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ORIGINAL ARTICLE

Investigation on the effect of different levels of dried sweet orange (*Citrus sinensis*) pulp on performance, carcass characteristics and physiological and biochemical parameters in broiler chicken

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Abstract Utilization of agricultural by-products in animal nutrition is a matter of great concern. Dried sweet orange (*Citrus sinensis*) pulp (DCSP) is a potential source of valuable nutrients and natural antioxidants for poultry feed. In the experiment, a feeding trial was conducted in order to investigate the effect of different levels of dried orange residues in diet on broiler growth performance, carcass characteristics, blood metabolites, humoral immunity, and cecum microbial population. A total of 200 one day experimental broiler chicks were distributed into a completely randomized design (CRD) which included 5 dietary treatments with 4 replicates per each treatment and 10 birds fed in each replicate. The experimental treatments consist of a control group (without additive), 0.5%, 1.0%, 1.5%, and 2% of DCSP (residue) in diet. Weight gain, feed intake and feed conversion ratio (FCR) were measured. Blood parameters and carcass traits were measured in the postnatal 35th day. The highest level of dried orange residues in treatment 5 (T5) had significantly increased the feed intake and body weight of broilers in groups and overall during the rearing period ($P > 0.05$). Different levels of dried orange residues had no significant effect on chicken FCR. Using of dried orange residues significantly decreased the liver and abdominal fat of broilers ($P < 0.05$). T5 has also significantly lower level of triglyceride than the control (T1) and treatment

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2 (T2) ($P < 0.05$). In conclusion, the use of dried orange residues improved some performance (e.g. feed intake and body weight gain), decreased liver and abdominal fat and also serum triglyceride level in broiler chicken.

