Microbial population and blood parameters of West African dwarf goats fed scent leaf (*Ocimum gratissimum*) as additive

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Abstract

A study was carried out to determine the rumen microbial population and blood parameters of 20 West African dwarf goats fed Ocimum gratissimum (scent leaf) as additive in a concentrate supplement. The goats were divided into four treatment groups (T1, T2, T3 andT4) with five replicates in a completely randomized design. Four concentrate supplements were formulated to contain scent leaf at levels 0,5,10, and 15g/kg DM. Panicum maximum was the basal diet. Chemical composition of scent leaf and the diets were determined. Rumen fluid and blood were collected from the goats before and after the experiment to determine microbial population and blood parameters. Results showed that scent leaf has moderate crude protein of 10.61% and rich in tannin, saponin and oxalate. At the end of the experiment, bacteria and protozoa counts were not significantly ($p>0.05$) influenced by the experimental diets, however there was a reduction in their values. Fungi count was significantly ($p<0.05$) affected by the diets and their values reduced as the level of scent leaf in the diets increased. The highest count (0.35×10^{6}cfu/ml) was obtained at T1 (control) and the lowest value (0.05×10^{6}cfu/ml) obtained in goats fed 15g/kg DM scent leaf (T4). Red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin values increased at the end of the study and were significantly ($p<0.05$) highest (15.03×10^{12}/l, 36.50% and 13.32g/dl respectively) in goats fed T3 (10g/kg DM of O. gratissimum). Serum urea and cholesterol levels were significantly lowest in goats fed T3 and T4. Alanine amino transferase (ALT) and aspartate aminotransferase (AST) values were reduced by the experimental diets. It can therefore be concluded that scent leaf can be included in the diet of West African dwarf goats up to 10g/kg DM for improved RBC, PCV and Hb, and regulation of serum enzymes, urea and cholesterol levels.

Keywords: *Ocimum gratissimum*, microbial population, haematology, biochemical parameters, goats, additives

Introduction

There is need for increase animal performance and protein intake in Nigeria in view of the population growth rate (Nworgu, 2016). Improvement of animal performance has usually been achieved through breeding and genetics but nutrition plays an important role. Antibiotics has been widely used in improving animal performance but in recent years there is restriction or outright ban in some countries on the use of feed antibiotics. This has shifted interest to the use of alternative products. A group of natural products known as phytogenics has been the focus of several studies in recent years (Windisch *et al*., 2008). Phytogenic feed additives include herbs, which are non woody flowering plants known to have medicinal properties; spices, which are herbs with intensive smell or taste, commonly added to human food; essential oils, which are aromatic oily liquids derived from plant materials such as flowers, leaves, fruits and roots; and oleoresins, which are extract derived by non-aqueous solvent from plant material (Windisch *et al.,* 2008). Some of the useful herbs and spices are indigenous to

Africa and include ginger (*Zingiber officinale*), garlic (*Allium sativium*), scent leaf (*Ocimum gratissimum*), bitter leaf (*Vernomnia amygdalia*), they have been reported to enhance the performance of livestock animals (Muhmmad *et al.,* 2009). *Ocimum gratissimum* belongs to the family Lamiaceae, the mint family. It is cultivated in many gardens around village huts and homes in the rural areas in Nigeria for its medicinal purposes. It is otherwise known as scent leaf or Clove Basil plant (Udeh and Mene, 2018). *O. gratissimum* is rich in alkaloids, tannins, favonoids, phytates and oligosaccharides. It has tolerable cyanogenic content (Ijeh, *et al*., 2004). The volatile aromatic oil from the leaves consists mainly of thymol (32-65%) and eugenol; it also contains xanthones, terpenes and lactones (Ezekwesili, *et al*., 2004). These chemical substances are antimicrobial, anti-oxidative, anti-inflammatory and immune-modulatory (Sofowora, 1993). Research effort has focused on the chemical constituents of scent leaf (Esvanszhuga, 1980; Edeoga and Eriata, 2001), its medicinal value to human and animals (Orafidiya *et al*., 2001; Ijeh *et al*., 2004,) use of its leaf and extract as additive in the diet of monogastric animals (Effraim *et al*., 2003; Nte *et al*., 2016). There is paucity of information of its use as additive in ruminants' diet. With the need to improve nutrient utilization in ruminants through the use of natural and safe substance, there is need for research into the use of scent leaves (*Ocimum gratissimum*) as phytogenic feed additive in the diet of West African dwarf goats.

