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ORIGINAL ARTICLE

Response of plasma constituents and body measurement in broiler chickens fed fish oil and green tea powder

Respuesta de los constituyentes del plasma y medición corporal en pollos broiler alimentados con aceite de pescado y polvo de té verde

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RESUMEN

Se realizó un experimento para examinar la influencia de la suplementación de la dieta de aceite de pescado (0, 15, o 20 g/kg), polvo de té verde (0, 10, o 15 g/kg), o sus arreglos de tratamiento factorial en componentes químicos de la sangre en pollos de engorda con 42 días de edad. El efecto del aceite de pescado no fue significativo para ninguno de los componentes de la sangre, excepto para el ácido úrico en plasma, el que experimentó una reducción con el nivel de suplementación de 20 g/kg. El polvo de té verde, en cantidad de 15 g/kg en las dietas, causó un aumento significativo en ácido úrico en plasma, pero en combinación con el aceite de pescado el ácido úrico en plasma fue menor que con 15 g/kg de polvo de té verde solo. Polvo de té verde en dosis de 15 g/kg causó una disminución significativa en el colesterol LDL en plasma, esto se atribuyó a una disminución significativa en la relación de LDL/HDL del plasma. El té verde mostró el potencial de mejorar la capacidad antioxidante debido al aumento de ácido úrico en plasma y perfil lipídico mejorado de plasma debido a la disminución de LDL. Los rendimientos de los pollos de engorde alimentados con polvo de té verde con y sin aceite de pescado en general disminuyeron, lo que se atribuyó a la disminución en el consumo de alimento y la disminución en la eficiencia de energía y proteínas.

Palabras clave: química sanguínea, pollos, aceite de pescado, té verde.

SUMMARY

An experiment was conducted to examine the influence of the dietary supplementation of fish oil (0, 15, or 20 g/kg), green tea powder (0, 10, or 15 g/kg), or their factorial treatment arrangements on selected blood chemical components in 42 days old broiler chickens. Fish oil as a main effect was not significant for any blood component except for decreased plasma uric acid, when fish oil was fed at 20 g/kg. Green tea powder at 15 g/kg in the diets was found to cause a significant increase in plasma uric acid, but in combination with fish oil, the plasma uric acid was lower than with 15 g/kg green tea powder alone. Green tea powder at 15 g/kg caused a significant decrease in plasma LDL, which was attributed to a significant decrease in plasma LDL/HDL ratio. Use of green tea appeared to have the potential to improve antioxidant status due to increased plasma uric acid and improved plasma lipid profile due to decreased LDL. In general, performance traits of broilers fed green tea powder with and without fish oil decreased, which was attributed to the decrease in feed intake and decreased energy and protein efficiencies.

Key words: blood chemistry, broilers, fish oil, green tea.

INTRODUCTION

Green tea is derived from minimally oxidized processed leaves of *Camellia sinensis*. On a global basis nearly five million tonnes of green tea are produced annually. It has been studied extensively in people and animals, and it has become the raw material for extracts, which are used in various beverages, health foods, and dietary supplements due to purported health benefits obtained from its consumption (Reich *et al* 2006, Cabrera *et al* 2006). Contained in the leaves are a variety of enzymes, amino acids, carbohydrates, lipids, sterols, polyphenols, carotenoids, tocopherols, vitamins, caffeine and related compounds, phytochemicals and dietary minerals (Cabrera *et al* 2006). Green tea has high flavonoid/polyphenol content, and these phytochemicals appear to be responsible for health benefits such as anti-oxidative and anti-carcinogenic functions[1].

Consumption of green tea is associated with reduced mortality caused by cardiovascular events (Kuriyama *et al* 2006), and it is believed that polyphenolic compounds in green tea help to prevent the cardiometabolic syndrome (Collins *et al* 2007, Kim 2007, Kim *et al* 2008, Potenza *et al* 2007, Reiter *et al* 2010). The major polyphenolic compounds in green tea include epicatechin (EC), catechins,

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epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG), and EGCG is the most abundant form found in green tea (Wolfram 2007, Kim 2008, Thielecke and Baschmann 2009). It has been suggested that EGCG has anti-diabetic, anti-obesity, and anti-inflammatory effects (Polychronopoulos *et al* 2008). EGCG improves endothelial function and insulin sensitivity while reducing blood pressure and protecting against ischemia reperfusion (I/R) injury in Spontaneously Hypertensive Rats (SHR) (Potenza *et al* 2007). In a randomized, triple cross-over controlled study, moderate and high doses of EGCG were given to patients with borderline hypertriglycerolaemia, and the treatment reduced plasma triacylglycerol levels by 15.1% (moderate dose) and 28.7% (high dose) (Unno *et al* 2005). Although green tea does not raise the metabolic rate enough to produce immediate weight loss, a green tea extract containing polyphenols and caffeine has been shown to induce thermogenesis and stimulate fat oxidation, boosting the metabolic rate 4% without increasing the heart rate (Dulloo *et al* 1999).

