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Original Research Article

Effects of Glucosinolates and their Hydrolysis Products on Energy Balances and Performance Parameters and Histological 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 aims of this study were to; investigate inexpensive animal food sources that both increases weight gain and provides 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 these glucosinolate containing diets. A total of 624 one-day-old Ross 308 broiler line chicks were divided into one control and three treatment groups. Cress seed (*Lepidium sativum*) was added 0.05% for the first treatment groups (Group 1, 10 g/kg), 0.10% for the second treatment groups (Group 2, 20 g/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. Performance parameters investigated included feed intake, live body weight and feed conversion ratio. . The villus length, number of goblet cells, crypt depth were determined for histological analyses. According to histological results, villus length was significant at $p < 0.05$ level between control and group 1 and at $p < 0.001$ level with groups 2 and 3 at 21 days. The depth of the crypts belonging to the control and experimental groups was not significant between the control group and the group 1 when the statistic was evaluated on the 21st day, whereas between the control group and the group 2, $p < 0.05$ and $p < 0.01$; statistical significance was found at $p < 0.001$ level between group 3 and control group. The results showed that dietary glucosinolate supplementation as feed additive (10, 20 and 30 g/kg) did not significantly improved the dietary performance, or carcass parameters of broiler chickens. Feed intake was the highest in group 2 (20 g/kg), female live weight was the highest in group 2 (20 g/kg) and 3 (30 g/kg). In conclusion, 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.

Keywords: Brassica, Glucosinolates, Broiler, Villus, Performance

INTRODUCTION

Glucosinolates are the main secondary, sulphur-containing metabolites found in Brassica crops. The glucosinolate molecule consists of a β-thioglucose unit, a sulphonated oxime unit and a variable side chain, derived from an amino acid. Glucosinolates with more than 120 variable side chain structures have been described (Fahey *et al.* 2001; Brignall 2011), although only about 16 of these are commonly found within crop plants. Several Brassica species are important feed ingredients and some species are also commonly used in human nutrition such as cauliflower, cabbages, broccoli, Brussels sprouts (Gardner and Adams 1986; Polat 2010).

Epidemiological studies suggest that glucosinolates provide health benefits, particularly with regard to a reduction in risk of cancer (Talalay and Fahey 2001). The presence of glucosinolates in the seeds of oilseed cruciferous crops on the other hand, significantly reduces the livestock feeding quality of the meal left following oil extraction from seeds. Glucosinolates are present in all parts of the plants, with the highest concentrations found in seeds. Data on the toxicity of individual glucosinolates for food-producing animal species are very limited, and in most cases only the total glucosinolate content in a given feed material, measured indirectly through quantification of hydrolysable glucose, is available (Bialy *et al.* 1990; Rahman and Sarkar 2002). Our hypothesis is 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.

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Aims of this study were 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, histological and performance status

MATERIALS AND METHODS

Animals and protocols

Birds, Housing and Feeding

A total of 624 one-day-old Ross 308 broiler line chicks were used in this study. Chicks were divided into 4 groups. The experimental study was designed with one control and three treatment groups. Each treatment group had 3 replicates and 2 sex groups. The chicks kept in 24 pens (26 animal/per 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 per day. Chicks were obtained from Uludag University, Animal Health and Production Research and Application Centre (Bursa, Turkey). Chicks were fed *ad libitum* with commercial broiler diets. Cress seed (*Lepidium sativum*) were added to the diets at 0.05% for the first treatment groups (Group 1; 10 g/kg), 0.10% for the second treatment groups (Group 2; 20 g/kg) and 0.15% for the last treatment groups (Group 3; 30 g/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 Uludag University and Research Centre, Bursa, Turkey.

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 D3, 30 mg Vitamin E, 5 mg Vitamin K3, 3 mg Vitamin B1, 6 mg Vitamin B2, 5 mg Vitamin B6 , 0.03 mg Vitamin B12 , 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, 2.5 mg

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 0.5 g of defatted meals were placed in a capped vial and extracted with between 2-5 ml of methanol. The vials were sonicated for 15 minutes 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.