1. Introduction

Currently, utilization of agricultural by-products in animal nutrition is a matter of public concern since agricultural by-products constitute an increasingly great part in the diet of domestic animals. Shortage of national feed resource particularly in developing countries has necessitated the investigation of novel sources of feedstuff. For instance, dried sweet orange (*Citrus sinensis*) pulp (DCSP) is exactly a potential source of some valuable nutrients for poultry feed as a source of natural antioxidants. Nevertheless, DCSP is a common by-product of extracting juice from citrus and produced in a large scale in Asian agricultural areas. DCSP is also a good source of calcium, but very low in phosphorus and carotene. Based on some observed data, dried and/or pelleted citrus pulp is one of the most desirable energy feeds and can be considered in feeding programs as a feed with high digestible nutrient content. It includes a mixture of citrus peel, pulp, and seed as by-product energy concentrate feed for domestic animals (Arthington et al., 2002). Arthington and Pate (2001) estimated that the waste from feeding wet citrus pulp could be as high as 30%. Although it is very palatable to grazing animals, it is typically uneconomical to feed wet pulp because of the increased cost of shipping (Kunkle et al., 2001). Furthermore, DCSP is used as a cheap ingredient to improve animal performance and to reduce cost of production in these animals, whereas fresh or dehydrated *Citrus sinensis* pulp (CSP) is mainly used in ruminant feeding (Lanza et al., 2001). Yang and Chung (1985) reported that use of 10% dried *C. sinensis* peel in laying hen diets had no significant adverse effect on its feed intake and egg production and egg weight, while the utilization of 5% citrus peel in broiler chicks gave similar results and reduced serum cholesterol concentration in comparison with the control group (Chaudry et al., 2004). Oluremi et al. (2006) reported that sweet orange (*C. sinensis*) rind can be used to replace maize in the diet of broiler up to 15% level without any adverse effect on performance. Mourão et al. (2008) showed that incorporating CSP (5–10%) reduced daily gain by 26% in birds of 10% citrus pulp treatment ($P < 0.05$), and feed intake increased in birds fed with diets with 5% or 10% CSP compared with the control group, which resulted in higher feed conversion ratio. Khempaka et al. (2009) found that dried cassava pulp (DCP) was appropriate to be used as an alternative feedstuff for broilers. Their experiment showed that broiler growth performance and nutrient digestibility decreased with increasing levels of DCP more than 8% in most cases. Hon et al. (2009) observed the effect of dried sweet orange (*C. sinensis*) fruit pulp meal on the growth performance of rabbits and concluded that sweet orange fruit pulp meal could be used as a replacement feedstuff for maize in the ration of grower rabbit up to a level of 20%. Nazok et al. (2010) studied the effect of different levels of dried citrus pulp on performance, egg quality, and blood parameters of laying hens in early phase of production. They found that utilization of DCP up to 16% significantly increased serum glucose and high-density lipoprotein and reduced cholesterol, low-density lipoprotein, and triglycerides ($P < 0.05$). Agu et al. (2010) evaluated the effect of sweet orange peel meal (SOPM) as feed resource in broiler production and found that dietary SOPM had no effect ($P > 0.05$) on the kidney, liver, heart, spleen, gall bladder and lung but had significant effect ($P < 0.01$) on proventriculus and gizzard as the SOPM levels increased. Oluremi et al. (2010) also studied the effect of fermentation of sweet orange (*C. sinensis*) fruit peel on the growth performance of broiler chicken in the starter period, but found a relatively negative conclusion. Ebrahimi et al. (2012, 2014) evaluated the effects of different levels of *Citrus sinensis* peel extract (CSPE) on the blood parameters of broilers and found that cholesterol, glucose, uric acid, low density lipoprotein (LDL) and high density lipoprotein (HDL) at the rearing period were significantly influenced ($P < 0.05$). Pourhossein et al. (2012a) found the positive effect of different levels of dried *Citrus sinensis* peel (DCSP) on broiler gastrointestinal microbial population stating that the mean of *Lactobacilli* in cecum on the postnatal 42 day indicated no significant results ($P > 0.05$). Later, Pourhossein et al. (2012b,c) carried out an experiment to evaluate the effects of different levels of *Citrus sinensis* peel extract (CSPE) on gastrointestinal microbial population of broilers. They revealed that the mean of *Escherichia coli* in the ileum on the postnatal 42 day was significantly different from the control ($P < 0.05$) too. Ebrahimi et al. (2013a) evaluated the effect of different levels of dried *C. sinensis* peel on broiler carcass quality. They found the effects of experimental treatments on FW, EBW weight as well as carcass percentage of broilers were not significantly different from the control ($P > 0.05$), but those of treatments on carcass characteristics and the jejunum and ileum were significantly different from the control ($P < 0.05$). Recently, Ebrahimi et al. (2013b, 2014) made experimental evaluations on the effect of different levels of dried sweet orange (*C. sinensis*) peel (DSOP) supplement on broiler growth performance in the first 21 days after hatch. To our knowledge, there are presently no more experiments reported about the effects of DCSP on chicken performance, egg quality, and blood parameters, etc. The objective of the present study was to evaluate the effect of different dietary levels of dried *Citrus sinensis* pulp (DCSP) on broiler growth performance, carcass characteristics, blood metabolites, humoral immunity, and cecum microbial population during the early six weeks after hatch.

2. Material and methods

2.1. Animals and experimental design

2.1.1. Birds

Before the feeding trial, the poultry rearing unit was thoroughly cleaned for any leftover refuse from the previous batch.

The building complex was then fumigated, washed and disinfected with 1% formalin solution. Birds were reared in land cages of 1.0 m (length) × 1.0 m (width) × 1.0 m (height). The rearing land cages, feeding and drinking units were installed and fumigated 24 h before introducing the broilers into the unit. Two hundred male Ross 308 (Aviagen, Newbridge, Scotland, UK 35805) chicks were purchased and transferred into the rearing area. During the experimental period the temperature of the building was thermostatically maintained using two gasoline rocket heaters. Water was regularly sprayed onto the floor and air relative humidity was maintained at 55–65%.