Materials and methods

Experimental site

The study was carried out at the small ruminant unit of the Directorate of University Farms and Animal Nutrition laboratory of the Federal University of Agriculture, Abeokuta (FUNAAB) Ogun State, Nigeria. Ogun State is in the rainforest zone of South West Nigeria. The area has an annual mean temperature of 34.7°C, a relative humidity of 82% and an annual mean rainfall of 1,037 mm. It is about 70 m above sea level and lies on latitude 7°5'-7°8'N and longitude 3°11.2'E.

Processing of test materials and formulation of diets

Scent leaves (*Ocimum gratissimum*) were harvested from villages around the University and air-dried for 3-5days after which they were ground to pass through 1mm sieve and used in diet formulation. The dietary treatments consisted of a basal diet (*Panicum maximum*) and concentrate supplement formulated to contain *O. gratissimum* as an additive at levels 0, 5, 10 and 15g/kg DM (Table 1). The diets; basal and concentrate supplement were offered to the animals at 5% of their body weight in a ratio of 60:40 respectively.

Table 1: Gross composition and chemical of concentrate diets (%)

	Experimental concentrate diets			
Ingredients	**T1 (0)**	**T2 (5)**	**T3(10)**	**T4 (15)**
Wheat offal	50.00	50.00	50.00	50.00
Maize bran	30.00	30.00	30.00	30.00
PKC	17.00	17.00	17.00	17.00
Bone meal	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00
*Scent leaf	-	+	++	+++
Total	100.00	100.00	100.00	100.00
Crude protein	12.80	12.80	12.80	12.80
Crude fibre	11.66	11.66	11.66	11.66

-, +, ++, and +++ means 0,5,10 and 15g/kg DM scent leaf inclusion levels

Experimental animals and management

Twenty (20) West African dwarf goats with an average live weight of 7.38 - 7.53kg were used for the experiment. Pens were cleaned and disinfected with Izal solution prior to the arrival of the animals. The goats were kept in the quarantine section for two weeks for proper prophylactic treatment which consisted of intramuscular application of oxytetracycline LA and vitamin B complex at the dosage of 1 mL/10 kg body weight of the animal. The animals were maintained on *Panicum maximum* grass and concentrate and clean water was supplied *ad libitum.* They were thereafter divided into four treatment groups of six animals each on weight equalization basis and housed in individual pens. The study comprises 14 days of adaptation followed by 12 weeks feeding trial and two weeks nutrient digestibility study. The diets were offered at 8:00am in the morning and 12.00pm in the afternoon. The concentrate diets were offered first and then the basal diet in separate feeding trough. Left over of the feed offered was weighed and recorded every morning to compute feed intake on dry matter basis. Initial weights of the animals were taken on the first day of the experiment and thereafter fortnightly throughout the experiment.

Chemical analyses

Oven dried feed samples were analyzed for their proximate compositions: crude protein, crude fibre, ether extract and ash according to AOAC (2000). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by the methods of Van Soest *et al.* (1991). Cellulose and hemicellulose were calculated as differences between ADF and ADL, and NDF and ADF respectively. Minerals and anti-nutritional factors were anal

Rumen fluid collection and analysis

Rumen fluid was collected from four goats in each treatment before and after the experiment. Samples of unstrained rumen fluid collected from each animal was fixed with 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin solution) for the determination of microbial population by total direct count of bacteria, protozoa and fungi (Galyean, 1989).

