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# PERFORMANCE CHARACTERISTICS, BLOOD PROFILE AND LIVER HISTOLOGY OF NEW ZEALAND WHITE RABBITS ADMINISTERED AQUEOUS *Ficus asperifolia* LEAF EXTRACT

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## ABSTRACT

Various pharmacological actions such as anti-ulcer, anti-diabetic lipid lowering and antifungal activities have been described for *Ficus asperifolia. It* has been widely used in albino rats but with limited use in rabbits. This study was, therefore, conducted to evaluate the effect of aqueous *Ficus asperifolia* leaf extract (FALE) on the growth performance, blood profile and liver of New Zealand white rabbits raised under tropical conditions. A total of 36 rabbits consisting of two groups of 18 bucks and 18 does were allotted to six treatment groups after weight equalization on the basis of sex and levels of FALE administration (0ml, 10ml and 20ml of aqueous FALE) in a 2x3 factorial arrangement, with each treatment consisting of 6 rabbits; each treatment was further subdivided into 3 replicates of 2 rabbits per replicate. Data on growth, haematology and serum biochemistry were collected and analysed using analysis of variance (ANOVA). Means, where significant, were separated using Duncan Multiple Range Test at ($P<0.05$). The livers were dissected and preserved in Bouin's fluid while routine haematoxylin and eosin method was used to stain them. Aqueous *Ficus asperifolia* leaf extract significantly ($P<0.05$) affected total weight gain, total feed intake and feed efficiency while sex effect showed that final weight and total feed intake were significantly reduced in bucks (2011.11g and 1170.11g, respectively) compared to the does (2258g and1474.61g, respectively). Effect of interaction between *Ficus asperifolia* leaf extract and sex significantly affected total weight gain, total feed intake and feed efficiency. Haematological parameters were not influenced by the main effect of aqueous *Ficus asperifolia* leaf extract and sex except for white blood cells (WBC) which were significantly higher ($7.90x10^9$/L) in bucks. Also, WBC and lymphocytes were significantly influenced by their interactive effect. Total protein, globulin, glucose, Alkaline phosphate and calcium were significantly affected by levels of aqueous *Ficus asperifolia* leaf extract while cholesterol and calcium were influenced significantly by sex. Haematoxylin and Eosin (H&E) stained sections of liver tissue of rabbit bucks showed no remarkable vascular changes. This study indicated that aqueous *Ficus asperifolia* leaf extract is anti-hyperglycemic and can support rabbit production without any negative influence on the immune status of rabbit.

**Keywords:** Growth performance, rabbits, *Ficus asperifolia*, liver histology

# INTRODUCTION

The bulk of proteins consumed in most developing countries like Nigeria are of plant origin and these sources lack certain essential amino acids. Proteins of animal origin are more balanced and complete in amino acids (Aduku, 2004). The production of animals like rabbits, with very short gestation period and production cycles, can be a solution to the problem of protein shortage in Nigeria. Rabbits play an important role in the supply of animal protein to the Nigerian populace (Amaefule *et al*., 2005). They are efficient converters of feed to meat and can utilise up to 30% crude fibre as against 10% by most poultry species (Egbo *et al*., 2001). According to Spore (2007) and Soyebo (2006), rabbits have the potential to improve on the diet and income of many poor households. This is due to its high growth and fecundity rates as well as low investment and labour cost. Nutritionally, rabbits have higher protein (20-21%) and lower fat content (10-11%) when compared with meat from other species. Rabbit meat has a cholesterol value of 169mg/100g (dry matter basis) when compared with beef (200mg) and chicken (220mg); and low sodium content (Janieri, 2003).

