Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2017.13717.1271

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Effects of Irradiated Flaxseed on Performance, Carcass Characteristics, Blood Parameters, and Nutrient Digestibility in Broiler Chickens

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Poultry Science Journal 2017, 5 (2): 153-163

Keywords
Broiler
Flaxseed
Performance
Electron irradiation
Nutrient digestibility

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Article history
Received: July 26, 2017
Revised: September 16, 2017
Accepted: September 28, 2017

Abstract
The objective of this study was to investigate the effects of feeding electron irradiated flaxseed (FS) on performance, carcass characteristics, blood parameters, digesta viscosity, and nutrient digestibility in broiler chickens. In a 2 × 2 factorial arrangement, 320 day-old broiler chicks were randomly assigned to one of five experimental diets, each with four replicates containing 16 chicks each. Dietary treatments included a corn-soybean meal-based diet (control), and diets containing 10% or 20% raw flaxseed (FS10, FS20), or 10% or 20% flaxseed irradiated at 20 kGy (RFS10, RFS20). Feeding irradiated flaxseed improved body weight gain in grower and finisher periods of the experiment ($P < 0.05$). Birds fed FS20 had a lower ($P < 0.05$) body weight gain in finisher period as well as lower breast muscle percentage in comparison to chicks fed FS10. Thigh percentage was greater in chicks fed FS20 compared to chicks fed FS10 ($P < 0.05$). Liver percentage decreased ($P < 0.05$) in birds fed RFS20 compared other treatments. Birds fed FS20 and RFS20 had significantly lower aspartate aminotransferase activity compared to birds fed FS10 and RFS10 ($P < 0.05$). Dry matter, organic matter, and ether extract digestibility decreased ($P < 0.05$) as the levels of FS increased. Apparent digestibility of dry matter, organic matter, and ether extract in irradiated FS increased in birds fed raw flaxseed. Irradiation of flaxseed significantly decreased digesta viscosity compared to diets containing raw irradiated flaxseed ($P < 0.05$). Results of this study demonstrated that irradiation increases the inclusion level of flaxseed in broiler diets without any negative impacts on broiler performance.

Introduction

Flaxseed contains high amounts of α-linolenic acid (52% of the total fatty acids), an essential fatty acid, making flaxseed a unique oilseed crop for oil production as well as for incorporation in foods (Chung *et al.*, 2005). Flaxseed is a good source of protein, oil, and α-linolenic acid, so it can be used for enrichments of poultry meat and eggs (Leeson and Summers, 2005). Due to anti-nutritional factors (ANF) present in flaxseed [non-starch poly saccharides (NSPs), cyanogenic glycosides, trypsin inhibitors, mucilages, linatine dipeptide (a vitamin B6 antagonist), and phytic acid], flaxseed has been shown to adversely affect broiler performance (Ajuyah *et al.*, 1993; Bhatty, 1995; Ortiz *et al.*, 2001; Alzueta *et al.*, 2003; Hernandez, 2013). These ANF and NSPs

Please cite this article as: Beheshti Moghadam MH, Rezaei M, Behgar M & Kermanshahi H. 2017. Effects of Irradiated Flaxseed on Performance, Carcass Characteristics, Blood Parameters, and Nutrient Digestibility in Broiler Chickens. Poult. Sci. J. 5 (2): 153-163.

are associated with increasing intestinal viscosity, reducing litter quality, and poor growth performance in broiler birds (Hall *et al*., 2006).

Gamma and electron radiation can denature proteins and decrease starch crystallinity (Chamani *et al*., 2009; Shawrang *et al*., 2013). Nayefi *et al*. (2015) showed an increase in feed intake and body weight gain in broiler chicks fed diets containing 12% irradiated (30 kGy) cotton seed meal compared to those fed control diet. Previous studies using gamma or electron irradiation show a reduction in anti-nutrient content of canola meal, barley, and cottonseed meal and an improvement in their utilization in broilers (Gharaghani *et al*., 2008; Chamani *et al*., 2009; Shawrang *et al*., 2013; Nayefi *et al*., 2015., Bahraini *et al*., 2017).

There is limited information on the use of irradiated flaxseed in broiler nutrition. Therefore, the present study was conducted to investigate the effects of FS diets irradiated by electron beam on performance, carcass characteristics, blood parameters, nutrient digestibility and digesta viscosity of broiler chickens.

Materials and methods

All experimental methods were in accordance with Sari Agricultural Sciences and Natural Resources University Research Policy on Animal Ethics and Welfare (Sari, Iran).