HPLC Analysis and Quantitation

For glucosinolate quantitation, a modification of a high-performance liquid chromatography (HPLC) method developed by Betz and Fox (1994) was used. The extract was run on a Shimadzu (Columbia, MD) HPLC System (two LC 20AD pumps; SIL 20A autoinjector; DGU 20As degasser; SPD-20A UV-VIS detector; and a CBM-20A communication BUS module) running under the Shimadzu LC solutions Version 1.25 software. The column was a C_{18} Inertsil reverse phase column (250 mm X 4.6 mm; RP C-18, ODS-3, 5u; with a Metaguard guard column; Varian, Torrance, CA). The glucosinolates were detected by monitoring at 237 nm.

Blood Parameters and Laboratory Analyses

Bloods were taken chicken's jugular veins from 10 animal for each group at days 1st, 21st and 42nd of the experiment. Serums 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 of serum adiponectin, leptin and growth hormone levels. Adiponectin (Cusabio Biotech Chicken Adiponectin ELISA Kit, Cat. No: CSB-EL001366CH, China), leptin (Cusabio Biotech Chicken Leptin ELISA Kit, Cat. No: CSB-E14082C, China) and growth hormone (Cusabio Biotech Chicken Growth Hormone ELISA Kit, Cat. No: CSB-E09866Ch, China were determined microplate reader (Biotek EL_x 808, USA) was used for all ELISA analysis. Biochemical parameters are presented in table 2.

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

Parameters	*Groups*	*Day 1*	*Day 21*	*Day 42*
	Sex		*Mean ± S.E.*	
Adiponectin	*Control M*	16.39 ± 2.73^{ab}	23.42 ± 0.96^{a}	13.44 ± 0.44^{b}
(µg/ml)	*F*	17.60 ± 2.07^{a}	17.73 ± 1.98^{a}	8.41 ± 1.55^{b}
	Group 1M	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 2M	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 3M	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	*Control M*	2776.81 ± 276.72^{a}	2855.13 ± 198.30^{a}	2135.13 ± 114.45^{a}
(pg/ml)	*F*	3088.69 ± 623.67^{a}	2982.45 ± 148.82^{a}	2632.62 ± 304.20^{a}
	Group 1M	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 2M	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 3M	2993.33 ± 05.46^{ab}	2871.16 ± 111.82^{a}	1786.17 ± 118.52^{b}
	F	2932.92 ± 45.55^{ab}	2627.90 ± 104.14^{a}	2067.39 ± 158.73^{b}
Leptin	*Control M*	3.37 ± 0.44^{a}	1.62 ± 0.26^{b}	7.95 ± 0.33^{c}
(ng/ml)	*F*	4.62 ± 1.84^{ab}	1.88 ± 0.16^{a}	7.88 ± 0.41^{b}
	Group 1M	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 2 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 3 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}

Performance Parameters

Chickens were weighed separately the days 1st, 7th, 14th, 21st, 35th and 42nd of the study. Live weight (LW), live weight gains (LWG) and feed intake (FI) were recorded weekly. After all data were recorded, feed conversion ratio (FCR) was calculated from feed intake and body weight gain. Performance parameters of broiler fed the different ratio of curly cress seed are shown in table 3.

Table 3. Performance Parameters of broiler fed the different ratio of cress seed.

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:*10 g/kg cress; *Group 2:*20 g/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 ($p < 0.05$)

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 color that is created by oxidation of unsaturatted fatty acids by using thiobarbituric acid (TBA) after heating malondialdehyde (MDA). 10 g meat samples were homogenized with distilled water in a blender and transferred to a Kjeldahl flask then 2.5 ml 4M HCl (Merck, Germany) and 1ml Antifoam A were added. 5ml TBA (Merck, Germany), was added to 5 ml distillated and the same amount of TBA was added to 5ml 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 that mg MDA per kg sample. The result of TBA analysis was shown in table 4.

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

Groups	***TBA Analysis***	***(mg MDA/ kg meat)***		
	Sex	***Day 1***	***Day 7***	***Day 15***
		Mean± S.E.		
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; *Group1*: 10 g/kg cress; *Group 2:* 20 g/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 ($P < 0.05$).

Histology and Histochemistry

On the 21 and 45th days of the investigations tissue samples were taken from duedonum, M. Femoralis and M. Pectoralis. Tissues were fixed in Bouin's solution consisting of 75% picric acid, 25% neutral buffered formalin solution, and 5% acetic acid for 24-48 h at 37 ºC. After dehydration in 70% ethanol, 80% ethanol, 96% ethanol, and absolute ethanol, the specimens were embedded in paraffin wax and 5 μm sections were cut and stained with Crossman's triple staining method (Crossman 1937).

