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Pedalium Murex Leaf Powder Supplemented in the Diet of Guinea Fowl: Effect on Immune Response, Haematology and Serum Biochemical Indices

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A B S T R A C T

This experiment was carried out to evaluate Pedalium murex leaf powder supplemented in the diet of Guinea fowl: effect on immune response, and serum biochemical indices. 300 -one- day-old guinea fowl (Pearl variety) mixed sex was randomly distributed into five treatment groups with six replicates (ten birds per replicate). A completely randomized design model was adopted and a basic diet was formulated according to the requirement of birds according to Nutritional Research Council's recommendation in 1994. Birds in group A which also served as the control, was fed basic diet only, group B received basic diet supplemented with Neomycin at 20 g/kg diet while those in group C, D and E were fed same diet supplemented with Pedalium murex leaf powder at 20 g, 40 g and 60 g/kg diet in that order. The experiment lasted for 42 days and animals were given unlimited access to water and feed. Values of immunoglobulin A, G and M were higher in birds fed Pedalium murex leaf powder (C, D and E), intermediate in group B and lower in group A ($p<0.05$). Hematocrit, heamoglobin, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration were not affected ($p>0.05$) except for white blood cell, lymphocytes and monocyte count. Values for total protein, albumin, globulin and total cholesterol were significantly influenced ($p<0.05$) except for glucose, uric acid, creatinine and aspartate amino-transferase ($p>0.05$). It was concluded that dietary supplementation of Pedalium murex leaf powder up to 60 g/kg had to detrimental effect on the blood profile of birds

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INTRODUCTION

Reducing the risk for antimicrobial resistance is an urgent priority as livestock producers across the globe strive to feed the growing population in a more sustainable way to safeguard animal protein (Adewale et al., 2021; John, 2024). To achieve sustainable success, medicinal plants are usually supplemented in Poultry diet because they have a wide range of applications and can be used as anti-bacterial, anti-inflammatory, antioxidant, cytotoxic (Adewale et al., 2021; Ojediran et al., 2024), anti-diabetic, immune-stimulatory, hepato-protective, anti-cancer, anti-helminthic, gastro-protective (Musa et al., 2020), antiviral and antifungal due to the presence of phytochemicals which have been reported to be non-toxic, effective and environmental friendly (Omokore and Alagbe, 2019).

LITERATURE REVIEW

Previous studies have shown that the dietary supplementation of medicinal plants can improve gut health, nutrient utilization and overall performance of birds (Singh et al., 2021; Agubosi et al., 2022). Among the potential herbs with therapeutic property is Pedalium murex is also called as Gokhru. The herb belongs to the family Pedaliaceae family includes 14 genera and over 60 species distributed in Asia, Africa and Europe (Anubu and Subramanian, 2011; Chitra et al., 2013). The plant is used in folk medicine for the treatment of gastrointestinal problems, skin infection, pyrexia, malaria, sexually transmitted infections, tooth ache, ulcer amongst others (Chaudhary and Kaushik, 2017; Imo et al., 2016). The leaves are alternate and occurs in pairs of about 5-8, it also contains a variety of compounds, including saponins, reducing sugars, phenolic compounds, alkaloids, triterpenoids, xantho-proteins, flavonoids and tannins (Thamizhmozhil et al., 2011; Thakar et al., 2011). Pharmacologically, the leaves have been investigated for anti-ulcerogenic (Suthar et al., 2008), nephron-protective, hypolipidemic, aphrodisiac, antimicrobial, analgesics, anti-inflammatory (Suresh et al., 2002), antidermatophytic, immuno-stimulatory, antioxidant, antimicrobial and insecticidal activities (Kalyani et al., 2011; Imran et al., 2012). Ethanolic and aqueous extract from Pedalium murex have been shown to inhibit the activities of pathogenic organisms such as, Escherichia coli, Staphylococcus spp, Salmonella spp, Aspergillus flavus, Aspergillus niger and Streptococcus spp (Arockiasamy et al., 2012; Babu et al., 2011).