Blood collection and analysis

About 10mL of blood sample were drawn from each animal via jugular vein puncture using hypodermic syringe before feeding the animals. Blood collection was done at the commencement and termination of the experiment. The blood sample from each of the goats was divided into two and one-half was emptied into labelled bottles containing Ethylene diamante tetra acetic acid (EDTA). The bottles were quickly capped and the content was gently mixed by rocking the bottles. Sample bottles were labelled and taken to the laboratory for haematological analysis such as packed cell volume (PCV), Haemoglobin, Red blood cell (RBC), or White blood cells (WBC) etc. while the second half was emptied into plain bottle for serum analysis of total protein, urea and liver enzymes activity.

Statistical analysis

All data collected were subjected to one-way analysis of variance for a completely randomized design using version 9.1 of SAS software (SAS, 2003) with the following model $Y_{ij} = \mu + T_i + eij$, Where Y_{ij}= Observed variation, μ = Population mean, T_i = effect of ith diets (1-4), eij = error term. Significant means were separated using Duncan's procedure of the same software. Mean differences were considered significant at $P < 0.05$

Results

Table 2 shows the chemical composition of *Ocimum gratissimum*. The dry matter content is high (82.48%) while it is low (10.61) in crude protein. It is however higher in minerals and anti-nutritional factors like tannin, saponin, oxalate phytate, alkaloids, phenols and trypsin inhibitor. No significant ($p>0.05$) difference was observed in all the parameters measured for rumen microbial count before the commencement of the study (Table 3). At the end of the study fungi count was significantly ($p<0.05$) influenced by the experimental diets. The highest (0.35×10^6 cfu/mL) count was obtained in goats fed T1 (control) while the lowest (0.05×10^6 cfu/mL) count was obtained in goats fed T4 (15g/kgDM scent leaf inclusion level). The count reduced as the level of scent leaf in the diets increased. Although no significant difference was observed in bacteria and protozoa count, there were reductions in the values for goats on scent leaf diets by the end of the study while the values obtained for goats on the control diet increased. Haematological indices of the experimental goats were presented in Table 4.

Table 2: Chemical composition of scent leaf *(Ocimum gratissimum)*

Proximate composition (%)	
Dry matter	82.48
Crude protein	10.61
Crude fibre	22.32
Ether extract	6.22
Ash	13.25
Anti-nutritional factors (%)	
Tannins	1.46
Saponins	1.07
Oxalate	2.11
Phytate	1.02
Alkaloids	2.88
Phenols	0.42
Trypsin inhibitor (TUI/mg)	30.87
Mineral content (mg/100g)	
Na	4.87
K	6.12
Ca	7.29
Mg	13.17
P	18.93
Fe	16.17
Cu	0.58
Zn	1.62

Table 3: Rumen microbial count of West African dwarf goats fed experimental diets

Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**
Before the experiment					
Bacteria count	3.03	2.73	2.53	3.10	0.14
Fungi count	0.15	0.13	0.15	0.12	0.03
Protozoa count	0.57	0.30	0.73	0.87	0.12
After the experiment					
Bacteria count	3.05	2.43	2.06	2.85	0.12
Fungi count	0.35^a	0.20^b	0.15^{bc}	0.05^c	0.04
Protozoa count	0.68	0.33	0.27	0.25	0.11

Means on the same row with different superscripts are significantly different ($P<0.05$). T1, T2, T3, and T4 represent 0, 5, 10 and 15g/kg D M levels of inclusion of scent leaf. Bacteria Count (10^6cfu/ml), Fungi Count (10^6cfu/ml), Total Protozoa Count (10^3cell/ml)

There were no significant ($p>0.05$) difference in all the parameters measured before the experiment. However, the diets significantly ($p<0.05$) influenced the packed cell volume (PCV), haemoglobin, red blood cell count (RBC), neutrophils and lymphocytes at the end of the study. PCV, haemoglobin and RBC values were highest in goats fed T3 (10g/kg DM scent leaf inclusion level) while statistically similar values were observed in the other treatments. Neutrophil value was also highest in goats fed T3. However, lymphocyte was lowest in T3. Serum biochemical parameters of the experimental goats were shown in Table 5. Except glucose, all other parameters measured before the commencement of the study were not significantly different ($p>0.05$). All the parameters measured at the end of the study were significantly affected by the experimental diets except total protein. Glucose was highest (71.83) in goats fed T1 (control) and lowest with statistically similar values in other treatments. Urea and cholesterol values were highest in goats fed T1 and T2 (5g/kg DM scent leaf inclusion level) and lowest in those fed T3 and T4 (15g/kg DM scent leaf inclusion level). Aspartate aminotransferase (AST) and Alanine aminotransferase (AST) values (90.50 and 48.17 respectively) were highest in goats fed T1.