On the first day, a total of 24 h of lighting was provided, which was followed by 23 h of lighting per day for the remaining experimental feeding period. Air conditioning was provided by two fans. During the first two weeks of rearing, one plastic feeding tray per cage was used. Then, all the feeding trays were collected and replaced by appropriate chute feeders since the 3rd week. For sanitation, drinkers were washed twice a day. A multi-electrolyte + electrolyte solution (1 in 1000 dilution in water) was introduced 24 h before and after vaccination to reduce the stress caused by vaccination.

2.2. Treatments

The experimental feeding lasted for a total of 35 days, after which one bird per replicate was slaughtered. The different treatments were set as following (see dietary composition in Table 1):

Table 1 Feed ingredients and nutrient analysis of used diets during the starter (postnatal 1st–21st day) and finisher periods (postnatal 22nd–35th day).

Ingredient (%)	Starter period (postnatal 1st–21st day)	Finisher period (postnatal 22nd–35th day)
Feed ingredients		
Corn	52.50	60.79
Soybean meal	37.50	29.50
Sunflower oil	4.00	4.00
$CaCO_3$	1.20	1.10
Ca%22P%18	1.60	1.50
NaCl	0.23	0.25
Vitamin mixture[1]	0.30	0.30
Mineral mixture[2]	0.30	0.30
Sodium bicarbonate($NaHCO_3$)	0.12	0.10
DL-Methionine	0.18	0.15
Lysine-hydro-chloride	0.07	0.01
Bentonite	2.00	2.00
Nutrient analysis		
Energy (kcal/kg)	3010	3100
Protein (%)	21.04	18.18
Lysine (%)	1.27	0.97
Methionine (%)	0.47	0.36
Calcium (%)	1.05	0.85
Available phosphorus (%)	0.50	0.42

[1] Vitamin A, 5000 IU/g; Vitamin D3, 500 IU/g; Vitamin E, 3 mg/g; Vitamin K3, 1.5 mg/g; Vitamin B2, 1 mg/g.

[2] Calcium Pantothenate, 4 mg/g; Niacin, 15 mg/g; Vitamin B6, 13 mg/g; Cu, 3 mg/g; Zn, 15 mg/g; Mn, 20 mg/g; Fe, 10 mg/g; K, 0.3 mg/g.

Treatment 1: Basal diet (control) during 1st–35th day.
Treatment 2: Basal diet included 0.5% dried *C. sinensis* pulp during 1st–35th day.
Treatment 3: Basal diet included 1.0% dried *C. sinensis* pulp during 1st–35th day.
Treatment 4: Basal diet included 1.5% dried *C. sinensis* pulp during 1st–35th day.
Treatment 5: Basal diet included 2.0% dried *C. sinensis* pulp during 1st–35th day.

2.3. Diets and water

The ingredient composition of the diets used as well as the nutrient composition is presented in Table 1. Feed and water were supplied *ad libitum* throughout the experimental period. The nutritional requirements of the broilers were provided based on the Ross 308 strain rearing catalog.

3. Sampling and data collection

3.1. Performance

Feed intake, refusals and weight gain were recorded daily. Feed conversion ratio was calculated using the conventional formula.

3.2. Blood parameters and metabolites

At the end of the study, on the postnatal 35th day, one bird per group, totaling 4 birds per treatment, was selected for blood sampling. Care was taken to choose representative male birds with respect to body weight compared to the group mean body weight. Blood samples (1 ml/bird) were collected into EDTA tubes from the wing veins. Samples were transferred to the laboratory for analysis within two hours of collection. After centrifugation (3000*g*, for 10 min at room temperature) plasma was harvested and stored in an eppendorf tube at −20 °C until assayed. Biochemical analysis was according to standard protocols (Jahanpour et al., 2013) using commercial laboratory kits.

3.3. Carcass characteristics parameters

The birds were humanely killed after 35 days of experimental feeding and following the pecking operations with the head and legs separated. Again, care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. Weight was recorded for carcass, stomach contents and empty body weight. Weights of the breast, thigh, abdominal fat, liver, pancreas, small intestine, and cecum were recorded too.

