



PRODUCTIVE PERFORMANCE, CAECA MICROBIAL POPULATION AND IMMUNE-MODULATORY ACTIVITY OF BROILER CHICKS FED DIFFERENT LEVELS SIDA ACUTA LEAF EXTRACT IN REPLACEMENT OF ANTIBIOTICS

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Abstract

The aim of the present study was to examine the productive performance, caeca microbial population and immune-modulatory activity of broiler chicks fed different levels Sida acuta leaf extract (SAL) in replacement of antibiotics. A total of 375 one-day old broiler chicks (Ross 307) were allotted to five treatments with five replicates consisting of 15 birds each in a completely randomized design. The experiment lasted for 56 days during which feed and water were given ad libitum. Basal diet was formulated to meet the nutrient requirements for birds according to NRC (1994) and all other management practices were strictly observed. Birds in treatment 1 (T1) were fed basal diet with 0 % administration of ciprofloxacin, T2 were fed a basal diet plus administration of 0.2 ml of ciprofloxacin/litre of water while T3, T4 and T5 were fed Sida acuta leaf extract (SAL) at 20 ml, 40 ml and 60 ml per litre of water to replace ciprofloxacin as antibiotics. Result obtained revealed that weight gain, average daily weight gain (ADWG) and feed:gain ratio were significantly ($P<0.05$) influenced among the treatments. Highest mortality was recorded in T1 (15.1 %) followed by T2 (10.0 %) none were recorded in the other treatments ($P<0.05$). Average daily feed and water intake were not significantly ($P>0.05$) different among the treatments. Antibody titres against Newcastle and Gumbro disease were significantly ($P<0.05$) different among the treatments. Birds fed SAL had the highest values of antibodies making them resistant against diseases. Similarly, caecal microbial population revealed that E.coli population decreases in T3, T4 and T5 as the level of SAL increases among the treatments. However, lactobacilli populations were highest in T3, T4 and T5, intermediate in T2 and lowest in T1 ($P<0.05$). It was concluded that SAL is loaded with several bioactive compounds can be used to bridge the gap between food safety and livestock production and its oral administration at 20 ml/litre of water had no deleterious effect on the performance and health status of broiler chicks.

Keywords: Antibiotics, broiler chicks, chemicals, *Sida acuta*

INTRODUCTION

The important of plants of various types cannot be over emphasized. Since the time immemorial, plant has been taking so important in various approaches. Several plant leaves are delicacy in the preparation of stew in some tribes, which when its number and types is not complete the stew look awkward and unacceptable to them. The belief is that different plant contributes different nutrient to the stew which make it delicious and nutritional. Therefore plenty plant is of important to many pharmaceutical companies manufacturing a wide range of allopathic medicines, due to their phytochemical properties. This has caused increasing consideration of natural drug to an individual and most companies producing most synthetic drug.

One of the common examples is the use of plant extractive in the production of antimalarial from the plant called *Artemisia annua*. The drug is proven in the cure of malaria. Mostafa et al., (2012) identified extract from *Artemisia annua* to be the most effective anti-malarial because it rich in artemisinin, which was extracted from the leaves of *Artemisia annua*. This and several others has make plant to be major source of breakthrough in the pharmaceutical industries.

It is an inevitable fact that increasing research on medicinal plants could pave the way for the discovery of novel therapeutic agents against many diseases that are outstanding diseases (Perumalsamy et al., 2019) which threatened human existence. Interestingly, WHO has recognized the significance of traditional medicine in the health-care sector and has assessed that approximately eighty percent of the population living in the developing countries depend on herbal medicines for their primary health care requirement (Negash et al., 2017). There are many plants in the tropical countries that have not been harnessed, knowing their phytoprofile will help to enable many people to be attracted to their usefulness as a result of their phytoproperties.