Many valuable therapeutic plants have been brought into limelight in livestock feed and production research. Treatment of various diseases using medicinal plants has increased globally due to its therapeutic efficacy and safety (Hakim *et al*., 2007). Their availability and increased usage throughout the world has led many researchers to study their various biological activities. Mansi and Lahham (2008) reported that medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethno-botanical communities. Ghasemi *et al.* (2012) also affirmed that 80% of the world's population use herbal medicine according to a World Health Organization report. *Ficus asperifolia* (Miq.), which is commonly known as sandpaper tree ("Eweipin" in Yoruba), is one of the many valuable therapeutic plants used in a wide range of plant preparations as wound-healing agents, antihelminthic and purgative (Sofowora, 1996.) as well as reverse cases of sterility and fertility (Ojo *et al*., 2016). It is also said to be effective in the treatment of piles, asthma, gonorrhea, hemoptysis and urinary diseases as a result of the phytochemicals. Phytochemical studies carried out on leaves of some Ficus species by Ojo and Akintayo (2014) showed the presence of alkaloids, saponins, tannins, cardiac glycosides, steroids, cardenolides and phlobtannins while terpenes, flavonoids, anthruinones and chalcones were not detected.

Previous work done by Omoniwa and Luka (2012) on the aqueous extract of *Ficus asperifolia* revealed that it exerted hypoglycemic and hypolipidemic effects on diabetic rats and the leaves have higher levels of protein, crude fibre and minerals than some Nigerian vegetables. This plant is used without the knowledge of its toxic potential. The liver is an organ that has an extensive range of functions including detoxification, plasma protein synthesis and generation of biochemicals.

Today, liver damage is one of the very common ailments in the world leading to series of severe health problems ranging from metabolic disorders to even death. One of the methods that contributes to the detection of some changes in health and physiological status which may not be apparent during physical examination but affects the fitness of an animal is haematological examination (Esonu *et al*., 2001; Bamishaiye *et al*., 2009). Examination of blood provides the oppor-

tunity to clinically investigate the presence of several metabolites and other constituents in the body. It plays a vital role in the assessment of physiological, nutritional and pathological status of an animal (Aderemi, 2004; Doyle and William, 2006). However, there is dearth of information on the response of rabbits to aqueous *Ficus asperifolia* leaf extract (FALE). Therefore, this study investigated the effect of *Ficus asperifolia* leaf extract on the growth performance, blood profile and histology of liver of New Zealand White rabbits.

## MATERIALS AND METHODS

### *Experimental Site*

The research work was carried out at the Rabbitry Unit of the Directorate of University Farms (DUFARMS) of Federal University of Agriculture, Abeokuta (FUNAAB), Ogun state, Nigeria. The region lies between latitude 7°10'N and longitude 3°2'E and an altitude of 830m above sea level. The experimental site is located in the derived savannah zone of southwestern Nigeria with an annual average rainfall of 1100 mm and peak temperature ranges from 28°C in December to 36°C in February with an average relative humidity of about 82% (Google Earth, 2019).

### *Experimental Animals and Management*

36 New Zealand White rabbits (18 bucks and 18 does) with average weight of 2.1kg were purchased from reliable farms in Abeokuta. Before the arrival of the animals, the stable was thoroughly washed and disinfected in readiness for stocking. The animals were housed under the same conditions, fed concentrate (Table 1) and supplied water *ad-libitum*. This was supplemented with *Tridax procumbens* twice a week to prevent bloating. On the day of arrival, the animals, were given an antistress (Maxiyield®) with a duration of acclimatisation of two weeks.

**Table 1: Nutrient composition of the commercial feed (as declared)**

| Ingredients | Composition |
|---|---|
| Crude Protein (%) | 16.00 |
| Fats/Oil (%) | 5.00 |
| Crude Fibre (%) | 7.00 |
| Calcium (%) | 1.60 |
| Available Phosphorus (%) | 0.45 |
| Lysine (%) | 0.75 |
| Methionine (%) | 0.36 |
| Salt (min) (%) | 0.30 |
| Energy (Kcal/kgME) | 2450 |

***Preparation of Test Ingredient***

Fresh leaves of *Ficus asperifolia* were harvested within the environment of Federal University of Agriculture, Abeokuta. The leaves were sorted to remove contaminants, dead matter, sand particles and were air-dried for 10 days (away from sunlight) so as to retain its nutrients. The air-dried leaves were finely ground using an electric blender. The leaf meal obtained was then stored until it was ready for use. 200 g of the leaf meal was measured into conical flasks and extracted with 1000 ml distilled water for 24 hours. The mixture was filtered into 500 ml conical flasks with Whatman filter paper no. 1. The solution was filtered, decanted and filtered three times using a sieve to achieve the aqueous *Ficus asperifolia* leaf extract (FALE).

