

## **Effect of scent leaf-extract (*ocimum gratissimum*) and neem leaf-extract (*Azadirachta Indica*) fed as antimicrobial feed additive on finishing broilers**

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**Target Audience:** *Animal Scientists, Poultry Producers, Animal Health Practitioners*

### **Abstract**

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100 Marshal White broiler chicks at 5-weeks old were fed on Scent leaf-extract (SLE) (*Ocimum gratissimum*) and Neem leaf-extract (NLE) (*Azadirachta indica*) to determine the effect of these leaf-extracts as antimicrobial feed additive. The Fresh leaf-extracts were administered on daily basis to the birds for four weeks at varying concentrations of 0ml, 10ml, 20ml, 30ml and 40ml per liter of water. Water intake, feed intake, growth performance and mortality rate of the birds were monitored during the period. Microbial levels of blood and fecal samples of the treatments were analyzed at the end of experiment. The results indicated a progressive decrease of water intake among chicks on NLE as the concentrations increased. The chicks on SLE did not show any significant water intake reduction ( $p > 0.01$ ) until 30ml concentration. Feed intake was significantly affected ( $P < 0.05$ ) by the leaf extracts' concentrations. The chicks on NLE had their feed intake progressively depressed as the concentration of the extract increased, while chicks on SLE had their feed intake depressed at 30ml/L concentration. The weight gain of the chicks on both extracts were higher than the weight gain without any extract up till 30ml/L for SLE and 10ml/L for NLE. The mortality rate of the chicks on SLE were less than chicks without the scent leaf extract up till the level of 30ml/L, while the mortality rate of those on NLE were not. There was continuous reduction of white and red blood cell per phial in the stool microscopy wet preparation in both NLE and SLE as the concentration of the extracts increased. The percentage of microbial isolates from blood and stool culture decreased progressively as the leaf-extract increased.

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**Key words:** *Scent leaf extract, Neem leaf extract, broiler chicks, microbial feed additive.*

### **Description of Problem**

Nigerian poultry industry, which is estimated at 137.6 million with 84% backyard poultry and 16% exotic poultry has issues among which is disease prevalence (1). In livestock production including poultry, issues of disease prevalence is of serious concern particularly in Nigeria where disease preventive measures and treatment forms a major part of farm expenditure in the production process. The expenditure in form of drugs and vaccines procurement especially with the foreign drugs and vaccines that are

usually costly, discourage small-holder farmers from adopting the best international standards of maintaining a healthy flock in poultry production (2).

It is a fact that one of the ways of disease management in poultry production process is through drug therapy. However, human health challenge due to indiscriminate drug therapy in the country is of serious threat to health of Nigerian populace. (3) This is because the uncontrolled drug usage leads to drug residues in poultry products. These drug residues could cause drug resistance in human, which is one

of the major health challenges associated with consumption of poultry products such as meat and eggs affected with drug residues (4, 5).

Many countries of the world including the European Union have prohibited the use of antibiotics and growth stimulants in poultry production because of human health complications (6). Health complications such as drug inactivation, drug modification, and even alteration of drug target sites and metabolic pathways as well as reduced drug accumulation could render drug therapy administration ineffective in human health management (7).

Reports have shown that organic extracts from plants can provide the medicinal remedy for disease therapy without the danger of drug resistance (8). This is because they contain active substances that can cure and prevent many diseases (9). Some plant extracts are of much benefit in fighting diseases in poultry because these extracts exhibit antioxidant and other bioactive properties (10). Efforts to identify such plant extracts are necessary for medical purpose to enhance efficient disease management in Nigerian poultry industry and at the same time increase the chances of producing wholesome poultry products for human consumption.

Earlier studies have reported that Neem plant, *Azadirachita indica* and scent plant, *Occimum grassitissimum* are two popular medicinal plants for human drug therapy in Nigeria (11,12). They are widely used because of their bioactive properties in fighting bacterial diseases, anti-inflammatory and immune modulatory agents (13, 14).

Moreover, the leaves of *Occimum grassitissimum*, can be used fresh or dried to serve as flavoring or spices, apart from its medicinal value. This is because of the reasonable level of vitamin, amino acid and mineral. The low level of phytates, oxalate, tannins and saponins in the fresh leaves make the leaves safe for consumption and hardly

cause any serious hazard even when consumed in a large quantity is a unique quality of the plant (15). The proximate composition of fresh neem leaves revealed 64.0% moisture, 15.5% crude protein, 12.7% crude fibre, 4.2% ether extract, 11.2% ash, 56.5% nitrogen free extract, 26.5% calcium and 0.24 phosphorus (13). However, Neem leaves were identified as having nutrients imbalance and improper metabolism in feeding layers birds because of anti nutritional factors and toxic elements as feeding ingredients (16). Nevertheless, Neem plant contains bioactive compound such as cyclic trisulphide, mamoodin, margolon and other bio actives that are bactericidal, anti-inflammatory and immune modulatory (17). The aim of the experiment is to assess the extracts of neem leaf and scent leaf on growth performance and microbial load in broiler finisher chickens.