Green tea supplements for poultry have been examined (Biswas and Wakita 2001, El-Deek and Al-Harthi 2004, Kojima and Yoshida 2008, Lee *et al* 2012, Huang *et al* 2013). Biswas and Wakita (2001) observed that green tea powder caused decreased feed intake and body weight, but feed conversion was improved. Further, Biswas and Wakita (2001) reported that liver weight of green tea fed birds was increased, but liver fat and cholesterol and serum total cholesterol were decreased significantly. Additionally, Biswas and Wakita (2001) found less thiobarituric acid reactive substances (TBARS), which are a byproduct of lipid peroxidation, in breast meat from green tea-fed birds. A lower TBARS level in meat is an indicator of improved antioxidant status in poultry meat (Fellenberg and Speisky 2006). El-Deek and Al-Harthi (2004) reported that green tea did not alter body weight in market birds compared to controls. El-Deek and Al-Harthi (2004) also found that green tea supplementation affected neither feed intake, feed conversion, dressing percentage, liver cholesterol, nor plasma cholesterol. Huang *et al* (2013) studied lipid metabolism and the genomics of lipid metabolism regulation in green tea-supplemented broiler chickens, reporting that green tea supplementation reduced body fat masses, serum triglycerides, serum low density lipoprotein (LDL) bound cholesterol, and total cholesterol along with an increase in high density lipoprotein (HDL) bound cholesterol. Very low density lipoprotein (VLDL), LDL, and HDL are three of the five major groups of lipoproteins found in plasma that carry most of the cholesterol, phospholipids and triglycerides (Kwiterovich 2000). Both VLDL and LDL are associated with atherogenic processes, and there is increasing evidence that HDL prevents atherogenesis (Kwiterovich 2000).

Huang *et al* (2013) reported that genes associated with lipid anabolism were down-regulated and genes associated with lipid transport and catabolism were up-regulated in green tea-fed broilers. Lee *et al* (2012) noted that green tea by-products fed to chickens had an antiviral effect. Laying hens given green tea powder experienced decreased egg production and performance with decreased egg weight, egg mass, decreased physical characteristics of the eggs, which was associated with green tea dose-dependent decreases in feed intake (Kojima and Yoshida 2008).

Poultry diets rich in fish oils containing n-3 polyunsaturated fatty acids (n-3 PUFAs) can have significant impact on physiological status, as the fish oils polyunsaturated fatty acids affect the nervous system (Mourot and Hermier 2001) and help maintain the immune system when fed at moderate levels (Prickett *et al* 1982, Sugano *et al* 2000, Wang *et al* 2000, Maroufyan *et al* 2012). Korver and Klasing (1997) noted that fish oil improved growth and feed efficiency in chickens experiencing an inflammatory response.

Given that green tea and fish oil extend certain benefits to vertebrate animals, we designed a study to ascertain the influence of dietary supplementation with various levels of fish oil and green tea powder on broiler chicken blood chemical parameters.

MATERIAL AND METHODS

ANIMAL WELFARE

The experimental procedures describe herein were approved by the Islamic Azad University Ethics Committee, and care was taken to minimize the number of animals used.

ANIMAL HUSBANDRY

This experiment was performed at a commercial poultry farm in Abkenar, Guilan, Iran near the Islamic Azad University, Rasht Branch, Iran. Before placement of chicks, the poultry barn, feeders, and drinkers were thoroughly cleaned and disinfected with a diluted multiphenolic (20% benzalkonium chloride). This was followed by fumigation of the barn with a volatilized formalin solution over a 48 hour period.

Oil-fired heaters were used for supplemental heat during the brooding phase. Ambient temperatures used in this experiment were standard for the growing of broiler chickens and consisted of starting temperature of 35 °C that was reduced gradually to 25 °C around 3 weeks of age. In order to provide atmospheric relative humidity between 55%-65% in the early growing period, the floor was sprayed periodically with water. Incandescent lamps, installed at a height of 2.2 meters above the floor, using ceiling-mounted incandescent lamps, provided 23 hours light and 1 hour dark, beginning at placement and continuing through 42 days after placement.

Drinkers were washed, and dead birds were removed, on a daily basis. A vaccination program used in this experiment has been published (Seidavie *et al* 2014) and

to alleviate potential stress associated with vaccination, 1:1000 multivitamin + electrolyte solution in drinking water was used during the 24 hours following vaccinations. The feed-delivered anticoccidial Salinomycin (0.5 kg/tonne) was used from 21-42 days of age. Once a week, residual food in the feeders was removed, and the feeders were cleaned, then fresh food was added to the feeders.