Discussion

The high dry matter (DM) value obtained for scent leaf (*Ocimum gratissimum*) is an indication of high nutrient content. The DM value is however lower than to 91.97% reported by Nworgu *et al*. (2013). The crude protein and crude fibre values obtained in this study are higher than 6.38% CP and 18.52 CF reported by Nworgu (2016) and 4.7% CP and 10.8% CF reported by Mensah *et al*. (2008) while the ether extract and ash values are comparable to those reported by the authors. The CP values is however comparable to 9.19-17.94% CP reported by Edeoga *et al*. (2006) The differences in values obtained can be attributed to differences in age of harvest, season or processing methods. Tannin, saponin, oxalate and phytate values are also higher than the values reported by Nworgu (2016). *O. gratissimum* from this study also contains alkaloids, phenols and trypsin inhibitor. This supports the reports of Ijeh *et al*. (2004) and Akujobi *et al*. (2004) that *O. gratissimum* is rich in tannins, alkaloids, flavonoids, phytates, saponins, oligosaccharides and tolerable cyanogenic content. *O. gratissimum* is rich in minerals like sodium, potassium calcium magnesium phosphorus iron, copper and zinc. This supports the findings of Nworgu *et al*. (2013). The reduction in the fungi count as the level of *O. gratissimum* in the diets increased affirms its antifungal property as previously reported by Lemos *et al*. (2005). Bacteria and protozoa count of goats offered scent leaves at varying levels reduced at the end of the experiment compared with what was obtained before the commencement of the study. This is indicative of its antibacterial and antiprotozoal property and corroborates the findings of Nakamura *et al*. (1999), Holetzl (2003) and Adebolu and Oladimeji (2005). These reductions can be attributed to the presence of some secondary compounds like tannin, oxalate and saponin in *O. gratissimum*. According to Sofowora (1993) these chemical substances possess antimicrobial, antioxidative, anti-inflammatory and immune-modulatory properties. Mohammed *et al*. (2016) opined that haematological and biochemical profiles constitute an index for measurement of health and nutritional status of the animals. Health of animals can be monitored by the assessment of nutritional effect on the haematological and serum biochemical indices. The blood analysis done at the onset of the experiment serves as a reference point and a basis to ascertain the health status of the animals for the experiment. Karesh and Cook (1995) reported that examining blood for their constituents is used to monitor and evaluate disease prognosis of animals. Blood values are therefore of importance in diagnosing many haemoparasitic infections in food animals (Adedeji *et al*., 2011; Daramola *et al.,* 2005) and assessing the health status of ruminants (Adedeji *et al*., 2011). The increased packed cell volume (PCV), haemoglobin (Hb) and red blood cell count (RBC) observed across the treatments indicates that all the diets did not waiver in protein quality and quantity. This observation is in line with the report of Adeyemi *et al.* (2008) who observed that red blood cells and haemoglobin are positively correlated with protein quality and protein level in the diet and that decreased in the red blood cell counts are associated with low protein or deficiency in the diet. It also indicates the absence of normocytic anaemia, leukopenia or leukocytosis which is only detected by a decreased number of RBC or PCV (Coles, 1986; Cullis, 2011). Animals on T3 (10g/kg scent leaf inclusion level) had the highest value for PCV, HB and RBC while animals on other diets have similar values. This is an indication that the secondary compounds in *O. gratissimum* did not induce any toxicity on the animals. It also implies that T3 promoted the utilization of iron for the production of Hb better than other diets. The result also validates the report of Ofem *et al*. (2012) that oral administration of *O. gratissimum* increased PCV, RBC and Hb in rats. The PCV values were within the normal range of 18-38% reported by Orheruata and Aikhuomobhogbe (2006). This further affirms that the oxygen carrying capacity of the blood was not hindered and the animals were free from anaemia. The heamoglobin (Hb) range fell within the range of 7-15g/dl and 8-16g/dl cited by Daramola *et al.* (2005) and Kaneko (1989) respectively. Neutrophils are known as the main defenders of the body against infections and antigens. If the count exceeds normal value, it resulted in an acute infection but if the count is considerably less, it may be due to a viral infection (Jelalu, 2014). The range of values obtained in this study is comparable to 30-48% documented by Merck (2012) for healthy goats. T1 and T3 had statistically similar values which confer a higher immunity on goats fed T1 and T3. Lymphocytes play important role in immune response; fight against virus infected cells and tumor cells. Parasitic invasion can be suspected when lymphocytes exceed the normal value (Etim *et al*., 2014). The values obtained were comparable to the physiological range of 50-70% reported by Merck (2012). The results suggest that the immune response of the animals was not hampered by the inclusion of *O. gratissimum* in the diets. Etim *et al.* (2014) reported that immune status is a function of leucocytes, lymphocytes and neutrophils

