

Research Article



Effects of Dietary Natural Antioxidants from Citrus Waste on Growth and Blood Antioxidants Status of the Broilers

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Abstract | Citrus waste is mostly wasted after juice extraction around the world which contains compounds that have positive effects on the health of animals and poultry. In the current studies the citrus waste is dried and mixed with the broiler feed replacing maize from the feed at 0%, 2.5%, 5%, 7.5% and 10%. 225 birds were reared in 5 treatments and 3 replications of each treatment having 15 birds in each replication. The chicks were being provided with the standard conditions for light, water, vaccination and experimental feed for 42 days. The body weight gain, feed intake and the feed conversion ratio was observed weekly. After 42 day of the growth, the birds were slaughter with the Islamic Halal Method and the blood samples of the birds were preserved for antioxidant enzymes assay analysis. The data showed that as the citrus waste percentage in the feed of the broiler was increased, its feed intake and the body weight decreased but the citrus waste has non-significant effect on the feed conversion ratio of the broiler birds. The results also showed that citrus waste in the feed have positive effect on the antioxidant status of the broiler birds. The activity of the superoxide dismutase, catalase and glutathione peroxidase in the broiler blood was increased as the citrus waste level in the feed of the broilers was increased. The citrus waste addition in the feed of the broiler have non-significant effects on the growth parameters, however it has positive effects on the antioxidant enzymes profile of the broilers.

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Introduction

More than 120 million metric tons of citrus is produced in the world and above 50% percent of the citrus is utilized in the juice production and after juice extraction about 60% of the citrus waste is lost which contains valuable antioxidants. The waste of the citrus can be utilized in the feed of the broilers and as the citrus peel is increased in the ration of the broiler, its weight increased, feed intake of the birds increased and the feed gain ratio decreased. Further-

more, broiler chicks fed on the higher levels of citrus peel ultimately decreased the total cholesterol and triglycerides of the broiler birds (Chaudry et al., 2004).

By addition of citrus peel extract in the feed of the broilers, all the blood parameters like cholesterol, uric acid, glucose, low density lipoprotein, low density lipoprotein were influenced significantly Ebrahimi et al. (2014). Mixed waste of different juices like, apple, mango, carrot, citrus, avocado, melons and tomato in the feed of the broilers as a corn replacement de-

creased feed conversion ratio in the broilers feed as the percentage of the juice waste was increased in the broiler ration. Abdominal fats of broiler birds which were fed on the higher levels of the juice waste were also reduced (Rizal et al., 2010). Feeding the broiler birds with the natural antioxidants from the different sources had found efficient in enhancing growth, microbiological and immunological parameters of the broiler. Beet roots extracts in the feed of the broilers improved overall body weight but tomato puree and ginger root extract group reduced the feed conversion ratio of the broilers. The natural antioxidants in the feed improved the antioxidant status of the broiler birds and depressed the bacterial count (Selim et al., 2013). Objective of the undergoing project was to see the effect of the citrus waste from the juice processing industries of the Sargodha region on growth parameters and antioxidant profile of the blood of the broilers.

Materials and Methods

The research was conducted in the National Institute of Food Science and Technology, University of Agriculture Faisalabad. A day old broiler chicks were purchased from the Aljadeed Hatchery Rawalpindi and were shifted to the broiler farm at the Department of Animal Nutrition, University of the Agriculture Faisalabad for further rearing. Chemical used in this study for the analysis were of analytical grade and were purchased from Sigma Lahore. Ethical guidelines for the rearing and slaughtering of the birds have been followed in the research. The feed of the broiler was prepared with the addition of the citrus waste from the juice processing plants. The citrus waste was dried at 60-65°C in the evaporator (R-5A) and mixed with the other ingredients of the feed. The feed ingredients and the treatment plan are presented in Table 1.

The birds were reared for 42 days and the optimum experimental feed and the standard conditions for the rearing of birds were provided to the birds. Measured the gain in body weight of experimental birds on weekly basis for each group during the trials in response to treatments in order to determine the effect of formulated feed supplemented with the citrus waste. Weekly basis measurement of the net feed intake of individual group of birds were through excluding spilled and left over diet from the total diet offered during a week. Feed conversion ratio was recorded during trials for each group of birds on weekly

basis to check the efficiency of feed, formulated according to the method of Sohaib et al. (2012).