CHICKENS AND EXPERIMENTAL TREATMENTS

A completely randomized design of 3 × 3 factorial arrangement with three levels of fish oil (0, 15, and 20 g/kg), and three levels of green tea powder (0, 10, and 15 g/kg) was used, which yielded nine treatments and three replicates per treatment. Two hundred and seventy one-day-old Ross 308 cockerels were weighed and assigned to 27 groups of 10 each in order that starting body weito start with similar body weights within each group. The 27 groups of chicks were placed randomly into floor pens and were provided feed and water for *ad libitum* consumption. Assessment of growth (weight gain-g/chick/day) and performance (feed intake-g/chick/day; feed efficiency-g feed.g gain; energy intake- kcal/chick/day; energy efficiency-kcal/g gain; protein intake- g/chick/day; protein efficiency-g/g gain) was made after the grower and finisher periods, respectively.

There were nine dietary treatments in this experiment. All diets, fed *ad libitum*, in each of the three rearing periods (starter [1-14 days of age], grower [15-28 days of age], and finisher [29-42 days of age]) were formulated with the same energy and protein content and met the nutritional requirements of the Ross 308 broilers used in this experiment as outlined in the Ross 308 Broiler Management Manual. The dietary treatments are described as follows:

Treatment 1: basal diet (no fish oil [0 g/kg] and no green tea powder [0 g/kg])

Treatment 2: basal diet plus green tea powder (10 g/kg)

Treatment 3: basal diet plus green tea powder (15 g/kg)

Treatment 4: basal diet plus fish oil (15 g/kg)

Treatment 5: basal diet plus fish oil (15 g/kg) and green tea powder (10 g/kg)

Treatment 6: basal diet plus fish oil (15 g/kg) and green tea powder (15 g/kg)

Treatment 7: basal diet plus fish oil (20 g/kg)

Treatment 8: basal diet plus fish oil (20 g/kg) and green tea powder (10 g/kg)

Treatment 9: basal diet plus fish oil (20 g/kg) and green tea powder (15 g/kg).

BLOOD SAMPLING AND ANALYSIS

At the termination of the study, at 42 days of age, one bird per group, totaling 3 birds per treatment replication, was removed randomly for blood collection. Blood samples (1 mL/bird) were collected from the ulnaris wing vein into EDTA tubes. Within two hours after blood samples were collected, they were centrifuged (3000 X *g*, for 10 min at room temperature) to separate plasma from blood cells, and plasma was then decanted and stored in Eppendorf safe-lock micro-centrifuge tubes at –20 °C until assayed.

Blood parameters analysed in this study were: glucose, uric acid, total cholesterol, triglycerides, very low density lipoprotein (VLDL), high density lipoprotein (HDL), low density lipoprotein (LDL), calculated LDL/HDL ratios, total protein, albumin, and globulin. The concentrations/ratios for these parameters were determined by routine methods using commercial laboratory kits (Teif Azmoon Pars, Co., Tehran, Iran) for each assay.

STATISTICAL ANALYSIS

The data were analyed using the general linear models procedure of SPSS statistical software (SPSS 1997). The model included fish oil and green tea powder as main effects. The interaction between main effects was included in the model. Means separation was accomplished using least significant difference (LSD). All statements of significance are based on $P \leq 0.05$.

RESULTS

The blood chemistry status of the broilers given diets supplemented with green tea powder/fish oil is shown in tables 1 and 2. Fish oil supplementation had no significant influence on any of the measured plasma chemical parameters, and there were no significant interactions between fish oil and green tea powder for the measured plasma chemical components in this investigation. However, examination of the treatment results (tables 1 and 2) suggested that in response to fish oil supplementation there was a small decrease in plasma uric acid when the broilers were given 15 g/kg fish oil, and there was a decrease ($P = 0.056$) in plasma uric acid when 20 g/kg fish oil was supplemented in their diets (table 2). Additionally, there was a downward trending plasma LDL in broilers given either 15 g/kg (treatment 4, $P = 0.012$) or 20 g/kg (treatment 7, $P = 0.038$) compared to 0% (treatment 1) fish oil in their diets (table 1).

Supplementation of green tea powder to the diets of the broilers in this study was associated with an upward-trending ($P = 0.068$) plasma uric acid concentration (table 2). This response to green tea powder supplementation was attributed to a significant increase ($P = 0.026$) in plasma uric acid in birds fed 15 g/kg green tea powder (0 g/kg = 2.3 mg/dl, 15 g/kg = 3.9 mg/dl). Among the various treatments, green tea powder supplementation at 15 g/kg was consistently associated with increased plasma uric acid (table 2). The green tea powder effect on plasma LDL approached significance ($P = 0.055$). Segregation of dietary treatment means revealed that green tea powder had produced a decreasing trend for plasma LDL when given alone and in combination with fish oil (table 1). At dietary levels of 10 and 15 g/kg green tea was effective

Table 1. Plasma lipid parameters (mean ± SEM) of Ross 308 broilers at 42 days of age fed diets supplemented fish oil and green tea.