Serum glucose level observed in the present study is similar to the normal reference value (48.2-75mg/dl) reported Fraser and Mays (1986). Hence, the observed decrease in serum glucose of goats fed *O. gratissimum* diets is not due to *O. gratissimum* intoxication as no clinical signs of ill-health were observed. Lower glucose level is an indication of hypoglyceamia (Olurunnisomo, 2012). Glucose is a relatively good indicator of energy balance. The increased level of glucose observed in animals on control diet might be attributed to increased levels of volatile fatty acids in the rumen (Jain *et al.,* 2005). Presence of secondary compounds especially tannin in *O. gratissimum* might hinder degradation of feed by rumen microbes which invariably lowered the volatile fatty acid produced.

According to Nworgu *et al*., (2013) urea is a function of protein quality and high urea level depicts low protein quality. Serum urea concentration range level obtained in the present study is in agreement with the normal range (12.6-25.8) revealed by Fraser and Mays (1986). Decreasing level of serum urea with increasing level of *O. gratissimum* in the diets may be attributed to reduced rumen protein breakdown which might be as a result of tannin present in *O. gratissimum*. Singh *et al*. (2015) reported that tannin in feed reduced protein breakdown in the rumen and increased essential amino acid absorption. Moderate tannin form protein-tannin complex and prevent excessive degradation of dietary protein by rumen. Condensed tannin prevents rapid protein degradation by rumen microbes and absorption of rumen protein, consequently furnished the lower gut with adequate rumen undegradable or by-pass protein for better absorption and utilization. Singh *et al*. (2015) explained that prevention of protein availability in the rumen for digestion and absorption by condensed tannin until it reaches the more acidic abomasum not only enhances nutrition by providing high-quality protein to the small intestines, but also has the potential to support the immunity and increase resisitance to gastrointestinal nematodes. The lower serum urea of *O. gratissimum* diets, therefore, indicates better utilization of protein by the animals.

The serum enzymes; Alanine amino transferase (ALT) and aspartate aminotransferase (AST) values were within the normal range (15.3-52.3 u/l and 66-230 u/l respectively) reported by Fraser and Mays (1986). Normal physiological serum enzyme values imply high quality protein in all the diets. The results also clearly suggest that the diets, particularly *O. gratissimum* diets which contained secondary compounds had no adverse effect on the liver of the experimental animals although their values decreased in *O. gratissimum* diets. Das *et al.* (2011) asserted that clinically significant rise of serum enzymes activities above normal ranges is an aberration and indication that the animals might have suffered from diseases of the liver. Both AST and ALT are associated with liver parenchymal cells (Patel *et al*., 2011). Increased level of ALT and AST in the control diet and their reduction in goats fed *O. gratissimum* diets did not implicate heptatic damage since they were within the normal ranges. Akah *et al*. (2009) revealed that reduced values of AST and ALT are an indication of improved renal and hepatic functions. Serum levels of AST, ALP, and cholesterol levels are those parameters conventionally used for diagnosing human and domestic animal hepatic damage (Silanikove and Tiomkin, 1992). The decrease in cholesterol values as the level of *O. gratissimum* in the diets suggests that *O. gratissimum* is capable of reducing cholesterol in the animals. Similar result was obtained by Matawalli *et al.* (2004) when rats were fed methanolic extract of *Adansonia digitata* and Akah *et al.* (2009) when diabetic rats were served leaf extract of *Vernonia amygdalina*.