The muscle specimens fixed in the Formol-Ca solution were kept at +4 °C and cross-sections were obtained with a freezing microtome at a thickness of 10-15 microns. Sudan Black-B staining technique was applied to the tissue sections (Herxheimer 1903).

Statistical Analysis

One-way ANOVA was used for compare carcass parameters (preslaughter 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 was 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).

Goblet cell number, crypt depth and villus length of the four groups were compared using the Univariate Analysis of Variance (one-way ANOVA test) with post hoc test Bonferroni. All analyses were performed using SPSS Windows 13.0.

RESULTS AND DISCUSSION

The Result of HPLC Analysis

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

The Results of Biochemistry Parameters

For serum adiponectin, a significant difference was observed ($p<0.05$) between control-group 1 and group 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 group 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 group 2 and group 3 in male and day 42. (Giachetto *et al.* 2003) found that growth hormone levels in broilers were as 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).

Performance Parameters

Live weights were similar until the 2nd 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 5th week. All male chicks had similar live weight each other, however heavier than all female chicks at 5th 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 6th week. Male chicks were heavier than female chicks inside group and there was no difference between female chicks in all groups for 3th and 4th weeks.

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

Male chicks were gained more live weight than females in same group except group 2 at 5th week. Male chicks were gained similar live weight each other as well as female chicks at 5th week. Similarly male chicks were 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 6th week. In general, male chicks were 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 each other as general.

There was no difference between groups and sex in all weeks except week 5 and overall for feed intake. Male chicks were 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 5th week.

As overall, male chicks consumed more feed than females in group 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.

FCR's were not differed for all groups and sex except 6th week. Male chicks had better FCR than female chicks at 6th 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.

The results of the present study indicate 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.

Pre slaughter live weights and carcass weights was different according to gender in groups ($p<0.05$). Male chicks were heavier than females in all groups for pre slaughter live weights and carcass weights ($P<0.05$). Additionally male chicks were 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 were shown in table 5.

Table 5. Results of Carcass Parameters 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; *Group1*: 10 g/kg cress; *Group 2:* 20 g/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 < 0.05).

The 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 d 1, 7 and 15 are shown in table 3. The extent of lipid oxidation as measured by MDA formation differed ($p<0.05$) between the control and experimental groups on d 1, 7 and 15. In this study the lowest MDA levels were determined in groups (Group 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.

Histological Results

As a result of the histological examinations, the villus length, number of goblet cells, crypt depth were determined by histometric analyzes and are shown in Table 6-7 and Figure (1-3).

Table 6. Histometric results of duodenum region of control and experiment groups on 21st day.

		n	*Control* *Female* *Male*	*Group 1* *Female* *Male*	*Group 2* *Female* *Male*	*Group 3* *Female* *Male*
Goblet	*Cells*	10	57,50±2,76	77,10±2,81***	123,30±3,04***	137,00±3,33***
(mm²)			56,80±2,15	75,10 ±3,16**	123,60 ±2,45***	132,70 ±3,70***
Crypt	*depth*	10	0,47±0,040	0,64±0,042	0,69±0,038*	0,75±0,043**
(mm)			0,51±0,046	0,66 ±0,043	0,72±0,055**	0,77±0,042***
Villus		10	88,10±1,69	98,00±2,39*	107,70±2,34***	105,70±2,62***
length(mm)			89,10 ±1,44	99,40 ±1,89*	106,40 ±2,32***	109,10 ±2,57***

Histological and Histometric Findings of Duodenum

On the 21st and 42nd days of the study, histological evaluations performed on the preparations of the duodenum region prepared from control and experimental groups done. Especially significant changes were observed in the tunica mucosa layers. Histomorphometric evaluations showed no difference between male and female animals in the same group. Therefore, the control and experimental groups without distinction between male and female are evaluated statistically. When the epithelial cells of the acidophilic cytoplasm forming the lamina epithelium in control and experimental groups were examined, it was observed that the cell boundaries were quite distinct and taller simple columnar epithelium especially in experimental groups (Figure-2). The epithelial cells of the control group are highly irregular and appear to be in the form of a thick layer or even a pseudostratified epithelium (Figure -1). There was no significant change in lamina epithelialis among the experimental groups. On days 21 to 42, the same findings were observed in control and experimental groups. The number of goblet cells among the epithelial cells forming lamina epithelium was found to be higher in the experimental groups on both 21st and 42nd days compared to the control (Figure-1) (Table 6-7). As a result of the evaluations, it was determined that the number of goblet cell counts belonging to the experimental groups were significant in the direction of the control group ($p < 0.05$).