Parámetros de lípidos del plasma (media ± SEM) en pollos de engorde Ross 308 a los 42 días de edad, alimentados con dietas suplementadas con aceite de pescado y té verde.

Treatment \ Plasma Lipid Parameter		Total cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (very low density lipoprotein) (mg/dl)	LDL Cholesterol (low density lipoproteins) (mg/dl)	HDL Cholesterol (high density lipoproteins) (mg/dl)	LDL/HDL Ratio
Fish oil (g/kg diet)	0	108±5.6^{a}	34.56±4.53^{a}	7.00±0.92^{a}	32.7±3.6^{a}	68.8±3.7^{a}	0.48±0.05^{a}
	15	114±5.6^{a}	39.67±4.53^{a}	8.00±0.92^{a}	27.6±3.6^{a}	78.4±3.7^{a}	0.35±0.05^{a}
	20	106±5.6^{a}	31.22±4.53^{a}	6.22±0.92^{a}	29.8±3.6^{a}	69.4±3.7^{a}	0.44±0.05^{a}
P-value		0.565	0.430	0.410	0.607	0.142	0.187
Green tea powder (g/kg diet)	0	114±5.6^{a}	30.33±4.53^{a}	6.11±0.92^{a}	36.7±3.6^{a}	70.9±3.7^{a}	0.52±0.05^{a}
	10	109±5.6^{a}	34.33±4.53^{a}	7.00±0.92^{a}	29.9±3.6^{ab}	71.9±3.7^{a}	0.42±0.05^{ab}
	15	106±5.6^{a}	40.78±4.53^{a}	8.11±0.92^{a}	23.4±3.6^{b}	73.9±3.7^{a}	0.32±0.05^{b}
P-value		0.595	0.283	0.329	0.055	0.841	0.034
Fish oil (0)-Green tea (0)		117±9.9^{a}	34.33±7.84^{a}	7.00±1.60^{a}	43.7±6.2^{a}	66.7±6.3^{a}	0.65±0.09^{a}
Fish oil (0)-Green tea (10)		108±9.9^{a}	33.33±7.84^{a}	6.67±1.60^{a}	27.3±6.2^{ab}	74.0±6.3^{a}	0.38±0.09^{b}
Fish oil (0)-Green tea (15)		100±9.9^{a}	36.00±7.84^{a}	7.33±1.60^{a}	27.0±6.2^{b}	65.7±6.3^{a}	0.41±0.09^{b}
Fish oil (15)-Green tea (0)		124±9.9^{a}	27.00±7.84^{a}	5.33±1.60^{a}	35.7±6.2^{ab}	83.3±6.3^{a}	0.43±0.09^{ab}
Fish oil (15)-Green tea (10)		107±9.9^{a}	38.33±7.84^{a}	7.67±1.60^{a}	27.7±6.2^{ab}	72.0±6.3^{a}	0.38±0.09^{b}
Fish oil (15)-Green tea (15)		110±9.9^{a}	53.67±7.84^{a}	11.0±1.60^{a}	19.3±6.2^{c}	80.0±6.3^{a}	0.24±0.09^{b}
Fish oil (20)-Green tea (0)		99±9.9^{a}	29.67±7.84^{a}	6.00±1.60^{a}	30.7±6.2^{a}	62.7±6.3^{a}	0.50±0.09^{a}
Fish oil (20)-Green tea (10)		111±9.9^{a}	31.33±7.84^{a}	6.67±1.60^{a}	34.7±6.2^{ab}	69.7±6.3^{a}	0.50±0.09^{a}
Fish oil (20)-Green tea (15)		106±9.9^{a}	32.67±7.84^{a}	6.00±1.60^{a}	24.0±6.2^{bc}	76.0±6.3^{a}	0.33±0.09^{b}
P-value		0.719	0.491	0.433	0.285	0.373	0.128

^{a,b,c} Means (± standard error) within each column of dietary treatments with no common superscript differ significantly (P ≤ 0.05).

Table 2. Plasma parameters (mean ± SEM) of Ross 308 broilers at 42 days of age fed diets supplemented fish oil and green tea.

Parámetros del plasma (media ± SEM) en pollos de engorde Ross 308 a los 42 días de edad, alimentados con dietas suplementadas con aceite de pescado y té verde.

