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Effect of Caffeine and *trans-cinnamaldehyde* on Growth Performance, Hematology, Stress Hormone, Immunity Response and Blood Parameters in Broiler Chickens

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Abstract

This experiment was conducted to evaluate the effects of caffeine and *trans-cinnamaldehyde* (TC) on growth performance, hematological parameters, stress hormones, immune response, and some blood parameters of broiler chickens. Three hundred fifty 1-d-old broiler chicks were randomly assigned to 7 dietary treatments (five pens/ treatment of 10 male broilers each) in a completely randomized design. A basal control corn-soybean meal diet for starter, grower and finisher periods were formulated and supplemented with appropriate levels of caffeine (0.5, 1 and 2%) or TC (0.5, 1 and 1.5%). Findings showed that caffeine at level of 2% decreased feed intake (FI), body weight (BW), body weight gain (BWG) and increased feed conversion ratio (FCR). TC supplementation had no significant effect on growth performance compare to control diet. Except caffeine which significantly decreased hemoglobin and hematocrit at level of 2% in 21 day old chicks ($P<0.05$), caffeine and TC had no significant effects on hematological parameters and stress hormone of broiler chickens in 21 and 42 d ($P>0.05$). Similar trend were observed for humeral and cell mediated immunity. Supplementation of 2% of caffeine significantly decreased glucose level in 21 d ($P<0.05$) and this effect was not observed for TC levels compare with the control treatment. On the other hand all levels of caffeine and TC at level of 1% significantly decreased cholesterol levels in 21 d compared to control treatment ($P<0.05$). Caffeine and TC levels were not change the blood parameters in 42 d. Findings showed that supplementation of diets with caffeine specially at level of 2% decrease growth performance and neither caffeine nor TC affect the hematological, hormonal and immunity response in broiler chickens.

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Introduction

Caffeine (1, 3, 7-trimethylxanthine) is one of the most important ingredients in food, and potential effects of caffeine associated with caffeine persist. Caffeine is a psychoactive substance and at very low concentration exerts physiological effects on different organisms from bacteria to human (Garattini, 1993). Caffeine affects cardiovascular activity including vasoconstriction, total peripheral resistance, blood flow and so forth (Fredholm *et al*., 1999). Furthermore, caffeine elevate systolic and diastolic blood pressure, and increase lipolysis and fat oxidation, reduce glycogen breakdown (Costill *et al*., 1978; Pasman *et al*., 1995), increase lysosyme concentration in blood of mice (Ramanaviciene *et al*., 2002), and inhibit carcinogenesis (Lu *et al*., 2002). Moreover, caffeine influence on pathways caused by DNA damage and checkrein repaired damage (Murnane, 1995). Increased caffeine concentration in plasma may caused erythrocyte sickling *in vivo* and result in slow recovery from a sickling crisis (Uwakwe *et al*., 2002).

Poultry immunity system was affected by many stressors (Ottaviani and Franceschi, 1996), and caffeine is the one the psychoactive substance that stimulate central nerve system (Gilbert, 1984). Increased calcium concentration is an essential incitation for T cells and lead to changes in antigen-specific immune responses. Ritter *et al*. (2001) suggested that caffeine alter calcium concentration in lymphocytes. Indeed, caffeine suppresses the activation of immune cells (Rosenthal *et al*. 1992; Ritter *et al*. 2005). Moreover, cloned T cell was employed to assess the effects of caffeine on antigen-specific T cell responses. Creation of antigen-specific T cell lines needs repeated stimulation with specific antigens. Therefore, there is difference between established immune cell lines from those of naive cells (Zhu *et al*. 2004). Caffeine improved glucose metabolism in short-term human studies (Keijzers *et al*., 2002). As respects of caffeinated and decaffeinated coffee decreased insulin sensitivity (a potential precursor to diabetes) (Van Dam, 2006), authors suggest that coffee consumption improves glucose tolerance.

