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Effect of different levels of *Citrus sinensis* peel extract on broiler performance, blood parameters, thyroid gland activity and bone ash

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ABSTRACT

A 6-week experiment involving 400 day-old Ross 308 chicks was carried out in a completed randomized design to evaluate the performance, thyroid gland activity, haematological parameters and bone ash of the broilers supplemented with *Citrus sinensis* peel extract (CSPE) in drinking water. The birds were allotted to 5 treatments containing basal diet (control), basal diet supplemented with 1000 ppm in drinking water for 1 to 21 days, 1000 ppm for 1 to 42 days, 1250 ppm for 1 to 21 days or 1250 ppm for 1 to 42 days. Each treatment was replicated four times, 20 chicks each. Results showed that the addition of CSPE in drinking water, irrespective of its concentration or the growth period when it is added, improves weight gain and feed conversion ratio in the 22 to 42 days of growth period. Red blood cells counts were higher for the four treatment groups compared to the control one and packed cell volume were higher for both groups added with 1250 ppm CSPE but no differences between treatment groups and control one were found in plasma T3 and T4 concentrations, Hg concentration or bone ash. So, the authors can conclude that the addition of orange could be positive for the poultry industry as well as a cheap substitute for the traditional antibiotics.

Key words: Broilers, *Citrus sinensis* peel extract (CSPE), Growth performance, Haematology, Thyroxin

The abusive use of antibiotics in animal production has led to the formation of resistance of certain bacteria, which, in turn, has carried out the ban of the antibiotic growth promoters and the need to find alternatives. *Citrus sinensis* can improve the bird´s health and production results (Whitehead and Keller 2003) and has also shown antimicrobial activity (Nannapaneni *et al.* 2008). This study investigated the effects of different levels of *Citrus sinensis* in Ross broilers performance.

MATERIALS AND METHODS

The experiment was conducted during 42 days in 2011 in a poultry farm in Sowme´eh, Guilan province, Iran. Four hundred one d-old chicks of Ross–308 strain were randomly located in 20 scaffoldings. Groups were divided in a completely randomized design into 5 studied treatments included:

Control group: Control treatment included standard diet with no additives.

Group 2: Standard diet +1000 ppm CSPE from 1 to 21 days (switched to regular water afterwards).

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Group 3: Standard diet +1000 ppm CSPE from 1 to 42 days.

Group 4: Standard diet +1250 ppm CSPE from 1 to 21 days (switched to regular water afterwards).

Group 5: Standard diet +1250 ppm CSPE from 1 to 42 days.

Each diet (treatment) was replicated four times, with each replicate comprising one pen of 20 birds.

All animals were maintained in the poultry facilities in good health and in conditions fully described in Ebrahimi *et al.* (2013).

A 2 phase feeding regime consisting of starter (1 to 21 day) and grower (22 to 42 day) was used in the study. The composition of nutrients in the feed during the starter and grower periods is shown in Tables 1, 2. Feed and water were provided *ad lib*. *C. sinensis* peel (40 g) was mixed in 320 ml of 72% ethanol and placed in a 50ºC water-bath for 3 h. The resulting suspension was centrifuged, collected, filtered and dried. The CSPE was added to drink. The chemical composition of CSPE was determined using methods of AOAC (2000) and resulted (in g/kg) as follows: fiber: 100.0, ether extract: 20.0, carbohydrate: 635.4, ash: 70.0, phosphorus: 0.5, calcium: 11.0, dry matter: 880.0, moisture: 120.0 and protein: 54.6.

The body weight and feed intake by replicate were determined weekly by pen. Average daily body weight gain, average total feed intake and feed conversion ratio (FCR) were then calculated.

