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Effects of *Carica Papaya* Leaf Extract on Blood Hematology, Serum Biochemistry and Immune Response of Broilers

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Abstract

Background: As an alternative of antibiotic growth promoters, medicinal plants are the most popular options. Papaya leaves are good source of vitamins, minerals, antioxidants and enzymes which provide aid in digestion of carbohydrates, proteins and fats and overcome the microbial load and used as a growth promoter and antibiotic in broiler performance. This study was carried out in order to evaluate the effect of papaya leaf extract in drinking water as a replacement of commercial antibiotic on blood hematology, serum biochemistry and immune response of broilers for the duration of 35 days.

Methods: In this experiment, 180 one-day old chicks were randomly distributed into 6 treatments. Each treatment was divided into 3 replicates having 10 chicks each. Feed was offered *ad-libitum* to broilers. Six treatments; G1 {(basal diet + antibiotic growth promoter (enramycin) + coccidiostat (salinomycin)}, G2 (basal diet without antibiotic growth promotor and coccidiostat), G3 (basal diet + 0.5% papaya extract), G4 (basal diet + 1.0% papaya extract), G5 (basal diet + 1.5% papaya extract) and G6 (basal diet + 2.0% papaya extract) were designed. Blood samples were taken for serum biochemistry, hematology and titer against Newcastle disease. Data were analyzed using analysis of variance technique by completely randomized design using SAS and Tukey's test were used for comparison of means.

Results: Birds received various level of papaya extract had no effect on serum biochemistry and hematology parameters except triglyceride levels which were significantly lower in G2 (15.00±15.00, commercial diet) and G6 (17.43±9.87, commercial diet + 2% papaya extract) at 17th day and 35th day where it failed to show significant difference. However, Newcastle disease and infectious bursal disease titers were not affected by the treatments.

Conclusion: It can be concluded that papaya leaf extract may be used in broiler diet as commercial antibiotics replacement.

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Introduction

Substances that are supplemented in poultry diet to destroy or inhibit growth of microbes especially bacteria are classified under the category of additives [1]. Many of synthetic drugs and growth promoters are used in poultry diets e.g. neomycin, bacitracin manganese, soframycin, tylosin, hygromycin-B, enramycin and erythromycin etc. [2]. However, their excess use develops resistance in bacteria and has adverse effects on human health [3]. Scientific research evidence proposes to reduce use of antibiotic growth promotors because uncontrolled use of these chemicals has increased issues related with antibiotic resistance and antibiotic residues having direct effect on and human and animal wellbeing [4-7]. As an alternative of antibiotic growth promoters, medicinal plants are most popular options. Medicinal plants are cheap and contain pharmacologically active substances which produce some specific chemicals that reduce growth of bacteria [8].

Carica Papaya is a species which belong to *Caricaceae* family. Its fruit is Papaya which is native fruit of America. It was firstly cultivated in Mexico and then cultivated in other countries [9]. *Carica papaya* seeds possess moisture 71.89, protein 8.4, carbohydrate 9.44, ash 1.47%, fatty oil-lauric 0.13, myristic 0.16, palmitic 15.13, stearic 3.61, arachidic 0.87, behenic 0.22, oleic 71.60, linoleic 7.68, linolenic 0.6%, Phospholipids-phosphatidylcholine, cardiolipin. Papaya leaves contain high crude protein and low fiber content. It contains about 30% crude protein only 5% crude fiber. It also contains calcium, potassium, magnesium, sodium and iron [10]. Papaya leaves and seeds are good source of vitamins, minerals, antioxidants and enzymes which provide aid in digestion of carbohydrates, proteins and fats and overcome the microbial load and used as a growth promoter and antibiotic in broiler performance [11]. Onyimonyi and Onu found that a 2% inclusion of papaya leaf meal in finishing broilers feed could improve performance, carcass and organoleptic indices [12].

Papaya leaves extract contain phytochemicals which have been reported to improve the platelet and red blood cell [13]. In the treatment of arthritis and intestinal worms, papain (a product which is produced from papaya fruit which mainly contains enzymes) is widely used. Because of the presence of phytochemicals, papaya is widely used to enhance immune system and release natural chemicals which attack on tumor cells [14]. Dried papaya leaves are medically important as a tonic and blood purifier [11].