Herbal medicines are plant-derived products which have been used as traditional folk medicine and food additives and their properties are under studies and became main part of alternative medicines. Their potential effects on cancer, allergy and diabetes are reported (Miller *et al*., 2008; Esmonde and long, 2008; Ernst, 2001; Laengler *et al*., 2008). Cinnamomum cassia bark is the outer skin of an evergreen tall tree belonging to the family Lauraceous. Cinnamomum extracts contain essential oils (cinnamic aldehyde and cinnamyl aldehyde), tannin, mucus and carbohydrates (Wijesekera, 1978; Tanaka, 2008) which have biological function including anti-oxidant, antimicrobial, anti-inflammation, anti-diabetic effects (Khan *et al*., 2003; Lee *et al*., 2003; Schoene *et al*., 2005; Kim *et al*., 2006; Matan *et al*., 2006; Youn *et al*., 2008), and anti-tumor activity (Kamei *et al*., 2000; Schoene *et al*., 2005).

This study has highlighted the potential effects of supplemented caffeine and *trans-cinnamaldehyde* on broiler stress hormones, hematological parameters, immune response, and some blood parameters.

Materials and Methods

Bird Management and Experimental Design

This experiment was conducted to assess the effect of caffeine and *trans-cinnamaldehyde* on growth performance, hematological parameters, hormonal concentration, immune response and some blood serum parameters in broiler chickens. A total of 350 day-old chickens of a commercial genotype (Ross 308) used in a completely randomized design (CRD) with seven dietary treatments. After incubation of chicks delivered from local institutions and entrance hall, weighed, and with the same average weight (36.5 ± 0.50 g) to ten pieces were distributed in 35, 1 × 1 m cages. Treatments consisted of a corn-soybean meal basal diet (Table 1) and six other treatments in which caffeine (at three levels of 0.5%, 1.0% and 2.0%) or *trans-cinnamaldehyde* that was prepared from Sigma-Aldrich Chemie GmbH (at three levels of 0.5%, 1.0% and 1.5%) supplemented to basal diet. Broiler chickens were monitored twice-daily for general health. Broilers were weighed by pen at days 0, 10, 24 and 42. Three birds with close to the average body weight (± 5 g) from each replicate of treatments were slaughtered at day 21 and 42 for carcass characteristics. Based on the remaining birds and feed at the end of each period, adjusted BWG, FI and FCR measured during starter, grower and finisher periods. The experimental protocol was reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran.

Hematological parameters

Blood samples were collected from the brachial vein of 10 hens in each treatment in K_3EDTA tubes. Each sample was coded and offered for manual microscopic count. For the relative microscopic differential leukocyte count (M-Diff), blood smears were made and stained with May-Grünwald-Giemsa stain. One hundred cells were counted, including heterophils, lymphocytes, monocytes, basophiles, eosinophils and heterophil to lymphocyte (H:L) ratios.

Corticosterone and Serotonin Measurement

Blood samples were taken at the age of 21 and 42 from the brachial vein of 10 hens in each treatment and placed in tubes containing EDTA to estimate the plasma corticosterone (CS) and serotonin (5-Hydroxy Tryptamine, 5-HT). The bleeding procedure was limited to 40 sec or less to minimize the influence of handling stress. Blood samples were collected at the same time in the evening (04:00 pm) and centrifuged. Plasma was frozen (-20°C) until analyzed for the determination of corticosterone and serotonin. Plasma corticosterone was measured by using enzyme-linked immunoassay method (EIA-CS kit. Assay

Designs Inc., Ann Arbor, MI)(de Jong *et al*., 2001). plasma serotonin concentrations were evaluated by using ELISA kit (Labor Diagnostika Nord Gmbh & Co. KG) (Mayer and Sturike, 1974).

Table 1. Feed Ingredients and chemical composition of basal diet

Ingredients (%)	Starter (0-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	51.41	53.82	55.51
SBM (44%)	39.91	36.52	34.77
Soy oil	4.45	5.80	6.29
Dicalcium Phosphate	1.87	1.64	1.53
Limestone	1.09	0.86	0.80s
Salt	0. 38	0.37	0.37
L-Lysine	0.29	0.15	0.01
DL-Met	0.38	0.29	0.22
L-Thr	0.10	0.05	-
Vitamin Mix[1]	0.25	0.25	0.25
Mineral Mix[2]	0.25	0.25	0.25
Calculated Analysis			
ME (Kcal/Kg)	3025	3150	3200
Protein (%)	22.57	21.06	20.19
Crude Fiber (%)	3.94	3.76	3.68
Crude Fat (%)	2.26	2.34	2.39
Calcium (%)	1.05	0.90	0.85
Available Phosphorous (%)	0.50	0.45	0.42
Sodium (%)	0.16	0.16	0.16
Lys (%)	1.43	1.24	1.09
Met (%)	0.71	0.61	0.52
Met + Cys (%)	1.07	0.95	0.86
Thr (%)	0.94	0.83	0.76
Trp (%)	0.32	0.30	0.29
Arg (%)	1.45	1.35	1.30