Table 1. Composition (g/kg) of diets used during the experimental periods. Starter: 1st – 21st days of age or grower: 22nd – 42nd days

Grower	Starter	Ingredient
586.9	543.2	Corn
318.7	394.3	Soybean meal
7.9	9.0	Oyster shell
58.3	21.6	Corn oil
2.2	2.0	DL-methionine
0.5	0.7	L-lysine
16.8	20.5	Dicalcium phosphate
3.7	3.7	Salt
2.5	2.5	Vitamin mixture[1]
2.5	2.5	Mineral mixture[2]

[1]Mineral mixture (mg/kg diet): manganese, 60 mg; iron, 13 mg; zinc, 40 mg; copper, 8 mg; iodine, 0.3 mg; cobalt, 1 mg; sodium selenite, 0.2 mg. [2]Vitamin mixture (per kg diet): vitamin A (retinil acetate), 10200 IU; vitamin D_3 (cholecalciferol), 2200 IU; vitamin E (D-a-tocopherol), 10 mg; vitamin K_3 (menadione), 2 mg; vitamin B_1 (thiamine), 2.4 mg; vitamin B_2 (riboflavin), 3.63 mg; vitamin B_3 (niacin), 35 mg; vitamin B_5 (calcium pantothenate), 12 mg; vitamin B_6 (pyridoxine), 3.5 mg; vitamin B_7 (biotin), 150 µg; vitamin B_9 (folic acid), 1.4 mg; vitamin B_{12} (cobalamin), 30 µg.

Table 2. Nutrient analysis of diets used during the experimental period. Starter: 1st – 21st days of age or grower: 22nd – 42nd days

Grower	Starter	Ingredient
13.4	12.1	Metabolisable energy (Mj/kg)
221.6	192.0	Crude protein (g/kg)
11.5	9.6	Lysine SID[1] (g/kg)
5.0	4.8	Methionine SID (g/kg)
8.3	7.8	Met+Cys (g/kg)
7.9	7.1	Thereonine (g/kg)
10.0	8.5	Calcium (g/kg)
5.0	4.2	Available phosphorus (g/kg)
236.00	202.00	Dietary cation anion balance (mEq/kg)

[1]SID (standardized ideal digestible).

At the end of the study, at 42 days of age, one bird per scaffolding, totalling 4 birds per treatment, was selected. Chickens were blood sampled before slaughtering. With a maximum delay of one hour after the extraction, the samples were centrifuged at 3000 rpm for 20 minutes. The red blood cell count (RBC) was estimated using haemocytometer. Haemoglobin (Hb) was measured using a commercial test kit. Packed cell volume (PCV) was calculated as ratio of red blood cells volume/blood volume. Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were calculated from Hb, PCV and RBC. Serum concentration of triiodothyronin (T3) and thyroxin (T4) were determined using commercial test kit.

The left femur was used for the determination of bone ash. Left femur of each bird was removed, cleaned and dried at 60º C during 24 h and ashed @ 600º C.

One-way ANOVA tested differences in the performance variables studied, blood parameters and bone ash, among treatment groups. Tukey's test was carried out to determine disparities among the groups. General lineal models examined the effects of the different treatments on performance variables (feed intake, weight gain and FCR) at different stages of growth (1–21, 22–42, 1–42 days of rearing period).

RESULTS AND DISCUSSION

A previous work of our group based on the addition of dried sweet orange peel on diet (solid) showed contradictory results with different trends depending on the concentration. In this sense, adding up to 3% seemed to depress feed intake, body weight gain and an increasing feed conversion ratio of both starter and growing broilers while in the proportion of 1.5% of feed seemed to promote these growth variables (Ebrahimi *et al.* 2013). These differences resulted in an improvement in carcass quality, concretely in weight of legs and wings (Ebrahimi *et al.* 2013). Authors concluded that, since dried orange peel could constitute a useful additive in the feeding of broilers, further research was needed to

Table 3. Effect of *Citrus sinensis* peel extract (CSPE) at different levels (1000 ppm or 1250 ppm) and experimental periods (starter, 1st to 21st rearing day; whole, 1st to 42nd rearing day) on chicken growth performance (mean±SEM)

