

## ***Ocimum gratissimum* oil as an alternative to in-feed antibiotics in animal agriculture**

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**Target Audience:** Poultry farmers, Feed millers and Poultry nutritionist

### **Abstract**

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*Digestive disorders as a result of withdrawal of in-feed antibiotics in poultry nutrition have resulted in the use and search for alternatives to alleviate the problem. In the light of this an experiment was carried out to determine the in-vitro antibacterial activity of *Ocimum gratissimum* (African basil or locally called scent leave) oil on bacteria isolated from the gut of 6 weeks old broiler birds. *Ocimum* oil was extracted from the aerial parts (leaves and flowers) freshly harvested from the plant. The oil was use tested against *Salmonella Spp* and *Clostridia perfringens* isolated from different sections of the gut. Results obtained indicated an inhibition of *Salmonella Spp* growth by  $14.5 \pm 1.92$  in the duodenum, jejunum and caecum. A value of  $14.5 \pm 2.06$  was recorded for *Clostridia* in the jejunum. Values recorded in the duodenum, ileum and caecum were  $12.5 \pm 2.52$ ,  $13.7 \pm 2.87$  and  $13.0 \pm 2.00$  respectively. There was no significant difference ( $p > 0.05$ ) regarding which bacteria the oil was most effective against. It can be concluded from results obtained that *Ocimum gratissimum* oil (from the aerial parts) of the plant has potential to inhibit the growth of either *Salmonella Spp* or *Clostridia* across different sections of broiler gut.*

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**Keywords:** Animal agriculture, Antibiotics, *Ocimum gratissimum* oil,

### **Description of problem**

Antibiotics have been added to poultry and pig diet to maintain health and production efficiency in the last few decades [1] but due to development of resistance by pathogenic bacteria which can impact on public health, antibiotics are being taken out of poultry and pig diets around the world. This ban began in Sweden in 1986 [2]. The research for alternatives to replace in feed antibiotics has gained increasing interest in animal nutrition in recent years. It was pointed out by [3] that the growth-promoting effects of antibiotics in animal diets are clearly related to the gut microflora because they exert no benefits on the performance of germ-free animals. Gut microflora has significant effect on host

nutrition, health and growth performance [4]. This is mediated via interaction with nutrient utilization and the development of gut-ecosystem of the host. This interaction is very complex and depending on the composition and activity of the gut microflora, it can have either positive or negative effect on health and growth of birds. For instance, when pathogens attach to the mucosa, gut integrity and function are severely affected and the immune system threatened [5]. The impact of gut health often comes from microbial imbalance in the gut, which will be exacerbated if antibiotics are withdrawn from feed. Any gut damage caused by pathogens will lead to poor gut health, which will ultimately affect nutrient utilization efficiency as reported by [6]. Chicks grown in

a pathogen free environment have been shown to grow 15% faster than those grown under conventional conditions where they are exposed to bacteria and viruses [7]. Furthermore, it is generally agreed that gut microflora is a nutritional "burden" in fast-growing broiler chickens [2]; [8], since an active microflora component may have an increased energy requirement for maintenance and a reduced efficiency of nutrient utilization.

The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved [9]. Antibiotics were used in feed for increasing the performance, preventing some diseases and for increasing the number of some useful micro-organism in the gut micro flora. The gut micro flora of poultry is a mixture of bacteria, fungi and protozoa, but bacteria are reported to be the predominant micro-organism [10]. This decision has therefore stimulated the search for alternative array of substances with beneficial health related biological properties [11]. The intestinal microflora may prevent infection by interfering with pathogens. The flora contains low population of potentially pathogenic organisms such as *Clostridium perfringens*. Necrotic enteritis (NE) is caused by *C. perfringens* type A and more uncommonly, by *C. perfringens* type C. Necrotic enteritis is one of the most common infectious diseases in poultry, resulting in an estimated annual economic loss of more than \$2billion, largely related to impaired growth performance [12]. This enteric infection is commonly well controlled by anticoccidials or antibiotic growth promoters, but the use of these compounds has been banned in animal feed in the European Union, and there is pressure to reduce their use worldwide because of the risk of multiple antimicrobial resistant strains [12]. Thus, Necrotic enteritis is re-emerging as an important disease in poultry, and there is concern about the increased risk of

contamination of poultry products for human consumption, as *C. perfringens* is one of the most common causes of food borne illness worldwide [13]. disease occurs mostly in animals between 2-6weeks of age and can vary from an acute to a subclinical form. The subclinical form is more prevalent, being responsible for the greatest economic losses in poultry production due to Necrotic enteritis, and is characterized by chronic intestinal mucosal damage, which leads to poor digestion and absorption [12]; [14].

