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Effect of Graded Levels of Dried Orange (*Citrus sinensis*) Byproducts on Production Efficiency, Blood Parameters and Antioxidant Status of Broiler Chickens

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ABSTRACT

Background: Orange wastes are bioactive compounds produced during the industrial transformation. The experiment was conducted to evaluate the effects of feeding dried sweet orange byproducts on broiler growth performance, serum metabolites and antioxidant status.

Methods: Two-hundred-day old experimental broiler chicks were distributed to 4 dietary treatments with 5 replicates of 10 birds each. In a complete randomized design, the dietary treatments included a control corn-based diet and three experimental diets containing 5, 10 and 15% Dried Orange Pulp to substitute corn. Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), carcass traits, serum components and blood antioxidant status were measured.

Result: FCR and BWG of chickens were improved with graded levels of DOP compared to the control group (P<0.05). Serum total protein and glucose were significantly increased (P<0.0001) in DOP groups compared to the control. Serum Cholesterol and triglyceride decreased significantly (P<0.0001) in the DOP groups. Higher Aspartate amino transferase, Lactate dehydrogenase and Creatine kinase activities were observed in control group compared to the other treatments.

Key words: Broilers, Dried orange pulp, Performance, Serum components, Stress.

INTRODUCTION

Broiler chicken meat production is a growing industry due to the rapid growth of chicks, the availability of high-quality meat at low prices and the chicken's rapid response to changes in ration composition (Alzawqari *et al*., 2016). Currently, there is a greater focus on consuming a diet that is rich in bioactive and antioxidants compounds (Abbas *et al*., 2022). In particular, *Citrus sinensis* known as sweet orange, is the major citrus crop produced worldwide and is a rich source of bioactive compounds and dietary fiber (Nieto *et al*., 2021). A total of 143 755.6 thousand tonnes of citrus fruits were produced worldwide in 2019; the largest producer was China, which produced 37 739.0 thousand tonnes.

Orange production constitutes approximately 54.84% of the entire world's production of citrus fruits, followed by mandarin production 24.70% and grapefruit production 6.47% (Nieto *et al*., 2021).

Algeria had a 1593 thousand tonnes of citrus production (2% of world production) with a 1199.5 thousand tonnes of oranges where 40% are utilized in fruit transformation (FAO, 2021). However, the orange fruit industry produces 390,000 tons of waste per year (Lagha-Benamrouche and Madani, 2013). Moreover, the rise in cereal prices, particularly for corn and soybean meal, coupled with the European Union's ban on antibiotics (Alzawqari *et al*., 2016), has prompted a search for new alternatives to animal diets. Citrus co-products obtained from juice production are composed of peel and pulp. Several bioactive substances, such as dietary fiber (pectin, cellulose and hemicellulose), minerals (potassium, calcium and magnesium), organic acids (citric, oxalic and malic), vitamins (vitamin C), phenolic compounds and flavonoids (hesperidin, narirutin) (Azizi *et al*., 2018) are abundant in these fractions.

The objective of the present study was to evaluate the effect of substituting maize by orange byproducts in the daily feed of broiler chickens and investigate the influence of dietary dried orange pulp on growth performance, carcass characteristics and blood metabolites of broiler chickens.

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MATERIALS AND METHODS

The preparation of dried orange pomace

Fresh orange byproducts were purchased from the local factory after juice processing and spread on a clean floor in a greenhouse for sun drying. The pulp was then transferred and processed into powder using a 1-mm screen and stored until it is used.

Animals, diets and experimental design

The study was conducted at the Agronomy Higher School Workshops in Mostaganem, Algeria, from March to June 2021. Two hundred male chicks (Arbor Acres), aged one day and weighing 45±5 g, were procured from a commercial hatchery. The chicks were fed a consistent starter diet (3035 kcal/kg) for a duration of 16 days and were provided with free access to food and water.

From the 16th day of age, the birds were individually weighed and then randomly assigned to one of four treatment diets and four floor pens. The treatment diets included a control diet formulated according to the nutritional requirements established by the Algerian West Poultry Group (ORAVIO) for broilers. Additionally, there were three experimental diets in which 5%, 10% and 15% dried orange pulp was substituted for corn in the diets, as detailed in Table 1.

