growth efficiency in broiler chicken, whereas, Cr supplementation reverses these negative effects. The 100 g FSM feeding reduces abdominal fat in broiler chicken and Cr supplementation linearly decreases it with minimum value at 1.5 ppm Cr. The 100 g FSM feeding favourably alters the lipid metabolism to improve the meat lipid profile and health indices and no such effects are exerted by Cr supplementation. The 100 g FSM feeding improves serum lipid profile and atherogenic indices and linear improvement occurs in these parameters with increasing Cr levels up to 1.5 mg/kg chicken diet. And, 100 g FSM feeding decrease the serum antioxidant status which is improved by 1.5 mg Cr /kg diet in broiler chicken.

Declarations

Finding: This research did not receive funding from any agency in public or private

Conflict of interests: The authors declare that they have no competing interest arising out of this manuscript

Ethics approval: The experimental procedures carried out in the study were approved by the Institutional Animal Ethics Committee (IAEC) following the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) 2012" established under the 'Prevention of Cruelty to Animals Act 1960' of Indian Penal Code (Approval number 604/09/bc/CPCSEA).

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