Blood is responsible for the transport of nutrients and gases round the body of animals, however factors like nutrition, presence of toxic compounds, concentration of phyto-constituents could influence their composition (John, 2024e). Accessing the blood parameters of birds could also give a clue on the clinical state of birds (Omokore and Alagbe, 2019). There is also a dearth of information on the effect of Pedalium murex on the blood profile of Guinea fowl. This research will help to promote livestock sustainability, food safety and provide natural alternatives to antibiotics.

METHODOLOGY

Materials and Methods

Experimental Site Description and Duration

The Poultry unit of Sumitra Research Institute, Gujarat was used for the experiment. The study was carried out between March to April, 2024. All animal management procedures adopted was in line with the approved guidelines approved by the ethics committee of the Institute (ethical reference number: FFR/09A/2024). The total experimental period is forty-two days.

Sourcing and Processing of *Pedalium Murex* Leaf Powder

Fresh *Pedalium murex* leaves were sourced from the botanical garden of Sumitra Research Institute, Gujarat and sent to the Department of Crop Science where it was authenticated and given a registration number HGO/22/2024. Collected leaves were rinsed in running tap water, placed in a plastic sieve and air dried for 15 days. Dried *Pedalium murex* leaves were grounded into powder using mortar and pestle sand kept in an airtight polythene bag under room temperature.

Animal Care and Experimental Design

300 -one- day-old guinea fowl (Pearl variety) mixed sex was sourced from a hatchery in Gujarat. Upon arrival, chicks were weighed with a digital sensitive scale on arrival to obtain their average initial weight before they were randomly distributed into five treatment groups with six replicates (10 birds per replicate). They were kept reared in a deep litter system, disinfected with Superguard® prior to the arrival of birds. Birds were placed on anti-stress with multivitamin in ratio 2:1 in ten liters of water for seven days and fed basic diet formulated to meet the nutritional requirements of birds as described by Nutritional Research Council in 1994. Brooding temperatures was properly monitored (36℃) and reduced weekly by 2℃ until a constant temperature of 27℃ was maintained. Birds in group A received basic diet supplemented without any additive (negative control), group B was fed same diet supplemented with antibiotics (neomycin) at 20.0 g/kg, group C, D and E were given basic diet supplemented with *Pedalium murex* leaf powder at 20 g, 40 g and 60 g respectively. A completely randomized design model was used and the trial lasted for forty-two days. Vaccination was administered according to the prevailing disease condition in the environment. Birds received unlimited access to clean water and diet. Feed intake was estimated as the difference between feed rejected and feed offered while body weight gain is the difference between final body weight and initial body weight.

Determined Parameters

Experimental diet was determined according to the methods outlined by Association of Analytical Chemist in 2000.

Haematological and Serum Biochemical Evaluation

At the end of the experiment, 3mL of blood sample was collected from the wing vein of ten randomly selected bird per treatment into labeled sample bottles. Blood for haematology (1.5 mL) was placed in bottles with anticoagulant while those for serum biochemical indices (1.5 mL) was transferred into bottles

without anticoagulant. After blood collection, samples were placed in an ice pack and transferred immediately to biochemistry laboratory of Sumitra Research Institute, Gujarat for further examination. Haematological parameters were analyzed using automated Rodimex analyzer (Model: HF/09D/201, Punjab) while those for serum biochemical indices was analyzed with Nutrure auto biochemical analyzer (model: RGT/06/2080C, China).

Analysis of Immune Parameters

On the 42nd day of the experiment, 1.5 mL of blood samples were collected from ten randomly selected bird per treatment (from samples used for serum biochemical indices). Immune parameters were analyzed using Immuno Rex automated analyzer (DS-2704 model, Netherlands).

Data Analysis

Data obtained were subjected to one -way analysis of variance using Statistical Package for Social Science (SPSS) (version 25). Significant differences among the groups were subjected to comparisons using the Duncan multiple range test of the same software. All differences were considered to be statistically significant when $p < 0.05$.

