

SELECTED PLASMA CONSTITUENTS OF BROILER CHICKENS FED DIFFERENT LEVELS OF DRIED SWEET ORANGE (*CITRUS SINENSIS*) PEELS

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ABSTRACT

An experiment was conducted to evaluate the effects of different levels of dried *Citrus sinensis* peel (DCSP) on selected plasma constituents of broilers. Data from five dietary treatments with four replicates per treatment were analyzed using a completely randomized design. Dietary treatments comprised: (1) control consisting of standard basal-diet without DCSP, (2) DCSP-1, basal starter diet supplemented with 1.5% DCSP from 1-21 days of age followed by standard basal grower diet with no DCSP from 22- 42 days of age, (3) DCSP-2, standard diets supplemented with 1.5% DCSP from 1-42 days of age, (4) DCSP-3, basal starter diet supplemented with 3.0% DCSP from 1-21 days of age followed by standard basal grower diet with no DCSP from 22- 42 days of age, and (5) DCSP-4, standard (basal starter followed by basal grower) diets supplemented with 3.0% DCSP from 1-42 days of age. Plasma constituents (glucose [GLU], cholesterol [CHOL], low density lipoprotein [LDL], high density lipoprotein [HDL], triglycerides [TGL], alkaline phosphatase activity [ALP] and uric acid [UA]) were measured at 42 days of age. DCSP decreased plasma CHOL, LDL, and TGL, but HDL was not affected significantly. Plasma GLU was decreased significantly at 42 days by 3.0% DCSP feeding 1-21 days. Plasma GLU was decreased similarly by continuous feeding of 1.5% and 3.0% DCSP from 1-42 days. Plasma ALP activity and UA concentrations were not altered significantly by DCSP feeding. Thus, using DCSP as an alternative feedstuff for broilers did not have overt negative effects on selected plasma constituents.

Keywords: *Citrus sinensis*, Dried peel, Broiler, Plasma traits.

INTRODUCTION

Sweet oranges (*Citrus sinensis*) are produced in many tropical and subtropical climates. Traditionally, the orange peel has been used for ruminant nutrition, fertilizer, essential oils extraction, ethanol production, marmalade production, pectin extraction, methane production, industrial enzyme production, and single cell protein production (López *et al.* 2010). Orange peel contains a variety of nutrients such as phenolic compounds, ascorbic acid, coumarin, volatile oils (Fernández-López *et al.* 2005) such as α -pinene (Azar *et al.*, 2011), flavonoids such as hesperidin, ferulic acid, tangeretin, sinensetin, nobiletin (Malterud and Rydland 2000; Luo *et al.* 2008) and naringin (Shafaghat 2010) and pectin (Kalapathy and Proctor 2001). Naringin has been shown to reduce significantly the levels of total cholesterol, low density lipoprotein cholesterol (LHD), very low density lipoprotein cholesterol (VLDL) and triglycerides, but naringin did not decrease high density lipoprotein (HDL) in chickens (da Silva *et al.* 2001). Many of the flavonoids from orange peel have been shown to inhibit *in vitro* enzymatic lipid peroxidation processes catalyzed by 15-lipoxygenase (Malterud and

Rydland 2000). It has been demonstrated that indigenous chicken cecum Lactobacilli are able to change flavonoid diglycosides into bioactive aglycones (Iqbal and Zhu 2009). However, coumarin does not appear to impart a health benefit in male chickens. Feeding coumarin in combination with a high fat diet to male chickens exacerbated the developing plasma hyperlipidemia and appeared to induce hyperlipidemia it earlier than in roosters given only the high fat diet (Mierzejewski 1975).

Thus, the many active principles in the sweet orange peel appear to be available for utilization in poultry, and the peel might have potential for use as a dietary ingredient in poultry production. Interest in the use of dried citrus pulp, which is the dried residue of peel, pulp and seeds of oranges, grapefruit and other citrus fruit as an alternative feed ingredient for poultry has been studied; however, Heuzé *et al.* (2012) concluded that even at low levels of inclusion, it had low value in poultry diets since it reduced growth performance in broiler chickens. Oluremi *et al.* (2006) and Agu *et al.* (2010) replaced up to 15 or 20%, respectively, of the corn content in broiler diets with dried *Citrus sinensis* peel (DCSP) and found no negative influence on broilers performance until these upper limits were exceeded in each of their respective investigations. Nevertheless,

research from the authors' laboratory, involving six weeks old broiler chickens fed diets supplemented with DCSP, revealed that diets supplemented with 1.5% DCSP promoted growth performance, but diets supplemented with 3.0% DCSP depressed slightly the growth performance (Ebrahimi *et al.* 2013; Pourhossein *et al.* 2015).