Preparing of irradiated flaxseed

One hundred ten kg of raw Canadian flaxseed (*Linum usitatissimum*) was purchased from Zarbal Company (Amol, Mazandaran, Iran). The flaxseed sample was packed in 35 × 25 cm^2 polyethylene bags and exposed to electron beam irradiation (Yazd radiation processing center, AEOI, Yazd center, Iran) at a dose of 20 kGy (fixed beam energy of 10 MeV) at room temperature by a Rhodotron accelerator model TT200 (Ion Beam Applications Company, Ottignies-Louvain-la-Neuve, Belgium). A dose rate of 180 kGy min^{-1} was used for irradiation as determined by cellulose triacetate film (ISO/ASTM 51650, 2015). Uncertainty for electron radiation was 5% and measured dose uniformity ratio (Dmax/Dmin) was 1.10.

Chemical analysis of flaxseed

We assessed the chemical composition of three samples of raw (RFS) and irradiated flaxseed (IFS) to determine content of organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), calcium, and phosphorus according to AOAC (2005) analytical methods in Sari Agricultural and Natural Resources University, Sari, Mazandaran, Iran.

Total cyanogenic glycosides (TCG, or cyanide) were measured using the Picrate method (Egan *et al*., 1998; Bradbury *et al*., 1999) using a Cyanide kit (courtesy of Howard Bradbury, The Australian National University, Canberra, Australia). About 25-100 mg ground flaxseed was mixed with phosphate buffer (0.5 mL, 0.1 M at pH=4-10) and cyanoglucoside enzyme. A picrate paper attached to a plastic backing strip (Bradbury *et al*., 1999) was inserted into the sample and after about 16 hrs at 30°C, the picrate paper was removed and immersed in 5.0 mL water for ~30 min. The absorbance of picrate solution was measured at 510 nm. The total cyanide content (ppm) determined as follows:

Total cyanide content (ppm) = 396 × absorbance × 100/z,

where z = sample weight (mg)

All the fatty acid (FA) analysis were done at the lipid laboratory (Oregon State University, Corvallis, USA). Total lipid was extracted from ~2 g of flaxseed using chloroform: methanol (2:1) following methods described by Folch *et al*. (1957). Fatty acid methyl esters were prepared from total lipid extract using boron trifluoride methanol (Beheshti Moghadam *et al*., 2017). Fatty acid analysis was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler, flame ionization detector, and SP-2360 fused silica capillary column. Samples in hexane (1 µL) were injected with helium as a carrier gas into the column programmed for ramped oven temperatures. Initial oven temperature was set at 150°C and held for 1.5 min, then ramped at 15°C /min to 190°C and held for 20 min, then ramped again at 30°C /min to 230°C and held for 3 min. Inlet and detector temperatures were both 250°C. Fatty acid methyl esters were identified by comparison with retention times of authentic internal or external standards (Nuchek Prep, Elysian, MN). Peak areas and percentages were calculated using Hewlett-Packard ChemStation software (Agilent Technologies Inc., Wilmington, DE). Fatty acid values are reported as percentage of methyl esters.

Birds and dietary treatments

A total of 320 day-old male broiler chicks (Ross 308 strain) were individually weighed and randomly distributed to five treatments, with four replicates of 16 chicks each (placed in 20 floor pens bedded with wood shavings). Light was continuous for the first three days post-hatch, after which a 23L:1D was applied. At one day of age, the room temperature was set at 33°C and subsequently reduced by 2 °C/week. Birds had free access to water and feed throughout the experiment.

Dietary treatments consisted of a corn-soybean meal based diet (control), and diets containing 10% raw FS (RFS10), 10% irradiated FS (IFS10), 20% raw FS (20RFS), or 20% irradiated FS (20IFS). Experimental diets were fed during the 3-phase feeding schedule: starter (0 to 10 d), grower (11-22 d), and finisher (23-42 d). All diets were mash and formulated to meet or exceed the minimum requirements for broiler chickens according to Ross 308 catalogue (2014). The composition of experimental diets is given in Table 1.

