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RESEARCH ARTICLE

Effect of feeding dried sweet orange (*Citrus sinensis*) peel and lemon grass (*Cymbopogon citratus*) leaves on growth performance, carcass traits, serum metabolites and antioxidant status in broiler during the finisher phase

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Abstract The current experiment was conducted to evaluate the effects of feeding dried sweet orange peel (SOP) and lemon grass leaves (LGL) as feed additives on broiler growth performance, serum metabolites, and antioxidant status. A total of 192-day-old (Ross 308) broiler chickens were distributed randomly into 4 dietary treatments with 4 replicates per each treatment. The dietary treatments included a control diet without any feed additive (T1), a diet containing 0.8 % SOP (T2), a diet containing 0.8 % LGL (T3), and a diet containing combination of 0.4 % SOP + 0.4 % LGL (T4) was fed during the growth period from 22 to 42 days. Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), carcass traits, serum components, and antioxidant status were measured. At the end of the experimental period, the results indicated that supplementation of SOP and LGL alone or in combination did not significantly ($P > 0.05$) affect BWG, FI, FCR, and carcass characteristics in broiler chickens. Serum total protein was increased significantly ($P < 0.05$) in T3 and T4 compared to the other treatments. Also, serum globulin increased significantly ($P < 0.05$) in the treated groups. Serum glucose, low density lipoprotein, triglyceride, and very low density lipoprotein decreased significantly ($P < 0.05$) in the treatment groups, while cholesterol and high-density lipoprotein decreased in T2 compared to the other groups. Significantly ($P < 0.05$) higher total antioxidant status was observed in T2 compared to the other treatments. In conclusion, these results indicate that SOP, LGL, and their combination may positively modify some serum components and the antioxidant status without any beneficial effect on growth performance and carcass traits in broiler chickens.

Keywords Broilers · Sweet orange peel · Lemon grass · Performance · Serum components · Antioxidant status

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Introduction

In the last few decades, a substantial increase in the usage of essential oils and aromatic herbs as feed additives has been observed in poultry nutrition (Chand et al. 2016; Raza et al. 2016). Animal performance can be improved by adding plant-derived products commonly known as phytogenics or phytobiotics or botanicals (Windisch et al. 2009; Raza et al. 2016). The extracts and oils of aromatic plants have a special advantage over the use of antibiotics due to no known antimicrobial resistance and residues in animal products (Brenes and Roura 2010; Varel 2002; Dhama et al. 2015). Due to the recent ban on antibiotics by the European Union, there is an increasing interest in the use of alternative feed additives in animal nutrition as alterative to antibiotics, ionophore, and anticoccidial drugs (Greathead 2003; Dhama et al. 2015; Khan et al. 2016a, b; Naz et al. 2016). Many plant derivatives such as quinines, flavonoids, polypeptide, and polyphenol are obtained from plants such as thymus, peppermint, garlic, rosemary, oregano, and others (Bampidis et al. 2005; Ocak et al.

2008; Botsoglou et al. 2009; Kadri et al. 2011; Ahmad et al. 2015).

Orange (*Citrus sinensis*) peel is one of the most common byproducts of the juice extraction and food industry (Ghasemi et al. 2009). The peel of the orange represents approximately one-fourth of the fruit and is derived after the extraction of the juice together with the removing of the pulp mechanically (Braddock 1999). The skin of sweet orange is very nutritious due to a rich source of vitamin C and energy and contains a high concentration of phenol (Hasin et al. 2006, Yang et al. 2011). Some studies have documented the cholesterol lowering effect of sweet orange and other citrus (Trovato et al. 1996; Parmar and Kar 2008). Lemon grass (*Cymbopogon citratus*) is a tall, aromatic perennial grass with dense tufted root and rhizomes (Carlini et al. 1986). Lemon grass contains a very high amount of vitamin C and its oil shows inhibiting activities towards phytopathogenic fungi (Nambiar and Matela 2012). The oil derived from lemon grass is used in human and other domestic animals to control the pathogens (Smith 2002). Important compounds obtained from this plant include luteolon and quercetin (Shah et al. 2011). Similarly, lemon grass extracts were efficacious in reducing cholesterol levels in the blood stream (Olorunnisola et al. 2014), probably due to the presence of active substance and crude fiber. In this study, dried orange peel powder and lemongrass leaves were incorporated into the broiler diets to investigate their effect on growth performance, carcass characteristics, serum components, and antioxidant status in broiler.

Materials and methods

Feed additives preparation

The sweet orange peel (SOP) and lemon grass leaves (LGL) powder were prepared as follows. Fresh sweet orange were purchased from the local market, peeled and chopped with a knife, and spread on a clean paper in the sun for drying. After drying, the peels were milled to the powder. After harvesting of the lemongrass, the leaves were separated from the stalks and air dried. After drying, the leaves were ground to powder in a hammer mill with a 1-mm screen and stored until used.

