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Original Articles

Vegetable Waste Inclusion in Broiler Diets and its Effect on Growth Performance, Blood Metabolites, Immunity, Meat Mineral Content and Lipid Oxidation Status

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ABSTRACT

The aim of present study was to evaluate the effect of feeding vegetable waste (VW) to broilers on their growth performance, serum chemistry, immune status, meat mineral content and lipid oxidation status. For this purpose, 100 one-day-old chicks (Cobb 500) were acquired from a commercial hatchery and allocated according to a completely randomized design into five dietary treatments with four replicates of five birds each. The dietary treatments included: T1 (100% commercial feed (CF) + 0% VW), T2 (75% CF + 25% VW), T3 (50% CF + 50% VW), T4 (25% CF + 75% VW) and T5 (0% CF + 100% VW). Experimental birds were subjected to dietary treatments from 5 to 7 weeks of age. At the end of week 7 (49 days), eight birds with uniform average body weight were selected per treatment (2birds/replicate), kept off-feed for 4 h and then manually slaughtered according to the Halal method to collect data for serum chemistry, meat minerals and lipid oxidation status. The results indicated better meat lipid oxidation status ($p \leq 0.05$) and lower meat mineral content ($p \leq 0.05$) when birds fed VW at 25, 50, 75, and 100% of the diets compared with 100% commercial feed. On the other hand, blood chemistry and antibody response parameters did not respond ($p>0.05$) to dietary intervention. In conclusion, dietary inclusion of vegetable waste had positive influence on meat quality in terms of meat lipid oxidation and meat mineral content, and may be replace up to 75% of commercial broiler feeds with beneficial effects.

INTRODUCTION

In comparison with beef, mutton and pork, broiler meat consumption has shown an increasing trend across the globe mainly due to affordable prices and high availability. In addition, its consumption has no religious bindings as, in particularly, in the case of beef (Hindus) and pork (Muslims) and has an overall acceptable nutritional profile and taste.

Due to the increasing availability and consumption of broiler meat, consumers have now started showing interest in its micronutrient profile, taste, juiciness and health benefits. Feeding commercial rations has supported the rapid growth rate broilers, but consumers often complain about poor juiciness and taste of chicken meat. Slowing the growth of commercial broilers has shown to improved meat quality and supplementing vegetable waste to slow down their growth rate, supplying only their maintenance requirements, without adding much to the production cost is an emerging trend (Hossain *et al*., 2013).

Additionally, vegetables are rich nutrient sources, potentially good for supplying essential amino acids, minerals and antioxidants to the birds, comparatively inexpensive, easily available, easy to process and pose less risk of disease contamination (Omenka & Anyasor, 2010). Consumers prefer the meat of broilers exclusively fed with vegetable sources (Mendes, 2003) as they feel it is more tasty, juicy, and tender. However, it should be also considered that vegetable sources contain numerous anti-nutritive factors (ANF), lower protein quality, lower digestibility and lower biological value compared with animal sources (Dublecz, 2003; Vieira *et al*., 2003) and can affect meat quality. Diets with vegetables may possibly be nutritionally inferior and broilers fed on such diets may or may not respond positively (Hossain *et al*., 2013).

Due to changes in life style across the globe, people are becoming more health-conscious, increasing the demand for functional and organic foods. Broiler meat with increased levels of natural antioxidants may be declared as a functional food. Chicken meat is rich in polyunsaturated fatty acids (PUFA), which are more sensitive to free-radical attack, leading to oxidative damage (Arshad *et al*., 2011). Meat quality is negatively affected when free radicals initiate the oxidation reaction and destroy the normal muscle fiber, accumulating harmful byproducts in the muscle (Brenes *et al*., 2008). Meat discoloration post mortem is predominantly caused by the oxidation of myoglobin (Faustman *et al*., 2010). It is assumed that vegetables/green leafy plants fed to broilers may improve the antioxidant status of meat and reduce the harmful effects of oxidative damage. Hence, this experiment was planned with multiple objectives, in particular, to study the effect of cheapest vegetable wastes available in the nearby market on overall growth performance, immune profile, serum chemistry and, most importantly, meat mineral and antioxidant status of broilers.