Table 1. Composition and nutrient content of starter, grower and finisher experimental diets (g/kg)

Ingredients	Starter (0-10d)			Grower (11-24 d)			Finisher (25-42 d)		
	Control	FS10, RFS10	FS20, RFS20	Control	FS10, RFS10	FS20, RFS20	Control	FS10, RFS10	FS20, RFS20
Corn	533.60	490.30	426.10	568.60	524.20	466.60	615.90	572.60	517.6
Soybean Meal	405.00	368.00	335.00	364.50	328.00	294.00	323.00	286.00	251.00
FS/RFS [1]	0.000	100	200	0.000	100	200	0.000	100	200
Vegetable oil	20.0	2.00	0.000	25.0	7.00	0.000	28.0	10.00	0.000
Salt	2.50	2.30	2.50	2.50	2.30	2.50	2.50	2.30	2.50
Bicarbonate sodium	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix [2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix [3]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Dicalcium phosphate	16.0	16.0	16.0	14.0	14.0	14.0	12.0	12.0	12.0
Limestone	9.00	8.00	7.00	8.00	7.00	6.00	7.00	6.00	6.00
L-Lysine. HCl	2.00	2.00	2.20	1.50	1.50	1.70	1.20	1.30	1.30
DL-Methionine	3.70	3.40	3.20	3.20	3.00	2.70	2.90	2.50	2.30
Choline chloride (60%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L-Threonine	1.20	1.00	1.00	0.700	1.00	0.500	0.500	0.300	0.300
Titanium dioxide	0.000	0.000	0.000	5.00	5.00	5.00	0.000	0.000	0.000
Nutrient values									
ME (Kcal/kg)	2900	2900	2900	2980	2980	2980	3050	3050	3050
Crud protein	220	220	220	205	205	205	190	190	190
Calcium	9.60	9.60	9.60	8.70	8.70	8.70	7.90	7.90	7.90
Available phosphorus	4.80	4.80	4.80	4.40	4.40	4.40	4.00	4.00	4.00
Lysine	11.8	11.8	11.8	10.5	10.5	10.5	9.50	9.50	9.50
Methionine	4.50	4.50	4.50	4.20	4.20	4.20	3.90	3.90	3.90
Methionine + Cystine	9.5	9.5	9.5	8.7	8.7	8.7	8.0	8.0	8.0

[1]Flaxseed/ Irradiated flaxseed.

Vitamin premix; Supplied per Kg feed: Vitamin A, 8000 UI; vitamin D3, 2000 UI; vitamin E, 30 UI; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg.

[3]Mineral premix supplied per Kg feed; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.

Broiler chicken performance, carcass characteristics and blood sample collection

During the experiment, body weight and feed intake (FI) were measured for each pen in three phases of the experiment (starter, grower and finisher), and then body weight gain (BWG) and feed conversion ratio (FCR) were calculated. At the end of the experiment (42 days), 12 birds per treatment (three birds/pen) were randomly selected, weighed, and then sacrificed by cervical dislocation. Tissue samples from each chicken including liver, breast and thigh muscle (pectoralis major and biceps femoris, without skin), and abdominal fat pad (including fat surrounding the gizzard, bursa of fabricius, and cloaca) were collected and weighed.

At days 21 and 42 of the experiment, eight birds from each treatment (two birds/replicate) were selected for blood analysis. Samples were collected from wing vein, collected in heparinized tubes and centrifuged for 10 min at 2000 × *g*. Plasma was collected, and stored at -20°C until analysis. Plasma was measured for triglycerides, cholesterol concentrations and aspartate aminotransferase (AST) activity using an auto-analyzer (HITACHI 902 automatic auto-analyzer) from specific commercial kits (Pars Azmoon, Tehran, Iran).

Nutrients digestibility and viscosity

Titanium dioxide (TiO_2) was added to the feed of all birds at a rate of 5 g/kg as a dietary marker from day 14 to 20. On day 21, two birds were randomly selected from each pen and euthanized by cervical dislocation. The content of the ileum (from Meckel's diverticulum to 1 cm above the ileocecal junction) was collected and pooled for two birds to yield four replicate samples per each treatment. The ileal digesta samples were frozen, freeze-dried, ground, and analyzed for TiO_2 using a UV spectrophotometer (Short *et al.*, 1996), and for DM, EE and OM as per AOAC (2005).

At day 42, ileal digesta (1.5 g) of two sacrificed birds in each replicate were collected and centrifuged at 12000 × *g* for 4 min. Viscosity of the supernatant was determined at 40°C using the Brookfield digital viscometer (model DVII+LV, Brookfield Engineering Laboratories, Mashhad, Iran). The ileal digesta samples were frozen, freeze-dried, ground, and analyzed TiO_2 using a UV spectrophotometer (Short *et al.*, 1996), and for DM, EE and OM as per AOAC (2005). Apparent ileal digestibility coefficients of OM, DM, and EE were calculated using TiO_2 in the diets and digesta by using the following equation: Apparent ileal digestibility coefficient = 1 – [diet TiO_2 / ileal TiO_2] × [ileal nutrient / diet nutrient].