RESULT AND DISCUSSION

Table 1: Nutrient Composition of Experimental Diet Fed to Guinea Fowl

Ingredients	Content (%)	Content (%)
Maize	56.16	57.30
Corn bran	3.04	3.00
Soybean meal	32.0	30.4
Fish meal (Imported brand)	3.00	2.00
Calcium carbonate	3.00	4.00
Di-calcium phosphate	1.50	2.00
L-Lysine	0.25	0.25
DL-Methionine	0.25	0.25
*Starter premix	0.25	0.25
Choline chloride	0.20	0.20
Sodium chloride	0.35	0.35
Total	100.0	100.0
Nutrient levels (%)		
Crude protein	23.12	21.05
Crude fibre	3.30	3.81
Crude fat	4.02	4.14
Calcium	1.13	1.20
Phosphorus	0.67	0.71
Lysine	1.37	1.38
Methionine	0.65	0.66
Energy (Kcal/kg)	2992.1	3040.2

Each 2.5 kg starter premix contains; vitamin A, 4,000,000 IU; vitamin K3, 800mg; vitamin E 4,000 mg; vitamin B1 800 mg; vitamin B2, 2000 mg; vitamin B5, 10,600 mg; vitamin B5, 4000 mg; vitamin B6, 1200 mg; vitamin B8, 40 mg; vitamin B9, 320 mg; vitamin B12; Iron, 20,000 mg; Copper, 4130 mg; Zinc, 250 mg; Manganese, 30,000 mg.

Each 2.5 kg grower premix contains; vitamin A, 2,000,000 IU; vitamin K3, 900mg; vitamin E 4,200 mg; vitamin B1 820 mg; vitamin B2, 3000 mg; vitamin B5, 10,000 mg; vitamin B5, 5000 mg; vitamin B6, 1500 mg; vitamin B8, 50 mg; vitamin B9, 350 mg; vitamin B12; Iron, 22,000 mg; Copper, 4000 mg; Zinc, 300 mg; Manganese, 35,000 mg.

Immune response of Guinea fowl fed diet supplemented with *Pedalium murex* leaf powder in Table 2. Immunoglobulin A (IgA) was higher in group E (2.12 mg/dL), group D (2.10 mg/dL), group C (2.08 mg/dL) than in group B (2.02 mg/dL) and group A (1.43 mg/dL) ($p<0.05$). Immunoglobulin G (IgG) values varied 0.37 - 1.05 mg/dL was lower in group A (0.37 mg/dL) and group B (1.00 mg/dL) than in group C (1.01 mg/dL), group D (1.03 mg/dL) and group E (1.05 mg/dL) ($p<0.05$). Immunoglobulin M values (0.74 - 1.25 mg/dL) were influenced by the treatments ($p<0.05$). It is noteworthy that IgA, IgG and IgM values follow similar pattern suggesting that dietary supplementation of *Pedalium murex* leaf powder in guinea fowl is capable of promoting the activity of immune cells and production of antibodies which plays an important role in body defense mechanisms against diseases and infections (John, 2024a; John, 2024b). Though immunoglobulins (A, G and M) values in group B were greater compared to group A, indicating that birds in group B have a higher tendency to resist disease compared to A. The high efficiency in *Pedalium murex* leaf powder could be attributed to the presence of phytochemicals, such as, flavonoids, saponins, tannins, alkaloids, phenolic compound, steroids amongst others (Daniel, 2024; Alagbe, 2024). These compounds possess anti-diarrhea, anti-helminthic (Alagbe, 2021), analgesics (John, 2024d), anti-inflammatory, immune-stimulatory, antiviral, gastro-protective, anti-cancer, anti-diabetic (John, 2024e), cytotoxic properties amongst others (Adewale et al., 2021; Shittu et al., 2023). The result obtained in this study is in consonance with the results obtained by Musa et al. (2020), when aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture was fed to broiler chickens.

Table 2: Immune Response of Guinea Fowl Fed Diet Supplemented with *Pedalium Murex* Leaf Powder

Variables (mg/dL)	GA	GB	GC	GD	GE	SEM	*P-value*
IgA	1.43^{c}	2.02^{b}	2.08^{a}	2.10^{a}	2.12^{a}	0.14	0.03
IgG	0.37^{c}	1.00^{b}	1.01^{a}	1.03^{a}	1.05^{a}	0.07	0.02
IgM	0.74^{c}	1.06^{b}	1.19^{a}	1.22^{a}	1.25^{a}	0.91	0.04