Papaya leaf extract administrating in drinking water can be replaced with antibiotics without compromising hematology, antibiotic titer and serum biochemistry. Therefore, present study was conducted to study the influence of papaya leaf extract on hematology, serum biochemistry and immune response of broilers.

Methods

Shed preparation

Shed was cleaned, whitewashed and wet cleaned. All holes in shed were closed in order to stop entering of rodents. One-inch layer of saw dust was spread in all pens. Bulbs were checked for proper light intensity in all pens. Brooder was arranged in order to maintain temperature. Shed was sealed properly (not to compromise ventilation) by polythene sheet in order to maintain temperature at brooding. Shed was preheated 24 hours before arrival of chicks. All fans and bulbs were checked before the flock arrival in order to remove any fault if present. Drinkers and feeders were washed and dried in sunshine, then placed in every pen before the arrival of flock. Chicks were received in chick boxes and placed in pens. Birds were weighed and counted. Glucose solution (4%) was supplemented in drinking water to chicks in order to ensure quick supply of energy. Brooding paper was spread over saw dust and feed was spread on it. Temperature and humidity were maintained according to the standard requirements. Feed was offered *ad libitum* to the birds. Clean and fresh water was provided to chicks.

Preparation of Papaya extract

Fresh papaya leaves were collected, washed and dried under shade and sliced. After grinding 5 g papaya leaf powder was mixed with 1000 ml of water and then boiled for two to three hours at constant 80°Ctemperature.

Bird Husbandry

In this experiment, 180 one-day old chicks were randomly distributed to 6 treatments. Each treatment was divided into 3 replicates having 10 chicks in each. Diets were offered *ad-libitum* to birds. Six treatments as shown in Table 1 were randomly allotted to the replicates the composition of commercial diet is given in Table 2.

Treatment Name	Treatment specification
G1 (Positive control)	basal diet + antibiotic growth promoter (Enramycin) + coccidiostat (Salinomycin)
G2 (Negative control)	basal diet with out antibiotic growth promotor and coccidiostat
G3	basal diet + 0.5% papaya extract
G4	basal diet + 1.0% papaya extract
G5	basal diet + 1.5% papaya extract
G6	basal diet + 2.0% papaya extract

Table 1: Specifications of treatment applied in the trial.

Items	Starter Ration 1-21 days	Finisher Ration 22-35 days
Metabolizable Energy (Kcal/Kg)	2900	3000
Crude Protein %	20	19.25
Ether Extract %	4	4
Crude Fiber %	5.51	5.5
Ash (Mineral content) %	5.3	5.5
Calcium %	0.9	0.8
Phosphorus (available) %	0.45	0.4
Sodium %	0.19	0.16
Lysine %	1.2	0.15
Methionine %	0.52	0.43
Arginine %	1.25	1.17
Methionine + Cystine %	0.89	0.9
Enramycine (Antibiotic growth promoter)	100 g/ton	100 g/ton
Salinomycine (Coccidiostat)	500 g/ton	500 g/ton

Table 2: Nutrient Composition of Experimental (basal) Diet.

Blood samples

Blood sample with anticoagulant (EDTA; 1 mg/ml) was collected (3-4 ml) from each bird at 17th and 35th day. Serum samples were separated and stored at -20°C. Blood samples were used for different parameters including red blood cell (RBC) count, hemoglobin

concentration (Hb%), hematocrit percent (Hc), total leukocyte count (TLC) and differential leukocyte count (DLC) [15]. Serum biochemical parameters were estimated spectrophoto-metrically according to methods described by Ghaffar *et al.,* [16] using commercially available Biomed 122-120, 122-190 and 122-220 kits.

Statistical analysis

Data were analyzed using analysis of variance technique by completely randomized design (CRD) using SAS [17] and Tukey's [18] test was used to compare the means.