***Experimental Design***

This study was a 2 x 3 factorial layout in a completely randomised design. The factors were sex (buck and doe) and levels of FALE administration (0ml, 10ml and 20ml). 36 New Zealand White (NZW) mature rabbit bucks and does were divided into two groups of 18 bucks and 18 does. Each group was randomly assigned to six experimental treatment groups after weight equalization on the basis of sex and levels of FALE administration (0ml, 10ml and 20ml of aqueous FALE) in a 2x3 factorial arrangement, with each treatment consisting of 6 rabbits; each treatment was further subdivided into 3 replicates of 2 rabbits per replicate. They were administered the prepared aqueous (FALE) orally and daily for 3 weeks consecutively.

***Data Collection***

***Performance Characteristics***

Body weight gain: data on body weight were recorded by taking their pre-experimental body weight and subsequently on weekly basis. Body weight gain was calculated as follows:

Body weight gain (g) = Final body weight (g) – Initial weight gain (g)

Feed intake: this was obtained by subtracting the feed leftover of each animal from the quantity of feed given during the week. Feed intake was calculated as:

Total Feed intake (g) = Feed offered (g) – Feed left over (g)

Feed efficiency: This was calculated by dividing the total feed intake by total weight gain.

$$\text{Feed efficiency (FE)} = \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}}$$

***Blood Collection***

At the end of the 3rd week of *Ficus asperifolia* administration, blood was withdrawn from the ear veins of one rabbit per replicate of each treatment by means of 2.5ml sterile needle and syringe into labeled sample bottles containing ethylene diamine tetra acetate (EDTA) to prevent blood coagulation while another set of blood samples was collected into 2.5ml plain bottles (without anticoagulant) for blood serum analysis. The blood samples in EDTA bottles were used to determine haematological parameters {packed cell volume, haemoglobin concentration, white blood cells, red blood cells, heterophil, lymphocyte, eosinophil, basophil, monocyte, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)} Blood samples in the plain bottles were tested for serum biochemical indices {total protein, albumin, globulin, glucose, cholesterol, alanine aminotransferase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), calcium, and phosphorus}.

***Liver collection***

At the end of the 3rd week, three rabbits were selected per treatment for liver

histology. The livers were removed from the rabbits after dissection and were preserved in sample bottles containing 10% formaldehyde, dehydrated in series of alcohol (70%, 90%, 100%), cleared in xylene, embedded in paraffin wax after which the tissues were sectioned (5 mm) and stained with H&E (haematoxylin and eosin).

***Statistical Analyses***

Data obtained were subjected to analysis of variance. Significant differences ($p<0.05$) between the means were separated using Duncan-Multiple Range Test as contained in SAS (2010), and the interactions were tested as shown in the statistical model:

$$\Upsilon_{ijk} = \mu + A_i + B_j + AB_{ij} + \sum_{ijk}$$

Where $\Upsilon_{ijk}$ = Expected output

$\mu$ = Population mean

$A_i$ = Effect due to $i^{th}$ sex (i = 1(bucks,2(does)

$B_j$ = Effect due to $j^{th}$ levels of FALE administration (j = 1(0ml),2(10ml),3 (20ml))

$AB_{ij}$ = Interactive effect between $i^{th}$ sex and $j^{th}$ levels of FALE administration

$\sum_{ijk}$ = Experimental error.