[1]Provided per Kg of diet: vitamin A, 3,600,000 IU; vitamin D_3, 800,000 IU; vitamin E, 7,200 IU; vitamin K_3, 800 mg; vitamin B_1, 720 mg; vitamin B_2, 2,640 mg; vitamin B_3, 4,000 mg; vitamin B_5, 12,000 mg; vitamin B_6, 1,200 mg; vitamin B_9, 400 mg; vitamin B_{12}, 6 mg; vitamin H_2, 40 mg; choline chloride, 200,000 mg.

[2]Provided per Kg of diet: Mn, 40,000 mg; Fe, 20,000 mg; Zn, 40,000 mg; Cu, 4,000 mg; Se, 80 mg.

Immune Response Measurements

Antibody production against sheep red blood cell (SRBC) was measured at the 30 and 36 d of age. For this purpose 10 chicks per treatment were injected with 0.1 mL/BW of 0.25% SRBC solution into the brachial vein. The same birds were vaccinated with SRBC at both time points. Six days after each immunization, blood was collected in nonheparinized tubes by puncturing the brachial vein. Serum was obtained by centrifuging at 1,500 × *g* for 15 min at 25°C, and stored at -20°C until assayed. Serum sample analyzed for anti-SRBC antibody titers at 405 nm on an

ELIZA reader. All titers were expressed as the log2 of the reciprocal of the serum dilution.

Coetaneous Basophile Hypersensitivity (CBH)

CBH elicited in 10 chickens in each treat at the day 14 and 40 by an intradermal injection of Phaseolus vulgaris (red kidney bean) Phytohemagglutinin **(**PHA-P) is mediated in part by T cells. PHA-P was dissolved in sterile phosphate buffer solution (PBS). CBH responses were studied at different concentrations of injected PHA-P the interdigital area of the foot. The CBH response to PHA-P was evaluated by measuring the skin thickness in the injection site 24 and 42 hrs post-injection by using low pressure calipers. Data are reported as the difference percent between skin thickness of the PHA-P and the PBS-injected sites.

Blood Parameters

Two birds from each pen were used for collecting blood samples. Blood samples were collected from the wing vein. Samples were then centrifuged at 2,000 × g for 30 min and serum was separated. Serum were collected and stored at -20 °C (for up to 2 d) for determination of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and cholesterol concentration. Concentration of this blood parameters were evaluated by Automatic Analyzer (Bio Systems S. A. – Costa Brava 30, 08030 Barcelona, Spain). Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction described by 12531 kit. Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction described by 12533 kit. Free and esterified cholesterol in the sample originates, by means of the coupled reactions can be measured by spectrophotometry described by 12505 kit.

Statistical Analyses

All data were analyzed by the analysis of variance (ANOVA) general linear models procedure of SAS software (SAS, 2001) in a completely randomized design and when treatment means were significant ($P<0.05$), Tukey's multiple range tests was used to compare means. Before analysis, the univariate test was used to assess the normality of all data.

Results

Performance

The effects of caffeine and TC levels on growth performance are shown in Tables 2 and 3. The results show that caffeine and TC levels had no significant effect on FCR, BW and daily weight gain during the starter period (*P>0.05*). TC supplementation (1%) significantly increased FI in the 0-10 d, although the 0.5 and 2% caffeine and 1.5% TC significantly decreased FI at this period (*P<0.05*). Moreover, there was no significant different between 1% caffeine and 0.5% TC when compared with that of control group. Higher dosage of caffeine supplementation (2%) significantly decreased FI at 11-24, 25-42 and 0-42 day (*P<0.05*). No significant changes were seen with the other caffeine and TC levels at 0-42 d. TC supplementation (0.5 and 1%) improved FCR when compared with the other groups (*P<0.05*). However 2% of caffeine addition increased FCR at 11-24, 25-42 and 0-42 d periods (*P<0.05*). Caffeine addition at the level of 2% significantly decreased body weight and daily weight gain when compared with other groups. The minimum BW was seen at 2.0% caffeine supplementation at 24 and 42 days of age (*P<0.05*). Caffeine supplementation decreased daily weight gain (DWG) when compared with the control and TC addition. The minimum DWG was seen in 2.0% caffeine supplementation (32.92) and the maximum BWG (49.13) seen at 1.0% TC addition at 0-42 d Period (*P<0.05*).