Results

White blood count (10^9/l) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% was 69.7, 96.4, 76.5, 92.6, 96.3 and 80.4, respectively. Lymphocyte (10^9/l) content of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% was 61.7, 69.5, 50.5, 69.8, 70.0 and 58.2, respectively. Cells other than Granulocyte or lymphocyte (10^9/l) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were 15.39, 15.24, 20.56, 16.48, 19.30 and 23.94, respectively. Granulocyte content (10^9/l) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were 8.56, 14.27, 6.68, 11.59, 9.53 and 9.34, respectively. Lymphocytes percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were 61.70, 69.47, 50.47, 69.77, 70.00 and 58.17, respectively. Cells other than Granulocyte or lymphocyte percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were 24.30, 15.77, 24.37, 17.70, 20.13 and 30.03, respectively. Granulocyte percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were 14.00, 14.80, 7.87, 12.53, 9.87 and 11.83, respectively. RBC content (10^{12}/l) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% was 1.9, 2.0, 1.6, 2.2, 2.1 and 2.1, respectively (Table 3).

Hemoglobin content (g/dl) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were 12.13, 13.20, 10.67, 13.87, 13.47 and 13.33, respectively. Hematocrit percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were 28.02, 29.91, 23.34, 31.75, 29.91 and 29.95, respectively. Mean Corpuscular Volume (fl) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 147.67, 147.33, 147.33, 147.00, 140.33 and 141.67, respectively. Mean Corpuscular Hemoglobin (+pg) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 64.00, 65.23, 67.87, 64.37, 63.20 and 63.03, respectively. Mean Corpuscular Hemoglobin Concentration (g/dl) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 43.33, 44.17, 46.07, 43.90, 44.97 and 44.47, respectively. Red cell distribution width (+fl) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 69.67, 73.57, 67.90, 69.33, 62.17 and 67.17, respectively. Red cell distribution width percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 10.40, 10.90, 10.20, 9.70, 10.13 and 10.73, respectively (Table 3).

Platelet count (10^9/l) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 11.33, 22.67, 12.00, 20.00, 18.67 and 9.33, respectively. Platelet percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 0.01, 0.01, 0.01, 0.01, 0.01 and 0.01, respectively. Mean Platelet Volume (-fl) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 6.90, 6.73, 6.70, 7.03, 7.10 and 7.00, respectively. Platelet distribution width (-fl) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 5.93, 6.40, 5.60, 6.83, 7.03 and 4.70, respectively. Platelet distribution width percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 29.00, 30.33, 28.57, 30.80, 32.03 and 25.63, respectively. Platelet Large Cell Count (10^9/l) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 1.00, 2.33, 1.33, 3.00, 2.00 and 1.00, respectively. Platelet Large Cell Ratio percentage of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 9.42, 11.25, 9.83, 9.92, 9.83 and 10.27, respectively. Lyse content (ml) of treatments PC, NC, 0.5, 1.0, 1.5 and 2.0% were, 0.90, 0.90, 0.90, 0.90, 0.90 and 0.90, respectively (Table 3).

At 17th day, highest triglycerides concentration (105.67±24.07) was recorded in the blood of group G3 while lowest concentration (15.00±15.00) was noted in blood of group G2 birds. Cholesterol level was highest (161.00±19.65) in group G4 and lowest (83.87±42.93) in group G3. Group G3 also showed highest high density lipoprotein (HDL) level (39.72±4.42) and group G5 recorded lowest (34.45±1.83) HDL. Highest Low density lipoprotein (LDL) concentration (109.92±17.85) was recorded in group G4 and lowest LDL concentration (23.01±44.07) was seen in group G3. Statically analysis of data revealed that blood chemistry parameters, (triglycerides, cholesterol, HDL and LDL) were non-significantly (P>0.05) different (Table 4).

At 35th day, group G5 revealed highest triglycerides concentration (80.53±20.55) while group G6 showed lowest concentration (17.43±9.87). Cholesterol level was also highest (173.33±10.41) in group G5 and lowest (101.67±13.17) in group G3. Group G6 had highest HDL level (30.34±0.87) and group G2 had lowest (24.90±1.28) HDL. Group G5 revealed highest LDL concentration (129.03±7.84) in blood while group G3 had lowest LDL concentration (60.66±17.29). Statically analysis of data revealed that blood chemistry parameters, (triglycerides, cholesterol, HDL and LDL) were non-significantly (P>0.05) different (Table 5).