## RESULTS

Oral administration of aqueous *Ficus asperifolia* leaf extract (FALE) to rabbits at various doses significantly affected total weight gain, total feed intake and feed efficiency (Table 2). These performance parameters in rabbits in the treated groups (T2 (rabbits administered 10 ml aqueous FALE): -0.25g, 1184.33g and -0.03; T3 (rabbits administered 20 ml aqueous FALE): -27.25g, 1231.17g and -0.04, respectively) were observed to decrease more rapidly than in the control group (T1(rabbits administered 0 ml aqueous FALE): 152g, 1551.08g and 0.08, respectively). Sex effect showed that final weight and total feed intake were significantly reduced in bucks (2011.11g and 1170.11g respectively) compared to the does (2258g and1474.61g, respectively).

Final weight was not significantly ($P>0.05$) influenced by the interaction between the levels of *Ficus asperifolia* leaf extract and sex (Table 3). However, total weight gain, total feed intake and feed efficiency were significantly affected. The values of these parameters were highest in does (210g, 1755.67g and 0.106, respectively) in the control group followed by bucks (94g, 1346.50g and 0.055, respectively) also in control group while the least values were observed in those (both sexes) administered the extract (Table 3).

**Table 2: Main effects of *Ficus asperifolia* leaf extract and sex on the performance parameters of New Zealand White rabbits**

| Parameters | Levels of administration of aqueous *Ficus asperifolia* leaves extract | | | Sex | |
|---|---|---|---|---|---|
| | 0ml | 10ml | 20ml | Buck | Doe |
| Initial weight (g) | 2041.67±66.35 | 2137.5±77.12 | 2100.00±89.21 | 2011.11±56.07 | 2175.00±56.52 |
| Final weight (g) | 2193.67±97.35 | 2137.25±88.33 | 2072.75±102.55 | 2011.11±72.15[b] | 2258.00±56.35[a] |
| Total Weight gain (g) | 152.00±41.82[a] | -0.25±29.36[b] | -27.25±38.53[b] | 0.00±34.36 | 83.00±40.64 |
| Total feed Intake (g) | 1551.08±139.25[a] | 1184.83±99.02[b] | 1231.17±80.27[b] | 1170.11±78.05[b] | 1474.61±97.93[a] |
| Feed efficiency | 0.08±0.02[a] | -0.03±0.04[b] | -0.04±0.04[b] | -0.02±0.03 | 0.03±0.03 |

[a,b] Means on the same row with different superscripts are significantly(P<0.05) different.

**Table 3: Interactive effect of *Ficus asperifolia* leaf extract and sex on the performance parameters of New Zealand White rabbits**

| Sex | Buck | | | Doe | | |
|---|---|---|---|---|---|---|
| Parameters/LoFALE | 0ml | 10ml | 20ml | 0ml | 10ml | 20ml |
| Initial weight (g) | 2000.00±118.15 | 2058.33±108.33 | 1975.00±101.04 | 2083.33±79.50 | 2216.67±108.33 | 2225.00±118.15 |
| Final weight (g) | 2094.00±134.09 | 2019.33±134.68 | 1920.00±136.86 | 2293.33±139.55 | 2255.17±83.45 | 2225.50±102.54 |
| Weight gain (g) | 94.00±16.49[ab] | -39.01.53±46.58[b] | -55.00±70.95[b] | 210.00±71.45[a] | 38.50±25.29[b] | 0.50±40.23[b] |
| Total feed Intake (g) | 1346.50±151.26[ab] | 1052.33±92.47[b] | 1111.33±132.15[b] | 1755.67±179.48[a] | 1317.33±151.40[b] | 1350.83±20.77[ab] |
| Feed efficiency | 0.055±0.011[ab] | -0.045±0.055[ab] | -0.071±0.069 [b] | 0.106±0.033[a] | -0.016±0.059[ab] | -0.002±0.037[ab] |

[a,b] Means on the same row with different superscripts are significantly(P<0.05) different.