The trial lasted 49 days during the period of March-April 2021 and used scaffoldings and pens with dimensions of 2,3×2, 2 m and a height of 1 m. Each treatment consisted of five replicated pens (1 m^2) with 10 birds each. The building complex was then fumigated, washed and disinfected.

The brooding temperature of the experimental broiler house was initially set at approximately 32-34°C. After three days of rearing, the temperature was gradually decreased by 0.5°C per day until a constant temperature of 20-22°C was reached. The temperature was then maintained at this level. The humidity was controlled at around 60-70%, and the chickens were reared under standard hygienic conditions. The poultry building had thermostatically controlled cross-ventilation and a lighting program. The lights were used to provide a 23-hour light and 1-hour dark cycle during the first 21 days of rearing and a 22-hour light and 2-hour dark cycle from day 22 to day 49 of the rearing period.

Sampling and data collection

Performance measurement

Feed intake, Body weight gain (BWG) and Feed conversion ratio (FCR) were calculated for each group on weekly basis.

Measurements at slaughter

After the 49-day duration of the experiment, ten birds were selected from each group or treatment. They were then weighed, slaughtered and eviscerated in a local commercial slaughterhouse. Carcass yield was determined by subtracting the weight of the eviscerated organs (liver, heart, gizzard, spleen, intestine, breast, thigh and abdominal fat) from the live weight.

Blood sampling and determination of serum components

At the end of the study, on the postnatal 49th day; one day before slaughter, two birds per replicate, were selected for the blood analysis; 5 ml of blood samples were collected into EDTA tubes from 10 birds in each treatment. The samples were then transferred to the laboratory and centrifuged at 3000×g for 10 minutes to separate the serum.

The serum was stored in an Eppendorf tube at -20°C until assayed for measuring blood parameters. Serum Total protein (TP), Glucose (Glu), Cholesterol (Chol), Triglycerides (TG), Aspartate Amino Transferase (AST), Alanine Amino

Table 1: Feed ingredients and nutrients composition of different diets.

Ingrédients (%)	Grower and finisher (15 to 49 days of age)			
	Control	DOP 5%	DOP 10%	DOP 15%
Corn	67.00	62.00	57.00	52.00
Soybean meal	27.00	27.00	27.00	27.00
Wheat bran	4.00	4.00	4.00	4.00
Calcium+Phosphorus	1.00	1.00	1.00	1.00
Vit-Min premix	1.00	1.00	1.00	1.00
Orange pulp	0	5.00	10.00	15.00
Analyzed composition (%)				
Moisture	87.97±0.007	90.26±0.12	90.49±0.21	90.81±0.22
Ash	3.07±2.28	3.20±0.98	3.51±2.73	3.82±1.99
Cellulose	5.09±0.68	6.43±0.54	6.59±0.34	6.68±0.79
Crude proteins	21.77±0.12	20.60±0.78	20.43±0.51	20.22±0.89
Energy (kacl)				
Metabolisable energy	3116.39	3134.44	3152.50	3170.55

*Vitamin-mineral premix: Provided (in mg kgG1 of diet), Vitamin E: 6, Vitamin K3: 0.80, Vitamin B1: 1, Vitamin B2: 3, Pantothenate of Ca: 6, Vitamin B6: 1.5, Vitamin B12: 0.006, Folic acid: 0.2, Nicotinic acid: 12, Copper: 5, Cobalt: 0.65, Manganese: 65, Zinc: 65, Selenium: 0.25, Iron: 50, Iode: 0.8, Magnesium: 100.

Transferase (ALT), Lactate Dehydrogenase, (LDH) and Creatine kinase (CK) were analyzed using reagent laboratory kits (BIOLABO SAS, France) using a spectrophotometer (SPECORD 210, Germany).

Stastical analysis

For determination of the statistical significance of the results, appropriate parametric test ANOVA were used. The results were presented as text and tables as mean values and standard error of mean (SEM). Data were statistically analyzed by SPSS statistical software (IBM SPSS version 26). The statistical comparison was made by Tukey test at the 95% probability level.