Table 7. Histometric results of duodenum region of control and experiment groups on 42nd day.

		n	*Control* *Female* *Male*		*Group 1* *Female* *Male*		*Group 2* *Female* *Male*		*Group 3* *Female* *Male*	
Goblet	*Cells*	10	139,20±3,13		170,70±8,6*		213,30±5,43***	214,30	217,40 ±5,69***	220,80
(mm²)			138,90 ±3,21		182,30 ±12,74**		±4,67***		±5,52***	
Crypt	*depth*	10	0,82±0,033	0,78	0,94±0,045	0,97	0,94±0,047	0,93 ±	0,88 ±0,047	0,92
(mm)			±0,053		±0,036		0,039		±0,059	
Villus	*length*	10	108,00±2,84		115,00±2,36		126,90±2,28***	126,80	120,80 ±3,60*	126,80
(mm)			107,30 ±2,71		116,10 ±2,40		±2,34***		±2,97***	

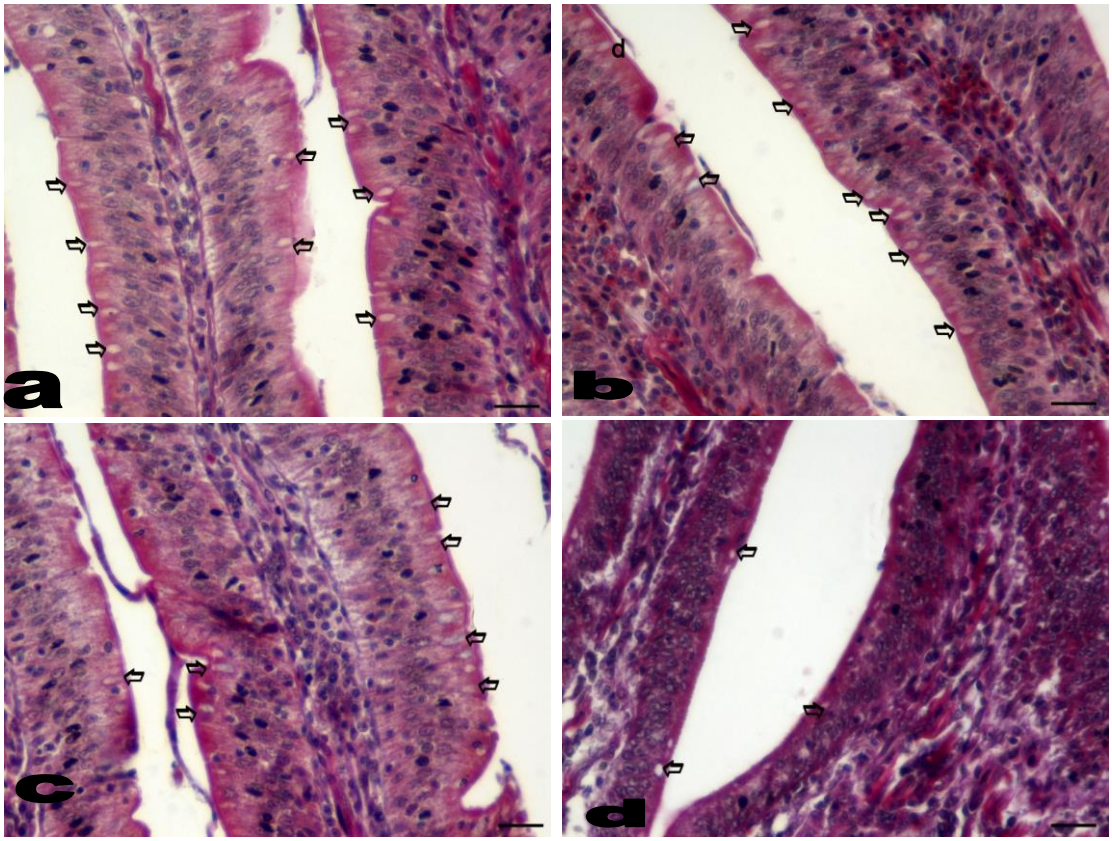

Figure 1. Experiment Group 1 (a); Group 2 (b); Group 3 (c) and Group 4(d); high prismatic epithelial cells and goblet cells (arrows) in the duodenal region, Triple Stain, Bar 50µ**.**