### Materials and Methods

One hundred Marshal White broiler chicks were randomly selected from five weeks old chicks raised on Teaching and Research farm, Ebonyi State University Abakaliki and fed with commercial feed from Master Feed Limited, Nnewi in Anambra state. Fresh Scent and Neem leaf extracts were obtained from the two plants by squeezing their leaves using *Jumbo Happy Home* mechanical extractor equipment with pure ethanol at one ml per gram. The extracts were exposed for 30minutes evaporation they were administered to birds orally through drinking water. The birds were fed with commercial finisher diet at estimated nutrient levels of 23.0% crude protein, 2850kcal/kg Metabolizable energy, 4.5% fiber, and 1.3% calcium among other nutrients without any other drug or additive.

### Experimental Design

The birds were randomly assigned to ten treatments involving 2 X 5 factorial

arrangements in a completely randomized design (CRD) at 0ml, 10ml, 20ml, 30ml, and 40ml leaf extract per liter of water concentration. Two treatment plants of Scent leaf extract and Neem leaf extract were involved in the trial. Each treatment comprises five replicates.

**Table 1: Feed and water intake, growth performance and mortality rate of finishing broilers on scent leaf extract (SLE) and Neem – leaf extract (NLE)**

LEAF EXTRACT	T <sub>0</sub>	T <sub>S1</sub>	T <sub>n1</sub>	T <sub>S2</sub>	T <sub>n2</sub>	T <sub>S3</sub>	T <sub>n3</sub>	T <sub>S4</sub>	T <sub>n4</sub>	SEM
5wk body weight (g)	1,200.00	1,205.00	1,203.05	1,200.50	1,201.00	1,209.00	1,201.00	1,203.00	1,200.00	33.11
9wk body weight (g)	2,633.04	3,182.08	2,751.28	3,307.14	2,545.84	2,983.08	2,124.16	2,352.68	1,586.26	41.67
Daily weight gain (g)	51.18 <sup>b</sup>	70.61 <sup>a</sup>	55.26 <sup>ab</sup>	75.25 <sup>a</sup>	48.03 <sup>a</sup>	63.36 <sup>a</sup>	32.97 <sup>d</sup>	41.06 <sup>c</sup>	12.81 <sup>c</sup>	3.82
Daily feed intake (g)	127.95 <sup>b</sup>	148.29 <sup>a</sup>	143.68 <sup>a</sup>	150.51 <sup>a</sup>	139.29 <sup>ab</sup>	134.32 <sup>ab</sup>	107.16 <sup>c</sup>	106.76 <sup>c</sup>	100.11 <sup>c</sup>	52.55
Daily water intake (ml)	247.86 <sup>a</sup>	216.67 <sup>a</sup>	188.59 <sup>ab</sup>	214.36 <sup>a</sup>	160.29 <sup>b</sup>	170.02 <sup>b</sup>	142.90 <sup>c</sup>	138.89 <sup>c</sup>	97.82 <sup>d</sup>	78.19
Feed/gain ratio	2.50	2.10	2.60	2.00	2.90	2.12	3.00	2.60	3.05	
Mortality %	15.20 <sup>c</sup>	7.30 <sup>d</sup>	14.90 <sup>c</sup>	6.50 <sup>d</sup>	19.39 <sup>c</sup>	10.37 <sup>cd</sup>	28.77 <sup>b</sup>	24.80 <sup>b</sup>	39.00 <sup>a</sup>	18.00

a,b,c,d: Means with different superscripts are significantly different (P<0.05)

- To = 0ml Scent and Neem leaf extract per liter of water
- T<sub>S1</sub> = 10ml Scent leaf extract per litre of water
- T<sub>n1</sub> = 10ml Neem leaf extract per liter of water
- T<sub>S2</sub> = 20ml Scent leaf extract per liter of water
- T<sub>n2</sub> = 20ml Neem leaf extract per liter of water
- T<sub>S3</sub> = 30ml Scent leaf extract per liter of water
- T<sub>n3</sub> = 30ml Neem leaf extract per liter of water
- T<sub>S4</sub> = 40ml Scent leaf extract per liter of water
- T<sub>n4</sub> = 40ml Neem leaf extract per liter of water

### Blood Microscopy examination

Five blood samples were taken randomly from each treatment at two weeks interval. They were collected by bleeding the wing vein of the chicks with sterile needle and introducing about 10mls into Bijou labelled bottle with *Ethylene Diamine Tetra Acetic acid (EDTA)* anti coagulant. The blood samples were used for broth culture, which were sub-cultured with *Mac-conkey agar* and *Deoxycholate Citrate agar* and incubated for 24hours. Motility test conducted with *Triple Iron agar* reaction for microbial identifications (18) was used.