Statistical analysis

The data were analyzed using Mixed procedure of SAS software version 9.4 (SAS, 2013) as a Control plus a 2 × 2 factorial with completely randomized design. The main factors were flaxseed and irradiation. Studentized residuals were calculated for both fixed and random effects and normality of studentized residuals was checked using Shapiro-Wilk test (Zar, 2009). Tukey and Dunnett comparison procedures used to compare means when F-test was significant. All statements of significance are based on $P < 0.05$.

Results

The effects of electron radiation on chemical composition, TCG, and FA of flaxseed are shown in Table 2. There were no significant differences among treatments, except for crude fiber content which was statistically lower in irritated flaxseed ($P < 0.05$).

Table 2. Analyzed chemical composition of raw and irradiated flaxseed (g/kg)

Composition	Raw flaxseed	Electron beam (20 kGy)*	*SEM*	*P*-value
Organic matter	965	965	0.142	0.1125
Crude protein	191	189	0.067	0.1248
Ether extract	342	362	0.056	0.5333
Crude fiber	210[a]	164[b]	0.086	0.04850
TCG [1] (ppm)	230	197.6	0.600	0.2074
C16:0	63.3	67.0	0.024	0.8728
C18:0	56.5	61.6	0.027	0.7664
C18:1 n-9	209	209	0.046	0.3862
C18:2 n-6	122	123	0.018	0.4694
C18:3 n-3	548	537	0.040	0.2556
Total SFA	119	128	0.033	0.9678
Total MUFA	209	209	0.046	0.3862
Total n-6 FA	122	123	0.018	0.4694
Total n-3 FA	548	537	0.040	0.2556

[1] Total cyanogenic glycosides

* Flaxseed ground after irradiation.

Total SFA = Total saturated fatty acids (14:0 + 16:0 + 17:0+ 18:0 + 20.0); Total MUFA = Total monounsaturated fatty acids (16:1 + 18:1 + 20:1 + 22:1); Total n-6 polyunsaturated fatty acids (18:2 n-6 +20:2 n-6 + 20:3 n-6 + 20:4 n-6 + 22:4 n-6 + 22:5 n-6); Total n-3 polyunsaturated fatty acids (18:3 n-3 + 20:5 n-3+ 22:5 n-3 + 22:6 n-3). Total LC n-6 FA = Total long chain n-6 fatty acids (20:2 n-6 + 20:3 n-6 + 20:4 n-6+ 22:4 n-6 + 22:5 n-6). Total LC n-3 FA = Total long chain n-3 fatty acids (20:5 n-3 + 22:5 n-3 + 22:6 n-3).
Values within a row with different superscripts differ significantly $P< 0.05$.

Table 3. Effect of experimental treatments on performance of broilers at starter, grower and finisher phases of the experiment

