Effect of Dried Orange (*Citrus sinensis*) Pulp on Growth Performance, Serum Biochemical Parameters, and Nutrient Digestibility in Broiler Chickens

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**ABSTRACT**

This study investigated the effect of dried orange pulp on growth performance, immunity, serum biochemical parameters, and nutrient utilization in broiler chickens. Two hundred and forty straight-run broiler chicks (Cobb 500) were allocated to 4 groups with 5 replicates of 12 chicks each in a completely randomized design. The treatment groups were control (CON), and 3 dried citrus pulps (DCP) e.g., the control group was fed a standard diet/basal diet and the other three groups contained control + 0.5g/kg citrus pulp (DCP-1), control + 1g/kg citrus pulp (DCP-2) and control + 2g/kg citrus pulp (DCP-3) respectively. Results showed a significantly overall higher body weight gain, carcass yield, and better feed conversion ratio (FCR) in the DCP-3 and DCP-2 diet groups as compared to the control and DCP-1 groups. Among serum biochemical parameters, alanine aminotransferase (ALT) in the CON but aspartate aminotransferase (AST) in the CON and DCP-1 diet groups were significantly higher than in the remaining groups. The digestibility of crude protein (CP), ether extract (EE), and apparent metabolizable energy were significantly higher for the DCP-3 and DCP-2 diet groups compared to the control and DCP-1 diet groups. In conclusion, the use of dry citrus pulp improved production performance, liver function, and nutrient digestibility in broiler chicken.

**INTRODUCTION**

Due to the rising costs of poultry feedstuffs like soybean and cereal grains, it has been observed that the cost of poultry has been rising steadily in recent years. Generally, about 60-80% of expenses in poultry production are related to feeding (Basir and Majid, 2017; Li and Chen, 2019). Cheaper feeding techniques are therefore necessary to reduce the costs associated with animal nutrition when using alternative feeding resources (Pisoschi and Pop, 2015). Since agricultural byproducts make up a larger portion of domestic animals’ diets, the use
of agricultural byproducts in animal nutrition is currently
a topic of public concern. The search for new feed sources
has become necessary due to a lack of domestic feed
resources, particularly in developing nations. As a source
of natural antioxidants, dried orange (Citrus sinensis)
pulp (DCP) has the potential to provide some important
nutrients for poultry feed. However, DCP is a typical by-
product of citrus juice extraction and is widely produced
in Asian agricultural regions. Different scientists reported
several types of chemical compounds in Citrus sinensis
which include volatile oil, flavonoids (e.g., flavanones,
flavanone glycosides, and polymethoxylated flavones),
carbohydrates, coumarins, peptides, fatty acids, steroids,
alkanes, hydroxyamides, carotenoids, carbamates,
vitamins and alkylamines (Dongre et al., 2023). Globally
more than 75.57 million tons of citrus are produced
(FAOSTAT, 2022). More than 50% of citrus is used to
make juice after the juice is extracted about 60% of the
citrus waste is lost which contains valuable antioxidants.
After juice extraction from citrus fruits, the leftovers are
dried and the pulp is then produced. It is a combination
of the peel, the interior sections, and the removed citrus
fruits that contain biologically active substances (i.e.,
flavonoids), which have favorable and helpful effects
on the human body (Bampidis, and Robinson, 2006).
Fruit and vegetable wastes can be added to animal feeds
to increase the diet’s palatability, which could increase
feed consumption and lower feed costs (Chaudry et al.,
2004). Citrus pulp, albedo, membranes, and pith
contain flavonoids (hesperidin, naringin, etc.), a class
of secondary plant phenolic with significant antioxidant
and chelating properties (Kamboh et al., 2015). Broiler
diets already include citrus pulp and its flavonoids,
with varying results in terms of growth performance metrics
but positive effects on the antioxidant properties of the
meat. After the dietary inclusion of dried Citrus sinensis
peels at the levels of 1.5 or 3.0%, no effect was seen on
the final weight, the hot carcass weight, or the carcass
yield (Ebrahimi et al., 2013). Feed intake and body
weight gain were improved when dried orange residues
at 2% or dried tangerine peel extract at 80–480 mg/
kg were used (Abbasi et al., 2015; Jiang et al., 2016).
Studies have shown that including citrus pulp in broiler
diets results in improvements in growth performance
metrics etc. (Huyghebaert et al., 2011), and this is a
stimulus for the implementation of research activities
in this direction. The objective of this study was to test
and investigate the possible effects of the dietary orange
pulp as supplements incorporated into broiler diets on
growth performances, serum biochemistry, and nutrient
utilization in broiler chickens.

