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Effects of glucosinolates and their hydrolysis products on biochemical and performance parameters in broiler chicken diets

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ABSTRACT

Glucosinolates are important bioactive molecules and widely found in Brassicaceae species (cress, brussels sprouts, mustard, broccoli, kale etc.). Depending on the amount of these vegetables consumed, both positive and negative metabolic effects from glucosinolate metabolites may occur. The aim of this study was to investigate inexpensive animal food sources that both increase weight gain and provide enhanced performance parameters without adversely affecting the animal's health and metabolism; to evaluate dose adjustment of food containing glucosinolates in animals; and to evaluate changes in the biochemical and performance status of chickens on the glucosinolate containing diets. Day-old Ross 308 broiler line chicks (624) were divided into 1 control and 3 treatment groups. Cress seed (*Lepidium sativum*) was added 0.05% for the first treatment group (group 1, 10g/kg), 0.10% for the second treatment group (group 2, 20g/kg) and 0.15% for the last treatment groups (group 3, 30 g/kg) to the diet. Serum samples were evaluated for serum glucose, adiponectin, leptin, growth hormone, estradiol and cortisol levels. Feed intake, live body weight gain and feed conversion ratio were investigated for performance parameters. The results showed that dietary cress seed supplementation as feed additive (10, 20 and 30 g/kg) did not significantly improve the dietary performance, or carcass parameters of broiler chickens. Feed intake was the highest in group 2 (20g/kg), female live weight was the highest in group 2 (20 g/kg) and 3 (30 g/kg).

Key words: Broiler, Cress seed, Diet, Glucosinolate

Glucosinolates in the nutrition are very important molecules and widely found in Brassicaceae species (cress, water cress, brussels sprouts, dijon mustard, broccoli, cress etc.). Depending on the amount of these kinds of vegetables consumed by humans and animals, some positive or negative metabolic effects of this molecule may occur (Polat 2010).

Glucosinolates are anticarcinogenic molecules with antioxidant and antibiotic activities and are able to induce the phase-2 detoxification enzymes. Berhow *et al.* (2013, 2014) indicated that Indol-3–Carbinol (I3C), a glucosinolate member hydrolysis product both stimulates and inhibits carcinogenesis. For example, while low doses of glucosinolates may be anti-estrogenic, high doses can act as an estrogen agonist and can cause abnormalities. Hydrolysis products of indole-GLSs, such as I_3C and DIM, may be responsible for the modulation of estrogen receptor activity, with both agonistic and antagonistic properties (Rahman and Sarkar 2002). Gardner and Adams (1986) reported that plant estrogens increased body weight in animals.

Still the lack of scientific data regarding the fate of glucosinolates in diets, the evaluation of their favorable and unfavorable effects on the target tissues, as well as on regulation of dietary energy balance remains largely incomplete.

The aim of this study was to investigate inexpensive animal food sources that result in healthy live weight gains and enhanced performance parameters; to evaluate dose effects in food that contains glucosinolates in animals for the first time and to evaluate the possible changes with the biochemical and performance status.

MATERIALS AND METHODS

Birds, housing and feeding: Day-old Ross 308 broiler line chicks (624) were divided into 4 groups. The experimental study was designed with 1 control and 3 treatment groups. Each treatment group had 3 replicates and 2 gender groups. The chicks kept in 24 pens (26 animal/pen). The groups were all housed under the same environmental conditions and the birds had continual access to water. The lights were set up to deliver 23 h light/1 h dark/day. Chicks were fed *ad lib.* with commercial broiler diets.Cress seed (*Lepidium sativum*) were added to the diets

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at 0.05% for group 1 (10g/kg), 0.10% for group 2 (20g/kg) and 0.15% for group 3 (30g/kg). The ingredients and chemical composition of the broiler starter, grower and finisher diets were shown in Table 1. All procedures in this study were approved by the Ethical Committee of the Animal Sciences group of the University.

HPLC analysis

Glucosinolate analysis sample preparation and extraction: Cress seeds and pressed cress seed meals were ground to a fine powder with a commercial coffee grinder. Weighed samples were placed in filter paper packets and defatted overnight in a Soxhlett extractor with hexane. After drying in the hood, the percent hexane extractable were determined by the difference in weight.

For HPLC analysis, typically between 0.25 and 0.5g of defatted meals were placed in a capped vial and extracted with between 2–5 ml of methanol. The vials were sonicated for 15 min in a sonic water bath then allowed to stand overnight. After another brief sonication, a portion of this extract was filtered through a 0.45 micron filter into an auto sampler vial.