Treatment \ Plasma Parameter		Glucose (mg/dl)	Uric acid (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Fish oil (g/kg diet)	0	195±11.1^{a}	3.78±0.47^{a}	3.1±0.16^{a}	1.34±0.06^{a}	1.78±0.12^{a}
	15	218±11.1^{a}	2.82±0.47^{ab}	2.9±0.16^{a}	1.26±0.06^{a}	1.66±0.12^{a}
	20	213±11.1^{a}	2.41±0.47^{b}	2.9±0.16^{a}	1.28±0.06^{a}	1.61±0.12^{a}
P-value		0.336	0.140	0.537	0.530	0.578
Green tea powder (g/kg diet)	0	200±11.1^{a}	2.31±0.47^{b}	2.9±0.16^{a}	1.27±0.06^{a}	1.63±0.12^{a}
	10	202±11.1^{a}	2.77±0.47^{b}	3.1±0.16^{a}	1.34±0.06^{a}	1.76±0.12^{a}
	15	223±11.1^{a}	3.93±0.47^{a}	2.9±0.16^{a}	1.27±0.06^{a}	1.66±0.12^{a}
P-value		0.300	0.068	0.635	0.549	0.729
Fish oil (0)-Green tea (0)		190±19.2^{a}	2.83±0.82^{b}	2.9±0.28^{a}	1.27±0.10^{a}	1.63±0.20^{a}
Fish oil (0)-Green tea (10)		183±19.2^{a}	3.43±0.82^{ab}	3.4±0.28^{a}	1.40±0.10^{a}	2.03±0.20^{a}
Fish oil (0)-Green tea (15)		213±19.2^{a}	5.07±0.82^{a}	3.0±0.28^{a}	1.37±0.10^{a}	1.67±0.20^{a}
Fish oil (15)-Green tea (0)		207±19.2^{a}	2.13±0.82^{b}	3.0±0.28^{a}	1.33±0.10^{a}	1.67±0.20^{a}
Fish oil (15)-Green tea (10)		217±19.2^{a}	2.83±0.82^{b}	2.9±0.28^{a}	1.27±0.10^{a}	1.67±0.20^{a}
Fish oil (15)-Green tea (15)		230±19.2^{a}	3.50±0.82^{ab}	2.8±0.28^{a}	1.17±0.10^{a}	1.63±0.20^{a}
Fish oil (20)-Green tea (0)		204±19.2^{a}	1.97±0.82^{b}	2.8±0.28^{a}	1.20±0.10^{a}	1.60±0.20^{a}
Fish oil (20)-Green tea (10)		207±19.2^{a}	2.03±0.82^{b}	2.9±0.28^{a}	1.37±0.10^{a}	1.57±0.20^{a}
Fish oil (20)-Green tea (15)		227±19.2^{a}	3.23±0.82^{ab}	2.9±0.28^{a}	1.27±0.10^{a}	1.67±0.20^{a}
P-value		0.729	0.259	0.866	0.728	0.860

^{a,b} Means (± standard error) within each column of dietary treatments with no common superscript differ significantly (P ≤ 0.05).

at reducing plasma LDL, and at a supplementation rate of 15 g/kg, green tea powder consistently caused plasma LDL to be decreased (P = 0.018). Green tea powder supplementation in a dose-dependent manner was associated with a significantly decreased (P = 0.034) plasma LDL/HDL ratio, which was due to the green tea powder associated decrease in LDL and maintenance of plasma HDL with or without fish oil supplementation (table 1). However, there were other plasma chemical parameters that were slightly, but not significantly affected by the green tea supplementation. Among these was a non-significant dose-dependent downward trending change in plasma total cholesterol (table 1).

The grower and finisher phase performance of the broilers are shown in tables 3 and 4, respectively. During the grower phase (table 3), none of the performance traits were affected by either fish oil or green tea powder either alone or in combination. Examination of the grower phase performance traits associated with the nine different dietary treatment revealed that none of these were altered by any of the dietary combinations of fish oil and green tea powder (table 3).

During the finisher phase (table 4), fish oil (20 g/kg diet) was found to support greater feed intake than either the 0 or 15 g fish oil/kg diet. Due to lower feed intake, the energy intake was less in the broilers consuming the 0 and 15 g fish oil/kg diets, and daily protein intake was also reduced in the broilers consuming the 0 and 15 g fish oil /kg diets. Nevertheless, neither weight gain, feed efficiency, energy efficiency, nor protein efficiency were affected by the fish oil diets. On the other hand, supplementation of green tea powder at 10 and 15 g/kg of diet was found to decrease feed intake, daily weight gain, energy intake and protein intake (table 4). Due to significant decrease in feed intake in those broilers consumming 10 or 15 g green tea powder/ kg of diet, the feed efficiencies, energy efficiencies, and protein efficiencies were elevated significantly (table 4). Examination of the finisher phase performance traits associated with the nine different dietary treatment revealed that the addition of green tea powder to the respective diets was associated with decreased performance traits except feed efficiency (table 4). Combining fish oil with the green tea powder in the various diets did not always correct the depressed performance traits associated with the green tea powder. One exception was with the 20 g fish oil/kg diet with 10 and 15 g green tea powder/kg of diet where feed intake was comparable to that of the negative control diet with neither fish oil nor green tea powder. In these diets, other performance traits such as energy intake (20 g fish oil +

Table 3. Performance parameters (mean±SEM) of Ross 308 broilers (15 to 28 days of age) fed grower diets containing the different levels of fish oil and green tea.