MATERIALS AND METHODS

Bird’s husbandry and treatments
Two hundred and forty day-old mixed sex broiler
chicks (Cobb 500) were purchased from a local hatchery,
weighed individually on arrival, and randomly allocated
to 4 treatments and 5 replicates of 12 chicks each in a
completely randomized design and reared in the open-
sided house for 35 days. The environmental conditions
and management practices were per the standard Cobb
guidelines. Feed in mash form and water were provided
ad libitum. The treatment groups were offered a starter (at
1 day to 21 days of age) and a finisher diet (at 22 to 35
days of age). One of the treatment groups was offered the
diet with no additive and it served as a control (CON),
whereas the other three treatment groups e.g., dried citrus
pulp (DCP-1, DCP-2, and DCP-3) were offered diets
further supplemented with dried orange pulp, at 0.5 g/kg,
1g/kg and 2g/kg, respectively. The treatment diets were
isocaloric and isonitrogenous. All chicks were vaccinated
as per the recommended schedule for broilers. Chicks
were housed in wire floor pens covered with paper rolls
and had free access to mash feed and water throughout
the trial. Experimental diets were formulated to provide
more or exceed nutritional requirements for broiler
chickens based on the Cobb Broiler Manual. The ambient
temperature was gradually decreased from 33 to 25°C on
day 21 and was then kept constant. Ingredients and nutrient
specifications of experimental diets are shown in Table I.
All experimental procedures were evaluated and approved
by the Ethics Committee Animal Care of the University of
Agriculture, Peshawar, Pakistan.

Performance parameters
Body weight (BW) and feed intake (FI) were recorded
weekly for each replicate using a digital weighing scale
with a measurement accuracy of two decimal points. Data
recorded on weekly BW and FI were used to calculate the
feed conversion ratio (FCR) (Islam et al., 2022).

Carcass characteristics and organ weight
On day 35 of the experiment, two birds close to the
mean body weight of the pen were selected, individually
weighed, and euthanized. Liver, gizzard, pancreas, and
cicum were collected, weighed, and expressed as a
percentage of live BW on day 35.

Serum biochemical parameters analysis
At the end of the study on day 35, four birds from
each group were randomly selected and fasted overnight.
Blood samples (1mL/bird) were collected into EDTA
tubes from the wing veins. Samples were transferred to the
Table I. Experimental diet composition and calculated nutrients during the starter and finisher periods.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
<th>Starter phase (1-21days)</th>
<th>Finisher phase (22-35days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>58.78</td>
<td>63.18</td>
<td></td>
</tr>
<tr>
<td>Soybean meal (46 %)</td>
<td>34.47</td>
<td>29.63</td>
<td></td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>2.590</td>
<td>3.830</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.370</td>
<td>0.370</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>1.470</td>
<td>1.360</td>
<td></td>
</tr>
<tr>
<td>Di calcium phosphate</td>
<td>1.080</td>
<td>0.730</td>
<td></td>
</tr>
<tr>
<td>Lysine sulfate</td>
<td>0.370</td>
<td>0.220</td>
<td></td>
</tr>
<tr>
<td>DL methonoine</td>
<td>0.300</td>
<td>0.240</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>0.110</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>Choline chloride (70%)</td>
<td>0.100</td>
<td>0.100</td>
<td></td>
</tr>
<tr>
<td>Mineral and vitamin premix</td>
<td>0.250</td>
<td>0.220</td>
<td></td>
</tr>
<tr>
<td>Phytase</td>
<td>0.010</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