Quantitation: For glucosinolate quantitation, a modification of a high-performance liquid chromatography (HPLC) method developed by Betz and Fox (1994) was used. The glucosinolates were detected by monitoring at 237 nm.

Blood parameters and laboratory analyses: Blood was taken from chicken's jugular veins (10 animals for each group) at day 1, 21 and 42 of the experiment. Serum were separated by centrifugation at 3,000×g for 5 min and stored at –20°C until the day of analyses. Samples were collected to determine serum glucose, adiponectin, leptin, growth hormone, estradiol and cortisol levels. Sera samples were analyzed for glucose using a colorimetric kit following the directions of the manufacturer. Concentrations of serum glucose were determined by measuring the colour change using a spectrophotometer. Adiponectin (Chicken Adiponectin ELISA Kit), leptin (Chicken Leptin ELISA Kit), growth hormone (Chicken Growth Hormone ELISA Kit), estradiol (Chicken Estradiol ELISA Kit), cortisol (ELISA Kit) were determined and microplate reader was used for all ELISA analysis. Biochemical parameters are presented in Table 2.

Performance parameters: Chickens were weighed separately on 1st, 7th, 14th, 21st, 35th and 42nd day of the study. Live weights (LW), live weight gains (LWG) and feed intakes (FI) were recorded weekly. After all data were recorded, feed conversion ratios (FCR) were calculated from feed intakes and body weight gains. Performance parameters of broiler fed the different ratio of cress seed are shown in Table 3.

Thiobarbituric acid (TBA) analysis: Breast meat samples were stored at 4°C to determine on day 1, 3 and 5 post slaughters. 2–thiobarbituric acid method [Ke *et al.* (1977)] was used and modificated to our samples. This method is based on the observation of a red colour that is created by oxidation of unsaturated fatty acids by using thiobarbituric acid (TBA) after heating malondialdehyde (MDA). Ten gram meat samples were homogenized with distilled water in a blender and transferred to a Kjeldahl flask then 2.5 ml 4 M HCl and 1 ml Antifoam A were added. Five millilitres TBA was added to 5 ml distillated and the same amount of TBA was added to 5 ml distilled water for blank. Each flask was incubated into boiling water bath for up to 30 min. The final solutions and a blank were measured in a spectrophotometer at 538 nm. The absorbance results were multiplied by 7.8. The final value was defined as mg MDA/kg sample. The result of TBA analysis was shown in Table 4.

Table 1. Ingredients and chemical composition of the broiler starter, grower and finisher diets

Ingredients (% of diet)	Starter diet (0-21d)	Grower diet (22-35 d)	Finisher diet (36-42 d)
Maize	20.45	25.51	28.05
Wheat	30.00	34.00	39.20
Full fat soy	24.95	24.11	16.37
Soybean meal	20.18	0.00	0.00
Sunflower meal	0.00	8.50	7.50
Poultry by product	0.00	3.20	3.50
Vegetable oil	0.80	0.92	1.80
Salt	0.22	0.12	0.11
Sodium bicarbonate	0.01	0.09	0.14
Limestone	1.29	0.66	0.62
Monocalcium phosphate	1.05	0.28	0.00
Methionine[1]	0.39	0.25	0.29
L-Lysine	0.14	0.32	0.44
Threonine	0.04	0.07	0.12
Choline chloride[2]	0.05	0.04	0.03
VMP[3]	0.33	0.33	0.33
Antioxidant[4]	0.10	0.10	0.10
Calculated nutrient concentration (%)			
Dry matter	88.93	88.92	88.92
Crude protein	21.85	19.65	19.48
Crude lipid	6.95	10.28	10.70
Starch	37.95	36.95	36.60
Glucose	7.10	5.80	5.30
Crude cellulose	5.15	4.80	4.70
Crude ash	5.50	5.48	5.30
Calcium, Ca	0.98	0.80	0.70
Phosphorus, P	0.48	0.42	0.38
Metabolic energy, ME, kcal/kg	3135	3230	3218

[1]Contained 88% liquid methionine; [2]Contained 75% liquid choline chloride; [3]VMP:Vitamin Mineral Premix providing per kg of diet: 12,000 IU Vitamin A, 1,500 IU Vitamin D_3, 30 mg Vitamin E, 5 mg Vitamin K_3, 3 mg Vitamin B_1, 6 mg Vitamin B_2, 5 mg Vitamin B_6 , 0.03 mg Vitamin B_{12} , 0.75 mg Folic acid, 10 mg calcium-D-pantothenate, 0.075 mg D-biotin, 40 mg nicotinamide, 0.08 mg manganese, 40 mg iron, 60 mg zinc, 5 mg copper, 0.5 mg iodide, 0.2 mg cobalt, 10 mg antioxidant, 70 mg niacin; [4]Providing per kg of diet: 2.5 mg BHA, 3.125 mg etoxyquine.