When the lengths of villi intestinalis belonging to the control and experimental groups were compared, it was found that they were longer and thinner in the experimental groups and shorter, but thicker in the control group (Figure 2). Villus length was statistically significant at $p < 0.05$ level between control and group 1 and at $p < 0.001$ level with groups 2 and 3 at 21 days. While there was no statistical significance between group 1 and control group, statistical significance was found between control group and groups 2 and 3 ($p < 0.001$).
The depth of the crypts belonging to the control and experimental groups was not significant between the control group and the group 1 when the statistic was evaluated on the 21st day, whereas between the control group and the group 2, $p < 0.05$ and $p < 0.01$; statistical significance was found at $p < 0.001$ level between group 3 and control group (Table 6-7).

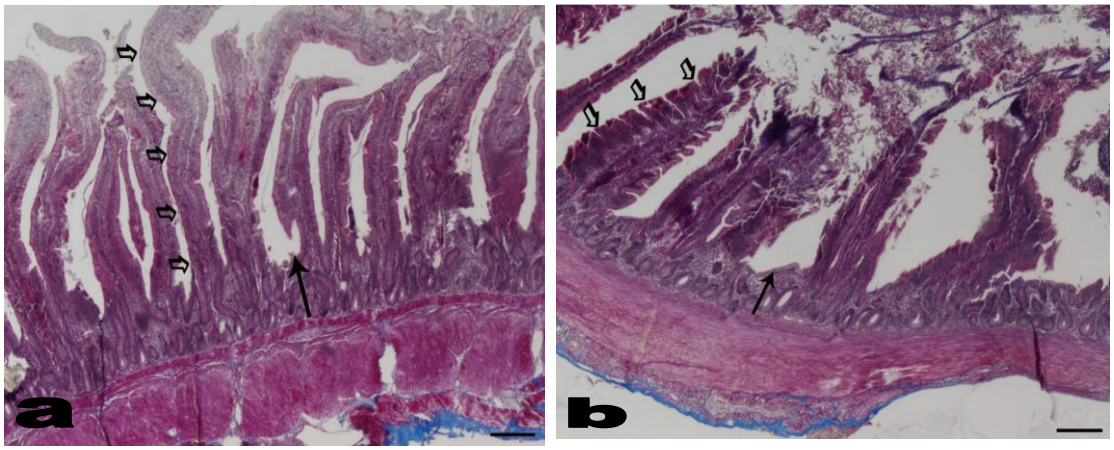

Figure 2. Villi intestinalis (thick arrows) and crypts (thin arrow) of the duodenum region of the experimental group (a) and control group (b). Triple Stain, Bar 100µ.

The lamina propria layer under the lamina epithelium is a tightly connective tissue, especially containing connective tissue fibril and free cells (lymphocytes, plasma cells, neutrophil granulocytes and macrophages). When this connective tissue is examined, lymphocyte infiltrations in control group preparations are very remarkable**.** The other layers of the mucosa are similar between control and experimental groups. Tunica muskularis, Tunica seroza did not show any significant difference between experiment and control groups.

Histological Findings of Musculus Femoralis and Musculus Pectoralis

When the transverse sections prepared from the control and experimental groups of the Musculus femoralis and Musculus pectoralis by the triple staining technique were examined on the 21st and 42nd days of the study, no significant difference was observed between the control and experimental groups and even between the female and male animals in the same group.

Musculus femoralis and Musculus pectoralis have been shown to have wider endomysium and perimysium and more blood vessels compared to Musculus pectoralis (Figure -3). In the preparations with the Sudan Black-B staining technique, endomysium and perimizyum were examined for lipid presence in muscle cells. In both groups of muscles, lipid- rich, lipid- medium and lipid-poor containing muscle fibers were detected at different rates (Figure 3).