MATERIALS AND METHODS

The study was conducted at the broiler experimental unit, Department of Poultry Production, University of Veterinary and Animal Sciences (UVAS), Ravi Campus, Pattoki, Pakistan.

In total, 100 one-day-old chicks (Cobb-500) were acquired from a local hatchery and fed a conventional diet (standard commercial broiler feed) for the first four weeks. Birds were vaccinated and received medical care according to the standard veterinary practices. At 28 days old, 100 birds with uniform body weight were selected and distributed into five dietary treatments according to a completely randomized design (Steel *et al*., 1997), with four replicates of five birds each. The treatments consisted of: T1 (100% commercial feed (CF) + 0% VW), T2 (75% CF + 25% VW), T3 (50% CF + 50% VW), T4 (25% CF + 75% VW) and T5 (0% CF + 100% VW).

Vegetable waste included alfalfa, spinach and cauliflower (fresh, on equal weight basis) collected in the local market. Representative samples of 10 g each of vegetable waste (alfalfa, spinach and cauliflower) were collected, stored, and their chemical composition was analyzed (AOAC, 2006). The nutritional composition of different vegetable wastes used in this study are presented in Table 1.

Table 1 – Chemical composition of the vegetable wastes fed with different levels.

Vegetable Waste	Dry Matter (%)	Crude Protein (%)	Ash (%)	Ether Extract (%)
Alfalfa	28.16	19.80	9.36	2.13
Cauliflower	11.64	10.63	6.56	1.38
Spinach	8.10	36.40	22.33	3.30

Housing and Management

The experimental birds were housed on litter-covered floor at a stocking density of 0.0604 m² per bird. They had free access to feed and water in pan feeders and nipple drinking system, respectively. Standard management practices were applied. House temperature and relative humidity (RH) were maintained at 35 ± 0.5°C and 62 ± 3%, respectively, for first week after hatch. After that, temperature was gradually decreased until it reached 24°C on day 21 with RH 65%. A 24-h continuous light schedule was applied throughout the study period.

Growth performance

Birds, feed offer and feed residues were recorded to calculate feed intake (g) and weight gain (g) on weekly basis. Feed efficiency was calculated as the ratio (g/g) between weight gain (WG) and feed intake (FI). Mortality (if any) was recorded on daily basis to calculate percent mortality. At 49 days of age, eight birds (2 birds/replicate) were randomly selected from each treatment. Birds were individually weighed, and tagged on the shanks according to dietary groups. The birds were then transported to the Department of Meat Science and Technology, UVAS, Lahore, and manually slaughtered according to the Halal method. Broilers were kept off-feed for four hours prior to slaughter.

Serum chemistry and antibody response

Blood biochemistry and antibody response analyses were conducted at the Quality Operations Laboratory and University Diagnostic Lab, UVAS, Lahore.

For blood biochemistry analyses, 5mL blood was collected from the jugular vein of three birds per treatment into a vacutainer tube with no anticoagulant. The blood was centrifuged at 3,000 rpm for 15 min and the serum was separated and stored at -20°C until analyses. The serum was then thawed, and the concentrations of cholesterol, triglycerides, glucose, total protein, albumin, globulin, uric acid and creatinine were determined in a spectrophotometer using commercially available kits at specific wave lengths. Antibody titers against Newcastle Disease (HI test; Allan & Gough 1974), Infectious Bronchitis (ELISA; Piela *et al*., 1984), *Salmonella* Pullorum (HA test; Minga & Wray 1992) and *Mycoplasma gallisepticum* (ELISA; Piela *et al*., 1984) were determined.