Treatment	Control	CSPE 1000 S	CSPE 1250 S	CSPE 1000 W	CSPE 1250 W
Total feed intake (g/bird)					
1–21 days	1065.8±3.6^{a}	1058.3±5.1$^{a, b}$	1037.4±4.8^{b}	956.9±2.7^{c}	1053.0±8.1^{a,b}
22–42 days	3523.8±2.5^{a}	3516.2±9.9^{a}	3546.6±20.5^{a}	3401.3±32.0^{b}	3675.3±18.0^{c}
1–42 days	4589.5±5.9^{a}	4574.4±14.5^{a}	4584.1±24.1^{a}	4358.2±33.0^{b}	4728.3±22.4^{c}
Total weight gains (g/bird)					
1–21 days	743.5±35.9^{a}	684.6±4.1^{a}	711.8±10.1^{a}	688.8±22.4^{a}	690.1±6.9^{a}
22–42 days	1664.3±52.2^{a}	1829.6±32.9^{a,b}	1798.9±35.8^{a,b}	1906.0±26.2^{b}	1937.1±88.0^{b}
1–42 days	2407.8±44.5^{a}	2514.3±30.2^{a}	2510.8±45.6^{a}	2594.8±45.4^{a}	2627.3±86.6^{a}
Feed conversion ratio (g/g)					
1–21 days	1.44±0.07^{a}	1.55±0.00^{a}	1.46±0.02^{a}	1.39±0.05^{a}	1.53±0.02^{a}
22–42 days	2.12±0.06^{a}	1.92±0.04^{a,b}	1.97±0.04^{a,b}	1.79±0.03^{b}	1.91±0.08^{a,b}
1–42 days	1.91±0.04^{a}	1.82±0.03^{a,b}	1.83±0.04^{a,b}	1.68±0.03^{b}	1.80±0.05^{a,b}

In each row, the averages with different letters indicate significant differences (P<0.05) between treatments.

assess the effects as a feed resource for poultry production.

Table 3 showed the average feed intake, weight gains and FCR by birds in the different treatments of the present study. Although the results did not show a clear trend towards increased feed intake by the supplemented animals, instead, show a clear trend towards increased live weight gain, and a lower conversion ratio of the groups added with orange peel extract in relation to the control one. In this sense, none of the GLM performed was significant for feed intake but regarding weight gains, the GLM reached significance for total weight gain (1–42 days; model F= 10.60, P<0.01, R^2, 37.1%), and weight gain in the second part of the growth period (22–42 days; Model F,14.66, P<0.001; R^2, 44.9%). Finally, the GLM performed for FCR were significant in the same periods of study that were conducted for live weight gain. Thus, they resulted significant and the conversion rate was affected by the type of diet for total growth period (1–42 days; Model F, 6.03; P<0.05; R^2 , 25.1%) and the second part of growth period (21–42; Model F,8.05; P<0.05; R^2 , 30.9%).

Since our results did not show differences in the first 21 days of the chicken growth, this seemed to be more effective in the second part of the growth period, i. e., from day 22nd to 42nd.

Table 4 showed the haematological values and bone ash concentration in the control and four treatment groups. There were no differences on T3, T4, Hg, MCH, MCHC and bone ash between groups.

Plasma T3, the hormone that plays an active role in energy metabolism and metabolic rate, is associated with temperature regulation and is an important growth promoter in broiler chickens (Sturkie 1986). The circulating T3 concentration appears to be linearity correlated with feed intake (Yahav 2000). So, since serum T3 and T4 levels was not significantly affected, the improvement in growth was not associated to the thyroid gland activity and must be mediated throughout the improvement in nutrient absorption linked by the orange antimicrobial properties on gut microflora (Nannapaneni *et al.* 2008). Another explanation or, part of the explanation, could be mediated throughout the increase in vitamin C supply of the birds supplemented. Both in broilers and turkeys have shown clear positive effects of widespread vitamin fortification in stress conditions (Deyhim and Teeter 1996). In particular, the role of vitamin C to relieve heat stress and improving the immune response is well established. Ascorbic acid promotes leukocyte function by increasing the number and size of the spleen lymphocytes (Gross *et al.* 1988).