Table 2. Biochemical parameters of broiler fed the different ratio of cress seed (n=10)

Parameter	Group	Sex	Day 1	Day 21	Day 42
			Mean± S.E.		
Adiponectin (µg/ml)	Control	M	16.39 ± 2.73^{ab}	23.42 ± 0.96^{a}	13.44 ± 0.44^{b}
		F	17.60 ± 2.07^{a}	17.73 ± 1.98^{a}	8.41 ± 1.55^{b}
	Group 1	M	19.09 ± 1.59^{a}	17.19 ± 1.47^{a}	8.65 ± 1.41^{b}
		F	16.84 ± 1.85^{a}	18.32 ± 2.63^{a}	8.81 ± 1.29^{b}
	Group 2	M	18.78 ± 2.51^{a}	12.82 ± 0.84^{ab}	11.66 ± 0.94^{b}
		F	20.75 ± 0.74^{a}	14.15 ± 1.10^{b}	12.71 ± 1.47^{b}
	Group 3	M	17.30 ± 1.34^{a}	13.16 ± 0.82^{b}	10.68 ± 1.50^{b}
		F	18.41 ± 1.09^{a}	12.39 ± 2.13^{b}	12.30 ± 0.65^{b}
Growth hormone (pg/ml)	Control	M	2776.81 ± 276.72^{a}	2855.13 ± 198.30^{a}	2135.13 ± 114.45^{a}
		F	3088.69 ± 623.67^{a}	2982.45 ± 148.82^{a}	2632.62 ± 304.20^{a}
	Group 1	M	3317.41 ± 288.74^{a}	3112.30 ± 511.12^{a}	1943.06 ± 154.79^{b}
		F	3781.56 ± 391.74^{a}	2993.10 ± 227.41^{a}	2265.66 ± 103.22^{b}
	Group 2	M	3758.84 ± 415.57^{a}	3136.41 ± 347.18^{ab}	2497.07 ± 306.03^{b}
		F	3787.33 ± 212.71^{a}	2990.11 ± 134.35^{b}	2011.24 ± 123.92^{c}
	Group 3	M	2993.33 ± 605.46^{ab}	2871.16 ± 111.82^{a}	1786.17 ± 118.52^{b}
		F	2932.92 ± 645.55^{ab}	2627.90 ± 104.14^{a}	2067.39 ± 158.73^{b}
Glucose (mg/dl)	Control	M	96.69 ± 1.47^{a}	107.70 ± 3.50^{a}	83.72 ± 4.15^{c}
		F	95.69 ± 3.07^{ba}	117.35 ± 3.68^{b}	91.28 ± 4.65^{c}
	Group 1	M	101.84 ± 7.69^{a}	123.91 ± 5.37^{b}	108.66 ± 5.69^{ab}
		F	114.03 ± 13.62^{ab}	114.97 ± 7.44^{a}	91.64 ± 2.83^{b}
	Group 2	M	101.92 ± 10.77^{a}	113.90 ± 5.41^{a}	89.88 ± 7.64^{a}
		F	95.48 ± 5.81^{a}	126.20 ± 7.54^{b}	76.86 ± 2.45^{a}
	Group 3	M	87.53 ± 6.60^{ba}	103.63 ± 7.09^{Ba}	86.07 ± 1.80^{a}
		F	86.32 ± 2.99^{a}	114.78 ± 4.77^{b}	76.89 ± 5.51^{a}
Cortisol (ng/ml)	Control	M	1.84 ± 0.11	2.38 ± 0.50	1.44 ± 0. 21
		F	2.19 ± 0.39	1.60 ± 0.41	1.78 ± 0.29
	Group 1	M	1.92 ± 0.37	1.06 ± 0.10	1.43 ± 0.26
		F	2.19 ± 0.37	1.25 ± 0.17	2.10 ± 0.86
	Group 2	M	2.84 ± 0.29^{a}	1.20 ± 0.11^{b}	1.32 ± 0.10^{b}
		F	3.23 ± 0.22^{a}	1.66 ± 0.17^{b}	1.11 ± 0.11^{c}
	Group 3	M	2.58 ± 0.42^{a}	1.18 ± 0.17^{b}	1.72 ± 0.28^{a}
		F	2.06 ± 0.51^{a}	1.01 ± 0.09^{b}	1.24 ± 0.13^{a}
Leptin (ng/ml)	Contro l	M	3.37 ± 0.44^{a}	1.62 ± 0.26^{b}	7.95 ± 0.33^{c}
		F	4.62 ± 1.84^{ab}	1.88 ± 0.16^{a}	7.88 ± 0.41^{b}
	Group 1	M	2.07 ± 0.19^{a}	1.69 ± 0.32^{a}	6.94 ± 0.59^{b}
		F	7.46 ± 1.11^{a}	1.91 ± 0.30^{b}	8.07 ± 0.97^{a}
	Group 1	M	7.68 ± 2.42^{ab}	2.04 ± 0.05^{a}	7.84 ± 0.48^{b}
		F	3.35 ± 1.15^{a}	1.54 ± 0.34^{a}	8.13 ± 0.96^{b}
	Group 1	M	5.05 ± 1.14^{a}	2.01 ± 0.40^{b}	8.46 ± 0.65^{c}
		F	2.02 ± 0.35^{a}	2.51 ± 0.37^{a}	7.24 ± 0.72^{b}
Estradiol (pg/ml)	Group C	F	52.86 ±11.38^{a}	178.41 ± 18.23^{b}	118.56 ± 10.51^{b}
	Group 1	F	46.89 ±15.15^{a}	90.43 ± 6.29^{c}	165.25 ± 10.06^{b}
	Group 2	F	47.86 ± 9.13^{a}	140.32 ± 23.81^{b}	75.12 ± 7.65^{c}
	Group 3	F	52.31 ±10.09^{a}	155.17± 19.20^{b}	77.95 ± 5.68^{c}