**Calculated nutrients**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Starter phase (1-21days)</th>
<th>Finisher phase (22-35days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>84.60</td>
<td>83.35</td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.95</td>
<td>18.78</td>
</tr>
<tr>
<td>Ash</td>
<td>5.349</td>
<td>4.726</td>
</tr>
<tr>
<td>Ether extract</td>
<td>5.450</td>
<td>6.735</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.791</td>
<td>2.674</td>
</tr>
<tr>
<td>AME (Kcal/Kg)</td>
<td>2975</td>
<td>3100</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.180</td>
<td>0.180</td>
</tr>
<tr>
<td>Chlorine</td>
<td>0.289</td>
<td>0.290</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.885</td>
<td>0.799</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.900</td>
<td>0.760</td>
</tr>
<tr>
<td>Ava. Phosphorus</td>
<td>0.450</td>
<td>0.38</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.220</td>
<td>1.020</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.397</td>
<td>0.507</td>
</tr>
<tr>
<td>Met+Cys</td>
<td>0.910</td>
<td>0.800</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.227</td>
<td>0.202</td>
</tr>
</tbody>
</table>

1Vitamin A, 5000 IU/g; Vitamin D3, 3000 IU/g; Vitamin E, 80 mg/g; Vitamin K3, 1.5 mg/g; Vitamin B2, 1 mg/g; Calcium Pantothenate, 4 mg/g; Niacin, 15 mg/g; Vitamin B6, 13 mg/g; Cu, 10 mg/g; Zn, 80 mg/g; Mn, 80 mg/g; Fe, 60 mg/g; AME, Apparent metabolizable energy.

**RESULTS**

The effect of different levels of dried orange (Citrus sinensis) pulp on growth performance during the starter period (1-21 days), finisher period (22-35 days), and total period (1-35 days) are presented in Table II. Feed intake during the starter, finisher, and overall phases was not affected among the groups. During the starter phase, significantly higher body weight gain (BWG) and better FCR were recorded in the DCP-3 diet group than in the remaining groups. At the finisher phase, a significantly higher BWG was calculated for DCP-3 and DCP-2 diet groups than the CON and DCP-1 groups while FCR was (p<0.05) higher in the DCP-1 diet groups as compared to all other groups. The overall phase showed a (p<0.05) higher BWG and better FCR in the DCP-3 and DCP-2 diet groups in comparison to the DCP-1 and CON groups. In addition, during the entire growing period, the better daily body weight gain was related to treatments including gradually increased DCP content during the total period, whereas the lowest gains were achieved in broilers fed with the control diet. The effect of feeding different levels of DCP on carcass yield and organ weight is presented in Table III. A significantly higher dressing percentage was observed in DCP-2, and DCP-3 diet groups as compared to the control and DCP-1 groups. Relative weights of gizzard, liver, pancreas, and cecum were not affected by the treatments during the entire experimental period. The effect of feeding different levels of DCP on blood parameters is presented in Table IV. The concentration of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased in groups DCP-2, and DCP-3 as compared to the control group. Concentrations of serum total protein and glucose were not affected (p>0.05). The results regarding the nutrient digestibility and AME are presented in Table V. The digestibility of crude protein, ether extract, and AME were recorded (p<0.05) higher for the DCP-3 and DCP-2 diet group as compared to the control and DCP-1 diet groups, while the digestibility of dry matter, crude fiber, and ash was not effected (p>0.05).

**Laboratory for analysis**

For analysis within two hours of collection and centrifuged at 3500 rpm for 15 min to harvest the plasma. The harvested plasma was stored in an Eppendorf tube at -20 °C until assayed. Biochemical analysis was done according to standard protocols using commercial laboratory kits (Sultan et al., 2023).

**Nutrient digestibility**

To calculate apparent total tract nutrient digestibility (ATD) on day 35 of the experiment, representative chicken from each treatment was transferred to metabolic cages. Chromium oxide (Cr₂O₃) was added to the experimental diet as an indigestible marker at the rate of 0.2% for six days. After processing on day 35, the ileal content was collected and stored at -20 °C for further analyses of nutrient content. Chromium concentrations were determined with a UV absorption spectrophotometer (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan) using the method while ATD was calculated by using the following formula (Islam et al., 2022).

\[
\text{ATD} (\%) = \frac{\text{conc. of marker in feed}}{\text{conc. of marker in digesta}} \times \frac{\text{conc. of nutrient in digesta}}{\text{conc. of nutrient in feed}} \times 100
\]
Table II. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on broiler production parameters.