In a unique research report dealing with the effects aromatic seeds and plant extracts on growth performance of broiler chickens, Khalaji *et al.* (2011) observed that there is often a good relationship between selected plasma chemical constituents and growth performance. As an adjunct to the aforementioned research from our laboratory (Ebrahimi *et al.* 2013), we had an interest in ascertaining if DCSP supplemented in diets of broiler chickens from 1 to 21 or 1 to 42 days of age might influence selected plasma constituents of market-age broiler chickens.

MATERIAL AND METHODS

Feed and *Citrus sinensis* peel treatments: Broilers in this investigation were fed starter feed from 1 to 21 days of age and grower diet from 22 to 42 days of age. The diets met or exceeded National Research Council (1994) Recommendations (Table 1). Our DCSP was purchased from Khazarnoush Co. (Chaboksar, Iran), with a chemically determined proximate analysis (AOAC, 1990) of crude protein 5.5%, crude fiber 10%, ether extract 2%, total carbohydrate 63.5%, ash 7%, calcium 1.1%, phosphorus 0.05%, dry matter 88%, was mixed as an additive into the basal starter and grower feeds at levels of 1.5 or 3.0% replacing corn by either 1.5 or 3.0% to formulate the experimental diets.

Animals and diet: The experiment was conducted in a field research poultry farm located in Sowme'eh Sara in the Gilan Province, Iran. Four hundred day-old Ross 308 mixed-gender broiler chickens from 38 weeks old breeders were purchased from a commercial hatchery and housed at the field poultry facility (Ebrahimi *et al.* 2013). The poultry house was a curtain sidewall house equipped with thermostatically-controlled curtains and used natural cross ventilation to maintain air quality in the poultry house. Light of 20 lux intensity was programmed to provide 18 hours light and 6 hour of darkness to the broilers from 1 to 42 days of age. The broilers were started with an ambient temperature of 33°C and reduced gradually to 21.5°C when birds were 21 days old. The broilers were reared to 42 days of age in tiered-cages with dimensions of 2 × 1 m and height of 1 m for each cage pen. Each pen had a wood floor covered by 13 cm of wood shavings. The broilers were divided in groups of 20 among 20 pens with floor space allocation of 1,000 cm² per bird. Feed was provided in trough feeders, and water was provided through gravity-fed drinkers. Feed and

water were provided for *ad libitum* consumption. The drinkers were washed and refilled with fresh water on a daily basis.

There were five dietary treatments, with each treatment utilizing 80 broiler chickens divided among four replicate pens containing 20 birds (10 males and 10 females) per pen. The dietary treatments are listed as follows: (1) Control- consisting of the standard basal (starter and grower) diets only, (2) dried *Citrus sinensis* peel-1 (DCSP-1): basal starter diet supplemented with 1.5% DCSP from 1- 21 days of age followed by basal grower with no supplemental DCSP through 42 days of age, (3) DCSP-2: standard (basal starter followed by basal grower) diets supplemented with 1.5% DCSP from 1 to 42 days of age, (4) DCSP-3: basal starter diet supplemented with 3.0% DCSP from 1 to 21 days of age followed by basal grower with no supplemental DCSP through 42 days of age, and (5) DCSP-4: standard (basal starter followed by basal grower) diets supplemented with 3.0% DCSP from 1 to 42 days of age.