Phytochemicals also called active principles or phytochemical substances (Ekpo and Etim, 2009) are the predominant substances present in many medicinal plants which responsible for many observed physiological actions. These phytochemicals are present in many forms such as alkaloids, steroids, tannins, glycosides, volatile oils, phenols, and flavonoids (Perumalsamy et al., 2019) while Ekpo and Etim, (2009) and Asaolu (2003) stated the active chemicals to consist terpenes, flavonoid, bioflavonoid, benzophenones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax-quinones which are non-specifically confined to various parts of a plant such as leaves, flowers, bark, seeds, fruits or roots (Perumalsamy et al., 2019). All these active ingredients are used in the phytotherapy and help to control or prevent many diseases. Ekpo and Etim, (2009) established that medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants.

Sida acuta is one of the plants with medicinal potential qualities and present in abundance in the tropics. It is drought resistance tropical weeds that are common in almost everywhere. *Sida acuta* is an erect, branched and perennial shrub with a woody tap root, hairy branched up to 1 m

high and is reproduced from their seeds. The stem is woody, rounded and slender, and is fibrous and hairy especially when young. The leaves are simple and alternate while the inflorescence is solitary and axillary with stalks up to 1.3 cm long jointed about half of the length. The flowers are yellow with five petals and the fruit is capsuled with 5 - 6 carpels (Ekpo and Etim, 2009). Therefore, this study was designed to examine the phytochemical properties of *Sida acuta* to make it uses popular to the populace.

MATERIALS AND METHODS

Study Area: The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of December to February, 2018.

Collection and processing of *Sida acuta* extract: Fresh and mature leaves of *Sida acuta* were collected within the farm premises and authenticated by a botanist (Liu Yung). The leaves were washed with running tap water, shade dried in a clean tray for 12 days and pulverized to coarse powder with a Panasonic electric blender Model WS-812 KJ and stored in an air tight container for further analysis. 100 g of the sample was soaked in 1000 ml water and agitated with an electric blender for 10 minutes, thereafter the mixture was turned into another container, kept in the refrigerator at 4°C for 72 hours and then filtered using filter paper (Whatmann No. 1) to obtain *Sida acuta* leaf extract (SAL).

Management of experimental birds: Two weeks before to the arrival of the animals, the deep litter pens were properly disinfected. Feeding and water troughs were washed; foot dip disinfectant was prepared at the entrance of the pen. A total of 375 broiler chickens of one-day-old (Ross-308) were purchased from a reputable hatchery in India and randomly allotted to five treatments in a Completely Randomized Design (CRD). Chicks were reared in deep litter house partitioned into pens as experimental units. Each treatment had 75 chicks in 5 replicates of 15 birds. Water and feed were offered ad libitum throughout the study period which lasted for 56 days. All the necessary routine management practices and vaccinations were administered according to the prevailing condition in the environment as presented in Table 2.

Formulation of experimental diets: Experimental diet was formulated to meet the nutritional levels of birds recommended by NRC (1991) as presented in Table 1. Broiler starter, grower and finisher diet contained 23 %, 21 % and 19 % crude protein respectively.

Treatment 1: Basal diet + 0.0 % Ciprofloxacin + 0.0 % SAL

Treatment 2: Basal diet + 0.2 ml Ciprofloxacin per liter of water

Treatment 3: Basal diet + 20 ml SAL per liter of water

Treatment 4: Basal diet + 40 ml SAL per liter of water

Treatment 5: Basal diet + 60 ml SAL per liter of water

Measurements and Calculations

- Daily feed intake (g) was calculated as a difference between feed offered and left-over.
- Weight gain = final weight (g) – initial weight (g)

- Feed conversion ratio (FCR) = $\frac{\text{Total feed intake (g/d)}}{\text{Total weight gain (g/d)}}$
- Average daily gain (g/d) = $\frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Number of experimental days}}$
- Mortality was also recorded as it occurs.
- Recommended daily allowance (RDA) = $\frac{\text{Concentrations of sample (mg)}}{\text{Requirement by animal}} \times 1000$