Tukey test	Dietary treatments[1]					Irradiation		Flax seed			*P*-value		
	Control	FS10	RFS10	FS20	RFS20	Yes	No	10%	20%	SEM [2]	FS [3]	R [4]	FS×R
Starter (0-10d)													
FI[5] (g)		194.72	186.25	188.75	188.99	187.12	191.74	190.49	188.37	5.90	0.726	0.449	0.526
BWG[6] (g)		162.85	163.82	146.39	168.61	166.22	154.62	163.33	157.50	5.70	0.326	0.064	0.087
FCR[7]		0.920	0.880	0.970	0.870	0.870^{b}	0.950^{a}	0.900	0.920	0.020	0.403	0.005	0.165
Grower (11-22d)													
FI (g)		1328.32	1314.79	1059.89	1358.27	1336.53	1194.10	1321.55	1209.08	155.23	0.482	0.376	0.334
BWG (g)		642.92	671.08	585.18	674.51	672.79^{a}	614.05^{b}	657.00	629.84	14.5	0.086	0.001	0.057
FCR		1.55^{b}	1.49^{b}	1.74^{a}	1.52^{b}	1.50^{b}	1.65^{a}	1.52^{b}	1.63^{a}	0.030	0.005	0.009	0.036
Finisher(23-42d)													
FI (g)		2793.22	2725.72	2894.86	2803.22	2769.47	2844.09	2759.47	2849.09	50.7	0.102	0.142	0.815
BWG (g)		1344.06^{a}	1419.69^{a}	1160^{b}.73	1344.83^{a}	1382.26^{a}	1252.40^{b}	1381.88^{a}	1252.78^{b}	21.7	0.001	0.001	0.028
FCR		1.27^{b}	1.18^{b}	1.49^{a}	1.25^{b}	1.22^{b}	1.38^{a}	1.22^{b}	1.38^{a}	0.022	0.001	0.001	0.006
Dunnett test													
Starter													
FI (g)	190.26	194.72	186.25	188.75	187.99					5.34	0.693		
BWG (g0	172.57^{a}	162.85^{a}	163.82^{a}	146.39^{b}	168.61^{a}					5.69	0.005		
FCR	0.860^{a}	0.920^{a}	0.880^{a}	0.970^{b}	0.870^{a}					0.020	0.005		
Grower													
FI (g)	1310.21	1328.32	1314.79	1059.89	1358.27					140.04	0.736		
BWG (g)	704.92^{a}	642.92^{b}	671.08^{a}	585.18^{b}	674.51^{a}					17.19	0.022		
FCR	1.41	1.55	1.49	1.38	1.52					0.180	0.734		
Finisher													
FI (g)	2738.28^{a}	2793.28^{a}	2725.72^{a}	2894.96^{b}	2803.22^{a}					50.3	0.043		
BWG (g)	1459.56^{a}	1344.06^{b}	1419.06^{a}	1160.73^{b}	1344.83^{b}					27.8	0.010		
FCR	1.14^{a}	1.27^{a}	1.18^{a}	1.49^{b}	1.25^{a}					0.024	0.001		

[1]Control, FS10, RFS10, FS20 and RFS20, represent corn-soybean meal basal diet, diet containing %10 raw flaxseed, diet containing %10 electron irradiated flaxseed, diet containing %20 flaxseed and diet containing %20 electron irradiated flaxseed, respectively.
[2]Standard error of means; [3]Flax seed; [4]Irradiation; [5]Feed intake; [6]Body weight gain; [7]Feed conversion ratio.
^{a,b} Values within a row with different superscripts differ significantly $P < 0.05$.

There were no significant interactions in FI, BWG, and FCR, during starter and grower periods, though this effect was significant for BWG and FCR during finisher period ($P < 0.05$; Table 3). Irradiation of flaxseed significantly increased ($P < 0.05$) BWG in grower and finisher periods, and improved FCR in all periods of the experiment. Significant differences were observed between the control and other treatments for FI in finisher periods, as well as for BWG and FCR in all three phases of the experiment ($P < 0.05$).

Relative weights of thigh, breast, liver, and abdominal fat of birds are shown in Table 4. F20 diets increased thigh and decreased breast percentage ($P < 0.05$). Feeding irradiated FS increased fat pad yield from 1.45% to 1.91% ($P < 0.05$). Flaxseed had a significant effect on liver weight. Chicks fed FS20 had lower liver percentage compared to chicks fed FS10 ($P < 0.05$). However, a significant interaction was observed in liver percentage ($P < 0.05$). There were significant differences between control and other treatments for thigh, fat pad, and liver percentage ($P < 0.05$).

Table 4. Effects of experimental diets on carcass characteristics of broilers at day 42 (% of live body weight)

Tukey	Dietary Treatments[1]					Irradiation		Flaxseed		*SEM* [2]	*P*-value		
test	Control	FS10	RFS10	FS20	RFS20	Yes	No	10%	20%		FS [3]	R [4]	FS×R
Thigh		27.1	27.6	29.3	28.3	27.9	28.2	27.4 ^{b}	28.8 ^{a}	0.510	0.008	0.556	0.163
Breast		34.5	34.6	32.6	32.5	33.6	33.6	34.6 ^{a}	32.6 ^{b}	0.600	0.003	0.950	0.887
Fat pad		1.55	1.91	1.35	1.91	1.91 ^{a}	1.45 ^{b}	1.73	1.63	0.105	0.540	0.006	0.515
Liver		3.08	3.20	3.06	2.80	3.00	3.07	3.14 ^{a}	2.93 ^{b}	0.090	0.039	0.458	0.050
Dunnett test													
Thigh	27.3^{b}	27.1^{b}	27.6^{b}	29.3^{a}	28.3^{b}					0.460	0.006		
Breast	33.8	34.5	34.6	32.6	32.5					0.560	0.242		
Fat pad	1.97^{a}	1.55^{b}	1.91^{a}	1.35^{b}	1.91^{a}					0.150	0.049		
Liver	3.26^{a}	3.08^{a}	3.20^{a}	3.06^{a}	2.80^{b}					0.080	0.009		

[1]Control, FS10, RFS10, FS20 and RFS20, represent corn-soybean meal basal diet, diet containing %10 raw flaxseed, diet containing %10 electron irradiated flaxseed, diet containing %20 flaxseed and diet containing %20 electron irradiated flaxseed, respectively.