Plasma chemical constituents: Before blood for plasma collections were made, when the broilers were 42 days old, feed was removed from all the birds for a period of four hours to allow stabilization of the various plasma constituents. Blood sampling was done in the morning to narrow the window for potential variability of the plasma constituents to be measured. A 5 mL volume of venous blood was collected from the ulnar vein in the wing of 20 birds randomly taken from each treatment group. The whole blood sample was transferred from the syringe into a tube coated with 10 mg of the anticoagulant ethylene diamine tetra acetic acid (EDTA). Blood samples were centrifuged at 3,000 rpm × 20 min to separate blood cells from the plasma. Plasma was collected and stored at -20°C until plasma constituent analyses were made.

The levels of plasma cholesterol and triglyceride were determined using enzymatic methods (TeifAzmoon Pars, Co., Tehran, Iran), and HDL cholesterol and LDL cholesterol were measured directly with HDL-C and LDL-C diagnostic kits (TeifAzmoon Pars Co, Tehran, Iran). The colorimetric determination of cholesterol in blood plasma samples involved the use of the cholesterol oxidase procedure of Barham and Trinder (1972), which is based on the formation of a colored red-purple quinoneimine dye, produced by oxidative condensation of a phenolic compound with 4-aminoantipyrine in the presence of hydrogen peroxide. The absorbance of the quinoneimine dye, measured spectrophotometrically, has a direct relationship with the amount of cholesterol in the sample.

Plasma triglycerides were measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. The glycerol is converted to pyruvate and then to lactate. Decreased absorbance, measured spectrophotometrically, is proportional to the

triglyceride concentration in the sample (Schmid and Forstner 1986).

A glucose oxidase kit (TeifAzmoon Pars, Co., Tehran, Iran), based on oxidase-peroxidase procedure, was used to measure plasma glucose. In this assay glucose is oxidized in the presence of the glucose oxidase catalyst into H₂O₂ and gluconic acid. The reaction among gluconic acid, hydrogen peroxide, a phenolic compound and 4-aminoantipyrine forms a red-violet colored quinoneimine and the absorbance of the quinoneimine chromagen, measured by spectrophotometry, is directly associated with the amount of glucose in the sample.

A uric acid-uricase enzyme kit (TeifAzmoon Pars, Co., Tehran, Iran), based on the oxidase-peroxidase procedure (Trinder 1969), was used to measure plasma uric acid. In this procedure uric acid is oxidized with the uricase, and in the presence of the generated hydrogen peroxide, a phenolic compound and 4-aminoantipyrine forms a red-colored quinoneimine and the absorbance of the quinoneimine chromagen, measured by spectrophotometry, is directly associated with the amount of uric acid in the sample (Thomas 1998).

Plasma alkaline phosphatase was determined enzymatically using commercial kits (TeifAzmoon Pars, Co., Tehran, Iran). In this procedure, alkaline phosphatase activity was determined colorimetrically by a modification of the Bessey *et al.* (1946) method, using p-nitrophenol phosphate as the enzyme substrate, which is converted to phosphate and p-nitrophenol by the alkaline phosphatase. The released p-nitrophenolis proportional to alkaline phosphatase activity.

Statistical Analysis: Data from a completely randomized experimental design, involving five treatments with four replicates per treatment, were subjected to statistical analysis using the General Linear Models procedures of SAS (2004). Differences among main effect means were assessed using Duncan's multiple range test. Statements of significance were based on P = 0.05.

RESULTS

The mean concentrations of selected plasma constituents, which include glucose (GLU), cholesterol (CHOL), triglyceride (TGL), high density lipoprotein-CHOL (HDL), low density lipoprotein-CHOL (LDL), alkaline phosphatase (ALP) and uric acid (UA) of the 42 days old broiler chickens are shown in Table 2. In 42 days old broilers, plasma GLU concentration was reduced significantly (P 0.05; Table 2) in broilers fed the 3.0% DCSP diet to 21 days of age, but the feeding of 1.5% DCSP diet to 21 days of age did not alter plasma GLU. Plasma GLU concentration in broilers fed DSCP at 1.5% and 3.0% from 1 to 42 days of age were intermediate to GLU concentrations in Control-fed and 1.5% DSCP fed to 21 days of age (Table 2). At 42 days of age, plasma CHOL of Control-fed broilers was increased significantly (P 0.05) compared to plasma CHOL at all levels of dietary DCSP supplementation for all feeding times (Table 2). Plasma TGL concentrations in all DSCP-fed broilers were lower than the concentration in Control-fed broilers and the plasma TGL concentration in broilers fed 3.0% DSCP from 1 to 42 days of age was significantly lower (P 0.05) than plasma TGL found in Control-fed broilers (Table 2). Plasma LDL concentrations in all of the DSCP-fed treatment groups (Table 2) were significantly (P 0.05) less than the LDL concentration found in Control-fed broilers at 42 days of age. Plasma HDL concentrations in the experimental treatment groups were not different (P 0.05) from plasma HDL concentrations in Control-fed broilers at 42 days of age (Table 2). The LDL/HDL ratios were found to be decreased significantly by the feeding of DCSP in all of the experimental diets (Table 2). At 42 days of age, the activities of plasma ALP in the experimental treatment groups were not different (P 0.05) from plasma ALP activity in Control-fed broilers (Table 2). The plasma UA concentrations in the experimental treatment groups did not differ significantly (P 0.05) from plasma UA concentrations in Control-fed broilers at 42 days of age (Table 2).