Immune Response: Birds were orally vaccinated against Newcastle on the 7th and 16th day and Gumboro disease on the 11th and 20th day. Five (5) birds were randomly selected per replicate to access the antibody response to Newcastle and Gumboro virus on the 28th and 45th day of the experiment. Blood samples were collected from the wing vein of the vaccinated birds into a test tube and sent into the laboratory for further analysis. Antibody titres against Newcastle and Gumboro viruses were measured using hemagglutination inhibition test according to the procedures outlined by Purchase et al. (1989).

Caeca microbial population: A 10-fold serial dilution method, in which 1% peptone solution was mixed with caeca samples collected from 3 birds per treatment for caeca microbial analysis. The mixture was poured on Mac Conkey agar plates and lactobacilli medium III agar plates to determine the colony forming unit (cfu) in each gram of caeca sample by means of pour plate method. E. coli was cultured on Eosin Methylene Blue (EMB) agar at 37°C for 48 hours. Lactobacilli were enumerated on Rogosa Sharpe agar at 37°C for 72 hours. Colonies with metallic green sheen colours were considered as E. coli while white colours signify colonies of lactobacilli. All records were computed according to the methods outlined by Suriya et al. (2014).

Laboratory procedures: Proximate analysis of experimental diet was determined by standard methods as described by AOAC (1990).

Statistical analysis: All data were subjected to one way analysis of variance (ANOVA) using SPSS (20.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

RESULTS AND DISCUSSION

Performance characteristics of broiler chickens are presented in table 3. The initial body weight values were similar and ranges from 43.40g (T5)- 43.50g (T1). Final body weights were significantly different ($p < 0.05$). Final weight increase with increase in the concentration of Sida acuta leaf extract. The control diet had 1896.40g final weight and this was smaller when compare with the other treatments. Weight gain (1852.90g T1- 2257.40g T5) were significantly higher ($p < 0.05$) in treatment 5 than the values obtained in T4, T3, T2 and T1. Although feed

intake was not affected significantly but in favour of treatment T1 (control diet) and treatment 5 consumed smaller quantity of feed to gain Kg weight. Mortality recorded were higher and similar under treatments T1 and T2 while there was no record of death in other treatments with higher inclusion of *Sida acuta* leaf extract (SAL). Improvement weight changes observed in the performance of birds placed on *Sida acuta* leaf extract may be as a result of the various phytonutrients in the SAL. The phytochemicals in SAL might have helped to either improve the feed utilization or act on some lock up nutrients in the feed. Also, the bioactive compounds might have enhanced the enzyme activities of the gut or inhibit the proliferation of harmful gut microbes. Also the gut physiology might have been induced to ensure good gut ecology in the process of digestion, which will reduce the inflammatory occurrence in the gut. The outcome of this research was in support of other researchers such as Fascina et al. (2017) when phytoadditive and organic acid were added to the feed of broiler chickens. Also, Dingfa et al. (2015) finding when Wenchang broiler chickens were exposed to turmeric extract. It has been reported by Yasmine and Timothy (2014) that microbiota play a fundamental role on induction, training and function of the host immune system. The adequately immune animal may have enough defensive mechanism against diseases which will enable them to concentrate the dietary nutrient on necessary grow factors. This was in line with the authors report that complex communities of microbes including bacteria, fungi, virus and other microbes provide tremendous enzymatic capability and play a fundamental role in controlling many aspects of the host physiology.