Antibodies titer against Newcastle disease were also assessed to study the effect of papaya leaves' extract on immune system of the broilers of treatments positive control (PC), negative control (NC), 0.5, 1.0, 1.5 and 2.0% was 2.33, 1.67, 3.33, 3.33, 3.00 and 3.33 at 17th day and 5.67, 5.67, 5.33, 6.00, 5.33 and 6.00 at 35th day, respectively. Antibodies titers against Newcastle disease were non-significantly different (P>0.05) in birds given various levels of papaya leaf extract in drinking water (Table 6).

Discussion

Blood hematology parameters were non-significantly (P>0.05) different in broiler birds received papaya extract in drinking water. Oloruntola in 42-day feeding trial reported similar results of feeding the effects of a diet supplemented with pawpaw leaf and seed meal composite mix (PCM) on hematological indices [19]. White blood cell counts of birds fed PCM supplemented diets were similar/comparable to that of the birds fed control diet. Hemoglobin concentration and packed cell volume of birds fed PCM supplemented diets were similar to those fed control diet, except for those fed diet

Treatments	PE (0%) PC	PE (0%) NC	PE (0.5%)	PE (1.0%)	PE (1.5%)	PE (2.0%)	P value
White Blood Cells* (10^9/l)	69.65	96.40	76.54	92.60	96.29	80.43	0.4347
Lymphocytes* (10^9/l)	45.69	66.23	49.29	64.53	67.47	47.17	0.5116
Cells other than Granulocyte or lymphocyte *(10^9/l)	15.39	15.24	20.56	16.48	19.30	23.94	0.8645
Granulocyte* (10^9/l)	8.56	14.27	6.68	11.59	9.53	9.34	0.7380
Lymphocytes* %	61.70	69.47	50.47	69.77	70.00	58.17	0.8139
Cells other than Granulocyte or lymphocyte * %	24.30	15.77	24.37	17.70	20.13	30.03	0.5488
Granulocyte* %	14.00	14.80	7.87	12.53	9.87	11.83	0.8466
Red Blood Cells (-10^{12}/l)	1.90	2.03	1.62	2.16	2.13	2.11	0.6309
Hemoglobin (g/dl)	12.13	13.20	10.67	13.87	13.47	13.33	0.6777
Hematocrit (-%)	28.02	29.91	23.34	31.75	29.91	29.95	0.6099
Mean Corpuscular Volume (+fl)	147.67	147.33	147.33	147.00	140.33	141.67	0.3953
Mean Corpuscular Hemoglobin (+pg)	64.00^{ab}	65.23^{ab}	67.87^{a}	64.37^{ab}	63.20^{ab}	63.03^{b}	0.2238
Mean Corpuscular Hemoglobin Concentration (+g/dl)	43.33^{b}	44.17^{b}	46.07^{a}	43.90^{b}	44.97^{ab}	44.47^{ab}	0.0664
Red cell distribution width (+fl)	69.67^{ab}	73.57^{a}	67.90^{ab}	69.33^{ab}	62.17^{b}	67.17^{ab}	0.1674
Red cell distribution width %	10.40	10.90	10.20	9.70	10.13	10.73	0.4428
Platelet count (-10^9/l)	11.33	22.67	12.00	20.00	18.67	9.33	0.1801
Platelet (-%)	0.01	0.01	0.01	0.01	0.01	0.01	0.8545
Mean Platelet Volume (-fl)	6.90	6.73	6.70	7.03	7.10	7.00	0.8509
Platelet distribution width (-fl)	5.93	6.40	5.60	6.83	7.03	4.70	0.6228
Platelet distribution width (-%)	29.00	30.33	28.57	30.80	32.03	25.63	0.6373
Platelet Large Cell Count (-10^9/l)	1.00	2.33	1.33	3.00	2.00	1.00	0.7737
Platelet Large Cell Ratio (-%)	9.42	11.25	9.83	9.92	9.83	10.27	0.9997
Lyse (ml)	0.90	0.90	0.90	0.90	0.90	0.90	0

Table 3: Average values of blood hematology parameters of broiler birds received different levels of papaya leaf extract.