RESULTS AND DISCUSSION

The results of the experimental trial on broiler performance, carcass characteristics and blood metabolites are summarized in Tables 2, 3 and 4.

Growth performance

The 5, 10 and 15% DOP based diets enhanced body weight at day 49 of rearing compared to the control group. There were significant differences in broiler weight gain and feed conversion ratio among treatments due to dietary DOP (P<0.05). Significant improvements in FCR of (5 and 10% DOP), except in 15% DOP. In the final week of the trial, the groups fed with 5% and 10% DOP had a decline in FCR of

Table 2: Effect of dietary dried orange pulp concentration on the performance of broiler chickens.

Attribute	Treatments				SEM	P value
	Control	5% DOP	10% DOP	15% DOP		
Grower period of (postnatal 21-28th day)						
Weight (g/chick)	1355^{b}	1390^{b}	1348^{b}	1288^{a}	21.29	<0.001
Feed conversion ratio	1.40^{a}	1.73^{b}	1.80^{b}	1.87^{b}	0.098	<0.0001
Weight gain (g/chick/duration)	773^{a}	837^{bc}	866^{c}	794^{ab}	19.93	<0.001
Finisher period of (postnatal 28th-35th day)						
Weight (g/chick)	1937^{bc}	1976^{b}	1935^{bc}	1918^{a}	21.63	0.064
Feed conversion ratio	1.84^{a}	1.89^{a}	2.01^{a}	1.92^{a}	0.090	0.325
Weight gain (g/chick)	582^{a}	586^{a}	587^{a}	630^{a}	27.50	0.270
Finisher period of (postnatal 35th -42nd day)						
Weight (g/chick)	2525^{a}	2654^{b}	2596^{bc}	2550^{a}	29.24	0.0002
Feed conversion ratio	2.09^{b}	1.92^{a}	2.07^{b}	2.29^{b}	0.112	0.016
Weight gain (g/chick)	588^{a}	678^{b}	661^{ab}	632^{ab}	30.53	0.024
Finisher period of (postnatal 42nd-49th day)						
Weight (g/chick)	2952^{a}	3224^{c}	3182^{bc}	3103^{b}	32.94	<0.0001
Feed conversion ratio	2.67^{a}	2.43^{a}	2.33^{a}	2.67^{a}	0.173	0.145
Weight gain (g/chick)	428^{a}	570^{b}	586^{b}	553^{b}	37.55	<0.0001

SEM, standard error of the means. Each mean represents 15 replicated pens (N=15).

$^{a-c}$ Means in each row with no common superscript differ significantly at (P<0.05).

DOP: Dried Orange Pulp.

Table 3: Effect of feeding dried orange pulp (DOP) on carcass yield, different organs and relative immune organs (expressed as a percentage of carcass weight) of broiler chickens at 49 days of age.

Items	Carcass weight (g)	Dressing Percentage		Gizzard (%)	Liver (%)	Heart (%)	Spleen (%)	Intestine (%)	Thigh (%)	Breast (%)	Abdominal at (%)
		A	B								
Control	2266^{a}	85.2^{a}	76.7^{a}	2.26^{a}	2.30^{a}	0.85^{a}	0.11^{a}	0.86^{a}	15.0^{a}	36.4^{a}	1.12^{c}
DOP 5%	2442^{a}	85.0^{a}	75.7^{a}	2.26^{a}	2.32^{a}	0.76^{a}	0.12^{a}	0.87^{a}	15.1^{a}	36.9^{a}	0.97^{b}
DOP 10%	2331^{a}	84.0^{a}	73.3^{a}	2.48^{a}	2.35^{b}	0.76^{a}	0.13^{a}	0.98^{a}	14.9^{a}	37.1^{a}	0.96^{b}
DOP 15 %	2435^{a}	88.5^{a}	78.5^{a}	2.22^{a}	2.85^{b}	0.70^{a}	0.13^{a}	1.48^{b}	15.4^{a}	38.7^{a}	0.82^{a}
SEM	62.68	3.49	2.66	0.134	0.13	0.11	0.013	0.12	0.89	0.70	0.15
P	0.057	0.608	0.156	0.053	0.0005	0.602	0.358	<0.0001	0.960	1.743	<0.001

SEM, standard error of the means.