Animals, diets, and experimental design

A total of 192-day-old straight run Ross 308 chicks was purchased from a local hatchery. At 22 days of age, birds were weighed on individual basis (842 ± 7.04 g) and randomly divided into four equal treatments diets as follows: control diet without feed additive (T1), a diet containing 0.8 % SOP (T2), a diet containing 0.8 % LGL (T3), and a diet containing of 0.4 % SOP + 0.4 % LGL, respectively (T4), as shown in Table 2. By using a completely randomized design, the experiment was conducted in 4 replicate pens (1 m^2), with 12 birds per replicate. A corn soybean meal in the form of mash was fed during the grower periods from 22 to 42 days of age. Diet was composed to meet the requirements suggested by the National Research Council (1994). The experimental feed and clean drinking water were available *ad libitum* throughout the experimental period. A continuous light was maintained throughout the experiment (Table 1).

Performance measurement

Feed intake was calculated on a daily basis by subtracting the amount of rejected feed from the offered feed. Feed intake (FI) and body weight gain (BWG) was calculated for each group. Feed conversion ratio (FCR) was computed for each group at the end of the study on the basis of FI and BWG.

Carcass traits parameters

At 42 day of age, one bird per replicate was randomly selected based on the average weight of the group and slaughtered.

Table 1 Composition and calculated analysis of the experimental diets during 22–42 days of age

Ingredients (%)	T1	T2	T3	T4
Yellow Corn	70	69.2	69.2	69.2
Soybean meal 48 %	20	20	20	20
Protein concentrate[a]	10	10	10	10
Sweet orange peel	0	0.8	0	0.4
Lemon grass leaves	0	0	0.8	0.4
Total	100	100	100	100
Calculated analysis[b]				
ME (Kcal/Kg)	2941.80	2946.91	2943.84	2945.38
Crude protein (%)	18.55	18.53	18.52	18.52
Crude fiber (%)	3.85	3.91	3.90	3.91
Calcium (%)	0.68	0.68	0.68	0.68
Available P (%)	0.31	0.31	0.31	0.31
Sodium (%)	0.18	0.17	0.18	0.17
Potassium (%)	1.01	1.01	1.02	1.02
Arginine (%)	0.99	0.99	0.99	0.99
Lysine (%)	0.94	0.94	0.94	0.94
Methionine (%)	0.44	0.44	0.44	0.44
Methionine + cysteine (%)	0.69	0.69	0.69	0.69
Threonine (%)	0.73	0.73	0.73	0.73
Tryptophan (%)	0.23	0.22	0.22	0.22

[a] Each kilogram of diet contains the flowing percentage 30 protein, 2000 Kcal/kg ME, 6.2 calcium, 1.5 available phosphor, 1.3 sodium, 2.4 potassium, 0.7 arginine, 2.2 lysine, 1.85 methionine, 2.4 methionine + cysteine, 1.1 threonine, 0.35 tryptophan, 7 crude fiber

[b] Values were calculated according to the nutrient composition to the NRC (1994)

Carcass yield was calculated by subtracting eviscerated (liver, heart, gizzard, spleen, and bursa) weight from the live weight. The weight of the liver, spleen, gizzard, heart, and bursa were calculated as a percentage of body weight.

Blood sampling and determination of serum components

On day 42, 5 ml of blood from the wing vein was collected from 4 birds from each treatment and centrifuged at 1500×*g* for 20 min to separate serum. Serum was stored at −20 °C until assayed for measuring blood parameters. Serum total protein (TP), albumin (Albu), glucose (Glu), cholesterol (Chol), high-density lipoprotein (HDL), and triglycerides (TG) were analyzed using reagent kits (United Diagnostics Industry, K.S.A) using a spectrophotometer (UDICHEM 310, K.S.A). The globulin concentration was calculated by subtracting serum albumin from total protein. Very low-density lipoprotein (VLDL) cholesterol was calculated from triglycerides by dividing the factor 5 according to the method (Panda et al. 2006; Ashayerizadeh et al. 2014). The low-density lipoprotein (LDL) cholesterol was calculated by subtracting HDL from Chol.

Measurement of antioxidant status and oxidative stress

Total antioxidant capacity (TAC) and malondialdehyde (MDA) in serum was assayed by ELISA kits (Cayman Chemical Company, USA) following the instruction of the manufacturer.

Statistical analysis

The data were analyzed with the help of general linear model procedure of the Statistical Analysis System (SAS 2004). Four treatments were arranged into 4 replicates in a completely randomized design using Tukey's test to compare the means. All statements of significance were based on probability $P<0.05$.