Antioxidant enzymes assay: At the age of 42 days, birds were slaughtered by the Halal Islamic method. The blood sample of slaughtered birds was collected using Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant. The blood samples were centrifuged at 3000 rpm to separate plasma for enzymes analysis. Antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase from broilers blood samples were analyzed.

Table 1: Composition of experimental feeds and treatment plan.

Ingredients	CW ₀	CW _{2.5}	CW ₅	CW _{7.5}	CW ₁₀
Maize	54	51	48	45	42
Wheat bran	5	5	5	5	5
Rice polishing	5	5	5	5	5
Fish meal	5	5	5	5	5
Blood meal	5	5	5	5	5
Citrus peel	0	2.5	5	7.5	10
Cotton seed meal	5	5	5	5	5
Soybean meal	5	5	5	5	5
Corn gluten	7	7	7	7	7
Molasses	5	5	5	5	5
Iodized salt	0.25	0.25	0.25	0.25	0.25
Rapeseed meal	0	0.5	1	1.50	2
Dicalcium Phosphate	1.25	1.25	1.25	1.25	1.25
Limestone	1.25	1.25	1.25	1.25	1.25
Vitamin mineral premix	1.25	1.25	1.25	1.25	1.25
Total	100	100	100	100	100
Protein (%)	22.22	22.35	22.41	22.31	22.27
ME (MJ/kg)	1.4	1.4	1.35	1.32	1.30

Superoxide Dismutase (SOD): activity was determined according to Ellerby and Bredesen (2000). Stock solution of 6-hydroxydopamine with the concentration of 15 ml were added to 1 ml of 0.05 M sodium phosphate, 0.01 mM diethylenetriaminepenta acetic acid (pH 7.4) and 100 ml serum. The auto oxidation of 6- hydroxydopamine was recorded at 490 nm in triplicate. One unit of enzyme activity is presented as mg of protein resulting in 50% inhibition of 6-hydroxydopamine autooxidation per min. A purified SOD used as standard and the SOD activity was expressed as unit/mg protein.

Catalase: was determined by the method of Block et al. (1980). Briefly, in a quartz cuvette containing

Table 3: Effect of treatments and growth period on feed utilization of broiler.

Treatment	7 days	14 days	21 days	28 days	35 days	42 days	Means
CW ₀	225.0o	722.3mn	1100.3l	1682.9hi	2364.4f	3434.6a	1588.3AB
CW _{2.5}	233.3o	717.6n	1089.2l	1709.5h	2452.5e	3389.2b	1598.6A
CW ₅	242.3o	755.2m	1142.3k	1655.3i	2343.4f	3316.0d	1575.8B
CW _{7.5}	229.5o	715.5n	1094.8l	1650.8i	2282.3g	3361.0bc	1555.7C
CW ₁₀	231.1o	723.5mn	1110.0kl	1460.7j	2360.5f	3337.4cd	1537.2D
Means	232.2F	726.8E	1107.3D	1631.9C	2360.7B	3367.6A	

Means carrying similar letters in a column are significantly identical; CW: Citrus waste at level of 0, 2.5, 5, 7.5, and 10%

Table 2: Effect of treatments and weeks on weight of broiler (g).

Treat-ment	7 days	14 days	21 days	28 days	35 days	42 days
CW ₀	146.4q	451.4op	675.1n	1011.8k	1364.1g	1887.2a
CW _{2.5}	147.3q	455.2o	677.9n	1015.5k	1383.0f	1876.8b
CW ₅	146.8q	450.9op	677.2n	999.1l	1355.0h	1856.9c
CW _{7.5}	144.1q	445.7p	676.8n	994.5l	1344.6i	1839.9d
CW ₁₀	146.3q	446.8p	677.3n	985.6m	1323.2j	1830.5e
Means	146.2F	450.0E	676.9D	1001.3C	1354.0B	1858.3A

Means carrying similar letters in a column are significantly identical; CW: Citrus waste at level of 0, 2.5, 5, 7.5, and 10%

100 ml 1 M Tris and 5 mM EDTA buffer (pH 8.0), 100 ml saturated thymol, 100 ml aminoantipyrine, 100 ml peroxidase (1 U/ml), 50 ml saturated thymol H₂O₂ and 540 ml H₂O, mixed with 10 ml serum sample, then measured at 505 nm for 5 min at 58°C by a spectrophotometer. One unit of catalase decomposes 1.0 mM of H₂O₂ per min. Purified catalase was used as standard and the enzyme activity was expressed as unit/mg protein.