3.4. Immunity parameters

A vaccination program against infectious bronchitis virus (on the postnatal first and 16th days), Gumboro virus (on the postnatal 14th and 23rd days), and Newcastle disease (on the postnatal 8th and 20th days) was practiced. Humoral immune response of chicken to the Newcastle vaccine was

tested on the postnatal 27th day, whereas gumboro virus on the postnatal 30th day and infectious bronchitis virus on the postnatal 23rd day were sampled and measured.

3.5. Microbiota parameters

At the end of the study, i.e. at the 35th day, one bird per group (totally 4 birds per treatment) was selected for sample collection. Care was taken to choose the most representative male birds with respect to body weight compared to group mean body weight. The cecum content was placed on agar plates for analysis. These samples were also used to determinate bacterial growth and colony counts. Collection tubes were weighted, wrapped in aluminum sheet and autoclaved for 10 min. The culture mediums were prepared and 24 h before collection samples were poured into petri dishes. MRS agar (Man Rogosa Sharpe agar, 1.10660.500) was used to culture Lactobacilli, Eosin Metilan Blou (EMB, 1.01347.0500) to culture *E. coli*, and Nutrient agar (1.05450.0500) was used to culture total aerobic bacteria counts respectively.

Samples were transferred to the laboratory and weighed again. The amount of sample in each tube was calculated to discern differences. Tubes were shaken for approximately 30 min. The action was performed for bacteria isolated from the cecum content and preparation of suspension. One ml was removed from the prepared suspension and added into 9 ml buffer phosphate saline (PBS) in the other tube. The suspension was prepared from dilutions 10^{-1} and serial dilutions were done (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). One hundred microliter was removed from (10^{-4}, 10^{-5} and 10^{-6}) dilutions and poured into petri dishes containing the medium. Lactobacilli bacteria were incubated at 37 °C in anaerobic conditions for at least 72 h. Total aerobic bacterium counts incubated at 37 °C in aerobic conditions were measured after 48 h. Counting bacteria in petri dishes was done by colony counter. Bacterial counts were reported as logarithm number of bacteria per 1 g sample.

3.6. Statistical analysis

The study was conducted based on a completely randomized design (CRD) with five treatments and four replicates per treatment. Data were analyzed by SPSS (1997) using the generalized linear model (GLM) procedure. The statistical comparison was made by Duncan test at the 95% probability level. The data recorded as ratio or percentage were adjusted within the range between 0% and 30% into their square root. The data recorded as ratio or percentage were transformed into $x0.5 + 0.5$. The mathematical model was as follows.

$$X_{ij} = \mu + T_i + \varepsilon_{ij}$$

where X_{ij} = value observed in each experimental unit, μ = mean population, T_i = the effect of each treatment, and ε_{ij} = the effect of experimental errors.

3.7. Ethic rules

The procedures have been approved by the Authors' Institutions' Ethic Committees, and care was taken to minimize the number of animals used.

4. Results and discussion

The overall results of the experimental trial on broiler performance, carcass characteristics, blood metabolites, humoral immunity, and cecum microbial population are summarized in Tables 2–6.