### Stool microscopy examination

Five fresh fecal samples randomly collected with scrub sticks from each of the treatments at two weeks intervals, incubated and cultured in *Selenite 'F'* broth at 37°C under anaerobic condition for 24hours and thereafter, sub-cultured using *Salmonella-Shigella agar* and *Deoxycholate Citrate agar* for the microscopy examination. *Triple Sugar Iron agar* reaction was involved for the motility test to identify the colony characteristics of the organisms.

**Table 2: Hemomicrobial load of broiler chicken on Scent Leaf and Neem leaf Extract Blood Culture**

Extract	T <sub>0</sub>	T <sub>S1</sub>	T <sub>n1</sub>	T <sub>S2</sub>	T <sub>n2</sub>	T <sub>S3</sub>	T <sub>n3</sub>	T <sub>S4</sub>	T <sub>n4</sub>
E.Coli	18	08	04	05	02	03	0	03	0
Klebsiella	12	4	5	3	0	0	0	0	0
Salmonella	45	12	10	5	4	4	0	0	0
Pasteulla	10	0	0	0	0	0	0	0	0
Clostridia	10	0	0	0	0	0	0	0	0
Pseudomonas	12	0	0	0	0	0	0	0	0
Emillia species	15	14	5	12	5	8	0	0	0
Helminthoozyte	0	0	0	0	0	0	0	4	0
Yeast	0	0	0	0	0	0	0	0	0

- To = 0ml Scent and Neem leaf extract per liter of water
- T<sub>S1</sub> = 10ml Scent leaf extract per litre of water
- T<sub>n1</sub> = 10ml Neem leaf extract per liter of water
- T<sub>S2</sub> = 20ml Scent leaf extract per liter of water
- T<sub>n2</sub> = 20ml Neem leaf extract per liter of water
- T<sub>S3</sub> = 30ml Scent leaf extract per liter of water
- T<sub>n3</sub> = 30ml Neem leaf extract per liter of water
- T<sub>S4</sub> = 40ml Scent leaf extract per liter of water
- T<sub>n4</sub> = 40ml Neem leaf extract per liter of water

**Data Collection**

Feed and water intake were monitored on daily basis, while the bird’s weight were determined weekly on each treatment. Data for water consumption were standardize to minimize errors due to water of evaporation and other wastages (19).

**Statistical Analysis**

Descriptive Statistics and analysis of variance were used to analyze the data while the means were separated by Fisher least significant difference (20)

**Result and Discussion**

Feed and water intake, growth performance and mortality rate of broiler chicks on scent and neem leaf-extract indicating decreasing water intake as the concentration of leaf-extract increased from 0ml to 40ml per liter of water was presented in Table 1. The process of water metabolism in birds, which determines the water requirement and the level of water consumption in a broiler

chick, is a complex phenomenon (21). This is because the internal and external reactivity of water intake in birds is a stimulus governed by many factors, among which is the level of concentration of bio-chemical substances in water. Such substances in water can affect the thirst bud of the animals. The thirst bud of animal, which is responsible for water consumption, can easily be altered by the quantity of chemical substance in water (22). Reports have indicated that leaf-extract from Neem plant contains some bioactive substances such as *nimbin*, *salanin* and *azadirachtin* while scent plant contains *phytales*, *tannins* and *oxalates* substance in their extract that could be responsible for water intake reduction (23). Such reduction in water intake increases as the concentration of the leaf-extracts increased in water.

The result indicated that feed intake was significantly different (P<0.05) among the chicks on the Scent and Neem leaf-extract. The chicks on scent-leaf-extract had increased feed consumption from 0ml to 30ml/L while the

chicks on neem leaf-extract had increased feed consumption from 0ml to 20ml/L, thus indicating an increased palatability with scent leaf-extract than neem leaf extract. *Ocimum gratissimum* plant has good aroma that can improve palatability of the feed by chicks on the extracts. Report shows that the level of bioactive substance such as *saponins*, *tannins* and *oxalates* in fresh scent leaves are very low

and could not cause health hazard even when consumed in a reasonable quantity (12). Nevertheless, the result shows that increase in palatability has a limit at 30ml per liter concentration of scent leaf-extract beyond which affected the performance of the birds. This is understandable because 30ml per liter is the upper limit for water consumption in the experiment.