Parámetros de rendimiento (media ± SEM) en pollos de engorde Ross 308 (15 a 28 días de edad), alimentados con dietas de crecimiento que contienen diferentes niveles de aceite de pescado y té verde.

Treatment	Trait	Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed Efficiency	Energy Intake (kcal/chick/day)	Energy Efficiency (kcal/g)	Protein Intake (g/chick/day)	Protein Efficiency (g/g)
Fish oil (g/kg diet)	0	102±1.3[a]	66.4±1.65[a]	1.67±0.047[a]	305±3.8[a]	5.00±0.142[a]	20.87±0.262[a]	0.34±0.010[a]
	15	102±1.3[a]	67.6±1.65[a]	1.63±0.047[a]	307±3.8[a]	4.89±0.142[a]	20.99±0.262[a]	0.34±0.010[a]
	20	102±1.3[a]	69.2±1.65[a]	1.60±0.047[a]	306±3.8[a]	4.82±0.142[a]	20.94±0.262[a]	0.33±0.010[a]
P-value		0.948	0.490	0.648	0.948	0.648	0.948	0.648
Green tea powder (g/kg diet)	0	102±1.3[a]	70.6±1.65[a]	1.56±0.047[a]	307±3.8[a]	4.70±0.142[a]	20.97±0.262[a]	0.32±0.010[a]
	10	103±1.3[a]	66.3±1.65[a]	1.70±0.047[a]	309±3.8[a]	5.10±0.142[a]	21.12±0.262[a]	0.35±0.010[a]
	15	101±1.3[a]	66.3±1.65[a]	1.64±0.047[a]	303±3.8[a]	4.91±0.142[a]	20.72±0.262[a]	0.34±0.010[a]
P-value		0.559	0.127	0.153	0.559	0.153	0.559	0.153
Fish oil (0)-Green tea (0)		104±2.2[a]	69.5±2.86[a]	1.63±0.082[a]	311±6.6[a]	4.91±0.245[a]	21.28±0.453[a]	0.34±0.017[a]
Fish oil (0)-Green tea (10)		98±2.2[a]	60.8±2.86[a]	1.73±0.082[a]	294±6.6[a]	5.20±0.245[a]	20.12±0.453[a]	0.36±0.017[a]
Fish oil (0)-Green tea (15)		103±2.2[a]	69.0±2.86[a]	1.63±0.082[a]	310±6.6[a]	4.90±0.245[a]	21.21±0.453[a]	0.34±0.017[a]
Fish oil (15)-Green tea (0)		103±2.2[a]	72.1±2.86[a]	1.52±0.082[a]	309±6.6[a]	4.57±0.245[a]	21.14±0.453[a]	0.31±0.017[a]
Fish oil (15)-Green tea (10)		106±2.2[a]	67.1±2.86[a]	1.77±0.082[a]	320±6.6[a]	5.33±0.245[a]	21.85±0.453[a]	0.36±0.017[a]
Fish oil (15)-Green tea (15)		97±2.2[a]	63.6±2.86[a]	1.59±0.082[a]	292±6.6[a]	4.79±0.245[a]	19.99±0.453[a]	0.33±0.017[a]
Fish oil (20)-Green tea (0)		100±2.2[a]	70.3±2.86[a]	1.53±0.082[a]	300±6.6[a]	4.61±0.245[a]	20.49±0.453[a]	0.32±0.017[a]
Fish oil (20)-Green tea (10)		104±2.2[a]	70.9±2.86[a]	1.59±0.082[a]	313±6.6[a]	4.78±0.245[a]	21.39±0.453[a]	0.33±0.017[a]
Fish oil (20)-Green tea (15)		102±2.2[a]	66.5±2.86[a]	1.68±0.082[a]	307±6.6[a]	5.05±0.245[a]	20.96±0.453[a]	0.35±0.017[a]
P-value		0.135	0.178	0.420	0.135	0.420	0.135	0.420

[*] Means (± standard error) within each column of dietary treatments with no common superscript differ significantly (P ≤ 0.05).

Table 4. Performance parameters (mean ±SEM) of Ross 308 broilers (29 to 42 days of age) fed finisher diets containing the different levels of fish oil and green tea.

Parámetros de rendimiento (media ± SEM) en pollos de engorde Ross 308 (29-42 días de edad), alimentados con dietas de acabado que contienen diferentes niveles de aceite de pescado y té verde.