The immune response potentials to Newcastle and Gumboro diseases is presented in table 3. Immune system is a networks that involve cells, tissues, organs, system and the substances produced by each solely and in combination to help the animal to fight infections and diseases. This may be innate, adaptive or passive in nature. Newcastle antibody titres value at both day twenty eight and day forty five measured increase with increased levels of *Sida acuta* leaf extract, so also the gumboro disease titres values. Newcastle antibody titres (Log₂) in day 28 ranged from 1.03 (T1) – 8.00 (T5) and 2.90 (T1) – 12.94 (T5) on day 45. Also, gumboro disease antibody titres values for both day 28 and day 45 were in favour of the birds exposed to *Sida acuta* leaf extract. Age also helped to influence the antibody potentials significantly, irrespective of the treatments. The bioactive compounds in *Sida acuta* might have helped to improve the passive immune response against Newcastle and gumboro diseases in this research. Shittu and Alagbe (2020) has reported that *Sida acuta* consists of phenol, flavonoid and many other bioactive compound. The phenol and flavonoid had been implicated to be a bioactive compound that is responsible for the increase serum antibody titres values (Musa et al., 2020). This might have been responsible for the increase antibody response from the broiler chickens fed with the higher concentration of *Sida acuta* leaf extract. Phenols are strong antioxidants which prevents the entry of diseases (Alagbe, 2019).

Table 5 shows the *E. coli* and *Lactobacillus* population of broiler chickens fed varied concentration of *Sida acuta* leaf extract. Although, Musa et al. (2020) reports had earlier reported that the immune system benefits greatly from proper nutrition referred to as phytogenics of the bird. Phytogenics is a safe growth promoter without side effects on birds, it enhances and modifying the gut physiology of the birds (Alagbe et al., 2020), this research

finding has discovered that the microbes also benefit from *Sida acuta* phytonutrients. This allowed the population of both *E. coli* and *Lactobacillus* to increase steadily as the concentration of the leaf extract increases. The result may be a warning to the nutritionist to take caution in administering the extract to avoid overshooting of microbes in the gut of the animal as a result of the nutritional modifications. Although immune system was suggested to compose of a complex network of innate and adaptive components endowed with an extraordinary capacity to adapt and respond to highly diverse challenges (Yasmine and Timothy, 2014), this might have helped the chickens to survive increase in the number of *E. coli* and *Lactobacillus* as the *Sida acuta* leaf extract increases. This support the report of Alagbe et al. (2020) that phytonutrients benefit intestinal microbiota by controlling potential pathogens and improvement of nutrient absorption and enzyme activity to enhance better weight gain and feed conversion efficiency due to the presence of several bioactive chemicals.

Table 1 Experimental diets

| Materials | Starter (1-21 days) | Grower (22-35 days) | Finisher (36-56 days) |
|---------------------|---------------------|---------------------|-----------------------|
| Maize | 50.00 | 56.00 | 60.50 |
| Wheat offal | 8.00 | 7.00 | 8.05 |
| Soya meal | 28.55 | 22.00 | 21.00 |
| Groundnut cake | 10.00 | 11.55 | 6.05 |
| Fish meal | 2.00 | 2.00 | 2.00 |
| Bone meal | 0.35 | 0.40 | 0.40 |
| Limestone | 0.20 | 0.20 | 0.20 |
| Lysine | 0.15 | 0.15 | 0.15 |
| Methionine | 0.20 | 0.20 | 0.20 |
| Premix | 0.25 | 0.25 | 0.25 |
| Salt | 0.30 | 0.30 | 0.30 |
| Total | 100.0 | 100.0 | 100.0 |
| Calculated analysis | | | |
| Crude protein | 23.08 | 20.11 | 19.33 |
| Ether extract | 5.03 | 4.87 | 4.28 |
| Crude fibre | 3.06 | 3.95 | 3.42 |
| Calcium | 0.81 | 0.96 | 0.80 |
| Phosphorus | 0.47 | 0.40 | 0.51 |
| Lysine | 1.17 | 1.29 | 1.60 |
| Meth +Cyst | 0.87 | 0.82 | 0.51 |
| ME (Kcal/kg) | 2936 | 3000.8 | 3100.2 |

* Premix supplied per kg diet: - vit A, 13,000 I.U; vit E, 5mg; vit D3, 3000I.U, vit K, 3mg; vit B2, 5.5mg; Niacin, 25mg; vit B12, 16mg; choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; folic acid, 2mg; Fe, 5g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg.

Table 2 Vaccination programme for broilers

| Vaccine | Days | Route of administration |
|-------------------------------|------|-------------------------|
| 1 st ND (Lasota) | 7 | Drinking water |
| 1 st IBD (Gumboro) | 14 | Drinking water |
| 2 nd ND (Lasota) | 21 | Drinking water |
| 2 nd IBD (Gumboro) | 28 | Drinking water |

Table 3 Performance response of broiler chickens fed varied concentration of Sida acuta leaf extract

| Parameters (g) | T1 | T2 | T3 | T4 | T5 | SEM |
|-------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-------|
| Initial body weight (g) | 43.50 | 43.42 | 43.45 | 43.47 | 43.40 | 0.03 |
| Final live weight (g) | 1896.4 ^c | 2000.8 ^b | 2216.0 ^a | 2230.4 ^a | 2300.8 ^a | 71.65 |
| Weight gain (g) | 1852.9 ^b | 1957.4 ^b | 2172.6 ^a | 2186.9 ^a | 2257.4 ^a | 91.06 |
| Daily weight gain (g) | 33.09 ^b | 34.95 ^b | 38.80 ^b | 39.05 ^a | 40.31 ^a | 3.52 |
| Feed intake (g) | 4200.3 | 4118.6 | 4110.8 | 4110.2 | 4110.0 | 52.17 |
| Average feed intake (g) | 75.01 | 73.55 | 73.41 | 73.40 | 73.39 | 9.13 |
| Feed:gain ratio | 2.27 ^a | 2.10 ^a | 1.89 ^b | 1.88 ^b | 1.82 ^b | 0.25 |
| Mortality (%) | 15.1 ^a | 10.0 ^b | 0.00 | 0.00 | 0.00 | 0.07 |
| Total water intake (ml) | 18,909 | 18,708 | 19,000 | 19,001 | 19,212 | 5.77 |
| Daily water intake (ml) | 337.7 | 334.1 | 339.3 | 340.0 | 343.0 | 6.33 |

Means in the same row with different superscripts are significantly different ($P < 0.05$)

Table 4 Immune response of broiler chickens fed varied concentration of *Sida acuta* leaf extract

| Parameter | T1 | T2 | T3 | T4 | T5 | SEM |
|---------------------------------|-------------------|-------------------|--------------------|--------------------|--------------------|------|
| Day 28 (Log₂) | | | | | | |
| Newcastle disease | 1.03 ^c | 4.11 ^c | 6.83 ^b | 7.03 ^b | 8.00 ^a | 0.10 |
| Gumboro | 2.61 ^c | 3.00 ^b | 4.72 ^a | 4.88 ^a | 5.01 ^a | 0.45 |
| Day 45 (Log₂) | | | | | | |
| Newcastle | 2.90 ^c | 8.63 ^b | 11.10 ^a | 12.08 ^a | 12.94 ^a | 0.12 |
| Gumboro | 3.41 ^b | 6.03 ^a | 7.01 ^a | 7.32 ^a | 7.88 ^a | 0.76 |

Means with different superscripts in the same row are significantly different ($P < 0.05$).

Table 5 Caeca microbial population of broiler fed varied concentration of *Sida acuta* leaf extract

| Parameter | 1 | 2 | 3 | 4 | 5 | SEM |
|----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|
| E. coli (Cfu/g) | 18.26 ^c | 27.12 ^b | 32.00 ^a | 33.60 ^a | 33.85 ^a | 4.71 |
| Lactobacilli (Cfu/g) | 8.06 ^c | 11.31 ^b | 20.54 ^a | 20.72 ^a | 21.50 ^a | 2.4 |

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