Table 1. Percentage composition of basal starter and grower diets and experimental starter and grower diets containing 1.5 or 3.0% DCSP and the calculated analysis of diet.

Ingredient, %	Starter	Grower	Starter	Starter 3.0%	Grower	Grower
	0.0% DCSP	0.0% DCSP	1.5% DCSP	DCSP	1.5% DCSP	3.0% DCSP
Corn	54.32	58.69	52.82	51.32	57.19	55.69
Soybean meal, 44% CP	39.43	31.87	39.43	39.43	31.87	31.87
Corn oil	2.16	5.83	2.16	2.16	5.83	5.83
Dicalcium phosphate	2.05	1.68	2.05	2.05	1.68	1.68
Oyster shell	0.90	0.79	0.90	0.90	0.79	0.79
Sodium chloride	0.37	0.37	0.37	0.37	0.37	0.37
DL-methionine	0.20	0.22	0.20	0.20	0.22	0.22
Vitamin mixture ¹	0.25	0.25	0.25	0.25	0.25	0.25

Mineral mixture ²	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl	0.07	0.05	0.07	0.07	0.05	0.05
DCSP ³	0.00	0.00	1.50	3.00	1.50	3.00
Calculated Analysis, %						
ME (kcal/kg)	2900	3200	2889	2878	3189	3178
Crude protein	22.16	19.20	22.12	22.08	19.16	19.12
Crude fiber	3.95	3.52	4.06	4.18	3.63	3.75
Lysine	1.15	0.96	1.14	1.14	0.95	0.95
Calcium	1.00	0.85	0.99	0.98	0.84	0.83
Available phosphorus	0.50	0.42	0.49	0.49	0.41	0.41
Methionine	0.50	0.48	0.49	0.49	0.47	0.47
Methionine + Cysteine	0.83	0.78	0.82	0.82	0.77	0.77
Threonine	0.79	0.71	0.78	0.78	0.70	0.70

¹Supplies the following per kilogram of feed: vitamin A, 12,000 IU; vitamin D₃, 2200 ICU; vitamin E, 10 mg; vitamin B₁₂, 30 mg; riboflavin, 3.63 mg; niacin, 35 mg; d-pantothenate, 12 mg; menadione, 2 mg; thiamine, 2.4 mg; pyridoxine, 3.5 mg; biotin, 150 mg; folic acid, 1.4 mg. ²Supplies the following per kilogram of feed: zinc, 40 mg; manganese, 60 mg; iron, 1280 mg; copper, 8 mg; iodine, 0.3 mg; cobalt, 1 mg; sodium selenite, 0.2 mg. ³DCSP-dried *Citrus sinensis* orange peel added to the basal starter and grower diets at the expense of corn.