[2]Standard error of means; [3]Flaxseed; [4]Irradiation.

[a,b]Values within a row with different superscripts differ significantly $P < 0.05$.

As shown in table 5, there were no interactions in cholesterol, triglyceride, and AST activity on days 21 and 42. Neither flaxseed nor irradiation had significant effects on serum cholesterol, triglyceride, and AST concentration at day 21, while the effect of flaxseed on AST activity on day 42 was significant ($P < 0.05$; Table 5). Broilers fed FS20 had significantly lower ($P < 0.05$) cholesterol and AST concentrations at 42 days of age. However, there were insignificant differences between the treatments for cholesterol, triglyceride and AST concentrations at 21 days of age.

The effects of irradiation dose and flaxseed levels on the apparent digestibility of EE, DM and OM in the ilea of broilers are shown in Table 6. Apparent digestibility of DM, OM, and EE increased in birds fed irradiated FS diets ($P < 0.05$). However, feeding FS20 reduced apparent ileal digestibility DM, OM, and EE when compared to FS10 ($P < 0.05$). There was a significant difference for EE, DM and OM between the control and FS treatments. Irradiation decreased ($P < 0.05$) digesta viscosity of birds fed FS diets (Table 6). Dietary FS levels had significant impact on digesta viscosity in birds fed FS20, as these birds had a higher viscous digesta than those fed FS10 ($P < 0.05$). There was a significant difference in digesta viscosity between the control and other treatments.

Table 5. Effects of experimental treatments on some blood parameters of broilers at 21 and 42 days of age

Tukey test	Dietary Treatments[1]					Irradiation		Flax seed		SEM [2]	*P*-value		
	Control	FS10	RFS10	FS20	RFS20	Yes	No	10%	20%		FS [3]	R [4]	FS×R
21 d													
Cholesterol (mg/dL)		122.50	121.25	110	116.20	118.73	116.25	121.88	113.10	8.08	0.300	0.766	0.654
Triglyceride (mg/dL)		45.1	52.4	64.0	50.0	51.2	54.5	48.7	57.0	8.05	0.322	0.679	0.205
AST[5] (IU/L)		62.2	51.1	53.8	60.5	55.8	58.0	56.7	57.1	9.27	0.962	0.817	0.353
42 d													
Cholesterol (mg/dL)		120.44	121.88	105.88	116.57	119.22	113.16	121.16	111.22	7.82	0.215	0.443	0.559
Triglyceride (mg/dL)		73.5	82.6	66.1	74.8	78.7	69.8	78.0	70.4	4.39	0.099	0.055	0.957
AST (IU/L)		53.2	59.5	26.8	34.5	47.0	40.0	56.4^{a}	30.7^{b}	6.34	0.004	0.279	0.914
Dunnett test													
21 d													
Cholesterol (mg/dL)	126.25	122.50	121.25	110	116.20					8.93	0.537		
Triglyceride (mg/dL)	49.2	45.1	52.4	64.0	50.0					7.89	0.658		
AST (IU/L)	41.5	62.2	51.1	53.8	60.5					8.02	0.208		
42 d													
Cholesterol (mg/dL)	134^{a}	120.44^{a}	121.88^{a}	105.88^{b}	116.57^{a}					7.31	0.010		
Triglyceride (mg/dL)	78.6	73.5	82.6	66.1	74.8					4.53	0.393		
AST (IU/L)	58.6^{a}	53.2^{a}	59.5^{a}	26.8^{b}	34.5^{b}					6.58	0.019		

[1]Control, FS10, RFS10, FS20 and RFS20, represent corn-soybean meal basal diet, diet containing %10 raw flaxseed, diet containing %10 electron irradiated flaxseed, diet containing %20 flaxseed and diet containing %20 electron irradiated flaxseed, respectively.

[2]Standard error of means; [3]Flax seed; [4]Irradiation; [5]Aspartate aminotransferase.

[a,b]Values within a row with different superscripts differ significantly $P < 0.05$.