Antibiotics that upset the balance of the normal flora can favor both infections by exogenous pathogens and overgrowth by endogenous pathogens. If the bowel wall is breached, enteric bacteria can escape into the peritoneum and cause peritonitis and abscesses [15] as the case may be. The objective of this study was to test the effectiveness of *Ocimum gratissimum* oil against broiler gut bacteria specifically *Salmonella spp* and *Clostridia perfringens*.

## Materials and Methods

### *Location of the Experiment*

Birds utilized for sample collection were obtained from three farms in Uyo metropolis of Akwa Ibom state. Sample collection was carried out by slaughtering three birds per replicate. Bacteria *spp* was taken as treatment. Collected digesta samples were placed in sterile sample containers on ice and sent to Microbiology Department of the University of Uyo Medical Center for isolation of pure cultures of *Salmonella spp* and *Clostridia*.

### *Extraction of Ocimum gratissimum Oil*

One kilogram (1.0kg) of the aerial parts (leaves and flowers) of the plant (*Ocimum gratissimum*) were cut directly from the farm at the early hours of 6.30am of the day and immediately put into a sample bag and sealed. It was taken to the laboratory and the leaves and flowers were removed from the stalk and

chopped. Two hundred milliliters (200ml) of petroleum ether was decanted into the container containing the chopped fresh aerial part of *Ocimum gratissimum* and left for 5 minutes to extract the oil. The filtrate separated from the extract by sieving using a cheese cloth. The extract was placed in a water bath to enable the solvent evaporate completely, leaving the oil in the container. A spatula was used to scrap the oil into a sterile container and stored.

#### *Digesta Collection and isolation of bacteria*

Three birds were slaughtered per replicate. Digesta samples collected from different sections of the small intestine namely duodenum, jejunum, ileum and lastly caecum. Pure cultures of *Salmonella spp* and *Clostridia* were isolated from these samples at the Microbiology Department, University of Uyo Medical Center. Each bacteria type was isolated on their specific agar on triplicate bases. *Salmonella spp* was isolated on *Salmonella Shigella* agar and *Clostridia* on Chocolate agar. The extracted *Ocimum* oil was used against the specific bacteria isolated on triplicate basis and the zone of inhibition measured with the aid of a translucent ruler in millimeters.

#### *Data analysis*

Data collected on zone of inhibition was subjected to statistical analysis using SPSS package volume 17 and significant means separated using LSD (least significant difference).

#### **Results and Discussion**

The antibiotic activity of *Ocimum gratissimum* oil against *Salmonella spp* and *Clostridia perfringens* is presented in Table 1. The mean value of zone of inhibition (ZOI)

recorded from each section of the gut was duodenum ( $14.5 \pm 1.92\text{mm}$ ); jejunum ( $14.5 \pm 1.92\text{mm}$ ); ileum ( $14.5 \pm 1.00\text{mm}$ ) and caecum ( $14.5 \pm 1.92\text{mm}$ ). The effect of *Ocimum gratissimum* oil tested against *Clostridia perfringens* isolated from the duodenum, jejunum, ileum and caecum as indicated in Table 1 showed that *Ocimum gratissimum* oil had bacteriostatic activity on *Clostridia* with the highest mean value of  $14.2 \pm 2.06\text{mm}$  recorded in the jejunum. The oil of *Ocimum gratissimum* tested against either bacteria specie isolated from different sections of six weeks old broiler birds gut indicated a ZOI higher than 10mm. There was no significant difference ( $p > 0.05$ ) regarding which bacteria the oil was most effective against.