Meat mineral profile and lipid oxidation status

The analysis of meat mineral profile was conducted at the Department of Environmental Science, UVAS, Lahore, while lipid oxidation tests of the meat samples were conducted at Lipid Chemistry lab, Department of Dairy Technology, UVAS, Ravi Campus, Pattoki.

Breast meat samples, weighing 50g, were collected from five birds per treatment and stored at -20ºC. Samples were then analyzed for macro and trace minerals, including calcium (flame photometer; Tuba, 2015), phosphorus (spectrophotometer; Adeyeye, 2009), magnesium (atomic absorption spectrophotometer; Adeyeye, 2009), sodium (flame photometer; Tuba, 2015), manganese and zinc (atomic absorption spectrophotometer; Adeyeye, 2009) and iron (atomic absorption spectrophotometer; Kilic, 2002).

In addition, breast meat samples, weighing 100-125g, were collected from each treatment and stored at -20ºC for 45 days. After 45 days, samples were analyzed for total phenolic contents (Al-Farsi *et al*. 2005), total flavonoid contents (Schieber *et al*., 2003) and thio-barbituric acid values (AOCS, 1995).

Statistical Analysis

The collected data were analyzed by one-way analysis of variance according to a completely randomized design (Steel *et al*., 1997) in SAS 9.4 statistical package (SAS Institute, 2013). Means were compared by Duncan's Multiple Range (DMR) test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance

In the present study, initial body weight (28 days of age) was not significantly different among treatments (Table 2), indicating that birds presented uniform body weight at the start of experiment. Birds fed 100% CF + 0% VW (T1) showed the highest final body weight and weight gain, and the best feed efficiency, followed by those fed 75% CF+ 25% VW (T2), 50% CF + 50% VW (T3), 25% CF + 75% VW (T4) and 0% CF + 100% VW (T5), respectively (Table 2). The gradual worsening of live performance parameters obtained as VW dietary levels increased may be attributed to the nutritional variation of the diets, as VW contains high fiber and low nutrient levels (Livingstone *et al*., 1980; Mustafa & Baurhoo, 2017). Therefore, high VW inclusion levels in diet resulted in lower nutrient intake and retarded growth. In addition, high fiber dietary content also causes poor feed efficiency (Thacker & Petri, 2009). The results of the present study are consistent with those reported by Westendorf & Myer (2009), who also observed slow broiler growth rate when vegetable waste was added to the diet. In a similar study, Oghenebrorhie & Oghenesuvwe (2016) obtained comparable final body weight (p>0.05) but worse feed efficiency when 10% of a conventional feed was replaced with vegetable sources. Bhuiyan *et al*. (2012a, b) fed broilers with diets supplemented with vegetable sources, but did not observe any significant differences in the growth performance. Onu (2010), on the other hand, obtained higher final body weight with the inclusion of 25% vegetable waste compared to control diet in broilers. Tasirnafas *et al*. (2015) supplemented ostrich chick diets with 0, 10, 20, or 30% vegetable waste, and obtained found significantly higher final body weight and weight gain in the 20% and 30% treatments as compared to control group.

Surprisingly, no mortality was observed in any of the treatment groups (Table 2) during the entire experimental period, which clearly indicates that the diets had no adverse effects on the birds' health status. Oghenebrorhie & Oghenesuvwe (2016) also reported no mortality during the entire experimental phase when broilers were fed diets with 5%, 10% and 20% of a vegetable source.

Table 2 – Growth performance of broilers fed increasing levels of vegetable waste (means±SEM).

Treatments	Initial weight (28 days) (g)	Final weight (at 49 days) (g)	Weight Gain (g)	Feed Efficiency[§]
Control (100% CF[+] + 0% WV[$])	1301.90±2.50	3089.45±4.08[a]	1787.55±4.06[a]	0.42±0.001[a]
75% CF[+] + 25% VW[$]	1304.20±2.32	2708.35±16.33[b]	1404.15±15.81[b]	0.24±0.006[b]
50% CF[+] + 50% VW[$]	1305.90±2.50	2287.90±11.02[c]	982.00±11.22[c]	0.11±0.001[c]
25% CF[+] + 75% VW[$]	1302.20±2.32	1784.30±12.53[d]	482.10±13.62[d]	0.03±0.005[d]
0% CF[+] +100% VW[$]	1310.65±2.58	1342.50±3.47[e]	31.85±1.66[e]	0.00±0.00[e]
p-value	0.0857	<0.0001	<0.0001	<.0001

*Means followed by different superscripts indicate significant differences ($p \leq 0.05$) among treatments.