Table 2. Effect of caffeine and *trans-Cinnamaldehyde* (TC) on Feed intake and Feed conversion ratio of broilers chickens

		Feed Intake (g/bird/day)				Feed Conversion Ratio			
		0-10d	11-24d	25-42d	0-42d	0-10d	11-24d	25-42d	0-42d
Control		23.01^{ab}	72.20^{a}	162.59^{a}	85.93^{a}	1.34	1.60^{ab}	2.19^{ab}	1.71^{ab}
	0.50	22.18^{b}	65.37^{a}	160.43^{a}	82.66^{a}	1.31	1.55^{ab}	2.34^{ab}	1.73^{ab}
Caffeine	1.00	23.53^{ab}	63.28^{a}	164.51^{a}	83.77^{a}	1.30	1.49^{ab}	2.47^{ab}	1.75^{ab}
	2.00	22.23^{b}	46.24^{b}	138.67^{b}	69.05^{b}	1.29	1.67^{a}	2.58^{a}	1.85^{a}
	0.50	23.24^{ab}	71.10^{a}	152.30^{ab}	82.22^{a}	1.32	1.48^{b}	2.00^{b}	1.60^{b}
TC	1.00	24.27^{a}	72.32^{a}	157.13^{a}	84.57^{a}	1.36	1.44^{b}	1.98^{b}	1.59^{b}
	1.50	22.47^{b}	73.48a	163.01^{a}	86.32^{a}	1.28	1.61^{ab}	2.19^{ab}	1.69^{ab}
SEM		0.20	1.72	1.85	1.03	0.015	0.019	0.054	0.02
P-value		0.04	<0.0001	0.0001	<0.0001	0.78	0.007	0.007	0.011

[a,b] Means in each column with different letters are significantly different (Tukey's test).

Hematological parameters

Supplementations of caffeine and TC in the diet significantly alter hemoglobin and hematocrit values at the day 21 (*P<0.05*). Maximum level of caffeine usage (2%) significantly decreased hemoglobin and hematocrit levels in 21 days old chicks (*P<0.05*). Caffeine addition (1.0%), as well as 0.5 and 1% TC addition show the highest levels of hemoglobin the same as control group. Although, there were no significant difference between 0.5 and 2.0 % caffeine and different levels of TC in hematocrit levels (*P>0.05*). No significant differences in the hematological

parameters were shown in the 42 days old chicks use different levels of caffeine and TC in the diets (*P>0.05*).(Tables 4 and 5)

Table 3. Effect of caffeine and *trans-Cinnamaldehyde* (TC) on body weight (gram) and body weight gain (gram/bird/day) of broilers chickens at 0-42 days of age

		Body Weight (g)			Daily Weight Gain (g/bird/day)			
		10d	24d	42d	0-10d	11-24d	25-42d	0-42d
Control		209.78	871.67^{a}	2228.50^{a}	17.25	45.04^{ab}	74.78^{a}	45.69^{ab}
	0.50	205.64	827.22^{a}	2063.70^{a}	16.91	42.05^{b}	68.69^{a}	42.55^{b}
Caffeine	1.00	219.64	836.56^{a}	2082.11^{a}	18.17	42.10^{b}	67.91^{ab}	42.73^{b}
	2.00	209.78	620.00^{b}	1586.71^{b}	17.17	27.55^{c}	54.04^{b}	32.92^{c}
	0.50	213.40	915.44^{a}	2293.90^{a}	17.59	47.99^{ab}	76.58^{a}	47.38^{ab}
TC	1.00	215.80	849.11^{a}	2381.00^{a}	17.83	50.02^{a}	79.54^{a}	49.13^{a}
	1.50	212.82	881.67^{a}	2225.40^{a}	17.54	45.53^{ab}	74.65^{a}	45.91^{ab}
SEM		2.09	20.37	48.29	0.20	1.25	1.73	0.94
P-value		0.70	<0.0001	<0.0001	0.76	<0.0001	0.0001	<0.0001

$^{a-c}$Means in each column with different letters are significantly different (Tukey's test).