<table>
<thead>
<tr>
<th>Production traits</th>
<th>Treatments</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>DCP-1</td>
</tr>
<tr>
<td><strong>Starter phase</strong></td>
<td>(d 1-21)</td>
<td></td>
</tr>
<tr>
<td>BWG (g)</td>
<td>688.0 ±0.63c</td>
<td>736.6±0.83a</td>
</tr>
<tr>
<td>FI (g)</td>
<td>1132±0.07</td>
<td>1114±0.01</td>
</tr>
<tr>
<td>FCR</td>
<td>1.56±0.02a</td>
<td>1.51±0.04a</td>
</tr>
<tr>
<td><strong>Finisher phase</strong></td>
<td>(d 22-35)</td>
<td></td>
</tr>
<tr>
<td>BWG (g)</td>
<td>1068.8±0.59a</td>
<td>1060±0.82a</td>
</tr>
<tr>
<td>FI (g)</td>
<td>2116±0.43</td>
<td>2114±0.53</td>
</tr>
<tr>
<td>FCR</td>
<td>1.97±0.02b</td>
<td>2.03±0.03a</td>
</tr>
<tr>
<td><strong>Overall phase</strong></td>
<td>(d 1-35)</td>
<td></td>
</tr>
<tr>
<td>BWG (g)</td>
<td>1757±0.09b</td>
<td>1777±0.79a</td>
</tr>
<tr>
<td>FI (g)</td>
<td>3248±0.40</td>
<td>3248±0.27</td>
</tr>
<tr>
<td>FCR</td>
<td>1.84±0.01a</td>
<td>1.82±0.02a</td>
</tr>
</tbody>
</table>

Different superscripts along the row indicate significant difference (p <0.05). CON, basal diet; DCP-1, basal diet + 0.5g of dried citrus pulp/kg diet; DCP-2, basal diet + 1g of dried citrus pulp/kg diet; DCP-3, basal diet + 1.5g of dried citrus pulp/kg diet. BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

Table III. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on broiler carcass trait and organ weight.

<table>
<thead>
<tr>
<th>Traits (%)</th>
<th>Treatments</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>DCP-1</td>
</tr>
<tr>
<td>Carcass yield</td>
<td>65.5±0.21c</td>
<td>68.8±0.24b</td>
</tr>
<tr>
<td>Gizzard</td>
<td>1.14± 0.01</td>
<td>1.13±0.01</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.28±0.03</td>
<td>0.27±0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>1.76±0.03</td>
<td>1.74±0.03</td>
</tr>
<tr>
<td>Cecum</td>
<td>0.57±0.02</td>
<td>0.56±0.01</td>
</tr>
</tbody>
</table>

Different superscripts along the row indicate significant difference (p <0.05). For composition of feed for different group, see Table II.

Table IV. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on blood metabolite at day-35.

<table>
<thead>
<tr>
<th>Liver enzyme test</th>
<th>Treatments</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>DCP-1</td>
</tr>
<tr>
<td>ALT (U/I)</td>
<td>42±0.40a</td>
<td>21.5±0.64a</td>
</tr>
<tr>
<td>AST (U/I)</td>
<td>30.25±0.47a</td>
<td>29.7±0.47a</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>255.5±4.31</td>
<td>256.3±4.21</td>
</tr>
<tr>
<td>Total Protein (mg/dl)</td>
<td>2.77±0.22</td>
<td>2.78±0.20</td>
</tr>
</tbody>
</table>

Different superscripts along the row indicate significant difference (p <0.05). For composition of feed for different group, see Table II. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

Table V. Effect of graded level of dried citrus pulp (*Citrus sinensis*) on nutrients digestibility and apparent metabolizable energy at day-35.