$^{a, b}$Different lower case letters indicate a statistical difference in the same column (P<0.05). Control, Control; Group1,10g/kg cress; Group 2, 20g/kg cress; Group 3, 30 g/kg cress. M, Male; F, Female.

Statistical analysis: One-way ANOVA was used for compare carcass parameters (pre-slaughter live weight, carcass weight and carcass yield) as well as weekly body weights. Homogenity of variances were tested and Tukey HSD test was chosen as post hoc multiple comparison test. Body weight gain, food intake and food conversion ratio were analyzed by Kruskal Wallis Test as well as MDA, glucose, adiponectin, leptin, growth hormone, cortisol and estradiol levels and Mann-Whitney U Test was chosen for paired comparisons. Differences were considered significant at P<0.05. All statistical analyses were carried out using SPSS software (Version 20.0, SPSS Inc, USA).

RESULTS AND DISCUSSION

This cress has only one glucosinolate, glucotropaeolin. The seed contains 43.83 ± 2.7 mg/g defatted dry weight glucotropaeolin (107.17 ± 6.61 µM/g).

Biochemistry parameters: A statistically significant (P<0.05) difference was observed in serum glucose levels between group 1 and group 3 in female and day 1; between

Table 3. Performance parameters of broiler fed the different ratio of cressseed

Groups		Weeks						Overall Mean± SE
Live Weight (g/d)	Sex	Week 1 Mean± SE	Week 2 Mean± SE	Week 3 Mean± SE	Week 4 Mean± SE	Week 5 Mean± SE	Week 6 Mean± SE	
Control	M	149.21 ± 2.05	437.37 ± 5.04^{a}	870.99 ± 10.24^{ae}	1569.39 ± 15.87^{a}	2284.54 ± 19.13^{a}	2879.82 ± 23.04^{a}	
	F	142.85 ± 2.29	399.99 ± 6.17^{b}	764.96 ± 9.67^{bf}	1345.63 ± 13.06^{b}	1935.91 ± 16.26^{b}	2386.59 ± 20.88^{b}	
Group 1	M	140.97 ± 1.94	406.18 ± 6.59^{b}	831.51 ± 12.55^{acf}	1475.70 ± 20.48^{c}	2178.57 ± 32.45^{a}	2718.64 ± 38.67^{cd}	
	F	141.18 ± 1.96	411.93 ± 4.28^{b}	801.48 ± 8.76^{bcf}	1378.18 ± 15.80^{b}	1984.03 ± 26.36^{b}	2503.95 ± 33.36^{b}	
Group 2	M	142.09 ± 1.94	417.01 ± 6.17^{ab}	845.21 ± 10.88^{ace}	1515.54 ± 16.13^{ac}	2243.36 ± 25.36^{a}	2813.73 ± 26.40^{ade}	
	F	141.38 ± 1.88	404.44 ± 5.49^{b}	801.40 ± 8.83^{cdf}	1364.84 ± 14.91^{b}	1961.03 ± 21.66^{b}	2465.33 ± 27.04^{b}	
Group 3	M	140.95 ± 1.91	409.29 ± 5.35^{b}	880.79 ± 10.72^{e}	1490.26 ± 18.81^{c}	2227.82 ± 27.96^{a}	2712.75 ± 31.74^{ce}	
	F	141.79 ± 1.87	394.85 ± 5.49^{b}	788.64 ± 12.27^{f}	1386.27 ± 18.11^{b}	1991.03 ± 28.11^{b}	2464.62 ± 37.31^{b}	
Live Weight Gain (g/d)								
Control	M	106.46 ± 1.18	287.92 ± 3.51^{a}	434.26 ± 13.37	697.86 ± 18.26	715.12 ± 10.92^{a}	596.09 ± 28.61^{ac}	2837,72 ± 22,77^{a}
	F	100.34 ± 0.16	257.04 ± 3.76^{bf}	364.30 ± 25.65	581.10 ± 16.60	590.24 ± 2.27^{b}	449.95 ± 41.86^{b}	2342,98 ± 66,13^{b}