4.1. Growth performance

The effect of Ross 308 broilers fed with different levels of DCSP on growth performance during starter period (postnatal 1st–21st day), finisher period (postnatal 22nd–35th days), and total period (postnatal 1st–35th days) is presented in Table 2. There was almost no difference existing in the records of broiler feed intake, weight gain, and feed conversion ratio in treatments compared to the control groups during starter period and total period. However, we observed significant differences in the performance parameters during the finisher period. In comparison with the control groups, every feed intake (i.e. feed consumption) and feed conversion ratio of groups fed with diet supplemented with 1.0% and 2.0% DCSP developed significantly better than the control group ($P = 0.04$ and $P = 0.07$), while broiler weight gain of each group was gradually increasing as expected ($P = 0.04$) in finisher period. On the contrary, the incorporation up to 1.0% DCSP in the mixtures resulted in lower feed intake and weight gain and unstable feed conversion ratio in the starter period which reflected a decreasing trend in growth and ineffective cases by much low feed conversion ratio. In addition, during the entire growing period, the better daily body weight gain was related to treatments including gradually increased DCSP content (e.g. 2.0% DCSP, Table 2) during the total period, whereas the lowest gains were achieved in broilers fed with 2.0% DCSP for the starter period. The result agreed with Ebrahimi et al. (2013b, 2014) who reported the effect of different levels of dried sweet orange (*C. sinensis*) peel (DSOP) supplement on broiler chicken growth performance and found the diet of 1.5% DSOP seems to promote broiler feed intake and weight gain in the period of the 1–21 days after hatch. However, Agu et al. (2010) conducted a study to evaluate the effect of sweet orange peel meal (SOPM) as feed resource in broiler production. They found that the dietary SOPM had no effect ($P > 0.05$) on the kidney, liver, heart, spleen, gall bladder and lung but had a significant effect ($P < 0.01$) on proventriculus and gizzard as the SOPM level increased. Moreover, Oluremi et al. (2010) also reported relatively negative results of the effect of fermentation of sweet orange (*C. sinensis*) fruit peel on the growth performance of broiler chicken in the starter period.

4.2. Carcass characteristics

The effect of Ross 308 broilers fed with different levels of DCSP on some carcass characteristics from first to 35th day is presented in Table 3. There were five poultry carcass characteristics measured in the experiments, i.e. eviscerated carcass, relative weights of breast, drumsticks (thighs), liver, pancreas, abdominal fat, small intestine, and cecum. However, there was merely a significant difference found in the statistics of relative weight of the liver compared to the control groups ($P = 0.04$) during the entire experimental period. Furthermore, there was

Table 2 Performance mean (±SEM) of Ross 308 broilers at starter, finisher and total periods of age fed with different levels of dietary dried *Citrus sinensis* pulp sampled from postnatal 1st–6th weeks*.

Treatment	Trait		
	Feed intake (g/chick/duration)	Weight gain (g/chick/duration)	Feed conversion ratio
Starter period of age (postnatal 1st–21st day)			
Control: No additive	844.2[a]	455.1[a]	1.85[a]
0.5% dried *Citrus sinensis* pulp	880.2[a]	443.9[a]	1.98[a]
1.0% dried *Citrus sinensis* pulp	858.5[a]	429.1[a]	2.00[a]
1.5% dried *Citrus sinensis* pulp	811.4[a]	439.1[a]	1.84[a]
2.0% dried *Citrus sinensis* pulp	701.7[a]	349.2[a]	2.00[a]
P	0.30	0.31	0.70
SEM (Standard error of mean)	60.40	35.71	0.10
Finisher period of age (postnatal 22nd–35th day)			
Control: No additive	2436.6[b]	957.8[b]	2.54[a]
0.5% dried *Citrus sinensis* pulp	2609.2[b]	1078.0[b]	2.42[a]
1.0% dried *Citrus sinensis* pulp	3260.6[ab]	1169.9[b]	2.78[a]
1.5% dried *Citrus sinensis* pulp	2742.8[b]	1196.7[b]	2.29[a]
2.0% dried *Citrus sinensis* pulp	3585.4[a]	1374.9[a]	2.60[a]
P	0.04	0.04	0.70
SEM (Standard error of mean)	531.5	210.0	0.2
Total period of age (postnatal 1st–35th day)			
Control: No additive	3280.8[b]	1412.9[b]	2.32[a]
0.5% dried *Citrus sinensis* pulp	3409.4[b]	1521.9[a,b]	2.24[a]
1.0% dried *Citrus sinensis* pulp	4119.1[a]	1599.0[a,b]	2.57[a]
1.5% dried *Citrus sinensis* pulp	3554.2[b]	1635.8[a,b]	2.17[a]
2.0% dried *Citrus sinensis* pulp	4287.1[a]	1724.2[a]	2.48[a]
P	0.08	0.08	0.60
SEM (Standard error of mean)	2.19	2.19	2.19