Table 4: Heamatological parameters of West African dwarf goats fed experimental diets

Parameters	Before the end of experiment					At the end of experiment				
	T1	T2	T3	T4	SEM	T1	T2	T3	T4	SEM
Packed cell volume (%)	26.67	22.10	22.50	25.90	1.93	30.50^{b}	30.83^{b}	36.50^{a}	31.50^{b}	1.23
Haemoglobin (g/dl)	9.73	8.47	8.57	10.00	0.19	11.30^{b}	12.55^{a}	13.32^{a}	11.75^{b}	0.24
Red blood cell (x10^{12}/l)	10.70	10.08	10.09	10.09	0.53	12.97^{b}	13.24^{b}	15.03^{a}	13.57^{b}	0.25
White blood cell (x10^{9}/l)	6.40	7.13	6.23	7.10	0.65	8.25	7.47	7.27	7.15	0.50
Neutrophils (%)	41.00	44.67	44.67	41.00	1.25	44.00^{ab}	39.50^{bc}	46.17^{a}	37.50^{c}	1.32
Lymphocyte (%)	50.00	51.67	48.00	52.33	2.13	57.00^{a}	56.50^{a}	52.00^{b}	58.00^{a}	0.82
Monocyte (%)	2.00	2.00	2.67	1.67	0.44	3.00	3.00	2.00	2.00	0.12
Eosinophil (%)	3.00	1.67	1.33	2.67	0.30	2.50	2.50	2.00	3.00	0.14
Basophil (%)	0.33	0.00	0.00	0.00	0.08	0.33	0.00	0.00	0.00	0.00

Means on the same row with different superscripts are significantly different (P<0.05). T1, T2, T3, and T4 represent 0, 5, 10 and 15g/kg DM levels of inclusion of scent leaf.

Table 5: Serum biochemical parameters of West African dwarf goats fed experimental diets

Parameters	Before the experiment					At the end of experiment				
	T1	T2	T3	T4	SEM	T1	T2	T3	T4	SEM
Glucose (mg/dl)	69.67^{a}	55.00^{b}	57.00^{b}	54.67^{b}	2.57	71.83^{a}	50.50^{b}	48.00^{b}	48.83^{b}	3.23
Total protein (g/dl)	4.87	4.23	3.40	3.87	0.30	6.70	6.45	6.35	6.35	0.32
Urea (mg/dl)	15.67	13.67	14.00	16.00	0.75	17.00^{a}	17.00^{a}	14.00^{b}	14.50^{b}	0.98
Cholesterol (mg/dl)	74.33	71.00	71.33	68.00	5.86	112.50^{a}	107.50^{a}	77.50^{b}	77.00^{b}	5.98
AST (u/l)	80.67	82.33	77.00	75.67	2.73	90.50^{a}	71.50^{c}	77.00^{b}	71.00^{c}	2.76
ALT (u/l)	46.67	45.33	43.00	46.67	1.19	48.17^{a}	37.00^{b}	41.50^{b}	46.00^{a}	1.50

Means on the same row with different superscripts are significantly different (P<0.05). T1, T2, T3, and T4 represent 0, 5, 10 and 15g/ kg DM levels of inclusion of scent leaf.

Conclusion

The study showed that *O. gratissimum* at 10g/kg DM increased packed cell volume (PCV), red blood cell count (RBC) and haemoglobin (Hb) and reduced fungi count, urea, aspartate aminotransferase (AST), alanine amino transferase (ALT) and cholesterol levels. This study has also shown that it can be used as a rumen modifier because of its content of secondary metabolites. However, its long term use in the diet of West African dwarf goats need to be studied because of its antimicrobial property which might interfere with degradation of feed in the rumen.

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