Group 1	M	98.56 ± 2.38	265.34 ± 3.77^{bcf}	548.55 ±146.28	520.89 ±119.68	702.13 ± 41.28^{ac}	540.52 ± 27.89^{ab}	2676,01 ± 91,46^{ad}
	F	97.12 ± 4.51	284.32 ± 14.46^{acd}	375.99 ± 24.43	576.62 ± 14.02	606.10 ± 20.26^{bc}	519.57 ± 12.72^{abd}	2459,73 ± 11,98^{b}
Group 2	M	99.23 ± 1.55	274.73 ± 4.70^{ce}	427.86 ± 20.59	670.33 ± 22.46	728.49 ± 12.97^{a}	570.48 ± 9.73^{cd}	2771,14 ± 14,65^{ac}
	F	98.24 ± 1.82	263.06 ± 6.32^{bde}	396.96 ± 14.34	563.66 ± 16.98	596.44 ± 20.91^{bc}	505.06 ± 28.36^{abe}	2423,44 ± 43,11^{b}
Group 3	M	97.44 ± 4.70	268.42 ± 5.39^{def}	471.62 ± 12.42	610.21 ± 30.94	737.94 ± 34.38^{a}	485.37 ± 20.97^{be}	2671,03 ± 86,92^{cd}
	F	99.53 ± 2.74	253.07 ± 3.77^{b}	393.80 ± 37.74	597.63 ± 14.96	604.75 ± 16.34^{bc}	473.59 ± 12.10^{ce}	2422,40 ± 56,58^{bd}
Feed Intake (FI) (g/d)								
Control	M	116.67 ± 3.20	396.53 ± 11.17	807.57 ± 22.31	965.28 ± 19.97	1186.24 ± 62.21^{ab}	1431.95 ± 44.13	4904,26 ± 136,55^{ac}
	F	121.44 ± 8.71	373.98 ± 15.88	700.70 ± 35.41	988.70 ± 67.21	1162.88 ± 71.87^{a}	1354.39 ± 83.71	4702,11 ± 188,17^{ac}
Group 1	M	114.71 ± 5.16	346.21 ± 34.37	782.60 ± 53.17	1068.79 ± 67.97	1280.02 ± 26.40^{ab}	1397.40 ± 29.89	4989,74 ± 17,04^{a}
	F	129.89 ± 9.85	352.11 ± 14.35	776.36 ± 37.70	972.40 ± 65.20	1175.43 ± 115.07^{a}	1425.43 ± 29.45	4831,64 ± 146,99^{ac}
Group 2	M	115.89 ± 0.05	372.45 ± 10.67	786.79 ± 65.57	1105.35 ± 15.70	1472.96 ± 17.28^{b}	1479.25 ± 43.59	5332,71 ± 85,33^{b}
	F	129.94 ± 2.97	363.22 ± 2.50	773.16 ± 59.80	1004.15 ± 26.06	1212.69 ± 65.14^{ab}	1280.59 ± 69.19	4763,76 ± 197,70^{ac}
Group 3	M	114.73 ± 5.86	343.35 ± 13.39	814.74 ± 16.52	1086.23 ± 19.90	1359.41 ± 17.15^{ab}	1375.40 ± 26.98	5093,88 ± 58,18^{a}
	F	113.16 ± 2.09	338.56 ± 23.72	699.89 ± 58.61	1040.22 ± 11.53	1212.35 ± 14.76^{ab}	1342.06 ± 40.43	4746,25 ± 117,15^{c}
Feed Conversion Ratio (FCR) (g/g)								
Control	M	1.10 ± 0.02	1.37 ± 0.05	1.86 ± 0.08	1.39 ± 0.06	1.66 ± 0.10	2.41 ± 0.05^{a}	1,73 ± 0.04^{a}
	F	1.21 ± 0.08	1.45 ± 0.06	1.92 ± 0.03	1.73 ± 0.11	2.06 ± 0.25	2.88 ± 0.18^{b}	2,01 ± 0.10^{b}
Group 1	M	1.16 ± 0.06	1.30 ± 0.12	1.65 ± 0.40	1.57 ± 0.08	1.83 ± 0.09	2.59 ± 0.10^{a}	1,87 ± 0.06^{ab}
	F	1.34 ± 0.13	1.25 ± 0.10	2.11 ± 0.02	1.71 ± 0.11	1.88 ± 0.20	2.75 ± 0.11^{bc}	1,96 ± 0.06^{b}
Group 2	M	1.17 ± 0.02	1.36 ± 0.06	1.86 ± 0.23	1.64 ± 0.08	2.02 ± 0.06	2.59 ± 0.07^{ac}	1,92 ± 0.02^{b}
	F	1.32 ± 0.05	1.39 ± 0.04	1.96 ± 0.21	1.78 ± 0.01	2.03 ± 0.04	2.54 ± 0.07^{a}	1,96 ± 0.05^{b}
Group 3	M	1.19 ± 0.10	1.28 ± 0.07	1.72 ± 0.02	1.79 ± 0.08	1.84 ± 0.08	2.84 ± 0.11^{b}	1,91 ± 0.06^{b}
	F	1.14 ± 0.04	1.33 ± 0.10	1.78 ± 0.06	1.74 ± 0.02	2.00 ± 0.07	2.83 ± 0.30^{b}	1,96 ± 0.03^{b}