Result and discussion

The effects of SOP and LGL on FI, BWG and FCR of broiler chickens are given in Table 2. The supplementation of SOP and LGL did no effect on BWG, FI, and FCR of the broiler chickens. Quantitatively higher value of body weight gain in T2 compared to other treatments was observed, but the difference was not significant. Similarly, Agu et al (2010) found that the dietary orange peel supplementation in both starter and finisher phases had no effect on mean FI, BWG, and FCR. Similar results were found in the study by Mmereole (2010) who reported that there was no significant differences in FI, BWG, and FCR of broiler chickens fed dietary lemongrass leaf meal (Thayalini et al. 2011). According to Oluremi et al (2006), the sweet orange rind can be added in broiler feed at the level of 15 % level with no adverse effect on body performance. Several reasons might explain these inconsistencies. The efficacy of plant extracts on animal performances depends on several factors such as the dose of the plant extract used, concentration, profile of active components present in the extracts, the physiological state of the animal, background diet, and housing conditions. The plant extract composition determined by extraction method, storage method, and soil and growth conditions of the plants (Lee et al. 2003a, b; Basmacioğlu Malayoğlu et al. 2010).

The effect of feeding SOP and LGL on the live body weight and carcass traits of broilers is shown in Table 3. No significant differences were observed in the carcass dressing percentage and relative weight of the liver, gizzard, spleen, heart, and the bursa between all treatments. The results were conformed to the findings of Mukhtar et al. (2012) who noticed no significant differences between all the treatments regarding carcass dressing percentages in response to the dietary

Table 2 Effect of feeding dried SOP and LGL on growth performance of broiler chickens during 22 to 42 days of age

	Feed intake (g/b/d)				Body weight gain (g/b/d)				Feed conversion ratio (g:g)			
Periods (day)	22–28	29–35	36–42	22–42	22–28	29–35	36–42	22–42	22–28	29–35	36–42	22–42
Treatments												
T1	125.33	154.63	168.97	144.86	88.80	83.90	66.98	79.90	1.40	1.85	2.60	1.83
T2	134.03	157.16	162.62	149.45	95.28	88.45	76.18	86.63	1.40	1.78	2.13	1.73
T3	124.26	149.35	157.68	140.98	88.30	75.65	72.95	78.95	1.40	1.98	2.15	1.78
T4	127.95	158.60	167.08	148.38	89.73	93.38	63.40	72.15	1.45	1.70	2.78	1.80
SEM	4.03	5.68	6.63	4.94	4.10	5.59	6.13	3.45	0.03	0.03	0.25	0.04
P value	0.300	0.680	0.641	0.626	0.610	0.179	0.478	0.456	0.6.30	0.235	0.233	0.291

Table 3 Effect of feeding dried SOP and LGL on the live body weight, carcass yield weight, gizzard, liver, heart, relative immune organs (percentage of live body weight) and other parts weight of broiler chickens at 42 days of age

Items	Live body weight (g)	Carcass weight (g)	*Dressing percentage		Gizzard (%)	Liver (%)	Heart (%)	Spleen (%)	Bursa (%)	Remaining parts (%)
			A	B						
Treatments										
T1	3203.80	2406.30	75.13	78.88	0.89	2.17	0.54	0.15	0.08	21.12
T2	2912.50	2130.00	73.14	77.25	1.06	2.39	0.50	0.16	0.09	23.01
T3	2890.00	2182.50	75.50	79.33	1.04	2.07	0.53	0.18	0.08	20.67
T4	3007.50	2280.00	75.82	79.46	0.90	2.06	0.48	0.19	0.11	20.54
SEM[a]	101.22	78.163	0.684	0.607	0.0823	0.256	0.024	0.0304	0.0589	0.6384
P value	0.1688	0.1172	0.0677	0.0848	0.3578	0.7871	0.2544	0.6507	0.6444	0.0612

Means with no common superscripts in each column differ significantly ($P < 0.05$)

[a] Standard error of mean

*Dressing percentage (A) = (carcass weight/live body weight)*100 & dressing percentage (B) = (carcass weight with edible parts/live body weight)*100

lemon grass oil. Also, the results of substituted sweet orange fruit peel with maize up to 20 % level had no negative impact on the growth traits and carcass quality in broiler (Agu et al. 2010; Abbasi et al. 2015). Similar findings were documented in rabbits (Hon et al. 2009). The final weight, empty body weight as well as carcass percentage of broilers feeding different levels of dried *Citrus sinensis* peel during the finisher period were not significantly different (Ebrahimi et al. 2013). Pourhossein et al. (2015) showed that feeding broilers with sweet orange peel extract had no significant effect on bursa of Fabricius and spleen weight during the 42 days of the rearing period.