<table>
<thead>
<tr>
<th>Nutrient (%)</th>
<th>Treatments</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>DCP-1</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>70.82±0.65</td>
<td>70.79±0.50</td>
</tr>
<tr>
<td>Crude protein</td>
<td>77.96±0.79a</td>
<td>77.37±0.46b</td>
</tr>
<tr>
<td>Dry matter</td>
<td>67.88±0.38</td>
<td>68.19±0.42</td>
</tr>
<tr>
<td>Ash</td>
<td>67.71±0.23</td>
<td>68.86±0.05</td>
</tr>
<tr>
<td>Ether extract</td>
<td>73.75±0.01a</td>
<td>75.82±0.19a</td>
</tr>
<tr>
<td>AME (Kcal/Kg)</td>
<td>275±1.10b</td>
<td>279±1.22a</td>
</tr>
</tbody>
</table>

Different superscripts along the row indicate significant difference (p <0.05). For composition of feed for different group, see Table II.
DISCUSSION

In the present study growth performance parameters like body weight gain and feed conversion ratio were improved by the supplementation of dried citrus pulp in the broiler diet. The current study’s findings are in agreement with those of Abbasi et al. (2015), who found that supplementing broiler chicken feed with 1.5% dried sweet orange peel promoted feed intake and weight gain in 1-21 days after hatch. Similarly, Faiz et al. (2017) observed improved production performance in broiler chicken supplemented with dried citrus pulp. The citrus pulp contains compounds with antibiotic-like properties that can reduce the population of harmful bacteria in the intestine; thereby improving overall gut health and potentially leading to better weight gain (Nannapaneni et al., 2018). The citrus pulp contains high levels of phenolic compounds, flavonoids, and limonoids, which have antioxidant properties that protect against oxidation in the aqueous medium of the cell (Vlaicu et al., 2020; Klimeczak et al., 2007) and these compounds have been shown to have antioxidant properties, meaning they can help neutralize harmful free radicals and protect cells from oxidative damage (Jayaprakasha and Patil, 2007). Among carcass characteristics, dressing parentage was significantly improved by the supplementation of citrus pulp in the broiler diet. Serum biochemical parameters can provide important information about a bird’s overall health status, including its immune function, organ function, and metabolic state. The use of *Citrus sinensis* in different concentrations yielded highly significant results for both liver enzymes i.e., ALT and AST and these results are in line of study with the findings of Christaki et al. (2011), who stated that 6% digestible crude protein has no impact on the performance of quail. Similarly, Ndelekwute and Enyenihi (2017) concluded significant results for EE and protein digestibility in broilers fed with lime juice but no significant effect on the dry matter, crude fiber, and ash levels of broilers. Similarly, Oluremi et al. (2006) demonstrated that citrus peel @ 30% could be used as an alternative source against corn. Ndelekwute and Enyenihi (2017) concluded significant results for ether extract and protein digestibility in broilers fed with lime juice but no significant effect on the dry matter, crude fiber, and ash levels of broilers. Moreover, Silva et al. (2013) reported that carbohydrates obtained from orange pulp especially pectin has a significant effect on the digestibility of broilers both in the starter and finisher phase. Similarly, Amaga et al. (2019) stated a significant effect of dried sweet orange pulp on the nutrient digestibility in broilers used at different levels. Silva et al. (2013) also checked the effect of pectin extracted from citrus pulp on the different digestibility parameters and the results revealed that pectin increased the intestinal viscosity while decreasing the excreta moisture content along with a significant change recorded in the levels of apparent metabolizable energy. Oluremi et al. (2019) studied the effect of dried sweet orange pulp on different digestibility and blood serum parameters and the results showed high levels of metabolizable energy which ultimately led to the suggestion of the use of sweet orange pulp as a source of energy. The slight variation in this research seems by chance and there is very limited data available regarding the use of sweet orange pulp in the literature. There may be multiple reasons for this variation, which include the sex, age, and heredity of the birds while the source of the sweet orange pulp may also contribute to this change. The higher crude fiber concentration in the pulp may determine the low performance of broilers.

CONCLUSION

Dietary supplementation of dried orange (*Citrus sinensis*) pulp has a useful effect on growth performance, ileal nutrient digestibility, and liver health in broilers and can be used in broiler diets up to 1.5g/kg feed without a negative effect on overall performance, nutrient digestibility, and liver health.
ACKNOWLEDGEMENT

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IRB approval

The departmental board of studies approved the experimental protocols (Approval number: 189-2022/PS/UAP.

Ethical approval

The ethical committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Pakistan approved all the experimental procedures adopted.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES


