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Original Article

Heamatology, Serum Biochemistry, Relative Organ Weight and Bacteria Count of Broiler Chicken Given Different Levels of Luffa Aegyptiaca Leaf Extracts

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ABSTRACT

The objective of this study was to evaluate the heamato-biochemical, relative organ weight and bacteria count of broiler chicken given different levels of Luffa aegyptiaca leaf extract (LUF). A total of 250 day old Ross 308 broiler chicks of mixed sex were divided into five treatments, each group was further divided into five replicates each of ten (10) birds in a completely randomized design. Birds in treatment A were given 1.20 g/litre of Neomycin in water, treatment B, C, D and E were given 5, 10, 15 and 20 mL/litre of LUF. Clean feed and water was given ad libitum and the experiment lasted for 3 weeks. The obtained data was used to evaluate the haematological parameters (PCV, RBC, Hb, WBC, MCV, MCH and MCHC), serum biochemical indices (Albumin, globulin, calcium, phosphorus, SGPT and SGOT) and organ weight (liver, kidney, spleen, heart, lungs, pancreas and small intestine) and bacteria count (*E. coli* and *Lactobacillus*). The obtained results revealed that there were no significant differences ($p>0.05$) in the haematological, relative organ weight, serum biochemical parameters and *E.coli* count. However, there was a significant difference ($p<0.05$) in the *Lactobacillus* count among the group. It was concluded that LUF could be given to broilers at 20 mL/litre without any deleterious effect on the blood profile and general performance of the animal.

Key words: Luffa aegyptiaca extract, Broilers, Haematology, Organ weight, Bacteria count.

Introduction

Medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or be regarded as precursors for the synthesis of useful drugs (Sofowora, 1993). Recently, there has been an increasing demand for organic or natural products to reduce or eliminate the use of antibiotics as growth promoters due to their high cost and negative human health issue of antibiotic resistance (Hassan, 2007). There have also been concerns on the contamination of animal products which made the European Union in 2006 to ban the use of antibiotics as growth promoters in livestock feed. Plants (herbs and spices) are therefore botanical alternatives to antibiotics because they possess phytochemicals or secondary metabolites. Phytochemicals also regarded as phytobiotics are a natural bioactive compound that are derived from plants and incorporated into animal feed or water as extract to enhance

productivity through the improvement of digestibility, nutrient absorption and elimination of pathogens resident in the animal gut (Rios and Recio, 2005; Sokovic *et al.,* 2010; Levic *et al.,* 2011; Rajasekaran *et al.,* 2013; Gadde *et al*., 2017). Plant extracts are used in animal nutrition as appetite and digestion stimulants, stimulants of physiological functions and treatment of certain pathological conditions, colourants and antioxidants (Tamara *et al.*, 2009). According to (Lina Šernaite, 2017) plants have the ability to synthesize secondary metabolites such as phenol, phenolic acids, flavones, flavonoids, flavonols, quinones, tannins, saponins, alkaloids and phytate. Phenolic compounds have been reported to exhibit antimicrobial and antioxidant activity (Martins *et al*., 2015), steroids have been reported to have antimicrobial and antifungal activity (Martins *et al*., 2015) and stimulation of bone marrow and growth. Saponins have been reported to perform antioxidant role and are useful in vaccine production (Asl and Hosseinzadeh, 2008). Flavonoids and tannin have been suggested to play a key role as antibacterial, antifungal, antidiarrheal and antioxidant (Adisa *et al.*, 2010). Alkaloids have been shown to have antipyretic activity (Faizi *et al.*, 2008).

Previous studies have been carried out on the use of plant extracts and their level of inclusion in the water of broilers, for instance, citrus sinensis peel extract (Abbas Ebrahimi *et al*., 2014; Akbarian, 2013; Pourhossein *et al.*, 2012), Morigold flower extract (Nuraini *et al*., 2017; Skrivan *et al*., 2016), Turmeric extract (Nuraini *et al*., 2019; green tea extract (Farahat, 2016; El-deek *et al*., 2012; Khalaji *et al*., 2011; Shahid *et al*., 2013), Neem leaf extract (Biu *et al.*, 2009; Chakravarty and Pasad, 1991; Nagalakshmi *et al*., 1996), Moringa olifera leaf extract (Alabi *et al*., 2017; Khan *et al*., 2017; Faluyi and Agbede, 2018). Yet, there is a dearth of information on the use of Luffa aegyptiaca extracts on the heamato-biochemical and bacterial count of broilers.