Values followed by different letters were significantly different ($p<0.05$); GA: basic diet only (control); GB: basic diet supplemented with Neomycin at 20.0 g/kg diet while GC, GD and GE: basic diet supplemented with *Pedalium murex* leaf powder at 20 g, 40 g and 60 g per kg diet respectively; SEM: standard error of mean

Haematological indices of Guinea fowl fed diet supplemented with *Pedalium murex* leaf powder (Table 3). Hematocrit, red blood cell, hemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were not affected ($p>0.05$) except for white blood cell, lymphocytes and monocyte count ($p<0.05$). Hematocrit, hemoglobin and red blood cell values obtained in this study (30.08 - 31.23 %), (120.9 to 128.9 g/L) and [(3.00 to 3.41($x10^{12}$/L)] was within the range (28.00 to 36.00 %); 100.0 - 18.00 g/L and ([1.90 - 4.00 ($x10^{12}$/L)] reported by Campbell (2012); Fair (2007). This result suggests efficient oxygen carrying capacity giving room for more absorbed nutrient absorption in the tissues of birds (Omokore and Alagbe, 2019). Decline in haematocrit and hemoglobin concentration is a sign of anaemia or iron deficiency in the blood (John, 2024e). The values of mean corpuscular haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentraions observed in the study were within the normal range (50.00−77.00 pg); (77.3 to 188.1 fl) and (39.1 to 58.0 %) reported by Etim et al. (2014); Islam et al. (2004); Ali et al. (2011). The outcome observed is in agreement with the reports of Muritala et al. (2022) when phytogenics was supplemented in the diets of broiler chickens. Low mean corpuscular volume and mean corpuscular haemoglobin concentration is an indication of deficiency in minerals and vitamins in the blood (Frandson et al., 2009); Nalubamba et al. (2010). The values for white blood cell, lymphocytes and monocytes which varied from [(9.88 to 23.00 ($x10^9$/L)], 55.92 to 66.07 % and 1.80 - 4.00 % was within the normal range [(10.00 to 25.00 ($x10^9$/L)], 50.00 to 75.00 % and 2.00 - 5.00 % cited by Keçeci and Çöl (2011); Olayemi (2009). White blood cells and their differentials are responsible for the production of antibodies for the prevention of diseases (John, 2024c), this explains why the immunoglobulin levels among birds fed group C, D and E were significant compared to those in group A and B. The result obtained is in agreement with the reports of Alagbe et al. (2020) when *Albizia lebbeck* stem bark aqueous extract was fed to birds.

Table 3: Haematological Indices of Guinea Fowl Fed Diet Supplemented with *Pedalium Murex* Leaf Powder

Variables	G1	G2	G3	G4	G5	SEM	*P value*
Hematocrit (%)	30.08	31.02	31.11	31.18	31.23	4.46	0.05
Red blood cell ($x10^{12}$/L)	3.00	3.18	3.31	3.38	3.41	0.78	0.03
Hemoglobin (g/L)	120.9	125.6	128.5	128.7	128.9	11.82	0.12
Mean corpuscular volume (fl)	151.2	156.1	158.7	159.1	159.6	14.42	0.18
Mean corpuscular haemoglobin (pg)	62.33	63.04	65.11	65.92	66.07	8.01	0.05
Mean corpuscular haemoglobin concentration (%)	31.04	32.84	33.08	33.12	33.50	2.34	0.06
White blood cell ($x10^9$/L)	10.08^b	19.32^a	19.41^a	19.59^a	19.68^a	1.67	0.02

Lymphocytes (%)	50.92[b]	64.11[a]	65.02[a]	65.90[a]	66.07[a]	8.32	0.05
Monocytes (%)	1.80[b]	3.87[a]	3.91[a]	3.98[a]	4.00[a]	0.06	0.01

Values followed by different letters were significantly different ($p<0.05$); GA: basic diet only (control); GB: basic diet supplemented with Neomycin at 20.0 g/kg diet while GC, GD and GE: basic diet supplemented with *Pedalium murex* leaf powder at 20 g, 40 g and 60 g per kg diet respectively; SEM: standard error of mean