[+]CF (Commercial Feed)

[$]VW (Vegetable Waste)

[§]Feed efficiency based only on commercial feed intake

Serum Chemistry

Total protein and globulin serum contents were significantly ($p \leq 0.05$) higher in the control group, followed by a gradual decrease as vegetable waste supplementation levels increased. No significant differences ($p > 0.05$) were observed in serum cholesterol, triglycerides, glucose, albumin, uric acid or creatinine levels among treatments (Table 3). The higher total protein and globulin contents obtained in the broilers of the control group may be attributed to their high growth rate (Table 2) as growth rate is associated with higher concentration of proteins in serum (Kapelanski *et al*., 2004; Krames, 2010). Moreover, the reduced total protein and globulin contents as the dietary vegetable waste level increased may be due to the defatted part of vegetables, due to their high fiber content (25.24 %) and lower CP content compared with the commercial feed, thereby decreasing absorption in the small intestine (Zhang *et al*., 2009) resulting in malabsorption and a decrease in total protein and globulin contents in the blood serum. Zhang *et al*. (2009) also found a trend of higher serum total protein levels in 21- and 42-d-old broilers fed a diet supplemented with dry ginger root compared with the control diet. However, Onu (2010) did not observe any differences in serum total protein, globulin, albumin, glucose, uric acid, triglyceride and creatinine levels between broilers fed diets supplemented with vegetable waste or a control diet, indicating that some serum biochemistry parameters, such as glucose, total cholesterol, and albumin are not necessarily dependent on vegetable waste inclusion levels (Sadeghi *et al*., 2016).

Table 3 – Serum chemistry of broilers fed increasing levels of vegetable waste (means±SEM).

Treatments	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)
Control (100% CF[+] + 0% VW[$])	120.00±36.77	40.00±20.07	188.33±33.62	2.10±0.47[a]	0.30±0.15	1.80±0.40[a]	4.30±1.55	0.27±0.03
75% CF[+] + 25% VW[$]	104.67±22.24	10.30±7.86	254.00±38.43	2.03±0.07[a]	0.27±0.09	1.77±0.12[a]	4.43±1.55	0.20±0.06
50% CF[+] + 50% VW[$]	78.33±14.08	10.33±4.06	170.00±21.79	1.60±0.36[ab]	0.47±0.07	1.13±0.30[ab]	2.30±0.51	0.30±0.06
25% CF[+] + 75% VW[$]	99.00±4.00	12.33±6.71	171.33±16.05	2.07±0.52[a]	0.70±0.25	1.37±0.43[a]	5.67±1.03	0.70±0.09
0% CF[+] + 100% VW[$]	86.00±15.10	35.67±19.65	160.67±0.88	0.53±0.09[b]	0.30±0.06	0.23±0.03[b]	6.13±0.20	0.27±0.09
p-value	0.6850	0.4508	0.1510	0.0493	0.2454	0.0242	0.2050	0.5024

*Means followed by different superscripts indicate significant differences ($p \leq 0.05$) among treatments.