Glutathione Peroxidase: was determined as the method described by Helen and Vijayammal (1997). 0.8 ml substrate contain 1 mM EDTA, 1 mM NaHCO₃, 0.2 mM NADPH, 1 U/ml glutathione reductase, 1 mM glutathione and 100 mM KH₂PO₄ (pH 7.0) were mixed with 25 ml serum sample and kept at room temperature for 5 min. The reaction was started by addition of 0.1 ml 2.5 mM H₂O₂ and then measured using a spectrophotometer at 340 nm for 3 min at 58°C. One unit is defined as 1 nM NADPH oxidized per mg protein per min.

Statistical Analysis

The data obtained from each treatment was subjected to statistical analysis to determine the level of significance by factorial design (two-way interaction) using Statistical Package (Statistic 8.1) following Stee et al. (1997).

Results and Discussion

Body weight of broilers

The broiler weight has been reduced significantly as the citrus waste supplementation in the feed on the broilers was increased (Table 2). The decreasing trend in final body weight may be because of increased fiber contents of feed as a result of citrus waste supplementation. The mean values for body weight during growth period increased significantly with the progression of growth period. This increase is a normal trend as with increase in age the body weight of birds also increases to get their maturity. These finding are in agreement with Oluremi et al. (2010) who studied the replacement of maize from feed with fermented sweet orange peel in broiler starter diet. They observed that replacement of the maize with the fermented dried orange waste reduced the body weight of the starter broiler. Anni and Zeidler (2004) observed a decrease in bird's growth parameter by supplementation of tomato pomace in broiler feed and stated a reason of high fiber content in supplemented diets. The broilers birds fed on flax seed based feed deceased body weight, while feed intake and feed conversion ratio of the birds was increased (Anjum et al., 2013). The body weight decreased and feed conversion efficiency increased in broilers receiving much higher dietary concentrations of natural antioxidants (Malayoglu et al., 2009).

Feed utilization

It has been observed that Feed utilization increased significantly with the passage of experimental weeks (Table 3). Feed utilization of the broiler birds decreased as the concentration of the citrus waste in the feed is increased and maximum feed utilization was observed in the treatment which was fed on 2.5% citrus supplemented feed (CW_{2.5}) and minimum feed utilization was observed in the treatment which was fed on 10% citrus supplemented feed (CW₁₀).

Table 4: Effect of treatments and growth period on feed conversion ratio of broiler.

Treatment	7 days	14 days	21 days	28 days	35 days	42 days	Means
CW ₀	1.54o	1.60k-m	1.63h-k	1.66d-g	1.73c	1.82a	1.66BC
CW _{2.5}	1.58mn	1.57mn	1.60 k-m	1.68d-f	1.77b	1.80a	1.67B
CW ₅	1.53no	1.58l-n	1.61j-l	1.65f-i	1.68d-f	1.81a	1.65C
CW _{7.5}	1.60klm	1.62i-k	1.64g-j	1.48p	1.75bc	1.81a	1.65C
CW ₁₀	1.65efgh	1.69d	1.68de	1.68d-f	1.77b	1.81a	1.71A
Means	1.59E	1.61D	1.63C	1.64C	1.74B	1.81A	

Means carrying similar letters in a column are significantly identical; CW: Citrus waste at level of 0, 2.5, 5, 7.5, and 10%

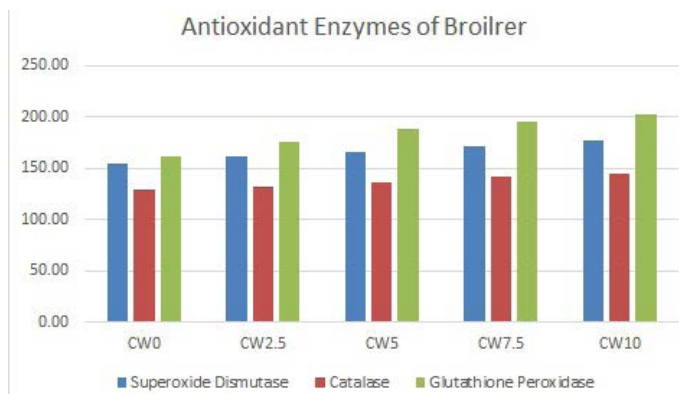


Figure 1: Antioxidant enzymes assay of the broiler blood, fed on citrus waste based feed