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

a near significant difference found in the statistics of relative weight of abdominal fat at the same time ($P = 0.08$). Other significant parameters were not observed in the study. For instance, the mean eviscerated carcass percentage between treatments was not significantly different ($P = 0.6$). The eviscerated carcass percentages dropped off with the increased dietary DCSP. The lowest eviscerated carcass percentage was related to treatments with 2.0% DCSP during the experimental period. The mean breast percentage between treatments was not significantly different yet ($P = 0.1$). The lowest breast percentages were related to treatments with 1.0% and 1.5% DCSP. The result partly agreed with that of Ebrahimi et al. (2013a) who stated that the effect of different treatments supplemented with dried *C. sinensis* peel on FW, EBW weight, and carcass percentage of broilers was not significantly different from the control groups ($P > 0.05$), but those of different treatments supplemented with dried *C. sinensis* peel on carcass characteristics and the jejunum and ileum were significantly different from the control groups ($P < 0.05$). This result was also in agreement with those of Torres et al., 2013 indicated that there were no significant differences among the treatments on ($P > 0.05$) in whole carcass or carcass part weights of broiler chickens fed on sorghum based diets on the 42 days after hatch.

4.3. Blood parameters

The effect of Ross 308 broilers fed with different levels of DCSP on some blood parameters from first to 35th day is presented in Table 4. It should be noted that citrus fruits are a particularly rich source of pectin, which is present both in the edible portions of fruit and the inedible residues such as peel, rag, and core. Use of DCSP in diets reduced broiler blood triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol, but kept the concentrations of glucose and aspartate amino transferase and alanine amino transferase from first to 35th day in the experiment. According to these results in Table 4, the triglyceride concentration with varying diets in the experimental treatment decreased very significantly in contrast to the control group ($P = 0.01$), and the concentrations of LDL and HDL cholesterol in the experimental treatments were significantly reduced too ($P < 0.05$). Results from this study showed that mean concentrations of total cholesterol, LDL cholesterol, and HDL cholesterol in the control group were more than the other treatments and in treatments adding DCSP. The result was partly consistent with the findings of Ebrahimi et al. (2012, 2014) who found cholesterol, glucose, uric acid, low density lipoprotein (LDL) and high density lipoprotein (HDL) in the rearing period were significantly influenced by experimental treatments ($P < 0.05$). This result agreed with that of Nazok et al. (2010) who studied the effect of different levels of dried citrus pulp on blood parameters of the laying hens in early phase and found utilization of dietary DCP more than 16% would significantly increase chicken blood serum glucose and high-density lipoprotein and reduced cholesterol, low-density lipoprotein, and triglycerides ($P < 0.05$). It also agreed with the findings of other researchers who showed that the extract obtained from garlic could create a positive correlation between enzyme activity and plasma cholesterol (Qureshi et al., 1988; Yu et al.,1994). Plasma

Table 3 Carcass yield mean (±SEM) of Ross 308 broilers fed with different levels of dietary dried *Citrus sinensis* pulp sampled from postnatal 1st–6th weeks[*].

Treatment	Trait							
	Eviscerated carcass (%)	Relative weight of breast (%)	Relative weight of drumsticks (thighs) (%)	Relative weight of liver (%)	Relative weight of pancreas (%)	Relative weight of abdominal fat (%)	Relative weight of small intestine (%)	Relative weight of cecum (%)
Control: No additive	65.50[a]	37.03[a]	28.83[a]	3.80[a]	0.23[a]	2.20[a]	3.31[a]	0.30[a]
0.5% dried *Citrus sinensis* pulp	65.62[a]	37.79[a]	29.56[a]	3.14[ab]	0.23[a]	1.75[a]	3.61[a]	0.42[a]
1.0% dried *Citrus sinensis* pulp	61.80[a]	34.53[a]	28.67[a]	2.91[b]	0.27[a]	1.79[a]	3.33[a]	0.43[a]
1.5% dried *Citrus sinensis* pulp	61.61[a]	34.53[a]	28.04[a]	3.94[a]	0.21[a]	1.40[a]	3.63[a]	0.32[a]
2.0% dried *Citrus sinensis* pulp	61.15[a]	37.10[a]	28.54[a]	2.89[b]	0.20[a]	1.30[a]	3.60[a]	0.39[a]
P	0.6	0.1	0.6	0.04	0.4	0.08	0.9	0.3
SEM (Standard error of mean)	2.7	0.9	0.6	0.2	0.02	0.3	0.3	0.05

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

Table 4 Blood metabolite mean (±SEM) of Ross 308 broilers fed with different levels of dietary dried *Citrus sinensis* pulp sampled from postnatal 1st–6th weeks[*].

Treatment	Trait						
	Glucose (mg/dl)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	LDL cholesterol (Low density lipoproteins) (mg/dl)	HDL cholesterol (high density lipoproteins) (mg/dl)	Aspartate amino transferase (AST) (S.G.O.T) (EC 2.6.1.1) (U/L)	Alanine amino transferase (ALT) (S.G.P.T) (EC 2.6.1.2) (U/L)
Control: No additive	251.67[a]	136.00[a]	162.33[a]	51.00[a]	102.33[a]	256.33[a]	4.33[a]
0.5% dried *Citrus sinensis* pulp	255.00[a]	111.00[b]	172.33[a]	49.33[a]	111.33[a]	219.33[a]	4.00[a]
1.0% dried *Citrus sinensis* pulp	267.33[a]	93.00[c]	130.00[a]	31.67[b]	86.00[b]	252.00[a]	4.00[a]
1.5% dried *Citrus sinensis* pulp	247.00[a]	60.00[d]	144.67[a]	34.00[b]	107.67[c]	223.33[a]	2.66[b]
2.0% dried *Citrus sinensis* pulp	251.00[a]	53.33[d]	147.00[a]	1.30[c]	97.67[c]	224.00[a]	3.33[c]
P	0.9	0.01	0.5	0.4	0.4	0.5	0.2
SEM (Standard error of mean)	16.0	15.7	17.8	8.6	10.3	20.11	0.5

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

Table 5 Humoral immunity parameter mean (±SEM) of Ross 308 broilers fed with different levels of dietary dried *Citrus sinensis* pulp sampled from postnatal 1st–6th weeks*.

Treatment	Trait			
	Relative weight of spleen (%)	Antibody titer against infectious bronchitis virus (IBV) (log 10)	Antibody titer against Newcastle (log 2)	Antibody titer against infectious bursal disease or Gumboro (IBD) (log 10)
Control: No additive	0.08[a]	3.27[a]	4.33[a]	3.44[a]
0.5% dried *Citrus sinensis* pulp	0.07[a]	3.16[a]	5.66[a]	3.50[a]
1.0% dried *Citrus sinensis* pulp	0.10[a]	3.22[a]	5.00[a]	3.60[a]
1.5% dried *Citrus sinensis* pulp	0.08[a]	3.19[a]	4.66[a]	3.34[a]
2.0% dried *Citrus sinensis* pulp	0.09[a]	3.12[a]	5.33[a]	3.53[a]
P	0.5	0.4	0.5	0.4
SEM (Standard error of mean)	0.01	1.36	1.71	0.92

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

Table 6 Cecum microflora mean (±SEM) of Ross 308 broilers fed with different levels of dietary dried *Citrus sinensis* pulp from postnatal 1st–6th weeks*.

Treatment	Trait		
	Total aerobic bacteria (log 10 cfu/gr)	*Escherichia coli* (log 10 cfu/gr)	Lactobacillus bacteria (log 10 cfu/g)
Control: No additive	8.21[a]	7.49[a,b]	7.46[b]
0.5% dried *Citrus sinensis* pulp	8.08[a,b]	7.34[b]	7.65[a,b]
1.0% dried *Citrus sinensis* pulp	8.26[a]	7.54[a,b]	7.70[a,b]
1.5% dried *Citrus sinensis* pulp	7.99[b]	7.62[a]	7.59[b]
2.0% dried *Citrus sinensis* pulp	7.94[b]	7.64[a]	7.79[a]
P	0.03	0.01	0.02
SEM (Standard error of mean)	0.21	0.89	1.32

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

cholesterol levels in birds are affected by factors such as heredity, nutrition, age, sex and environmental conditions. Some compounds such as carbohydrates, vitamin C and some plant compounds can reduce cholesterol in birds. Furthermore, the detailed mechanism of the cholesterol lowering by DCSP is worthy of further study.

4.4. Humoral immunity and cecum microbial population

The effects of Ross 308 broilers fed with different levels of DCSP on humoral immunity and cecum microbial population from first to 35th day in the experiment are presented in Tables 5 and 6 and respectively. There were four avian humoral immunity carcass indexes measured in the experiments, i.e. relative weight of the spleen, antibody titer against infectious bronchitis virus (IBV), antibody titer against newcastle, and antibody titer against infectious bursal disease or gumboro (IBD). In comparison with the control group, the experimental statistics were significant at the levels of 0.5, 0.4, 0.5, and 0.4 respectively (Table 5). Although it was shown that all the humoral immunity parameters were not significant between treatments, there were trends that suggested additives of dietary DCSP might increase body immune function of broilers compared to the control groups. There were three cecum microbial population indexes measured in the experiments, i.e. the counts of total aerobic bacteria, *E. coli*, and *Lactobacillus bacteria*. The statistics of Ross 308 broilers fed with different levels of DCSP on counts of cecum microbial population were all significant (Table 6). Generally, fungi and herb extract used in the diet greatly reduced the species of *Bacteroid*, and *Enterococcus* populations, but also increased the number of *E. coli* and *Lactobacilli* populations. The total aerobic bacterium count was significantly reduced ($P = 0.03$), whereas the counts of *E. coli*, and *L. bacteria* increased significantly ($P = 0.01$ and $P = 0.02$) with the gradual increases of dietary DCSP. The results of mean comparisons of *E. coli* and *L. bacteria* in the ileum showed significant differences compared to the control groups. In comparison with the control groups, the lowest mean of *E. coli* in the ileum was related to the treatment with 0.5% DCSP, while the lowest mean of *L. bacteria* in the ileum was related to the control group and next the treatment with 1.5% DCSP. These results partly agreed with those of Pourhossein et al. (2012a,b,c) who found a positive effect of different levels of dried *Citrus sinensis* peel (DCSP) affecting on broiler gastrointestinal microbial population. Pourhossein et al. (2012a,b,c) found that the mean of Lactobacilli in the cecum on the postnatal 42 day showed no significant result ($P > 0.05$), while the mean of *E. coli* in the ileum on the postnatal 42 day showed a significant difference from the control ($P < 0.05$). The highest was produced in the control group and the lowest was related to the *C. sinensis* peel extract (CSPE) treatment of 1250 ppm up to the end of the experimental rearing period (Pourhossein et al., 2012b).

5. Conclusion

In the present study, broiler weight gain, feed intake and feed conversion ratio (FCR) were measured. Blood parameters and carcass traits were measured in the postnatal 35th day. The highest level of dried orange residues in treatment 5 (T5) had significantly increased the feed intake and body weight of broilers in groups and overall during the rearing period ($P > 0.05$). Different levels of dried orange residues had no significant effect on chicken FCR. Using of dried orange residues significantly decreased the liver and abdominal fat of broilers ($P < 0.05$). T5 has also significantly lower level of triglyceride than the control (T1) and treatment 2 (T2) ($P < 0.05$).

In conclusion, the use of dried orange residues improved some performance (e.g. feed intake and body weight gain), decreased liver and abdominal fat and also serum triglyceride level in broiler chicken. Based on the results stated above, utilization of 2% DCSP in diets of broiler chicken had a significant positive effect of dried sweet orange residues in diet on performance, carcass characteristics, blood metabolites, humoral immunity, and cecum microbial population of broilers. It was also observed that the small number of levels of DCSP used in this study might lead to some bias in conclusion. Therefore, more researches are needed in the future.

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