There is a correlation between nutrition and immune response, an animal that is not properly managed will undergo stress, poor health and finally death during serious conditions. Hence, the presented study is focused to investigate the effect of Luffa aegyptiaca leaf extracts on the haematology, serum biochemistry and bacteria count of broiler chicken.

Materials and methods

Experimental site

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of January to March, 2019.

Collection and processing of Luffa aegyptiaca leaves (LUF)

Luffa aegyptiaca leaf was identified and authenticated by a botanist on the research farm. Thereafter, fresh disease free leaves of *L. aegyptiaca* were harvested from the farm, the leaves were thoroughly washed with running tap water to remove the debris and allowed to dry under shade for 11 days, and they were then hammer milled into *L. aegyptiaca* powder (LUF). The extract was prepared by soaking 200 grams of LUF in one litre of water and kept in an air tight plastic container and the mixture kept in the refrigerator at 4 ^{o}C for 48 hours and then sieved with a with cheese cloth, then with What Man No 1 filter paper (24 cm).

Pre-experimental operations

A deep litter poultry house was used for the experiment, the pen was swept, cleaned and well disinfected with Cid 2000, feed and water troughs were also washed. The electrical

fittings (bulb) 200 watts were properly fixed and a vaccination programme was designed before the commencement of the study.

Animal management and experimental set-up

One day old 200 (Ross 308) broilers of mixed sex were obtained from a commercial hatchery in India. The chicks were weighed individually at the beginning of the experiment and wing banded. They were assigned into five treatments, each group was further divided into five replicates each of ten (10) birds. Anti-stress was added in the drinking water of the birds. The light (electric bulb) was continuous and the initial brooding temperature was 34 ^{o}C for the first week of age and it was gradually reduced by 2 ^{o}C per week. Vaccines were administered according to the prevailing vaccination schedule in the environment. Vitamins (Miavit) were added in water a day before and after each vaccination. Clean feed and water were provided unrestricted throughout the experimental period which lasted for 3 weeks.
Diets were formulated to meet the nutritional requirements of birds according to NRC (1994).
Treatment 1: 1.20 g/liter Neomycin (drinking water)
Treatment 2: 5.0 mL/liter of LUF
Treatment 3: 10 mL/liter of LUF
Treatment 4: 15 mL/liter of LUF
Treatment 5: 20 mL/liter of LUF

Blood analysis

At the end of the experiment, ten (10) birds were randomly selected from each treatment, two from each replicate for blood analysis. Blood was collected from the wing vein with a syringe and needle. Samples meant for hematology were put in tubes containing EDTA to prevent coagulation while those for serum biochemical parameters were put in bottles without EDTA. Hematological parameters covered pack cell volume (PCV), red blood cell (RBC), hemoglobin concentration (Hb), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Serum parameters included albumin, globulin, total protein, cholesterol, calcium, phosphorus, serum glutamic oxaloacetate transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT). PCV, RBC, Hb, MCV, MCHC, WBC and its differentials were determined using standard techniques as reported by Jain (1986). The serum total protein, Albumin and Globulin were computed according to (Doumas and Briggs, 1972). Glutamic oxaloacetate transaminase (SGOT) and Glutamic phosphatase transaminase (SGPT) were determined according to Scott (1965).

Carcass evaluation and bacteria count evaluation

At the end of the 3rd week, five birds were randomly selected per treatment; they were fasted overnight and given only water, weighed and slaughtered. After evisceration, the weight of the visceral organs and other parts of the birds were recorded. One gram of each sample of the jejunal content was collected and transferred into the test tube and was then diluted with 9ml of 1% peptone broth and homogenized. Counts of bacteria and lactobacillus were determined according to (Farmer 2003).