Results show a positive effect of orange peel extract on red blood cells, as well as, on a directly related variable as the PCV but only statistically higher for both groups of 1250 ppm (starter or whole growth period). On the other hand, the addition did not affect haemoglobin concentration. Nowaczewski *et al.* (2006) founded higher number of erythrocytes, haemoglobin and haematrocrit of pheasants supplemented with vitamin C. Blood parameters have been shown to be major indices of physiological, pathological and nutritional status of an organism and changes with the quality of feed (Babatunde *et al.* 1992), so our results suggest that the addition of CSPE helps in RBC formation. Similarly as we have discussed with the improvement in growth, due to the results on plasma T3 and T4, these increase in red blood cells could be mediated by a greater feed absorption which would lead to an increase availability of blood constituent minerals and proteins as well as the greater vitamin C supply specially important in young organisms because of their lower biosynthesis of this vitamin and their higher metabolism (Seeman 1991).

Theoretically, an increase in the number of erythrocytes of the same volume (MCV) always leads to an increase in PCV and, as a consequence of it, an increase in MCV of the same number of red blood cells, increases PCV too. This would explain the higher PCV percentage of both groups added with 1,250 ppm related to those added with 1,000 ppm and hence the decrease in MCHC index which is observed in our results for these two groups (not reaching significance differences). These results shows that the addition of CSPE to drink water can be offered to broiler

Table 4. Effect of *Citrus sinensis* peel extract (CSPE) at different levels (1000 ppm or 1250 ppm) and experimental periods (starter, S, 1st to 21st rearing day; whole, W, 1st to 42nd rearing day) on thyroid gland activity, haematological values and bone ash (mean±SEM)

Haematological values	Control	CSPE 1000 S	CSPE 1250 S	CSPE 1000 W	CSPE 1250 W
T3 (ng/dL)	2.31±0.39^a	4.99±1.43^a	3.21±0.46^a	4.19±1.24^a	2.51±0.54^a
T4 (µg/dL)	0.40±0.01^a	0.42±0.06^a	0.43±0.02^a	0.44±0.02^a	0.40±0.01^a
RBC (×10^6/µL)	2.08±0.05^a	2.68±0.04^b	3.05±0.02^c	2.75±0.07^b	2.53±0.04^b
PCV (%)	27.8±0.6^a	31.0±1.0^a	41.9±0.7^b	29.2±1.0^a	35.6±1.1^c
Haemoglobin (g/dL)	12.8±1.0^a	15.7±0.2^a	15.8±1.1^a	15.0±2.2^a	13.5±0.6^a
MCV (fL)	133.9±3.8^a	115.7±5.3^b	137.6±3.1^a	106.4±1.8^b	140.8±2.6^a
MCH (pg)	62.0±6.2^a	58.6±0.3^a	51.7±3.5^a	54.6±7.9^a	53.5±6.2^a
MCHC (g/dL)	46.1±3.7^a	50.9±2.0^a	37.7±3.0^a	51.1±6.7^a	38.1±4.8^a
Bone ash (%)	53.1±0.4^a	52.0±0.3^a	53.0±0.7^a	50.7±0.6^a	53.6±0.3^a

T3, triiodthyronine; T4, thyroxin; RBC, red blood cells; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular of haemoglobin; MCHC, mean corpuscular hemoglobin concentration. In each row, the averages with different letters indicate significant differences (P<0.05) between treatments.

chicken not only without any adverse effect on blood constituents, but also, the addition of 1250 ppm (starter or whole growth stages) improves PCV percentage. An increment in PCV percentage could lead to an improved oxygen supply which in these fast-growing chicken breeds could protect against ascites syndrome.

In conclusion, the effects of sweet orange peel extract addition in drinking water showed that weight gain and as a consequence of it, FCR were improved with the supplementation especially in the second part of the growth period and with the addition of 1,000 ppm CSPE during the whole growth period. In addition, orange peel extract improves blood formation too, which makes that we can conclude that the addition of orange could be positive for the poultry industry, as well, as cheap, health and safety for humans.

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