Treatment / Trait		Feed intake (g/chick/day)	Weight gain (g/chick/day)	Feed Efficiency	Energy Intake (kcal/chick/day)	Energy Efficiency (kcal/g)	Protein Intake (g/chick/day)	Protein Efficiency (g/g)
Fish oil (g/kg diet)	0	185±1.98^{b}	72.7±2.12^{a}	2.67±0.101^{a}	565±6.0^{b}	8.15±0.307^{a}	35.92±0.383^{b}	0.52±0.020^{a}
	15	190±1.98^{b}	73.5±2.12^{a}	2.79±0.101^{a}	581±6.0^{b}	8.51±0.307^{a}	36.90±0.383^{b}	0.54±0.020^{a}
	20	201±1.98^{a}	78.5±2.12^{a}	2.71±0.101^{a}	612±6.0^{a}	8.26±0.307^{a}	38.90±0.383^{a}	0.53±0.020^{a}
P-value		0.000	0.144	0.701	0.000	0.701	0.000	0.701
Green tea powder (g/kg diet)	0	205±1.98^{a}	85.5±2.12^{a}	2.46±0.101^{b}	627±6.0^{a}	7.51±0.307^{b}	39.82±0.383^{a}	0.48±0.020^{b}
	10	185±1.98^{b}	68.9±2.12^{b}	2.91±0.101^{a}	565±6.0^{b}	8.88±0.307^{a}	35.92±0.383^{b}	0.56±0.020^{a}
	15	186±1.98^{b}	70.2±2.12^{b}	2.80±0.101^{a}	566±6.0^{b}	8.54±0.307^{a}	35.98±0.383^{b}	0.54±0.020^{a}
P-value		0.000	0.000	0.015	0.000	0.015	0.000	0.015
Fish oil (0)-Green tea (0)		197±3.42^{b}	82.1±3.68^{abc}	2.49±0.174^{a}	602±10.4^{b}	7.59±0.532^{a}	38.27±0.663^{b}	0.48±0.034^{a}
Fish oil (0)-Green tea (10)		184±3.42^{cd}	68.0±3.68^{de}	2.89±0.174^{a}	561±10.4^{cd}	8.82±0.532^{a}	35.65±0.663^{cd}	0.56±0.034^{a}
Fish oil (0)-Green tea (15)		175±3.42^{d}	68.0±3.68^{de}	2.64±0.174^{a}	533±10.4^{d}	8.05±0.532^{a}	33.85±0.663^{d}	0.51±0.034^{a}
Fish oil (15)-Green tea (0)		210±3.42^{a}	88.9±3.68^{a}	2.40±0.174^{a}	640±10.4^{a}	7.33±0.532^{a}	40.70±0.663^{a}	0.47±0.034^{a}
Fish oil (15)-Green tea (10)		179±3.42^{d}	65.1±3.68^{e}	2.97±0.174^{a}	547±10.4^{d}	9.07±0.532^{a}	34.77±0.663^{d}	0.58±0.034^{a}
Fish oil (15)-Green tea (15)		182±3.42^{d}	66.4±3.68^{de}	3.00±0.174^{a}	554±10.4^{d}	9.14±0.532^{a}	35.22±0.663^{d}	0.58±0.034^{a}
Fish oil (20)-Green tea (0)		209±3.42^{a}	85.6±3.68^{ab}	2.50±0.174^{a}	637±10.4^{a}	7.62±0.532^{a}	40.49±0.663^{a}	0.48±0.034^{a}
Fish oil (20)-Green tea (10)		192±3.42^{bc}	73.6±3.68^{cde}	2.87±0.174^{a}	587±10.4^{bc}	8.75±0.532^{a}	37.32±0.663^{bc}	0.56±0.034^{a}
Fish oil (20)-Green tea (15)		200±3.42^{ab}	76.2±3.68^{bcd}	2.76±0.174^{a}	612±10.4^{ab}	8.42±0.532^{a}	38.87±0.663^{ab}	0.54±0.034^{a}
P-value		0.000	0.001	0.175	0.000	0.175	0.000	0.175

* Means (± standard error) within each column of dietary treatments with no common superscript differ significantly (P ≤ 0.05).

15 g green tea powder only), protein intake, and protein efficiency were comparable to the negative control diet (0 fish oil and 0 green tea powder).