Treatments	Triglycerides mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl
G1	45.83±20.84	88.33±34.30	37.91±2.06	41.25±36.61
G2	15.00±15.00	130.00±35.23	36.60±1.30	90.41±35.27
G3	105.67±24.07	83.87±42.93	39.72±4.42	23.01±44.07
G4	60.10±17.28	161.00±19.65	39.07±2.22	109.92±17.85
G5	72.50±15.05	107.67±20.83	34.45±1.83	58.71±22.72
G6	21.50±12.93	97.33±17.67	37.25±0.71	55.78±15.58
p value	0.034	0.483	0.688	0.414

Table 4: Effect of papaya leaf extract on blood chemistry parameters at 17th day.

Treatments	Triglycerides mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl
G1	53.83±43.03	167.33±58.07	25.40±2.65	131.17±65.14
G2	36.70±10.64	142.67±35.87	24.90±1.28	110.42±34.06
G3	58.30±29.39	101.67±13.17	29.35±4.04	60.66±17.29
G4	54.93±6.06	105.33±43.68	28.86±1.46	65.49±45.67
G5	80.53±20.55	173.33±10.41	28.20±1.59	129.03±7.84
G6	17.43±9.87	108.33±23.96	30.34±0.87	74.51±22.87
p value	0.566	0.541	0.480	0.607

Table 5: Effect of papaya leaf extract on blood chemistry parameters at 35th day.

Treatments	At 17th day	At 35th day
G1	2.33	5.67
G2	1.67	5.67
G3	3.33	5.33
G4	3.33	6.00
G5	3.00	5.33
G6	3.33	6.00
P-Value	0.462	0.93

Table 6: Mean of ND titer of broiler birds received various levels of papaya leaf extract.

1.0% PCM had significantly (P<0.05) lower Hbc and PCV. Similar results were noted by Mahejabin *et al.,* who observed that papaya extract had no effect on blood parameters [20]. Results are not in line with the previous study who found that papaya leaf had higher total erythrocyte count and erythrocyte sedimentation rate. Similarly, Ogbuokiri *et al*., showed that higher PVC, lower RBC, higher Hb and lower Neutrophil were recorded in birds received 2.5% Papaya leaf [22]. Ameen *et al*., also found that birds received *Carica papaya* extract had higher packed cell volume, haemoglobin concentration, lymphocyte counts, red blood cells and lower eosinophil counts [23]. Contrary results were reported by Sorwar *et al*., who found that papaya leaf had higher total erythrocyte count (2.57±0.23 million/mm^3) and erythrocyte sedimentation rate (6.13±0.64 mm in first hour) [21].

Papaya extract had no effect on blood chemistry parameters (triglycerides, cholesterol, HDL and LDL) and ND titer. Oloruntola *et al*., reported the effect of pawpaw leaf meal inclusion and enzyme supplementation in the diet of broiler chickens. Enzyme reduced ($p < 0.05$) low-density lipoproteins (LDL), whereas PLM reduced ($p < 0.05$) cholesterol and LDL [24]. Similar results were noted by Hussain *et al*., who observed that papaya extract had no effect on blood parameters line total erythrocyte count, hemoglobin, pack cell volume and erythrocyte sedimentation rate [15].

Present study concluded that papaya extract can be used in drinking water of poultry without any adverse effect on hematology, antibiotic tire and serum biochemistry.

Authors' Contribution

Muhammad. Khalid Bashir, Muhammad Ashraf, Sadia Razzaq, Shahid Ur Rehman and Muhammad Qamar Bilal planned, conducted and took data for the trial. Muhammad. Khalid Bashir, Muhammad Ashraf, Sadia Razzaq, Shahid Ur Rehman and Muhammad Qamar Bilal written and refined the manuscript. Sadia Razzaq, Sayeda Mariam and Madiha Tabbasum analyzed the data statistically.