Table 6. Effects of experimental treatments on ileal nutrient digestibility (at d 21 d), and digesta viscosity (at 42 d) of broilers

Tukey test	Dietary Treatments[1]					Irradiation		Flax seed		SEM[2]	*P*-value		
	Control	FS10	RFS10	FS20	RFS20	Yes	No	10%	20%		FS [3]	R [4]	FS×R
EE[5] (%)		55.6^{c}	69.8^{a}	50.9^{d}	68.5^{b}	69.1^{a}	53.2^{b}	62.7^{a}	59.7^{b}	0.240	0.0001	0.0001	0.0001
DM[6] (%)		74.5^{c}	78.5^{a}	69.3^{d}	77.3^{b}	77.9^{a}	71.9^{b}	76.5^{a}	73.3^{b}	0.130	0.0001	0.0001	0.0001
OM[7] (%)		70.4^{c}	79.5^{a}	65.5^{d}	78.6^{b}	79.0^{a}	68.0^{b}	74.9^{a}	72.0^{b}	0.130	0.0001	0.0001	0.0001
Viscosity (centipoise)		3.78	2.99	5.20	3.40	3.20^{b}	4.49^{a}	3.39^{b}	4.30^{a}	0.340	0.0164	0.0016	0.1563
Dunnett test													
EE	71.4^{a}	55.6^{b}	69.8^{b}	50.9^{b}	68.5^{b}					0.240	0.0001		
DM	79.4^{a}	74.5^{b}	78.5^{b}	69.3^{b}	77.3^{b}					0.130	0.0001		
OM	85.4^{a}	70.4^{b}	79.5^{b}	65.5^{b}	78.6^{b}					0.130	0.0001		
Viscosity	2.83^{b}	3.78^{a}	2.99^{a}	5.20^{a}	3.40^{a}					0.400	0.0224		

[1]Control, FS10, RFS10, FS, and RFS20, represent corn-soybean meal basal diet, diet containing %10 raw flaxseed, diet containing %10 electron irradiated flaxseed, diet containing %20 flaxseed and diet containing %20 electron irradiated flaxseed, respectively.

[2]Standard error of means; [3]Flax seed; [4]Irradiation; [5]Ether extract; [6]Dry matter; [7]Organic matters.

[a,d]Values within a row with different superscripts differ significantly $P < 0.05$.

Discussion

Feed intake was not affected by FS inclusion level which is in agreement with previous findings that supplemented corn-soybean meal diets with FS (2.5-10%) was not effective (Mridula *et al*., 2011; 2015). In the present study, inclusion of 20% FS in grower and finisher phases decreased BWG by 16% and 21% compared to the control group, respectively. Ajuyah *et al*. (1993) reported a 17% decline in body weight of birds fed 15% FS compared to chicks fed corn-soybean diet. Birds fed diets containing flaxseed had greater FCR compared to chicks fed control corn-soy diet. This result is in agreement with findings of Beheshti Moghadam *et al*. (2017) who reported that FCR was higher in chicks fed diets containing 15% FS compared to corn-soy diet. Mridula *et al*. (2015) also observed increases in FCR in broilers fed diets containing 15% flaxseed. The depression in bird performance of broilers fed raw flaxseed appears to be due to poor energy availability (Rodriguez *et al*., 2001) and ANF (Hernandez, 2013) such as cyanide. High levels of fiber and cyanide adversely affect digestibility and utilization of nutrients by birds (Esonu and Udedibie, 1993). Irradiation improved FCR and BWG of birds fed F20 compared to control, FS10 and RFS10 diets.

Nayefi *et al*. (2015) reported that broilers fed diets containing 10% electron irradiated cotton seed meal had higher feed intake and BWG compared to birds fed diet containing raw cotton seed meal. The greater BWG was attributed to the reduction in gossypol by irradiation. Previously, Chamani *et al*. (2009) reported that gamma irradiation (15-45 kGy) decreased glucosinolate and improved apparent ileal digestibility of amino acids in canola meal fed to broiler breeders. The observed effects of irradiated diets on birds' performance in the current study could be due to the effects of ionizing radiation on linatine, though these effects have not previously been reported in the literature. It is possible that using the high levels of irradiated flaxseed can have positive impacts on performance indices by decreasing the cyanide content in broiler chickens fed FS diets.