Control, Control Group; Group 1, 10g/kg cress; Group 2, 20g/kg cress; Group 3, 30 g/kg cress. M, Male, F, Female.

$^{a, d}$, Different lower case letters indicate a statistical difference in the same column ($P<0.05$).

Table 4. Result of thiobarbituric acid (TBA) analysis (n=10)

Groups	TBA sex	Analysis (mg MDA/kg meat) Day 1 Mean ± SE	Day 7 Mean ± SE	Day 15 Mean ± SE
Control	M	0.34 ± 0.06^{a}	0.49 ± 0.01^{a}	1.10 ± 0.07^{b}
	F	0.22 ± 0.01^{a}	0.45 ± 0.01^{b}	1.08 ± 0.06^{c}
Group 1	M	0.22 ± 0.01^{a}	0.43 ± 0.03^{b}	1.07 ± 0.10^{c}
	F	0.20 ± 0.01^{a}	0.41 ± 0.02^{b}	1.02 ± 0.04^{c}
Group 2	M	0.18 ± 0.02^{a}	0.41 ± 0.01^{b}	0.77 ± 0.02^{c}
	F	0.15 ± 0.01^{a}	0.40 ± 0.01^{b}	0.49 ± 0.04^{c}
Group 3	M	0.15 ± 0.01^{a}	0.36 ± 0.02^{b}	0.57 ± 0.07^{c}
	F	0.12 ± 0.01^{a}	0.35 ± 0.01^{b}	0.58 ± 0.03^{c}

Control, Control group; Group 1, 10g/kg cress; Group 2, 20g/kg cress; Group 3, 30 g/kg cress. M, Male; F, Female. $^{a, b}$, Different lower case letters indicate a statistical difference in the same column ($P \leq 0.05$).

group 1 and group 3 in male and day 21; between control and group 1 in male and day 42, and between group 1 and group 3 in male and day 42. In normal broiler chickens, the reference levels of serum glucose were 136–260 mg/dl (Karagul *et al.*1999). Our results showed that glucose levels declined with relative increase in cress seed percentage in the meals. This is in agreement with the findings of Jaiswal *et al*. (2009) who reported that blood glucose level decreased after adding glucosinolate leaf extract to feed. This suggested that glucosinolates may have an insulin-like effect on peripheral tissues either by promoting glucose uptake and metabolism or by inhibiting gluconeogenesis. The aqueous leaf extracts may have some effect in increasing the tissue utilization of glucose by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues (Ghebreselassie *et al.* 2011).