[+]CF (Commercial Feed)

[$]VW (Vegetable Waste)

Antibody Response

In the present study, antibody titers against Newcastle Disease (ND), Infectious Bronchitis (IB) and *Mycoplasma gallisepticum* (MG) were not influenced ($p > 0.05$) by the dietary treatments. All treatment groups were negative for *Salmonella* Pullorum (SP) (Table 4). The lack of significant differences in immune response parameters among different dietary treatments suggests that reducing the intake of commercial feeds by feeding higher concentrations of vegetable waste did not negatively affect the broilers' immune status (Table 4). Zhang *et al*. (2009) observed reduced growth rate due to lower nutrient intake, but no differences in the immune profile broilers fed ginger root. Yu *et al*. (2009) did not find any significant differences in the immune response of broilers receiving vegetables with their diet, and concluded that the addition of either 1 or 3% level in the diet has no beneficial effect on

Table 4 – Antibody response of broilers fed increasing levels of vegetable waste (means±SEM).

Treatments	ND titer	IB titer	SP detection	MG detection
Control (100% CF+ + 0% WV$)	3.67±1.20	5.69±4.69	Negative	0.40±0.17
75% CF+ + 25% VW$	4.33±0.88	1.00±0.00	Negative	0.11±0.01
50% CF+ + 50% VW$	6.67±0.88	112.51±40.74	Negative	0.12±0.00
25% CF+ + 75% VW$	6.33±0.33	184.63±183.63	Negative	0.15±0.03
0% CF+ + 100% VW$	7.00±0.58	37.50±36.50	Negative	0.017±0.05
p-value	0.0591	0.5268	Negative	0.1562

*Means followed by different superscripts indicate significant differences ($p \leq 0.05$) among treatments.

+CF (Commercial Feed)

$VW (Vegetable Waste)

the antibody production in broilers (Jafari *et al*., 2008). Moreover, blood biochemistry values obtained in the present study were within the normal range for chickens i.e., blood glucose levels in the range 200 to 500 mg/dL, blood protein 2.5 to 4.5 g/dL, albumin and globulin in the range of 0.5 to 1.8 g/dL (Thrall, 2007).

Meat Mineral Profile

Magnesium (Mg) levels were significantly ($p \leq 0.05$) higher in the meat of birds fed the control diet, whereas sodium, phosphorus, calcium, zinc, manganese and iron levels were not affected by the dietary treatments (Table 5). After potassium, magnesium is second most abundant mineral present in the muscle tissue (Suttle, 2010). The birds in the control group showed the highest weight gain weight (Table 2) and highest Mg meat content, suggesting the higher Mg level of the commercial feed resulted in higher Mg deposition. In line with these findings, it was reported that supplementation of vegetable sources during the growing phase may result in lower mineral contents in meat of broilers (Teeter & Deyhim, 1994). Vegetable waste is usually considered as rich source of minerals (Omenka & Anyasor, 2010). However, in the present study, no significant differences were observed for most of the evaluated minerals. Commercial poultry feeds typically contain mineral levels to supply the birds' requirements (Leeson & Summers, 2005). Therefore, the obtained results may be correlated as the birds fed the diets with higher VW supplementation had lower feed intake and vice versa, resulting in comparable mineral contents among all dietary treatments.

Table 5 – Meat mineral profile of broilers fed increasing levels of vegetable waste (means±SEM).

Treatments	Na(mg/L)	P(mg/L)	Mg(mg/L)	Ca(mg/L)	Mn (mg\L)	Zn(mg/L)	Fe(mg/L)
Control (100% CF+ +0% WV)	577.50±91.39	505.83±7.41	134.75±0.38[a]	5159.17±45.51	Zero	4.33±0.85	31.92±0.98
75% CF+ + 25% VW$	456.67±18.78	462.50±13.77	132.42±0.46[ab]	5051.67±43.21	Zero	4.83±1.46	38.08±0.87
50% CF+ + 50% VW$	420.00±6.61	449.17±23.02	131.00±0.38[bc]	5162.50±35.56	Zero	4.25±1.44	42.33±6.21
25% CF+ + 75% VW$	429.17±28.22	460.83±14.53	129.75±1.51[cd]	4964.17±119.18	Zero	3.42±1.20	38.17±4.41
0% CF+ + 100% VW$	759.17±172.16	470.83±4.41	128.41±0.36[d]	4683.33±267.16	Zero	5.17±1.42	27.92±1.10
p-value	0.0980	0.1287	0.0014	0.1492	Zero	0.8949	0.0940

*Means followed by different superscripts indicate significant differences ($p \leq 0.05$) among treatments.