Means with no common superscripts in each column differ significantly (N=10).

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

$^{a-c}$ Means in each column with no common superscript differ significantly at (P<0.05).

Table 4: Effect of Dried orange pulp (DOP) on Blood Serum Constituents of Arbor Acres Broiler Chickens at 49 Days of Age.

Attribute	Treatments				SEM	P value
	Control	5% DOP	10% DOP	15% DOP		
Glucose and lipid fractions						
Glucose (mg/dl)	175^{a}	163^{a}	209^{b}	253^{c}	13.32	<0.0001
Triglycerides (mg/dl)	137^{c}	144^{c}	71.8^{a}	113^{b}	8.90	<0.0001
Total cholesterol (mg/dl)	212^{c}	144^{b}	102^{a}	137^{b}	11.96	<0.0001
Total protein (mg/dl)	37.5^{a}	45.9^{b}	47.4^{bc}	51.7^{c}	1.69	<0.0001
Liver function						
Aspartate amino transferase (AST) (S.G.O.T) (U/L)	260^{b}	251^{ab}	236^{ab}	223^{a}	11.30	0.014
Alanine amino transferase (ALT) (S.G.P.T) (U/L)	6.18^{a}	6.32^{a}	6.24^{a}	2.27^{b}	0.60	<0.0001
Stress status						
Lactate dehydrogenase (LDH) (UI/L)	3336^{c}	3665^{c}	2606^{ab}	1852^{a}	172.12	<0.0001
Creatine kinase (CK) (UI/L)	2960^{b}	2060^{a}	2049^{a}	1722^{a}	129.06	<0.0001

SEM, standard error of the means.

Each mean represents 10 replicated pens at the end of rearing period.

$^{a-d}$ Means with different superscripts within the same row are significantly different (P<0.05), (N=10).

9% and 13%, respectively, compared to the control group. Additionally, during the period of 35-42 days, the 5% DOP group had better FCR values compared to the control group, with a statistically significant difference (P<0.05). The trial also found a significant enhancement in BWG of chicks after the incorporation of orange byproducts to their diets.

Our results are related to the findings of (Vlaicu *et al*., 2020), who showed that adding orange pulp or essential oil (*Citrus sinensis* L) to broiler diets had a significant impact on weight growth and feed conversion ratio. The improvement in FCR values observed in DOP-diets is consistent with the results reported by (Abbasi *et al*., 2015), where hesperidin was found to enhance the activity of digestive enzymes (Kamboh and Zhu, 2013). The composition of gut microbiota can also influence FCR since colonic bacteria enzymes can affect the structure of polyphenols, increasing their bioavailability (Luca *et al*., 2019).

Our findings are similar to those reported by (Oluremi *et al*., 2010), who demonstrated that orange pulp had a positive effect on body weight and feed intake throughout the experiment. However, our results partially agree with the experiments of (Boumezrag *et al*., 2018 and Alzawqari *et al*., 2016), where supplementation with orange peel or a combination of orange peel and lemon grass leaf had no effect on BWG, FI and FCR.

Carcass parameters

The inclusion of dietary dried orange pulp (DOP) in the diets resulted in a significant increase in liver and intestine yields (P<0.01). The liver efficiency in the 15% DOP group showed a 28% increase compared to the control broilers. Additionally, a significant decrease of over 26% in abdominal fat yield was observed in the 15% DOP broilers compared to the control group (P<0.05).

The increase in liver and intestine yields of broilers fed orange waste is attributed to the antioxidant property of flavonoids like naringin (Goliomytis *et al*., 2015), also feeding soluble fiber-rich meals result in high-viscosity digesta and elevated cell proliferation (González-Alvarado *et al*., 2010). These findings were partially in line with (Akbarian *et al*., 2013 and Ebrahimi *et al*., 2014); where none of the dietary interventions had a significant influence on the mean weight of gizzard or liver. Our findings of abdominal fat yield are in agreement with those of (Abbassi *et al*., 2015).