Table 2. Mean (\pm pooled SEM) concentrations of plasma constituents in 42 day-old broiler chickens fed different levels of DCSP

Blood Constituents ¹	Control	1.5% DCSP	1.5% DCSP	3.0% DCSP	3.0% DCSP
		1-21 days	1-42 days	1-21 days	1-42 days
GLU, mg/dL	176 \pm 7.2 ^a	183 \pm 7.2 ^a	162 \pm 7.2 ^{ab}	160 \pm 7.2 ^b	163 \pm 7.2 ^{ab}
CHOL, mg/dL	147 \pm 5.2 ^a	130 \pm 5.2 ^b	125 \pm 5.2 ^b	125 \pm 5.2 ^b	120 \pm 5.2 ^b
TGL, mg/dL	110 \pm 6.4 ^a	101 \pm 6.4 ^{ab}	97 \pm 6.4 ^{ab}	97 \pm 6.4 ^{ab}	81 \pm 6.4 ^b
LDL, mg/dL	76 \pm 5.7 ^a	57 \pm 5.7 ^b	52 \pm 5.7 ^b	52 \pm 5.7 ^b	57 \pm 5.7 ^b
HDL, mg/dL	44 \pm 3.8	53 \pm 3.8	54 \pm 3.8	55 \pm 3.8	47 \pm 3.8
ALP, IU/dL	466 \pm 64	538 \pm 64	473 \pm 64	554 \pm 64	391 \pm 64
UA, mg/dL	4.2 \pm 0.3	4.0 \pm 0.3	4.4 \pm 0.3	3.5 \pm 0.3	3.7 \pm 0.3

^{a, b}In a row, means with unlike lower case superscripts differ significantly ($P < 0.05$).

¹GLU: glucose; CHOL: total cholesterol; TGL: triglycerides; LDL: low density lipoprotein; HDL: high density lipoprotein; ALP: alkaline phosphatase; UA: uric acid.

DISCUSSION

In this investigation, changes in selected plasma constituents in broilers given the experimental diets were related to the feeding of DCSP at 1.5% and 3.0%. At 42 days of age, plasma GLU was decreased significantly with continuous feeding of experimental diets, containing either 1.5% or 3.0% DCSP, to 42 days of age and by feeding 3.0% DCSP from 1 to 21 days of age. These observations suggested that there might be some threshold effect and/or cumulative effect on plasma GLU due to continuous feeding of 1.5% or 3.0% DCSP to broilers from 1 to 42 days of age. Whether this hypoglycemic effect was due to increased insulin secretion from beta cells or decreased glucagon secretion from alpha cells in the pancreas (Hazelwood and Langslow 1978) or via some other mechanism was not addressed in this investigation. Rats given aqueous extracts of *Citrus sinensis* peel also experienced decreased serum glucose along with a decrease in serum triiodothyronine (T₃) and increased insulin (Parmar & Kar, 2008). Rosemary leaves contain α -pinene essential

oil at concentrations ranging from 15-20% of the total essential oils and feeding rosemary leaves (0.5, 1.0 and 2.0% of diet, respectively) to broilers decreased plasma glucose in broilers (Ghazalah and Ali, 2008). Thus, in this trial there is a possibility that α -pinene in DCSP, which was not quantified, might be responsible for either increased plasma insulin or decreased plasma glucagon that would lead to decreased plasma glucose in our broiler chickens. We do not think that the small fraction of fiber found in the added DCSP was sufficient to change either GLU or plasma lipid profiles observed in this investigation.

Plasma lipids (CHOL, TGL and LDL) were generally decreased in response to dietary supplementation of 1.5 and 3.0% DCSP. In our study, plasma HDL was not affected significantly by DCSP. Chaudry *et al.* (2004) reported that feeding up to 7.5% DCSP to broilers caused a decrease in plasma CHOL and non-significant decreases in TGL and GLU, which was attributed to pectin in DCSP. Further, Bok *et al.* (1999) reported decreased blood lipid levels in rats fed citrus peel extract and attributed those changes to the flavonoids

in the peel. Decreased plasma total CHOL was found in broiler chickens fed rosemary leaves and the rosemary essential oils, which contained 25% α -pinene (Polat *et al.* 2011), and Ghazalah and Ali (2008) also reported that rosemary leaves and rosemary essential oils decreased total lipids in broilers. Thus, some agent in *Citrus sinensis* peel, possibly the unquantified α -pinene, flavonoids, or other substances, singly or in combination, in DCSP in this study, had the potential to modify plasma lipid profiles in broiler chickens. However, further research is required to ascertain the mechanism by which DCSP affects lipid metabolism in chickens.