+CF (Commercial Feed)

$VW (Vegetable Waste)

Meat Lipid Oxidation

The replacement of the commercial feed with increasing levels of vegetable waste had a marked effect on the meat antioxidant capacity of broilers (Table 6). Vegetable waste inclusion improved both total phenolic contents (TPC) and total flavonoid contents (TFC) in the meat as the highest TPC and TFC were determined in the meat of birds fed 100% VW, followed by those of fed diets containing 75% CF + 25% VW, 50% CF + 50% VW and 75% CF + 25% VW and 100 CF + 0% VW (Table 6). This increase in meat TPC and TFC contents may be explained by the fact that vegetables are rich sources of carotenoids, which have potent antioxidant capacity (Osterlie & Lerfall, 2005) and are mainly responsible for the protection against oxidative damage at cellular level (DiMascio *et al*., 1989; Rao & Agarwal, 1999). Carotenoids are also the main pigments present in meat, and provide resistance against rancidity, allowing long-term meat storage (Peters *et al*., 2011).

The thiobarbituric acid value (TBA value) is an indicator of meat oxidation status and it also used to describe the shelf life of foods. Low meat TBA values

Table 6 – Meat lipid oxidation of broilers fed increasing levels of vegetable waste (means±SEM).

Treatments	Total phenolic content (TPC) mg/g GAE	Total flavonoid content (TFC) mg/g QE	Thiobarbituricacid value (TBA)
Control (100% CF⁺ + 0% WV$)	43.88±0.56[e]	27.98±2.23[e]	0.0176±0.0012[a]
75% CF⁺ + 25% VW$	62.26±0.33[d]	132.67±4.06[d]	0.0123±0.0012[b]
75% CF⁺ + 25% VW$	73.52±0.29[c]	158.19±8.96[c]	0.006±0.0008[c]
75% CF⁺ + 25% VW$	80±0.50[b]	244.08±8.25[b]	0.004±0.0005[c]
0% WV$ + 100% VW$	93.26±0.32[a]	295.07±6.94[a]	0.0008±0.0001[d]
p-value	<0.0001	<0.0001	<0.0001

*Means followed by different superscripts indicate significant differences ($p \leq 0.05$) among treatments.

⁺CF (Commercial Feed)

$VW (Vegetable Waste)

indicate greater antioxidant status and storage stability. The treatment groups which meat presented higher phenolic and flavonoid contents had lower TBA values. In present study, the highest TBA values were found in control group, and steadily decline as the dietary level of waste vegetable increased (Table 6). These results may be attributed to the activity of antioxidants found in abundant quantity in vegetable sources (Wang & Yang, 2010) as compared to the commercial diet. Similarly, Naeem & Khan (2013) also reported higher antioxidant activity after supplementation of plant origin ingredients in diet.

CONCLUSIONS

The replacement of a commercial diet by increasing levels of vegetable waste had negatively affected broiler growth performance as broiler weight gain decreased as dietary vegetable waste inclusion increased. Meat mineral profile was not influenced by vegetable waste inclusion levels, except for magnesium, which level also decreased as vegetable waste inclusion increased. Broiler immune profile of was not affected by the treatments. The most significant change was observed in meat lipid oxidation status, which improved with increasing dietary vegetable waste levels. The highest vegetable waste level promoted the best meat antioxidant status, as demonstrated by the highest total phenolic and flavonoid contents and the lowest TBA value. Therefore, it may be concluded that meat quality, particularly in terms of shelf life, may be enhanced by the dietary inclusion of vegetable waste, due to a significant reduction in the probability of rancidity.

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CONFLICT OF INTEREST

No potential conflict of interest was found by the authors.

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