Haematological indices considered in this study were not significantly affected by FALE (Table 4). Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, and Mean corpuscular Haemoglobin Concentration values (60fl, 20pg, and 33g/dl, respectively) were the same for all the treatments. Packed cell volume and lymphocytes recorded the highest mean values of 40.50% and 67.50%, respectively for rabbits administered 10ml of the extract. Only white blood cell was significantly influenced by sex as highest WBC ($7.90x10^9$/L) was recorded for the bucks (Table 4).

**Table 4: Main effect of *Ficus asperifolia* leaves extract and sex on haematological parameters of NZW rabbits**

| Parameters | Levels of administration of aqueous *Ficus asperifolia* leaf extract | | | Sex | |
|---|---|---|---|---|---|
| | 0ml | 10ml | 20ml | Buck | Doe |
| Packed cell volume (%) | 38.83±1.08 | 40.50±1.45 | 40.33±0.88 | 38.89±0.84 | 40.89±0.93 |
| Haemoglobin (g/dl) | 12.57±0.67 | 13.88±0.86 | 14.05±0.58 | 13.44±0.78 | 13.56±0.36 |
| RBC ($x10^{12}$/L) | 6.27±0.34 | 6.95±0.43 | 7.03±0.30 | 6.72±0.39 | 6.78±0.18 |
| WBC ($x10^9$/L) | 7.02±0.73 | 6.72±0.75 | 7.50±0.71 | $7.90±0.49^a$ | $6.26±0.53^b$ |
| Heterophil (%) | 32.17±2.40 | 31.50±2.38 | 33.67±1.38 | 32.89±1.81 | 32.00±1.56 |
| Lymphocytes (%) | 66.67±2.22 | 67.50±2.50 | 64.17±1.42 | 65.33±1.77 | 66.89±1.64 |
| Eosinophil (% | 0.33±0.33 | 0.33±0.21 | 0.33±0.21 | 0.56±0.24 | 0.11±0.11 |
| Basophil (%) | 0.33±0.21 | 0.17±0.17 | 0.67±0.21 | 0.44±0.18 | 0.33±0.17 |
| Monocytes (%) | 0.50±0.22 | 0.50±0.34 | 1.17±0.40 | 0.78±0.32 | 0.67±0.24 |
| Mean corpuscular volume (fl) | 60.00±0.00 | 60.00±0.00 | 60.00±0.00 | 60.00±0.00 | 60.00±0.00 |
| Mean corpuscular haemoglobin (pg) | 20.00±0.00 | 20.00±0.00 | 20.00±0.00 | 20.00±0.00 | 20.00±0.00 |
| Mean corpuscular Haemoglobin Concentration (g/dl) | 33.00±0.00 | 33.00±0.00 | 33.00±0.00 | 33.00±0.00 | 33.00±0.00 |

White blood cells (WBC) and lymphocytes were significantly influenced by the interactive effects of levels of aqueous *Ficus asperifolia* leaf extract (FALE) and sex (Table 5). White blood cells (WBC) of bucks administered 0ml ($8.33x10^9$/L) and 10ml ($8.17x10^9$/L) were statistically similar but significantly higher than that of does administered 10ml FALE ($5.27x10^9$/L). Conversely, highest ($p<0.05$) lymphocytes were recorded for does administered 0ml (71.00%) while the least value of 62.33% was recorded for bucks also in the control group (Table 5).