**Table 3: Faecal microbial loads of broiler chicken on SLE and NLE Stool microscopy wet preparation**

Cell phf	T <sub>0</sub>	T <sub>S1</sub>	T <sub>n1</sub>	T <sub>S2</sub>	T <sub>n2</sub>	T <sub>S3</sub>	T <sub>n3</sub>	T <sub>S4</sub>	T <sub>n4</sub>
Red Blood Cell	3 – 4	1 – 3	3 – 4	0 – 2	3 – 4	0 – 1	0 – 2	1 – 2	0
White Blood Cell	0 – 2	0	0	0	0	0	0	0	0
Helminth (Oozyte/ml)	35	15	5	10	0	5	0	0	0
E. Coli	32	8	4	5	2	3	0	2	0
Klebsiella	25	12	7	8	3	5	3	2	0
Salmonella	33	18	8	15	04	5	3	0	0
Pastuella	8	3	0	0	0	0	0	0	0
Clostridia	15	4	0	0	0	0	0	0	0
Pseudomonas	5	0	0	0	0	0	0	0	0
Emeria species	38	25	8	12	0	5	0	0	0
Fungi species	0	0	0	0	0	0	0	0	0

- T<sub>0</sub> = 0ml Scent and Neem leaf extract per liter of water
- T<sub>S1</sub> = 10ml Scent leaf extract per litre of water
- T<sub>n1</sub> = 10ml Neem leaf extract per liter of water
- T<sub>S2</sub> = 20ml Scent leaf extract per liter of water
- T<sub>n2</sub> = 20ml Neem leaf extract per liter of water
- T<sub>S3</sub> = 30ml Scent leaf extract per liter of water
- T<sub>n3</sub> = 30ml Neem leaf extract per liter of water
- T<sub>S4</sub> = 40ml Scent leaf extract per liter of water
- T<sub>n4</sub> = 40ml Neem leaf extract per liter of water

The growth performance of the birds on the leaf-extracts in terms of weight gain shows that the chicks on scent leaf-extract has better gain from 10m to 30m per liter of water than those without the leaf-extract, while those on Neem leaf-extract perform better only with 10ml/L and 20ml per liter. This shows that there is correlation between feed intake and weight gain. The adverse effect of leaf-extracts on feed consumed also showed the same effect on weight gain. The superior weight gain in

scent leaf-extract could be because of the nutritional values of scent leaf-extract. It was reported that scent leaves contain some levels of nutrients such as amino acid, vitamins, sodium, potassium, calcium and other micronutrient that affected the weight gain of the broiler chicks (12, 24). However, such could only be possible at a limited concentration level of scent leaf-extract. The adverse effect of the leaf-extract is much

evident in Neem leaf-extract as could be seen in the result.

The results indicated that feed conversion ratio of birds on leaf-extract from 10ml/L to 30ml/L was better than those birds without the leaf-extract. In addition, the mortality rate of the broiler chicks on scent leaf extract from 10ml to 30ml per litre of water was lower than those on water free scent leaf extract, indicating that the maximum limit of scent leaf-extract was at 30ml/L.

Table 2 shows the blood microbial load while Table 3 indicates the fecal microbial load of the broiler chicks on the leaf-extracts. There were decreasing and low percentage microbial isolates per milligram in both stool and blood culture of broiler chicks on the leaf-extracts.

There were also decreasing white and red blood cells per phial as the concentration of the leaf-extracts increased. The medicinal properties of scent and neem plants can be suggested to be responsible for that (19, 25). Reports have shown that *sodium nimbidate* was one of the bioactive substances that possess significant dose dependent of anti-inflammatory activities in some animals (26).

Ocimum oil was reported as being active against several pathogenic organisms such as bacteria, fungi and protozoa (27). Other bioactive substances such as *eugenol*, *methyl cinamate* and *thymol* in neem leaf extracts were also identified as the beneficial substances that protect the body against some diseases. The substances can also reduce stress and increase the efficiency use of oxygen in the body. They can as well boost immune system of the birds (28). It was observed that oral administration of *nimbidin*, an active substance in neem leaf-extract had significant hypoglycemic effect on feeding, thereby assisting the prevention of gastric ulcer. Other active substances in neem leaf-extracts that have been identified as antibacterial, anti-fungal and anti-inflammatory agent are

*memoodim* and *cyclic tetra- animbidate sulphide*. This compound is also anti-tumor agent. However, Neem leaf-extract has been associated nutritional defects like nutritional imbalance and improper metabolism. The antinutritional factors and other toxic elements in neem plants could affect performance of the birds (22) hence be a limiting factor in its usage by broiler finishers.

### Conclusion and Application

1. Scent leaf extract and Neem leaf extracts can be recommended as antimicrobial feed additives for birds at low levels of concentration
2. The antimicrobial properties of neem leaf extract influence protozoans while scent leaf-extract appeared to be more effective against bacterial pathogens.
3. Since the leaf-extract of *Ocimum gratissimum* (scent leaf) and *Azadirachta indica* (Neem leaf) reduces water intake and gut microbes, poultry farmers should therefore fortify poultry feed with vitamins and other nutrients as well as increase in quantity of water available for the birds to enhance feed efficiency.

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