The increase in the relative weight of thigh of birds fed FS20 compared to FS10 is in contrast with that of Mridula *et al*. (2011; 2015) who indicated no changes in the thigh percentage at 2.5-15% level of flaxseed in the diets of broilers. Feeding FS20 led to decrease in fat deposition which is in line with findings of Najib *et al*. (2011). However, this is in contrast to Arshami *et al*. (2010) who reported no adverse effects of flaxseed (5-10%) on breast weight and abdominal fat of chickens. In the present study, the experimental diets were iso-energetic, so the reduction of fat deposits could be due to fatty acid composition of the diets. Esteve-Garcia (2012) stated that poultry diets that are high in polyunsaturated fatty acid can improve FCR and reduce fat deposition compared to diets containing monounsaturated fatty acid from tallow or vegetable oil sources. In other words, there is an optimum ratio of n-6 and n-3 fatty acid that leads to a decrease in adipose tissue. In the present study, feeding diets containing flaxseed (at 10% and 20%) to broilers had no effect on liver weight. Feeding RFS20 diet to broilers decreased liver percentage compared to other diets. Similar results were observed when 5-10% flaxseed were fed to pullet chicks (Arshami *et al*., 2010). The reduction of liver weight may be a result of reduction in cyanogenic glycosides content as a function of irradiation. Gharaghani *et al* (2008) observed that liver weight decreased in broilers fed irradiated (10-30 kGy) canola meal. In contrast, Nayefi *et al*. (2015) observed no significant effect of irradiation of cotton seed meal at doses of 30 kGy on the relative weight of liver and other organs of broiler.

The current study did not show any impacts of experimental diets on blood plasma lipid parameters. Our findings are in agreement with results of Nayefi *et al*. (2015) who reported that feeding electron irradiated cotton seed meal had not significant effect on blood parameters including triglyceride and cholesterol concentrations. The activity of AST at day 42 decreased as the dietary flaxseed levels increased to 20% in the diets. Similarly, Omer *et al*. (2013) reported that AST activity decreased in rabbits receiving 15% flaxseed in their diets. In contrast to this result, Yassein *et al*. (2015) showed that feeding moderate levels of flaxseed (5-10%) had no significant effect on AST activity on laying hens. However, high dose of flaxseed (12-16%) increased blood AST activity in broilers (Al-Nawass, 2015). These researchers attributed this effect to the presence of hydrogen cyanide in flaxseed, which can lead to the accumulation of toxins in the liver and increase secretion of liver enzymes into the blood.

We found that irradiation decreased 15% and 22% of total cyanide and fiber contents of flaxseed, respectively. These results could confirm one of the reasons for increasing digestibility of irradiated flaxseed diets. Although in the present study, irradiation improved apparent DM, OM and EE digestibility of flaxseed containing diets, RFS diets still had lower digestibility compared to the control group. Chamani *et al*. (2009) fed gamma irradiated (15-45 kGy) canola meal to the broiler breeders and showed an increase in apparent digestibility of amino acids with increasing doses of irradiation. Glucosinolate levels also decreased by 58% in gamma-irradiated canola meal at 45 kGy. Shawrang *et al*. (2013) conducted a digestion trail and showed an increase in energy and protein digestion of electron irradiated barley (10-30 kGy) as compared to control group.

In the present study, feeding FS20 to broilers increased ileal viscosity compared with FS10 and control diets. Irradiation decreased intestinal viscosity of broilers fed FS20. Studies using gamma or electron radiation showed a decrease in viscosity of rice and barley (Wu *et al*., 2002). Shawrang *et al*. (2013) reported that electron irradiation (10-30 kGy) linearly decreases viscosity of barley grain by 78%. Byun *et al*. (2008) showed that radiolysis of the glycosidic bonds of beta-glucan decreased the molecular weight, water solubility of polysaccharides, granule size and viscosity. Gamma irradiation at doses lower than 50 kGy could change β-glucan (purified from black yeast) with high solubility and low viscosity (Byun *et al*., 2008). The depolymerization of starch (Wu *et al*., 2002) has also been associated with the decrease in viscosity caused by ionizing irradiation.

Conclusion

In summary, results of the current study show that electron beam irradiation at 20 kGy of flaxseed is an effective way to improve performance, ileal digestibility, and reduce digesta viscosity of broiler chickens. Further research on the use of irradiated flaxseed in combination with carbohydrase enzymes and its impact on broiler performance and nutrient utilization are warranted.

Acknowledgements

This study was financially supported by Sari Agricultural Sciences and Natural Resources University, Sari, Iran. Authors wish to thank Mrs. Nayefi and Zarbal Company (Amol, Iran) for providing technical assistance to carry out this work. Also, a special thanks to Professor Howard Bradbury that provide Cyanide kit from the Australian National University, Canberra, Australia.

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