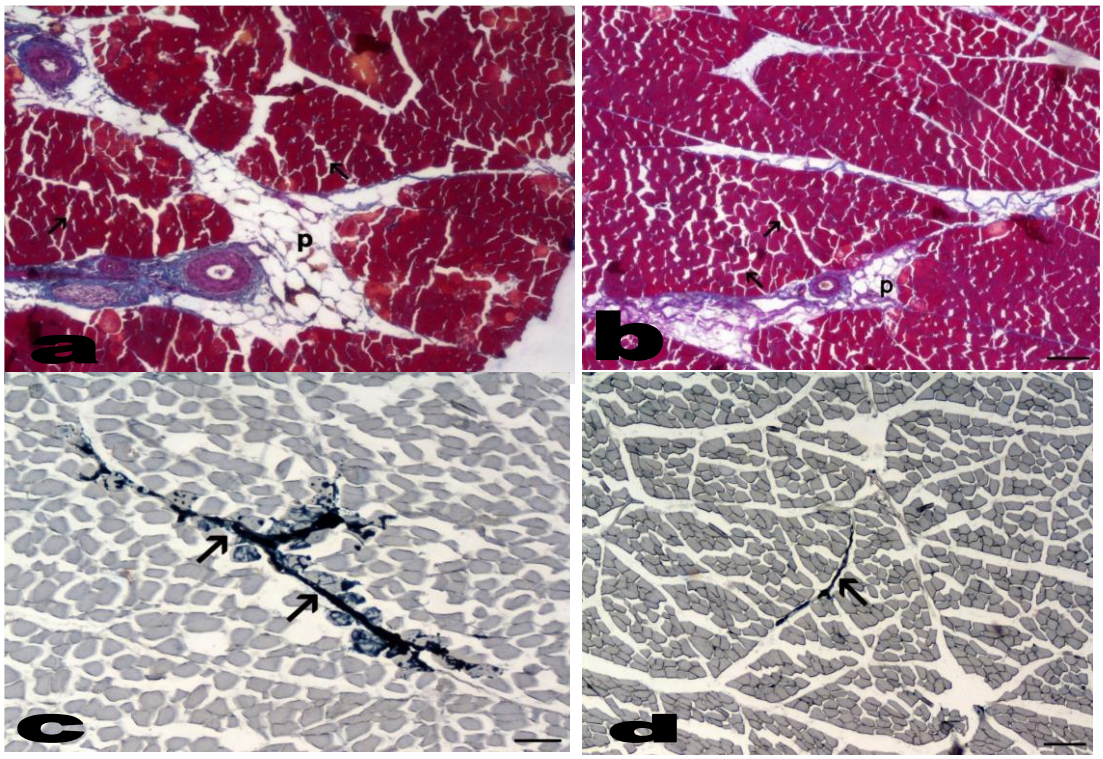

Figure 3. Musculus pectoralis (a) and Musculus femoralis (b) endomysium (arrow) and perimysium (p). Triple Stain, Musculus femoralis (c) and Musculus pectoralis (d) lipid demonstration. Sudan Black Stain, Bar 50µ.

Musculus pectoralis were evaluated for their content of lipids, and it was found that the number of lipid rich cells was few and small in diameter. In addition, it was found that the lipid-poor muscle fibers are more numerous and larger in diameter. While endomisium is poor in terms of lipids, it has been observed that fat cells form groups in perimisum. Musculus femoralis more numerous in lipid-rich muscle cells compared to Musculus pectoralis (Figure-3).

CONCLUSIONS

In conclusion, the study that examined glucosinolates and hydrolysis prouducts on energy balances 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. In the all experimental groups, it was observed that villus intestinalis were morfologically longer, thinner and more regular than the control group. The surface epithelial cells in the experimental group were observed to have simple columnar epithelium with a very distinctive cell bounders whereas the control group surface epithelial cells were observed to have pseudostratified epithelium. As a result of the examinations, goblet cells as considered more numerous in the experimental groups. Lymphocyte infiltration was found fewer. At the same time, villus length and crypt depth was statistically significant. When the cross sections prepared from M. Femoralis and M. Pectoralis by triple staining were examined, histopathological result was not observed in the experimental groups. In conclusion, that supplementation broiler diets with cress seed may help prolong the shelf-life of the commercial broiler meat by reducing lymphocyte infiltration and carcass quality can be affected positively.

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