Serum adiponectin was found significantly ($P<0.05$) different between control-group 1 and groups 2–3 in male and day 21; between control and group 1 in male and day 42, and between group 1 and group 3 in female and day 42. Hendricks *et al.* (2009) indicated that 8-wk-old chickens have significantly lower plasma adiponectin levels at a time when their body weight and abdominal fat pad mass increased by 2– and 1.5-fold, respectively. Changes associated with age or rapid growth may have also led to the decline in circulating adiponectin levels. Both adiponectin levels in the present study were identical (detection range: 31.25–500 ng/ml; Cusabio) and there was a decline among day 1, 21 and 42. Serum leptin levels had a statistical significance at the levels of $P<0.05$ between group 1 and groups 2–3 in female and day 1.

While there was no significant difference between day 1 and 21 in male and female for serum growth hormone levels, significant differences ($P<0.05$) were detected between groups 2 and 3 in male and day 42. Giachetto *et al.* (2003) found that growth hormone levels in broilers was 27.20–193.88 ng/ml but our values are significantly different. For serum cortisol levels, there were differences ($P<0.05$) between control and group 2 in male and day 1; between group 1 and group 2 in female and day 1, and between group 2 and group 3 in female and day 21.The values recorded in this study are different than those reported by Jadhav *et al.* (2013).The cortisol levels in the present study were higher in control group (male) than other groups. This result indicated that dietary glucosinolates do not seem to effect serum cortisol levels. Increases in serum cortisol (control/male in day 21), may be the result of stress and has been associated with decreases in neutrophil function, as well as humoral immune response by reducing the synthesis and secretion of antibodies and cell-mediated immune response (Kalaycioglu *et al.* 2000).

For serum estradiol, we found a significant difference ($P<0.05$) between control-group 1 and groups 2,3 in day 21, and between control-group 1 and groups 2,3 in day 42. In present study, serum estrogen levels in control group are higher than in groups 1–3. Some plants have estrogenic effects. When *Brassica* vegetable extracts are taken in low doses, they behave as antiestrogenic effect. However, in the high doses, they have estrogenic properties. Plants are activated by metabolized following ingestion by animals and exhibit estrogen-like effects (Rahman and Sarkar 2002). Estrogens in plants reduce the activities of substances with higher estrogenic effects when these substances and estrogenic plants are used in combination. This effect occurs due to estrogen receptors are occupied by plant estrogens. Because of having higher activity of estrogens cannot find receptors, they exhibit the anti-estrogenic effects. Brignall (2001) determined that *Brassica* vegetables are effective on increasing estrogen concentrations. Conversely, Stoner *et al.* (2002) demonstrated that they did not have any measurable estrogenic effects. Estrogenic plants stimulate body weight gain of the growing animals that determined in the other studies by Gardner and Adams (1986). Brassica extracts especially I3C and DIM indicate responsibility for the regulation of the estrogen receptor's activity owing to the agonistic and antagonistic properties.

Performance parameters: Live weights were similar until the second week for all groups and sex. Control group male chicks were heavier than the other group and sex for all groups except group 2 male chicks and fifth week. All male chicks had similar live weight each other, however, heavier than all female chicks at fifth week. Female chicks had similar live weight and they were lighter than male chicks and control group male chicks were heavier than the other group male chicks except group 2 male chicks at sixth week. Male chicks were heavier than female chicks inside group and there was no difference between female chicks in all groups for third and fourth week.

Live weight gains between groups and sex were not significant for week 1, 3 and 4. Male chicks gained more live weight than females at second week except group 1 and 2. Control group male chicks gained more live weight than the other group male chicks and group 1 female chicks gained more live weight then the other group female chicks except group 2 female chicks at second week.

Male chicks gained more live weight than females in

same group except group 2 at fifth week. Male chicks gained similar live weight each other as well as female chicks at fifth week. Similarly, male chicks gained more live weight than females in control and group 3 and live weight gains were similar in male and female chicks in group 2 and 3 at sixth week. In general, male chicks gained more live weight than females in same group, except group 3. There was no difference between female chicks in point of live weight gain in all groups. Also male chicks had similar live weight gain.