**Table 5: Interactive effect of *Ficus asperifolia* leaf extract and sex on haematological parameters of New Zealand White rabbits**

| Sex | Buck | | | Doe | | |
|---|---|---|---|---|---|---|
| **Parameters/LoFALE** | **0ml** | **10ml** | **20ml** | **0ml** | **10ml** | **20ml** |
| Packed cell volume (%) | 37.33±0.88 | 40.67±2.19 | 38.67±0.33 | 40.33±1.67 | 40.33±2.40 | 42.00±1.00 |
| Haemoglobin (g/dl) | 11.57±0.59 | 14.33±1.68 | 14.43±0.28 | 13.57±0.94 | 13.43±0.81 | 13.67±0.20 |
| RBC ($x10^{12}$/L) | 5.77±0.29 | 7.17±0.84 | 7.23±0.62 | 6.77±0.48 | 6.73±0.39 | 6.83±0.09 |
| WBC ($x10^{9}$/L) | 8.33±0.93$^{a}$ | 8.17±0.49$^{a}$ | 7.20±1.17$^{ab}$ | 5.70±0.21$^{ab}$ | 5.27±0.69$^{b}$ | 7.80±1.03$^{ab}$ |
| Heterophil (%) | 36.33±3.18 | 28.33±2.40 | 34.00±2.52 | 28.00±1.15 | 34.67±3.53 | 33.33±1.76 |
| Lymphocytes (%) | 62.33±2.19$^{b}$ | 70.33±2.60$^{ab}$ | 63.33±2.67$^{ab}$ | 71.00±1.00$^{a}$ | 64.67±4.06$^{ab}$ | 65.00±1.53$^{ab}$ |
| Eosinophil (% | 0.67±0.67 | 0.33±0.33 | 0.67±0.33 | 0.00 | 0.33±0.33 | 0.00 |
| Basophil (%) | 0.33±0.33 | 0.33±0.33 | 0.67±0.33 | 0.33±0.33 | 0.00 | 0.67±0.33 |
| Monocytes (%) | 0.33±0.33 | 0.67±0.67 | 1.33.±0.67 | 0.67±0.33 | 0.33±0.33 | 1.00±0.58 |
| Mean corpuscular volume (fl) | 60.00±0.00 | 60.00±0.00 | 60.00±0.00 | 60.00±0.00 | 60.00±0.00 | 60.00±0.00 |

$^{a,b}$ Means on the same row with different superscripts are significantly($P<0.05$) different

Total protein, globulin, glucose, alkaline phosphate and calcium were significantly affected by levels of aqueous *Ficus asperifolia* leaf extract while cholesterol and calcium were influenced significantly by sex (Table 6). Total protein (5.60g/dl, 5.87g/dl and 6.65g/dl), globulin (2g/dl, 2.07g/dl and 3.42g/dl) and calcium (6.97mg/dl, 7.25mg/dl and 8.55mg/dl) values increased significantly with increasing inclusion levels of aqueous *Ficus asperifolia* leaf extract; rabbits administered 0ml and 10ml of the extract recorded the highest mean values for glucose (138 mg/dl) and alkaline phosphate (81.67u/l) respectively. Cholesterol level was significantly higher in the bucks (83.69 mg/dl) compared to the does (43.52mg/dl) while calcium was significantly higher in the does (8.49mg/dl) than in bucks (6.69 mg/dl). Does administered 20ml of *Ficus asperifolia* leaf extract recorded highest mean values for globulin (3.67g/dl), glucose (142.67mg/dl) and calcium (10.10mg/dl) while highest mean value of 83.33u/l was recorded in does administered 20ml of *Ficus asperifolia* leaf extract (Table 6).

**Table 6: Main effect of *Ficus asperifolia* leaf extract and sex on serum biochemical indices of New Zealand White rabbits**