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Islam MR, Mostofa M, Mondal KS, Roy RR, Sorwar MG, Islam S. Effect of probiotic on beneficial and pathogenic microorganisms in the gastrointestinal tract of commercial broiler with BCRDV vaccine. Progressive Agriculture, (2013); 24: 107-115.
2. Castanon JIR. History of the Use of Antibiotic as Growth Promoters in European Poultry Feeds. Poultry Science, (2007); 86(11): 2466-2471.
3. Butaye P, Luc A, Devriese F, Brouck H. Antimicrobial growth promoters used in animal feed: Effects of less well known antibiotics on gram positive bacteria. Clinical Microbiology Review, (2003); 16: 175-188.
4. Forgetta V, Rempel H, Malouin F, Vaillancourt Jr, Topp E, Dewar K, Diarra M.S. Pathogenic and multidrug-resistant Escherichia fergusonii from broiler chicken. Poultry Science, (2012); 91(2): 512-525.
5. Carvalho IT, Santos L. Antibiotics in the aquatic environments: a review of the European scenario. Environment International, (2016); 94: 736-757.
6. Ronquillo MG, Hernandez JCA. Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control, (2017); 72: 255-267.
7. Mehdi Y, Montminy MPL, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, Brar SK, Cote C, Ramirez AA, Godbout S. Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition, (2018); 4(2): 170-178.
8. Basile A, Giordano S, Lopez-Saez JA, Cobianchi RC. Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry (1999); 52: 1479-1482.
9. Satrija F, Nansen P, Bjorn H, Murtini S, He S. Effect of papaya latex against Ascarissuum in naturally infected pigs. Journal of Helminthology, (1994); 68: 343-346.
10. Maisarah AM, Asmah R, Fauziah O. Proximate analysis, antioxidant and antiproliferative activities of different parts of *Carica Papaya*. Journal of Nutrition and Food Sciences, (2014); 4: 267.
11. Nath DD, Rahman MM, Akter F. Effects of tulsi, black pepper and cloves extract as a growth promoter in broilers. Bangladesh Journal of Veterinary Medicine, (2012); 10: 33-39.
12. Onyimonyi AE, Onu E. An assessment of papaya leaf meal as protein ingredient for finishing broiler. International Journal of Poultry Science, (2009); 8: 995-998.
13. Dharmarathna SLCA, Wickramasinghe S, Waduge RN, Rajapakse RPVJ, Kularatne SAM. Does Carica papaya leaf-extract increase the platelet count? An experimental study in a murine model. Asian Pacific Journal of Tropical Biomedicine, (2013); 3(9): 720-724.
14. Sarker EH, Khokon JU, Rahma MA. Comparative efficacy of probiotic papaya leaf leaves and vitamin AD3E as a growth promoter on broilers. International Journal of Natural and Social Sciences, (2014); 1: 26-32.
15. Hussain R, Khan A, Mahmood F, Rehan S, Ali F. Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (Coturnix japonica). Pesticide biochemistry and physiology, (2014); 109: 58-63.
16. Ghaffar A, Hussain R, Khan A, Abbas RZ. Butachlor induced clinico-hematological and cellular changes in fresh water fish Labeorohita (Rohu). Pakistan Veterinary Journal, (2015); 35: 201-206.
17. SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2002-2004.
18. Tukey JW. Exploratory data analysis. Addison-Wesley, Reading (1977).
19. Oloruntola OD. Effect of pawpaw leaf and seed meal composite mix dietary supplementation on haematological indices, carcass traits and histological studies of broiler chicken. Billiton National Research Centre (2019); 43: 129.
20. Mahejabin N, Mostofa M, Akter F, Das S, Alam M. Effects of neem, turmeric and papaya leaf extract mixture on growth performance of broilers. International Journal of Natural and Social Sciences, (2015); 2: 17-21.
21. Sorwar MG, Mostofa M, Hasan MN, Billah M, Rahman MT. Effect of kaloJeera seeds and Papaya leaf supplementation on the performance of broiler. Bangladesh Journal of Veterinary Medicine, (2016); 14: 37-42.
22. Ogbuokiri UDE, Iheanacho A, OsujiA I, Okoli IC, Ekenyem BU. Effect of papaya leaf (*Carica Papaya, linn*.) meal on some performance attributes of starter broiler chicks. Journal of Animal Science Advances, (2014); 4: 826-832.
23. Ameen SA, Adedeji OS, Ojedapo LO, Salihu T, Fakorede OL. Anthelmintic efficacy of pawpaw (Carica papaya) seeds in commercial layers. African Journal of Biotechnology, (2012); 11: 126-130.
24. Oloruntola OD, Ayodele SO, Oloruntola DA. Effect of pawpaw (*Carica papaya*) leaf meal and dietary enzymes on broiler performance, digestibility, carcass and blood composition. Ressources Alimentaires *Et al*imentation, (2018); 71(3): 1-10.