Table 4. Effect of caffeine and *trans-Cinnamaldehyde* (TC) on hematological parameters in 21 day old chicks

		Lymphocyte (%)	Heterophile (%)	Monocyte (%)	Basophile (%)	Eossinophile (%)	Hemoglobin (g/dl)	Hematocrit (%)	H:L[1]
Control		64.00	31.33	2.33	1.33	1.01	8.66^{a}	31.16^{a}	0.49
	0.50	66.00	30.00	2.00	1.00	1.00	8.50^{ab}	31.43^{a}	0.45
Caffeine	1.00	62.66	33.00	2.33	1.00	1.01	8.63^{a}	30.76^{a}	0.52
	2.00	62.66	32.33	2.66	1.33	1.02	6.96^{b}	28.33^{b}	0.51
	0.50	61.33	32.66	2.66	2.33	1.02	8.66^{a}	31.50^{a}	0.53
TC	1.00	65.00	30.00	2.66	1.33	1.01	8.76^{a}	30.76^{a}	0.46
	1.50	63.00	32.66	2.33	1.00	1.01	8.50^{ab}	30.40^{a}	0.51
SEM		0.52	0.42	0.17	0.14	0	0.16	0.25	0.01
P-value		0.25	0.25	0.96	0.13	0	0.02	0.001	0.23

[1]Heterophile to lymphocyte ratio.

^{a,b}Means in each column with different letters are significantly different (Tukey's test).

Corticosterone and serotonin Measurement

Corticosterone and serotonin concentrations have been illustrated in table 6. The results shown that different levels of caffeine and TC had no significant effects on corticosterone and serotonin concentrations in the 21 and 42 days of age (*P>0.05*).

Table 5. Effect of caffeine and *trans-Cinnamaldehyde* (TC) on hematological parameters in 42 day old chicks (%)

		Lymphocyte (%)	Heterophile (%)	Monocyte (%)	Basophile (%)	Eossinophile (%)	Hemoglobin (g/dl)	Hematocrit (%)	H: L[1]
Control		63.00	33.00	2.00	1.00	1.00	8.66	30.66	0.52
	0.50	60.66	34.66	2.00	1.66	1.02	8.76	30.43	0.57
Caffeine	1.00	64.30	31.00	2.33	1.33	1.04	8.63	31.56	0.47
	2.00	64.66	31.66	1.66	1.00	1.02	8.73	31.43	0.48
	0.50	64.00	31.66	2.00	1.33	1.01	8.66	31.80	0.49
TC	1.00	62.66	32.66	2.00	1.66	1.02	8.90	31.46	0.52
	1.50	63.33	32.33	2.00	1.33	1.01	8.76	30.36	0.51
SEM		0.41	0.45	0.18	0.10	0	0-2.00	0.20	0.01
P-value		0.06	0.45	0.99	0.27	0	0.99	0.29	0.24

[1]Heterophile to lymphocyte ratio.

No significant difference was observed between treatments in each trait (*P>0.05*).

Table 6. Effect of caffeine and *trans-Cinnamaldehyde* on corticosterone and serotonin concentration in 21 and 42 day old chicks

		Corticosterone (pg/dl)		Serotonin (ng/dl)	
		21d	42d	21d	42d
Control		639.41	902.13	2218.41	2230.00
	0.50	699.42	902.13	2214.37	1606.40
Caffeine	1.00	609.34	819.46	2100.60	2214.91
	2.00	556.82	870.11	2100.60	2197.64
	0.50	738.78	888.78	2279.76	2214.91
TC	1.00	752.13	759.34	2229.76	2104.50
	1.50	666.23	890.54	2214.87	2214.91
SEM		22.25	19.03	25.17	96.88
P-value		0.18	0.36	0.42	0.64

No significant difference was observed between treatments in each trait (*P>0.05*).

Immune Response Measurements

Antibody production in response to SRBC and coetaneous basophile hypersensitivity was shown in tables 7 and 8, respectively. There were no significant differences between serum IgM and IgG levels at 21 and 42 days of age (*P>0.05*). In the other hand, caffeine and TC levels did not affect CBH responses in the 14 and 40 day (*P>0.05*).