The improvement in abdominal fat yield for dietary orange pulp treatments may be due to presence of naringin in DOP and its role as a powerful antioxidant that be able to prevent oxidation and accumulation of fat by the inhibition of adipocyte proliferation and modulating fat metabolism (Guo *et al*., 2016). The trial results were partially related to (Pourhossein *et al*., 2015), proved that adding essential oil isolated from orange peel to broiler meals results in a higher live weight, showed no significant effect on the spleen weight and the broilers fed with sweet orange peel diet had a considerable rise in liver weight.

Blood parameters

The substitution of corn with orange byproducts in bird's diets at 16 days of rearing had a significant (P<0.05) effect on serum glucose, total protein, cholesterol, triglycerides, Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Lactate dehydrogenase (LDH) and Creatine kinase (CK).

Serum glucose levels were significantly higher (P<0.05) in all dietary groups, except for the 5% DOP group, compared to the control group. Feeding the control diet resulted in a decrease in blood glucose levels by more than 68% compared to the 15% DOP-based diet. The elevated glucose concentration may be attributed to the main glycosylated flavanones, hesperidin and narirutin, found in citrus peel (Chen *et al*., 2017).

Serum lipid fractions showed significant reduction (P<0.05) in total cholesterol and triglycerides, in broilers fed with DOP-based diets in comparison to control. An increase of more than 48% in TG blood concentration was noticed in the serum of control group compared to the 10% DOP group. The 10% DOP meal decreased the cholesterol in animal serum by more than 52% compared to the control diet.

Our findings are in agreement with (Alefzadeh *et al*., 2016 and Abbasi *et al*., 2015) concluded that dietary dried orange peel and orange pulp lowered blood cholesterol and triglycerides. This effect may be attributed to the hypocholesterolemic properties of citrus fruits. The flavonoids have been shown to inhibit the synthesis of cholesterol in the liver by inhibiting the activity of the hepatic enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (Gilani *et al*., 2018).

Feeding DOP-diets led to an increase in the serum total protein level ($P<0.001$) compared to the control diet; which can be ascribed to the transamination of phenolic and flavonoid compounds present in the orange wastes (Akbarian *et al*., 2013).

Feeding DOP-based diets presented a significant decrease in AST levels compared to the control diet ($P=0.014$). Specifically, the serum activity of AST was reduced in 15% DOP group, which is more than 29% lower than the control group activity.

However, the results of ALT obtained in this trial were similar to (Alagawany *et al*., 2021 and Suliman *et al*., 2019), showed that orange byproducts have positively affected the liver enzymes in broiler chickens and quail. The decrease in AST serum levels observed in this study could be linked to naringin, a compound found in orange peel and byproducts which may reduce the liver cell enzyme leakage (Jiang *et al*., 2020 and Gibson *et al*., 2017).

The inclusion of DOP reduced the activity of LDH and CK ($P<0.05$). The lowest activities of (CK) were observed in DOP-based diets compared to the control group. A reduction of more than 46% was observed in the 15% DOP compared to the basal diet. The relative concentrations of LDH were significantly lower in DOP 10 and 15% than in the control group ($P<0.01$).

In the current investigation, the reduction of CK and LDH levels in the serum indicated that dietary flavonoids have beneficial effects against stress. This is in line with (Akbarian *et al*., 2013), who found that dietary antioxidants such as hesperidin and dietary genistein are defenders against oxidative damage in poultry rearing and with (Hao *et al*., 2019), who reported that naringenin has cardioprotective potential and reduces myocardial biomarkers such as AST, ALT, CK and LDH. Furthermore, DOP at different percentage in the diets from (21 to 49 days) age had a significant positive effect on chicken antioxidant status. These results are in agreement with those showed by (Alzawqari *et al*., 2016), because citrus byproducts are a source of phenolic molecules and α-tocopherol with antioxidant properties (Delgado-Pertíñez *et al*., 2021).

CONCLUSION

The inclusion of dried orange pulp in broiler diet is effective in reducing serum triglycerides and cholesterol. The use of DOP in broiler diets increased the serum total protein content. Broilers fed DOP- based diets presented an increase in productive performance, improved feed conversion ratio and liver enzyme activity and reduced stress enzymes activity.

Conflict of interest: None.

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