There was no difference between groups and sex in all weeks except week 5 and overall for feed intake. Male chicks consumed similar feed with female chicks in same group, just group 2 male chicks had more feed intake than female chicks in control and group 1 at fifth week.

As overall, male chicks consumed more feed than females in groups 2 and 3, male and female chicks had similar feed intake in control and group 1, the highest feed intake was obtained in group 2 male chicks. There was no difference between female chicks for feed intake in all groups. Also, there was a similar result for male chicks except group 2 in all groups.

FCRs did not differ for all groups and sex except sixth week. Male chicks had better FCR than female chicks at sixth week for control and group 1 ($P<0.05$). Male chicks had similar FCR except group 3 and male chicks in group3 had worst FCR ($P<0.05$). The better FCR was calculated for group 2 female chicks ($P<0.05$) and there was no difference between the other groups female chicks.

Our results indicated that control group male chicks had better FCR than other male and female group chicks as overall except group 1 male chick. There was no difference between female chicks in all groups as overall.

Table 5. Results of carcass parameters of broiler fed the different ratio of cress seed (n=15)

Groups	Sex	Pre-slaughter live weight (g)	Carcass weight (g)	Carcass yield (%)
Control	M	3015.33 ± 46.07[a]	2459.47 ± 38.23[a]	81.86 ± 0.46
	F	2418.20 ± 44.69[b]	1980.00 ± 38.88[b]	81.58 ± 0.56
Group 1	M	2873.33 ± 67.25[a]	2318.67 ± 53.85[a]	80.71 ± 0.37
	F	2372.80 ± 61.61[b]	1951.47 ± 58.39[b]	82.12 ± 0.59
Group 2	M	2863.13 ± 58.32[a]	2331.53 ± 53.25[a]	81.41 ± 0.64
	F	2441.73 ± 30.85[b]	2002.93 ± 28.93[b]	82.01 ± 0.42
Group 3	M	2720.87 ± 82.21[a]	2203.13 ± 75.83[a]	80.82 ± 0.62
	F	2400.27 ± 42.49[b]	1967.93 ± 34.65[b]	82.01 ± 0.53

Control, Control group; Group 1, 10g/kg cress; Group 2, 20g/kg cress; Group 3, 30 g/kg cress. M, Male; F, Female. [a, b], Different lowercase letters indicate a statistical difference in the same column ($P<0.05$).

Pre-slaughter live weights and carcass weights were different according to gender in groups ($P<0.05$). Male chicks were heavier than females in all groups for preslaughter live weights and carcass weights ($P<0.05$). Additionally, male chicks did not differ each other in different groups as well as female chicks. Statistical significance were not determined between groups and sex for carcass yield. The carcass parameters are shown in Table 5.

Effects of cress seed supplementation on breast meat MDA levels in broiler: Many plant extracts are an excellent source of natural antioxidants that can improve meat's shelf-life and quality mainly by retarding lipid oxidation and microbial growth (Botsoglou *et al.* 2002). The effects of dietary treatment on TBA development in raw breast meat during refrigerated storage at 1, 7 and 15 d are shown in Table 3. The extent of lipid oxidation as measured by MDA formation differed ($P<0.05$) between control and experimental groups on d 1,7 and 15. In this study, the lowest MDA levels were determined in groups 2 and 3, including high doses of cress seed which contain glucosinolate and hydrolysis products. The researches that related to supplementation of cress seed to broiler diets are limited. But cress seed is rich in antioxidants such as glucosinolates (Souri *et al.* 2004). Diwakar *et al.* (2010) reported that the essential oil derived from cress seed contained tocopherol, carotenoid, oleic acid and α-linolenic acid, while Zia-Ul-Haq *et al.* (2012) showed that cress seed extract, had a good antioxidant capacity that could reduce different types of radicals.

In conclusion, the study that examined glucosinolates and hydrolysis products on energy balance and performance parameters was performed to investigate the cress seed will be used for cheap feed additive or will be not in broiler chickens. According to the results, the rates of the cress seed (0.05, 0.10 and 0.15%) that contain glucotropaeolin were not affected for feed additive on performance (especially live weight and live weight gain) and carcass parameters. However, it was determined that feed intakes were decreased in female group 2 added 0.10% cress seed and feed conversion ratios were increased significantly in male group 3 added 0.15% cress seed.

At the same time, it was considered that MDA levels were reduced in group 2 (0.10) and 3 (0.15). These rates particularly would help to extend the shelf life of the chicken meat by preventing lipid oxidation commercially.

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