Laboratory analysis

Phytochemical analysis was carried out on the plants leaf extract using standard methods Sofowora, 1993; AOAC, 2000). Percentage composition of flavonoids, saponin, phytate, alkaloids, tannin and oxalate were carried out according to procedures outlined by (Harbone, 1984; Boham and Kocipai-Abyazan, 1974). Mineral analysis was carried out using Atomic Absorption Spectrophotometer (AAS). Proximate analysis of crude protein, ash, ether extract and crude fibre in the experimental diet was carried out in accordance with the Association of Official Analytical Chemists (AOAC, 2000).

Statistical analysis

All data were analysed using (Snedecor and Cochran, 1989). The difference in treatment means was separated using Duncan's multiple range test as outlined by (Obi, 2002).

Table 1. Percentage composition of experimental diets (0-3 weeks)

Ingredients	Quantity
Maize	52.00
Soya meal	38.60
Groundnut cake	3.00
Fish meal (72%)	1.00
Bone meal	3.00
Limestone	1.50
Lysine	0.15
Methionine	0.20
Toxin binder	0.01
Premix	0.25
Salt	0.30
Total	100.0
Calculated analysis	
ME (Kcal/kg)	2801.9
Crude protein (%)	23.23
Ether extract (%)	6.11
Crude fibre (%)	3.14

* Premix supplied per kg diet :- Vit A, 10,000 I.U; Vit E, 5 mg; Vit D3, 3000I.U, Vit K, 3 mg; Vit B2, 5.5 mg; Niacin, 25 mg ; Vit B12, 16 mg ; Choline chloride, 120 mg ; Mn, 5.2 mg ; Zn, 25 mg ; Cu, 2.6 g ; Folic acid, 2 mg ; Fe, 5 g ; Pantothenic acid, 10 mg ; Biotin, 30.5 g ; Antioxidant, 56 mg

Table 2. Phytochemical components of Luffa aegyptiaca leaf extract

Parameters	% Composition	Recommended safe
Saponin	6.22	7.02
Flavonoids	3.02	6.11
Phytate	1.01	11.33
Alkaloids	2.81	3.50
Tannin	3.11	31.20
Oxalate	1.01	1.30
Phenol	11.43	-

Vikas Kumar *et al.,* (2009)

Table 3. Mineral composition of Luffa aegyptiaca leaf meal

Components	Quantity (mg/g)
Calcium	34.31
Phosphorus	0.12
Potassium	2.16
Magnesium	7.44
Sodium	0.31
Manganese	0.01
Zinc	0.001
Iron	0.03

Table 4. Relative weight of internal organs of broilers given Neomycin and LUF

	Group					
Parameters (g)	**A**	**B**	**C**	**D**	**E**	**SEM**
Heart	3.51	3.47	3.44	3.57	3.50	0.34
Liver	1.22	1.26	1.27	1.19	1.49	0.22
Pancreas	2.26	2.27	2.22	2.24	2.26	0.18
Gizzard	3.17	3.13	3.09	3.00	3.10	0.27
Proventriculus	1.47	1.44	1.41	1.52	1.49	0.29
Intestine (cm)	81.2	81.1	79.3	90.4	91.9	14.2

Table 5. Bacteria and *Lactobacillus* count of broiler given Neomycin and LUF

Parameter	A	B	C	D	E	SEM
E. coli (cfu/g)	22.11	21.81	21.26	21.17	21.11	6.77
Lactobacillus	10.44^{c}	17.44^{c}	20.44^{b}	20.51^{a}	20.73^{a}	4.11