We feel that the changes in the plasma lipid profiles in broilers fed DCSP are important for growing broiler chickens. The decrease in plasma CHOL, LDL and TGL might have long-term influence on the cardiovascular health of broilers. Metabolic disease in broiler chickens has many contributory factors, but among the systemic problems associated with metabolic disease in poultry, one can find significant cardiovascular impairment often times related to elevated intake of high energy feed (Siddiqui *et al.* 2009; Sahraei 2014). Restricted high energy feed intake for rapidly growing high-yielding broilers during the starter period alleviates many of the signs of metabolic disorder (Sahraei 2014). High energy feeds with saturated fats can produce hyperlipidemia in chickens (García-Fuentes *et al.* 2002), which is characterized by elevated plasma concentrations of CHOL, LDL, and TGL (Castillo *et al.* 2000; Martín-Castillo *et al.* 2010). Dyslipidemia in chickens can be associated with elevated liver lipogenesis, which is directly related to increased plasma concentrations of CHOL, LDL, and TGL (Du and Ahn 2003).

In our study, feeding DCSP decreased the plasma concentrations of CHOL, LDL and TGL, but plasma HDL concentrations were not affected by DCSP. Plasma HDL carries 75-80% of the total cholesterol in broiler chickens (Peebles *et al.* 1997) and HDL concentrations along with CHOL, TGL and LDL increase significantly in broilers fed high energy diets associated with high saturated fat content (Martín-Castillo *et al.* 2010). In this investigation, the LDL/HDL ratio in all of our experimental animals was lower than the LDL/HDL ratio in control-fed broilers. The implication of the lower LDL/HDL ratios in DCSP-fed modern fast-growing high-yielding broilers, conceivably, could be an increased potential for improved cardiovascular health. Low levels of supplemental DCSP in conventional diets might be a means to control metabolic disease in modern fast-growing high-yielding broiler chickens. In this study, plasma ALP activity was not affected by the feeding of DCSP to broiler chickens. This observation is consistent with the report that broilers fed a hyperlipidemic diet did not alter plasma APL compared to Control-fed broilers (Martín-Castillo *et al.* 2010). Plasma ALP, which originates in the bone, reflects increased osteoblastic

activity associated with skeletal growth and calcification in young animals (Bell 1960). Plasma UA in this investigation was not altered significantly by the feeding of DCSP. Plasma UA is a naturally occurring product of purine metabolism and plays a critical role in down regulating oxidative stress (Klandorf *et al.* 2001). Simoyi *et al.* (2002) report that plasma UA plays a significant role plasma lipid resistance to oxidation. Extract of DCSP has been shown to inhibit lipid peroxidation in broiler hearts, liver and kidneys (Parmar and Kar 2008), which supports our contention that DCSP feeding to broilers might provide health benefits by reducing the potential for development of metabolic disease associated with oxidative stress. The maintenance of normal plasma UA in DCSP-fed broilers in this investigation would be contributory to the anti-oxidative processes that protects against lipid peroxidation. The levels of plasma UA found in this study are consistent with observed plasma UA concentrations in well-fed chickens (Okumura and Tasaki 1969), and the concentration of plasma UA reported in this report would suggest that broilers in all DCSP treatment groups were healthy. Darsi *et al.* (2012) observed that plasma UA concentration was reduced in parallel with dietary crude protein reduction, and decreased plasma UA in low crude protein diets might result in blood electrolyte imbalance.

In overall, DCSP feeding in this study did not appear to alter overtly any of the selected plasma constituents quantified in our broiler chickens. Selected plasma constituents assessed in this study were in a normal range for chickens held without feed for a mean period of four hours. These selected plasma constituents suggest that the broiler chicken responds to the feeding of DCSP similarly to mammals and that certain factors in DCSP can have beneficial influences on plasma lipid concentrations and the lipid's potential resistance to peroxidation and also can lower plasma glucose concentrations via an unknown process at this time. Results for plasma ALP activity support the idea that DCSP does not appear to inhibit normal skeletal growth and calcification. Thus, from our findings, the dietary supplementation of 1.5 to 3.0% DCSP would appear to be a feasible alternative feedstuff for modern fast-growing high-yielding broiler chickens.

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