The broilers fed on orange waste replacing yellow corn have bad effects on body weight gain, feed intake and feed conversion ratio (Abdel-Moneim et al., 2014). Oluremi et al. (2010) while replaced maize from feed with fermented sweet orange peel in broiler starter diet observed that replacement of the maize with the fermented dried orange waste reduced the body weight and feed utilization of the starter broiler.

Feed conversion ratio

The feed conversion ratio of the broiler increased as the citrus waste in the feed is increased and maximum feed conversion ratio was observed for the treatment with 10% citrus waste (CW₁₀) in the feed and minimum feed conversion ratio was observed for CW₀ (Table 4). The feed conversion ratio of the broilers increased as the production period of the broiler proceeded. Rezaeipour et al. (2011) observed in their study that feed conversion ratio of the broiler birds increased as total phenolic in the feed of broilers increased. Sohaib et al. (2012) recorded higher feed conversion ratio in the broiler groups which were fed on the alpha tocopherol supplementation in the feed of broilers. It has been observed that feed conversion ratio of the meat was less at 20% inclusion of the sweet orange rind in the diet as this group needed more feed per unit gain of the broiler (Oluremi et

al., 2006). Feed consumption, weight gain, and feed conversion ratio of the broilers was improved as the percentage of the dried juice wastes of different fruits (carrot, apple, mango, avocado, orange, melon and tree tomato) were used in the ration (Rizal et al., 2010).

Antioxidant enzymes assay of the broiler blood serum

The results for superoxide dismutase, catalase and Glutathione peroxidase of the broiler blood fed on citrus waste supplemented showed highly significant effect. The mean values of superoxide dismutase, catalase and glutathione peroxidase are expressed in Figure 1, which showed that broilers fed on CW₀ (control feed) showed the lowest value of these enzymes in their serum and an increasing trend was observed with increase in amount of citrus waste supplementation in broiler feed. The highest value of antioxidant enzymes was observed for treatment CW₁₀ (broilers which were fed on 10% citrus waste supplemented feed).

Addition of vitamin E + selenium significantly raised the level of superoxide dismutase in the blood serum of the broilers which increased from 106.65 in control to 265.5 in the Vitamin E + selenium group in the broilers (Tras et al., 2010). The activities of catalase and glutathione were independently influenced by concentrations of antioxidants and α-tocopherol (Srilatha et al., 2010). The glutathione and catalase levels were significantly increased in the feed containing antioxidants at 60 mg/kg. Ciftci et al. (2010) fed cinnamon oil in addition to the basal feed to broiler to see the effect of this on antioxidant enzymes and cholesterol level of the serum and the meat of the broiler. The antioxidant enzymes like glutathione peroxidase and the catalase were increased in the groups of the broilers which were fed on 1000ppm cinnamon oil. Glutathione peroxidase level of the birds was increased when the feed supplemented with the lemon and peel extracts was provided to the broilers (Akbar-

ian et al., 2014).

Conclusion

Pakistan is a major producer of the citrus fruit. The fruit waste is lost after juice extraction which has valuable antioxidants which are utilized in the ration of the broiler meat in replacement with the corn in the meal. The citrus waste replacement with the corn not only reduced the cost of the feed but also have good effect on the antioxidants properties of the broiler. The results showed that the body weight gain and feed intake of the broiler groups decreased as the more amount of citrus waste in the broiler feed but the feed conversion ratio of the broilers remained non-significant. The positive effects of citrus waste on the antioxidants profile of the broiler birds is an indication that the broiler meat consumption should have positive effects on the human being.

Authors Contributions

Furukh Faiz had performed all the practical work, from rearing of the birds to the analysis of blood samples. Muhammad Issa Khan had designed the project worked on designing the experimentation and approval of the version for publication. Masood Sadiq and Haq Nawaz had helped on management of the broiler farm for rearing of the birds and performed the statistical analysis. Furukh Faiz had written the draft manuscript and Muhammad Issa Khan had corrected it. Finally all the authors of the paper read the paper and approved the final manuscripts.

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