Table 6. Haematological parameters of broilers given Neomycin and LUF

	Group					
Parameters (g)	**A**	**B**	**C**	**D**	**E**	**SEM**
Pack cell volume (%)	29.22	29.88	30.01	30.67	31.71	1.31
Haemoglobin (g/dl)	11.51	12.13	12.61	12.98	13.11	1.34
Red blood cell $(10)^{6}$/µl	2.06	2.11	2.21	2.29	2.30	0.31
MCV (fl)	103.5	141.2	158.2	161.7	161.9	35.7
MCH (pg)	48.34	48.37	50.33	50.88	53.33	3.00
MCHC %	39.67	40.61	41.58	41.77	42.01	2.91
WBC $(10)^{6}$ /µl	19.31	20.21	20.89	21.12	21.56	2.07
Heterophils (%)	6.13	7.02	7.23	8.10	8.15	0.47
Monocytes (%)	0.89	0.94	1.02	1.10	1.12	0.21
Lymphocytes (%)	9.04	9.31	9.51	9.64	9.85	0.45
Eosinophils (%)	1.00	1.03	1.10	1.11	1.12	0.91

a, b, c means being different superscripts in the same row are significantly different (P<0,05).

WBC- White blood cells; MCV – Mean corpuscular volume; MCH- Mean corpuscular haemoglobin; MCHC – Mean corpuscular haemoglobin concentration

Table 7. Serum biochemical parameters of broilers given Neomycin and LUF

Parameters (g)	Group A	B	C	D	E	SEM
Total protein (g/dl)	3.45	3.61	3.63	3.65	3.68	0.25
Albumin (g/dl)	1.83	1.87	1.90	1.91	1.95	0.24
Globulin (g/dl)	1.62	1.74	1.73	1.74	1.73	0.25
Cholesterol (mg/l)	68.1	68.3	65.1	65.3	65.1	8.72
Calcium (mg/l)	6.91	6.98	7.13	7.61	7.71	2.01
Phosphorus (mg/l)	4.01	4.13	4.32	4.33	4.41	1.10
SGPT (iu/l)	33.1	30.6	30.1	28.4	28.1	2.34
SGOT (iu/l)	63.2	61.4	59.3	58.1	55.1	3.21

SGPT- Serum glutamic pyruvate transaminase
SGOT- Serum glutamic oxaloacetate transaminase