The effect of feeding SOP and LGL on serum components in broiler chickens is presented in Table 3. Serum TP increased significantly ($P < 0.05$) in T3 and T4 compared to the other treatments. Also, serum glob increased significantly ($P < 0.05$) in the treated groups. Serum Glu, LDL, VLDL, and TG decreased significantly ($P < 0.05$) in the treatment groups, while Chol and HDL decreased in T2 compared to the other groups. These results were in agreement with Nobakht (2013) who found that dried citrus pulp had desirable effects on the reduction of blood Chol, LDL, because the citrus fruit is a rich source of pectin (Hong et al. 2012) (Table 4). Reduced LDL, HDL, and triglyceride with no effect on blood Glu and Chol was reported in broiler in response to dietary treatment of *C. sinensis* pulp by Abbasi et al. (2015) and suggested that vitamin C and other components present in the pulp of the citrus fruits may be responsible for the altered blood metabolites. In contrast, Ojabo et al. (2013) did not find any effect on the inclusion of sun-dried sweet orange peel in broiler diet upto the level of 40 % on most of the blood biochemical parameters such as TP, Alb, Glob, Glu, and Chol. The increased TP and Glob in the present study may be due to the different phenolic and flavonoid compounds present in the citrus fruits (Akbarian et al. 2013).

Table 5 shows the effect of feeding SOP and LGL on the antioxidant status of broilers. Results indicated that TAC increased significantly ($P < 0.05$) in T2. Orange peel is a good source of phenols (Manthey 2004), beta-carotene (Ghazi 1999), and vitamin C (Abbasi et al. 2015). In addition, Manthey (2004) and Anagnostopoulou et al. (2005) reported

Table 4 Effect of feeding dried SOP and LGL on serum components of broiler chickens during 22 to 42 days of age

Items	TP g/dl	Albu g/dl	Glob g/dl	Gluc mg/dl	Chol mg/ dl	HDL mg/ dl	LDL mg/ dl	VLDL mg/ dl	TG mg/ dl
Treatments									
T1	3.85^{b}	3.45	0.40^{b}	187.25^{a}	172.00^{a}	59.35^{c}	80.90^{a}	31.75^{a}	158.75^{a}
T2	4.23^{ab}	3.49	1.50^{a}	110.00^{b}	125.50^{b}	55.35^{c}	42.95^{b}	27.20^{b}	136.00^{b}
T3	5.20^{a}	3.65	1.55^{a}	118.75^{b}	139.00^{a}	95.63^{a}	16.83^{b}	26.55^{b}	132.75^{b}
T4	5.05^{a}	3.36	1.70^{a}	116.00^{b}	143.00^{a}	76.73^{b}	37.48^{b}	28.80^{b}	144.00^{b}
SEM[a]	0.32	0.12	0.16	3.32	7.93	2.64	8.20	0.70	3.51
P value	0.0336	0.4388	0.0003	0.0001	0.0093	0.0001	0.0011	0.0010	0.0010

TP total protein, *Album* albumin, *Glob* globulin, *Gluc* glucose, *Chol* cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very low-density lipoprotein, *TG* triglyceride

[a–c] Means with different superscripts within the same column are significantly different ($P < 0.05$)

[a] Standard error of mean

Table 5 Effect of feeding dried SOP and LGL on antioxidant status and oxidative stress of broiler chickens during 22 to 42 days of age

Items	TAC μM/L	MAD μM/L
Treatments		
T1	3.82[b]	11.08
T2	5.56[a]	8.82
T3	4.21[b]	9.72
T4	4.18[b]	9.12
SEM[a]	0.37	0.71
P value	0.0477	0.1962

TAC total antioxidant capacity, *MAD* malondialdehyde

[a–b] Means with different superscripts within the same column are significantly different ($P < 0.05$)

[a] Standard error of mean

that citrus peel contains substances showing antioxidant activity that is attributable to the flavones. Hesperidins is one of the most important flavanone isolated from orange peel has shown diuretic and antioxidant effects in experimental rats (Galati et al. 1996; Tirkey et al. 2005). It has been suggested that antioxidant effect of SOP is associated with 3OH-groups, where it can donate H atom to reduce the number of free radicals (Jeon et al. 2001). Patel and Metha (2006) proved the presence of antioxidants in lemon grass and further suggested that the dry lemon grass contains more phenol and flavonoids than fresh lemon (Vanisha and Hema 2012).

According to the results of the present study including SOP alone or in combination with LGL in the diets during grower period had no significant effect on chicken performance and carcass traits; however, some of the blood metabolites and antioxidant status were positively modified.

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