*Salmonella spp* isolated from duodenum, jejunum, ileum and caecum gave a similar ZOI value of  $14.5 \pm 1.92\text{mm}$ , that recorded in the ileum was ( $14.5 \pm 1.00\text{mm}$ ). The result also indicated that *Ocimum gratissimum* oil had bacteriostatic activity against *Clostridia* with a high mean value ZOI of  $14.5 \pm 2.06\text{mm}$  recorded in the jejunum. The use of underutilized medicinal plants in the control of animal diseases in animal health has been found to be effective, cheap and practicable [16]. Ethno veterinary medical applications have also been demonstrated for the control of animal diseases of small ruminants ([17], cattle [18], poultry [19] and pig [20]). In a previous study, [21] a ZOI of 11.8mm was reported against *Salmonella* isolated from the ileum of broilers when *Ocimum gratissimum* extract was used. This value was numerically lower than that obtained in the current study which the oil and not extract was utilized. This might be an indication that the oil was probably more effective than the extract from sun dried leaves.

**Table 1: Anti – bacterial (mm) effect of *Ocimum gratissimum* oil on *Salmonella spp* and *Clostridia spp* isolated from Broiler Gut.**

Gut section	<i>Salmonella</i>	<i>Clostridia</i>	<i>P value</i>
Duodenum	14.5 ± 1.92	12.5 ± 2.52	0.252 <sup>ns</sup>
Jejunum	14.5 ± 1.92	14.5 ± 2.06	0.861 <sup>ns</sup>
Ileum	14.5 ± 1.00	13.7 ± 2.87	0.671 <sup>ns</sup>
Caecum	14.5 ± 1.92	13.0 ± 2.00	0.444 <sup>ns</sup>

ns: means along the same row are not significantly (ns) different at ( $p>0.05$ )

In an earlier report, [22] a ZOI of 7 – 15mm was reported using a concentration range of 25 – 75% *Ocimum* water extract. Although the results of this study corroborate the report of [22] however, concentrations of 20 – 30mg/ml water extract of *Ocimum* resulted in lower ZOI of 10mm against *Salmonella* as reported by [23]. Other authors have reported values higher than 14mm and 7mm at varied concentrations [24]; [25]; [26]. It has been reported that the greater the ZOI, the stronger the antimicrobial effect [27]. This may indicate a probable improvement with regards to *Ocimum* oil utilized in this study against the extract utilized in a previous study [21].

*Ocimum* oil against *C. Perfringens* gave a numerically high ZOI of 14.5mm in the jejunum. This can be attributed to the antibiotic activity of *O. gratissimum* oil against *C. perfringens*. The mode of action of *Ocimum* oil against *Clostridia* was not determined in this study being an in – vitro study but the ZOI recorded could be attributed to the sum effect of the active ingredients in *Ocimum* oil. Phytochemical analysis of fresh and dried leaf extracts of *O. gratissimum* revealed the presence of antimicrobial principles such as resins, tannins, glycosides, alkaloids, flavonoids saponin, anthraquinone, cardiac glycoside, steroidal ring, steroidal terpenes and carbohydrates at different concentrations [28]. The findings from the study [28] seem to provide in -vitro evidence that might justify *O. gratissimum* as a good candidate medicinal plant. Later studies [29,30], indicated the

presence of eugenol, cis-ocimene, trans-ocimene,  $\alpha$ -pinene and camphor as the major chemical compounds in *O. gratissimum* essential oil. The compounds were reported to be effective against *S. aureus*, *Bacillus spp.*, *E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae*. Evidence based in – vivo research using monogastric animals have shown that botanicals facilitate useful changes on the gut environment and bacteria [31]; [32]. As previously stated, the greater the ZOI, the stronger the antimicrobial effect, however the activity and efficacy of plant extracts on gut microbes can be quite variable as recorded in this study and previous study [33]; [21]. This variability can be dependent on the plant source, extraction process, quality and consistency of the product as asserted by [27].

### Conclusion and Application

1. It is evident that *Ocimum gratissimum* oil extract has antimicrobial effect on *Clostridia* and *Salmonella spp.*
2. Nutritional and medicinal use of the oil of *Ocimum gratissimum* should be investigated further on *Clostridia*.
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