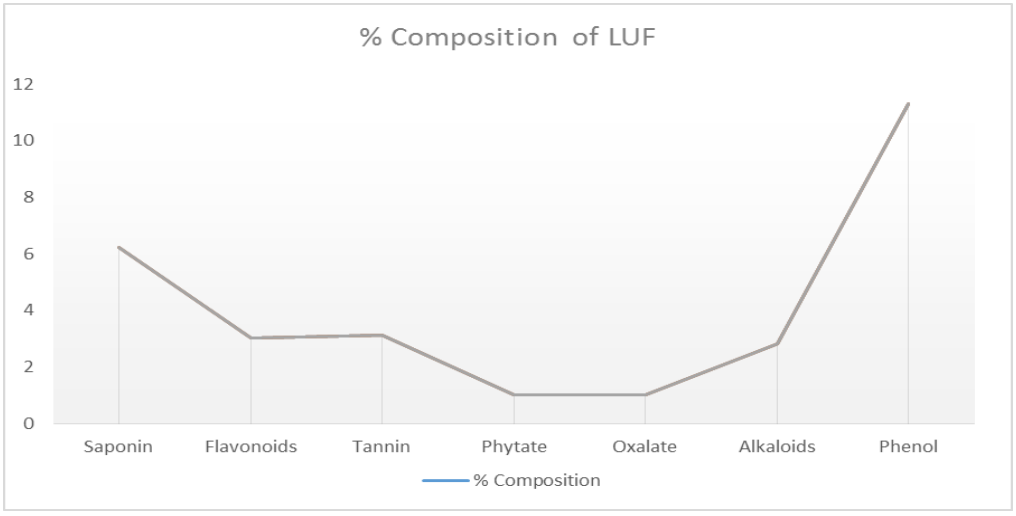

Figure 1. Phytochemical composition of LUF

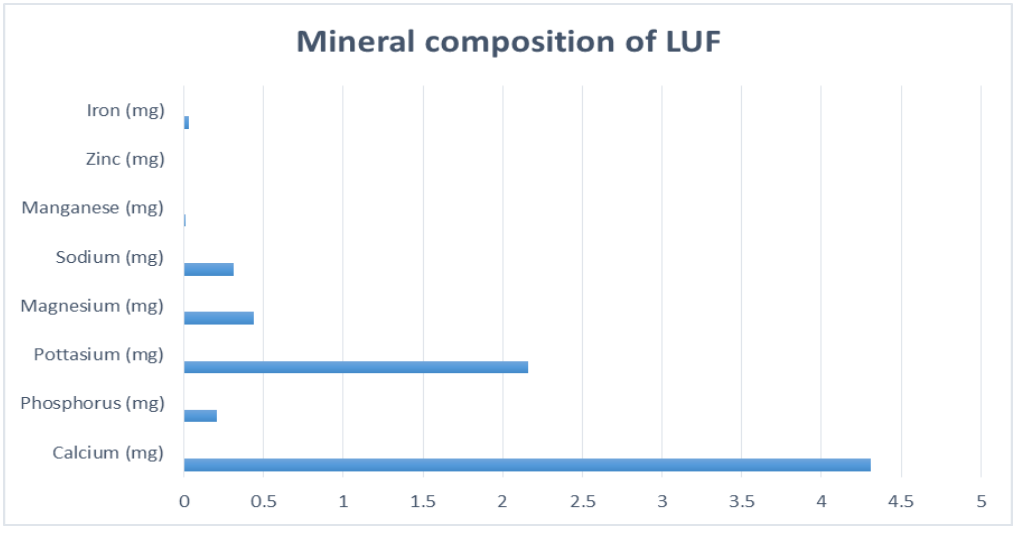

Figure 2. Mineral composition of LUF

Results and discussion

Table 1 shows the percentage composition of experimental diet. The diets were formulated to meet the nutritional requirements of the animals according to (NRC, 1994) specifications. The result on the phytochemical analysis of LUF revealed the presence of several bioactive compounds at different concentrations as presented in Table 2. The phytochemical components are saponin, flavonoids, phytate, alkaloids, tannin, oxalate and phenol at 6.22%, 3.02%, 1.01%, 2.81%, 3.11%, 1.01% and 11.43% respectively. The result in the current study was consistent with the findings of (Shakeri *et al.,* 2012; Jong *et al.,* 2010; Mabhiza *et al.,* 2016). However, all values falls within the safe recommended limit as proposed by (Vikas Kumar *et al.,* 2009). According to (Igwe *et al*., 2010), alkaloid helped in repelling parasites and predators in plants and, when ingested at high level, could result in a negative effect on the thyroid stimulating hormone and some enzymes (Okaka *et al*., 1992). Flavonoids perform several functions such as antibacterial, antioxidant, anti-inflammatory, anti-viral and anti-carcinogenic (Middleton *et al*., 2000).

The mineral composition of LUF revealed the followings: calcium (34.31 mg/g), phosphorus (0.12 mg/g), potassium (2.16 mg/g), magnesium (7.44 mg/g), sodium (0.31 mg/g), manganese (0.01 mg/g), zinc (0.001 mg/g) and iron (0.03 mg/g). Calcium had the highest quantity followed by magnesium, zinc had the lowest value, the result obtained in this experiment is contrary to the reports of (Alagbe Seyi Valerie *et al.,* 2017) who recorded a higher value for calcium (58.6 mg/g) and magnesium (12.4 mg/g). The reported higher value can be attributed to differences in the variety of *L. aegyptiaca* leaf, environment and stage of growth of the plant before it was harvested. The presence of different minerals in LUF facilitates various physiological activities in animals. According to (Murray *et al.,* 2000), minerals are structural components of the body tissues which are involved in the maintenance of acid base balance, regulation of body fluids, transport of gases and in muscle contractions.

Calcium plays a vital role in providing rigidity and support to animals (Ibrahim *et al*., 2001). Magnesium, zinc, iron and manganese are important co-factors found in the structure of certain enzymes and are indispensable in numerous biochemical pathways (Soetan *et al*., 2010).