Serum biochemical indices of Guinea fowl fed diet supplemented with *Pedalium murex* leaf powder is presented in Table 3. Total protein, albumin and globulin values were lower ($p<0.05$) in group A (5.50 g/dL; 2.50 g/dL; 3.00 g/dL) than in group B (6.68 g/dL; 3.00 g/dL; 3.68 g/dL), group C (6.70 g/dL; 3.00 g/dL; 3.70 g/dL), group D (6.73 g/dL; 3.00 g/dL; 3.73 g/dL) and group E (6.88 g/dL; 3.00 g/dL; 3.88 g/dL) in that order. The birds in group A had values of albumin lower than 2.88 - 3.00 g/dL reported by Okoro et al. (2011). However, the values of total protein and globulin were within the normal range cited by Kerr (2002); Harr (2006). This result suggests protein adequacy across the treatments for proper body maintenance (Alagbe et al., 2020). The values of cholesterol (89.44 to 119.7 mg/dL) observed in the study were within the values (50.00−150.00 mg. dl-1) reported by Agubosi et al. (2022) for broiler chickens fed diet supplemented with Sunflower essential oil. However, values obtained was lower than 100.8 - 203.2 mg/dL reported by Alagbe (2024) *Megaphrynium macrostachyum* leaves was supplemented in the diet of Japanese quails. The disparity in these results can be attributed to the nature and concentrations of phyto-constituents in the test ingredient. According to John (2024d), high serum cholesterol can damage the arteries and also cause cardiovascular disease. Glucose level (73.09 - 78.11 mg/dL) was within the range reported by Nikolov et al. (2017); Hochleithner (2013). Uric and creatinine levels (3.91 - 4.01 mg/dL); 0.60 - 0.69 mg/dL were within the range reported by Hrubec (2002); Baudouin et al. (2021). Values obtained were not significantly ($p>0.05$) influenced by the treatment. This result suggests the absence of liver or kidney dysfunction in the body of birds (Omokore and Alagbe, 2019). Aspartate amino transferase value (73.09 - 78.11 U/L) is within the range (60.40 - 105.6 U/L) reported by Christev et al. (2011). This result suggests that dietary supplementation of *Pedalium murex* leaf powder up to 60 g/kg diet is safe and non-toxic to birds. This result is in agreement with the report of Daniel (2024), when doum palm pulp extract was fed to broiler chicks.

Table 3: Serum Biochemical Indices of Guinea Fowl Fed Diet Supplemented with *Pedalium Murex* Leaf Powder

Variables	GA	GB	GC	GD	GE	SEM	*P-value*
Total protein	5.50[b]	6.68[a]	6.70[a]	6.73[a]	6.88[a]	0.02	0.51
Albumin	2.50[b]	3.00[a]	3.00[a]	3.00[a]	3.00[a]	0.07	0.62
Globulin	3.00[b]	3.68[a]	3.70[a]	3.73[a]	3.88[a]	0.05	0.36

Cholesterol (mg/dL)	119.7[a]	97.12[b]	95.06[b]	94.85[b]	89.44[c]	8.44	0.63
Aspartate amino-transferase (U/L)	78.11	75.02	74.66	74.15	73.09	5.39	0.41
Glucose (mg/dL)	128.6	128.9	130.5	130.9	131.6	10.36	0.88
Uric acid (mg/dL)	3.91	3.93	3.98	4.00	4.01	0.03	0.40
Creatinine (mg/dL)	0.60	0.61	0.67	0.69	0.65	0.02	0.01

Values followed by different letters were significantly different ($p<0.05$); GA: basic diet only (control); GB: basic diet supplemented with Neomycin at 20.0 g/kg diet while GC, GD and GE: basic diet supplemented with *Pedalium murex* leaf powder at 20 g, 40 g and 60 g per kg diet respectively; SEM: standard error of mean

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, *Pedalium murex* leaf powder was found to contain abundant medicinal properties which contributes significantly to the health of birds especially when it is supplemented up to 60 g/kg in the diet of guinea fowl. Blood parameters examined were within the established ranges for guinea fowl suggesting that birds were healthy throughout the experimental period.

FURTHER STUDY

This research still has limitations so further research is still needed on this topic "Pedalium Murex Leaf Powder Supplemented in The Diet of Guinea Fowl: Effect on Immune Response, Haematology and Serum Biochemical Indices"

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