Table 7. Effect of caffeine and *trans-Cinnamaldehyde* on SRBC response in 21 and 42 day old chicks

		21d			42d		
		Ig T	IgM	IgG	IgT	IgM	IgG
Control		4.00	1.00	3.00	3.00	0.50	2.50
	0.50	4.00	1.00	3.00	4.00	2.00	2.50
Caffeine	1.00	2.50	0.50	2.00	5.50	1.00	4.50
	2.00	4.00	1.00	3.00	4.00	1.00	3.00
	0.50	6.00	1.00	5.00	6.00	1.00	5.00
TC	1.00	4.50	0.50	4.00	7.50	0.50	7.00
	1.50	5.50	1.00	4.50	6.00	2.00	4.00
SEM		0.50	0.09	0.44	0.49	0.20	0.51
P-value		0.73	0.58	0.67	0.15	0.22	0.08

No significant difference was observed between treatments in each trait (*P>0.05*).

Table 8. Effect of caffeine and *trans-Cinnamaldehyde* on CBH in 14 and 40 day old chicks (% differences)

		14 d		40 d	
		24 h	48 h	24 h	48 h
Control		114.99	103.76	111.17	106.31
	0.50	124.81	116.60	120.47	110.68
Caffeine	1.00	121.16	107.35	124.22	104.05
	2.00	112.78	97.94	133.94	112.27
	0.50	119.51	111.96	128.24	121.00
TC	1.00	113.27	102.75	120.72	105.95
	1.50	121.66	106.37	139.34	116.74
SEM		2.47	2.46	4.00	2.44
P-value		0.88	0.58	0.68	0.56

No significant difference was observed between treatments in each trait (*P>0.05*).

Blood parameters

The effects of different levels of caffeine and TC on some blood parameters in broiler chickens on 21 and 42 days of age are shown in table 9. Results illustrated 2% addition of caffeine significantly decreased blood glucose levels in 21 day old chicks (*P<0.05*). No significant differences were show in the blood glucose level with usage of different levels of TC (*P>0.05*). Moreover, supplemented 2% of caffeine significantly decreased cholesterol concentration as compare with the other treatments (*P<0.05*). Maximum cholesterol level was shown in the control groups (176.35). No significant differences were shown due to addition of caffeine and TC in the 42 day old chick's blood parameters (*P>0.05*). As we show, inclusion of caffeine up to 2% into diet of broilers reduced nutrients intake and this may causes lower blood glucose concentration in chicks.

Table 9. Effect of caffeine and *trans-Cinnamaldehyde* on blood parameters in 21 and 42 day old chicks

		21 d				42 d			
		Glucose (mg/dl)	AST (U/L)	ALT (U/L)	CHL (mg/dl)	Glucose (mg/dl)	AST (U/L)	ALT (U/L)	CHL (mg/dl)
Control		226.90[a]	140.88	14.75	176.35[a]	234.72	208.29	23.19	149.34
	0.50	232.18[a]	152.50	18.27	153.55[bcd]	251.22	192.02	19.36	163.82
Caffeine	1.00	219.63[ab]	138.34	11.77	150.94[bcd]	243.57	168.66	11.05	154.39
	2.00	178.96[b]	140.30	17.31	142.63[d]	235.06	148.09	20.01	162.82
	0.50	235.06[a]	154.67	18.39	170.75[abc]	240.39	140.30	17.31	142.62
TC	1.00	227.89[a]	159.51	14.28	149.18[dc]	235.34	221.68	14.43	162.84
	1.50	231.91[a]	148.59	17.30	172.77[ab]	236.19	171.09	12.24	136.05
SEM		5.53	5.45	0.89	3.86	2.36	11.99	1.80	4.51
P-value		0.02	0.96	0.41	0.03	0.52	0.57	0.62	0.63

[a-d]Means in each column with different letters are significantly different (Tukey's test).