The relative organ weights of broilers as given by Neomycin and LUF are presented in Table 4. The relative weights of heart, liver, pancreas, gizzard, proventriculus and intestine were not ($P>0.05$) significantly different among the groups. The weight of the heart ranged from 3.44–3.57 g, liver (1.26-1.49 g), pancreas (2.22–2.27 g), gizzard (3.00–3.17 g), proventriculus (1.41–1.52 g) and intestine (79.3–91.9 cm). According to (Madhusadha *et al.,* 1986), antinutrients are causes of internal organs enlargement in birds. The non-significant differences in the organs' weight can be simply regarded as a reflection that the test materials are non-toxic. The results are in agreement with the findings of (Bolu *et al*., 2009) but contrary to the findings of (Nderi *et al*., 2014).

Table 5 revealed the bacteria and Lactobacillus count of broiler given by Neomycin and LUF. The bacteria count ranged from 21.11-22.11 (cfu/g) while those of lactobacillus ranged from 10.44–20.73 (cfu/g). There was a significant difference ($p<0.05$) in the E.coli count among the groups, the values slightly increased from group 1 to 2 before it eventually decreased though not at a significant rate. This was comparable to the findings of (Al-Mashhadani, 2014). There was no significant ($p>0.05$) difference in the lactobacillus count among the groups, thus acting as prebiotics. The current study is also in line with the

findings of (Bird *et al*., 2002; Tiwari and Jyoti, 2008) who reported that Lactic acid bacteria produce several bactericidal/antibiotic like substances, which have been found to be effective against enteric pathogens. These bacteriocins can kill pathogenic bacteria, prevent colonization actions and also perform competitive exclusion which refers to the physical blocking of opportunistic pathogen (Watkins and Kratzer, 1983).

Haematological parameters of broilers given by Neomycin and LUF are presented in Table 6. The PCV values ranged from (29.22-31.71%), Hb (11.51-13.11 g/dl), RBC (2.06-2.30 10^6/µl), MCV (103.5-161.9 fl), MCH (48.34–53.33 pg), MCHC (39.67–42.01%), WBC (19.3121.56 10^6/µl), heterophils (6.13–8.15%), monocytes (0.89-1.12%), lymphocytes (9.04–9.85%) and eosinophils (1.00-1.12%). There were not significant ($p>0.05$) differences in all of the haematological parameters. In this sense, the haematological values slightly increased from group A to E but not at a significant level. The trend in the values could be as a result of intestinal microbial balance and a reflection that the animals were well nourished. However, all values are within the range reported by (Talebi *et al*., 2005; Islam *et al*., 2004). Changes in the RBC, MCH, MCHC and PCV values have been attributed to adaptation to adverse weather condition (Minka and Ayo, 2007), PCV and Hb variations which could also be linked to differences in the breed of animals (Adili and Melizi, 2013). The result obtained in the current study was consistent with the finding of (Akintomide *et al*., 2018) when neem leaf meal was fed to cockerels. (Adeyinka and Bello, 2013) also reported that WBC and its differentials helped to fight infections and produced antibodies to protect the body. Herbs, spices, and their extracts have high antioxidant capacity (Wojdylo *et al*., 2007).

The serum biochemical parameters of broiler chicken given by Neomycin and LUF revealed that albumin values ranged from (1.83-1.95 g/dl), globulin (1.62-1.83 g/dl), cholesterol (65.1–68.3 mg/l), calcium (6.91-7.71 mg/l), phosphorus (4.01–4.41 mg/l), SGPT (28.1-33.1 mg/l) and SGOT (55.1–63.2 mg/l). All the serum biochemical traits were not significantly ($p>0.05$) among the groups. Albumin values in the blood can be easily influenced by protein shortage, the results obtained is an indication that the experimental diets contained enough protein to support the normal protein reserves across the group. The values for all the parameters fall within the normal range values established for birds by (Albokhadaim, 2012). Reports have also shown that when probiotic is supplemented in the diets broilers they tend to have lower total cholesterol, VLDL cholesterol and triglyceride concentrations in the serum.

Conclusion

It can be concluded from the experiment that LUF could be given to broilers at 20ml/litre without any deleterious effect on the blood profile and general performance of the animal. LUF has proven to play the significant role of a competitive exclusion i.e. physical blocking of opportunistic pathogen, thus maintaining a balanced gut microbial ecosystem making it a botanical alternative to antibiotics for use in organic poultry production.

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