DISCUSSION

It has been long-noted that green tea confers certain beneficial effects (Thavanesan 2011), which are associated with anti-lipogenesis and weight control (Hasegawa *et al* 2003), anti-diabetogenic (Tsuneki *et al* 2004), anti-carcinogenic (Zaveri 2006), anti-bacterial (Shiota *et al* 1999) and anti-viral (Nance *et al* 2009) properties for humans. Kaneko *et al* (2001) reported that the feeding of green tea powder to broilers in a dose-dependent manner caused reduced broiler chicken body weight associated with an interruption in lipid metabolism and deposition in adipose tissue. Recently, Huang *et al* (2013) have reported that green tea polyphenols are capable of preventing obesity in chickens via interference in lipid metabolism. Green tea powder and extracts also have been shown to have anti-influenza viral effects in chickens (Lee *et al* 2012). Additionally, green tea has been credited with anti-inflammatory, anti-oxidative, and anti-mutagenic effects (Benelli *et al* 2002), which are believed to contribute to reduction in heart disorders (Weisburger and Chung 2002).

In this investigation, fish oil supplementation had little effect on the measured plasma chemical components in broiler chickens. In rats, Popović *et al* (2102) reported that fish oil supplementation decreased many parameters associated with oxidative stress in rats, but they also noted that dietary fish oil was associated with significant increases in plasma uric acid and plasma HDL. Results from this investigation with broiler chickens were contrary to those reported for rats (Popović *et al* 2102). Furthermore, Popović *et al* (2102) reported that fish oil supplementation was associated with significant decreases in plasma triglycerides and LDL, but we found no significant change in either plasma triglycerides or LDL in broilers given fish oil. Castillo *et al* (1999) have reported that, after seven days feeding of 10% menhaden oil, chicks exhibited a plasma hypocholesterolemic response involving decreased VLDL, decreased triacylglycerol contents in HDL, but no change in LDL. In our investigation a maximum of 20 g/kg fish oil was fed, and the possibility exists that we did not reach a minimal threshold to alter plasma lipid profiles in our broilers. We observed that 15 g/kg green tea powder decreased plasma LDL leading to a significantly altered plasma LDL/HDL ratio. The decreased LDL/HDL ratio was indicative of a green tea powder related shift in plasma lipid profiles (Table 1). The

decreased plasma LDL/HDL ratio and increased plasma uric acid suggested a possibility for improved anti-oxidant status in association with the feeding of 15 g/kg of green tea powder (Klandorf *et al* 2001, Carro *et al* 2010). The possibility for improved anti-oxidant status in green tea powder fed broilers is supported in earlier scientific reports of improved anti-oxidant status in broilers fed either green tea powder (Biswas and Wakita 2001) or green tea extracts (Sahin *et al* 2010, Sahin *et al* 2013).

Dietary green tea powder has potential to decrease plasma LDL and LDL/HDL ratios and increase plasma uric acid in broiler chickens. On the other hand, dietary fish oil decreased plasma uric acid profile but did not significantly affect plasma lipid profiles in this investigation. When both fish oil and green tea powder were combined in the broiler diets, the green tea effect on plasma LDL, LDL/HDL ratio, and uric acid was maintained. Our results suggest that green tea powder exerts a positive influence on plasma lipid (LDL and LDL/HDL ratio) and uric acid, which is related to improved anti-oxidant status in broiler chickens (Simolyi *et al* 2002).

Appetite appeared to have been suppressed by the presence of green tea powder in the experimental diets, which could be considered the causative factor for the negative influence of green tea powder on performance traits in the broilers during the finisher feeding phase in this investigation. The presence of green tea powder in the respective experimental diets appeared to exert negative influences on several performance traits related to energy and lipid metabolism as shown by decreased LDL and LDL/HDL ratios. Ashwell and McMurtry (2003) reported that metformin acts as an appetite suppressor in broiler chickens, and along with this suppression in appetite, the chickens had reduced daily weight gain, a transitory decrease in plasma glucose that returned to normal levels, decreased insulin, increased glucagon, increased nonesterified fatty acids, a transitory increase in triglycerides and an increase in plasma uric acid, which was also observed in this investigation. Changes in glucose regulating pancreatic hormones could have altered the lipogenic and lipolytic activies of the liver on both short and long term bases. In this investigation we noted decreased LDL and LDL/HDL ratios which would be in line with effects caused by decreased feed intake. Biswas and Wakita (2001) observed that green tea powder caused decreased feed intake and body weight, and improved feed efficiency, which was not observed in this study. Biswas and Wakita (2001) also noted that green tea-fed birds had less hepatic lipids and cholesterol along with decreased serum total cholesterol.

It is concluded that the use of green tea powder in broiler diets appeared to have beneficial effects on maintenance of plasma LDL, LDL/HDL ratios, and uric acid at levels that would support a more healthful status. The improved health status of green tea powder-fed broilers possibly could be reflected in improved anti-oxidant status, associated with elevated plasma uric acid, or a more healthful cardiovascular system, associated with lower LDL cholesterol. Performance traits of broilers fed green tea powder with and without fish oil generally were decreased, which we attributed to the decrease in feed intake and decreased energy and protein efficiencies. Further studies are needed on the effects of green tea powder on performance of broilers.

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