Discussion

Circulating glucagon-like peptide-1 (GLP-1) concentrations was significantly enhanced after consumption of decaffeinated diet, later in the postprandial time period. These opposing effects of incretin hormones would have minimized any effects of caffeine on insulin secretion that regulate fed consumption in chicks. Sheffield (1991) reported that 500 mg caffeine per mice, cusses increased weight at day 15. The increased litter weight apparently was due to increased growth rate of offspring during lactation, because litter weight at birth was not significantly affected by caffeine. In other research, Mcgruder *et al*. (2011) had *in ovo* injection of caffeine and expressed that caffeine had no significant effects on settable egg weight (SEW), but caffeine at concentration of 1×10^{-3} mM decreased proportional embryo body weight (PEMBW) in day 18 ($P<0.05$).

The values obtained for all hematological parameters are almost uniform across the treatments and was within the normal range (Weiss and Wardrop, 2011), which influenced by genetic factors and depended on poultry hematological constituents reflect the physiological responsiveness of the animals to its internal and external environment including the type of feed the animal consumed and feeding practices (Esonu *et al*., 2001). Although, hematocrit and hemoglobin slightly decreased with the high level of caffeine, but did neither caffeine nor TC had any adverse effects on the other factors. In this study H:L ratio was not significantly different due to caffeine and TC usage it could be explain that in the current research broiler brooding in the no stress condition as this index has been accepted as a reliable index for determining stress in poultry (Siegel, 1995).

In the peripheral system, however, biological roles of serotonin in behavioral adaptation and motivational regulation are unclear. Decreased, increased, and unchanged blood serotonin concentrations have been found in association with behavioral dysfunctions, including aggressiveness (Hanna *et al*., 1995; Moffitt *et al*., 1998). The conflicting data from different investigations could be related to

different genetic selection programs, species, behavioral evaluations, and stressors used as well as duration and frequency of stressor presentation (Cheng *at al*., 2001a, b). Adrenocorticotropic hormone (ACTH) a hormone secreted by the pituitary gland that causes the adrenal glands to synthesize and secrete corticosterone (Frankel, 1970). In fact, 42 different responses were studied, and blood corticosterone concentration was shown to be the most predictable indicator of the stress condition in broilers. The commercial production practices used in the present study are representative of industry norm, and these practices did not causes a stress condition that change blood concentrations of corticosterone and serotonin (Thaxton *et al*., 2005).

Dietary bioactive food components such as caffeine and TC that interact with the immune response have considerable potential to reduce susceptibility to infectious diseases (Kogut, 2009). The hallmark of an immune system is the diverse and variable responsiveness to potential pathogens. It is this diversity and variability that allows the immune response to defend against multiple types of infectious agents and to eliminate those agents from the host, especially in genetically homogenous populations of today's commercial poultry industry. Interestingly, selection of today's modern chickens for growth and egg production has resulted in a diminished inflammatory response (Leshchinsky and Klasing, 2001), in this study chicks were not against pathogen or stressor factors that changes the SRBC titters or CBH responses.

Lower cholesterol concentration could be explained by the reduction of synthetic enzyme activity because caffeine and TC would be expected to have a much higher antioxidant capacity or biological effects than control, among which are suppressing the formation of free radicals in 21 day (Chowdhury *et al*., 2002). Similar results have been observed in laying hens and broiler chicks; in which dietary plant extract reduced serum cholesterol concentration or total cholesterol oxidation products (Chowdhury *et al*., 2002; Kim *et al*., 2006). In contrast, other studies have shown that 0.02% essential oil did not have any significant effect on serum cholesterol (Reddy *et al*., 1991).

Several cross-sectional studies have found coffee intake to reduce serum γ-glutamul transferase (GGT) activity, an indicator of liver injury (Higdon and Frei, 2006; Dórea and da Costa, 2005). Recently, Ruhl and Everhart (2005) analyzed the data from the U.S. National Health and Nutrition Examination Survey (NHANES) (1988-1994), and found that consumption of either coffee or caffeine decreased the risk of abnormally elevated ALT activities.

Conclusions

In summary, base on growth performance results of our study caffeine supplementation at level of 2% cusses caffeinism symptoms in broilers and not recommended. Moreover, there are no differences between 0.5 and 1% caffeine supplementation in broilers growth performance. An addition of 1% *trans-*

cinnamaldehyde shows the better growth performance as compare with the 0.5 and 1.50%. Although, no differences were shown for the other characteristics measured in this experiment in different levels of caffeine and *trans-cinnamaldehyde*.

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