| Parameters | Levels of Administration of Aqueous *Ficus asperifolia* Leaves Extract | | | Sex | |
|---|---|---|---|---|---|
| | 0ml | 10ml | 20ml | Buck | Doe |
| Total protein (g/l) | 5.60±0.24$^{b}$ | 5.87±0.28$^{ab}$ | 6.65±0.23$^{a}$ | 5.96±0.24 | 6.12±0.26 |
| Albumin (g/dl) | 3.57±0.15 | 3.78±0.47 | 3.25±0.17 | 3.82±0.28 | 3.24±0.16 |
| Globulin (g/dl) | 2.00±0.30$^{b}$ | 2.07±0.46$^{b}$ | 3.42±0.28$^{a}$ | 2.12±0.37 | 2.87±0.31 |
| Glucose (mg/dl) | 138.00±3.99$^{a}$ | 101.33±10.70$^{b}$ | 122.50±10.76$^{ab}$ | 113.00±8.06 | 128.00±8.92 |
| Cholesterol (mg/dl) | 57.68±12.38 | 60.38±13.62 | 72.75±11.27 | 83.69±5.82$^{a}$ | 43.52±8.37$^{b}$ |
| Aspartate amino transferase (u/l) | 47.50±,57 | 50.67±2.65 | 47.83±2.30 | 49.33±2.11 | 48.00±1.45 |
| Alanine amino transferase (u/l) | 45.17±1.96 | 41.33±1.02 | 41.50±0.62 | 41.67±0.90 | 43.67±1.38 |
| Alkaline phosphate (u/l) | 71.33±1.33$^{b}$ | 81.67±2.85$^{a}$ | 72.83±2.30$^{b}$ | 74.33±2.11 | 76.22±2.61 |
| Calcium (mg/dl) | 6.97±0.13$^{b}$ | 7.25±0.43$^{b}$ | 8.55±0.76$^{a}$ | 6.69±0.18$^{b}$ | 8.49±0.45$^{a}$ |
| Phosphorus(mg/dl) | 7.97±1.69 | 8.77±1.18 | 6.58±1.03 | 8.32±1.17 | 7.22±0.99 |

$^{a,b}$ Means on the same row with different superscripts are significantly($P<0.05$) different.

Globulin, glucose, cholesterol, aspartate amino transferase, alkaline phosphate and calcium were significantly ($p<0.05$) influenced by the interaction between levels of aqueous *Ficus asperifolia* leaf extract (FALE) and sex while other indices were not significantly affected (Table 7). Alkaline phosphate was significantly highest in NZW rabbit does administered 10ml of the extract and lowest in rabbit does administered 0ml.

**Table 7: Interactive effect of *Ficus asperifolia* leaf extract and sex on serum biochemical indices of New Zealand White rabbits**

| Sex | Buck | | | Doe | | |
|---|---|---|---|---|---|---|
| Parameters/ LoFALE | 0ml | 10ml | 20ml | 0ml | 10ml | 20ml |
| Total protein (g/l) | 5.60±0.45 | 5.80±0.29 | 6.47±0.47 | 5.60±0.31 | 5.93±0.56 | 6.83±0.09 |
| Albumin (g/dl) | 3.87±0.15 | 4.27±0.83 | 3.33±0.09 | 3.27±0.07 | 3.30±0.42 | 3.17±0.35 |
| Globulin (g/dl) | 1.67±0.50$^{b}$ | 1.53±0.57$^{b}$ | 3.17±0.47$^{ab}$ | 2.33±0.29$^{ab}$ | 2.60±0.68$^{ab}$ | 3.67±0.32$^{a}$ |
| Glucose (mg/dl) | 135.67±2.73$^{ab}$ | 101.00±14.73$^{b}$ | 102.00±12.99$^{b}$ | 140.33±8.17$^{ab}$ | 101.67±18.84$^{b}$ | 142.67±1.86$^{a}$ |
| Cholesterol (mg/dl) | 84.17±6.38$^{a}$ | 86.30±13.85$^{a}$ | 80.60±12.87$^{a}$ | 31.20±4.88$^{b}$ | 34.47±7.98$^{b}$ | 64.90±20.18$^{ab}$ |
| Aspartate amino transferase (u/l) | 48.00±2.00$^{ab}$ | 55.00±4.04$^{a}$ | 45.00±2.65$^{b}$ | 47.00±2.89$^{ab}$ | 46.33±0.33$^{ab}$ | 50.67±3.38$^{ab}$ |
| Alanine amino transferase (u/l) | 43.67±1.45 | 41.00±2.08 | 40.33±0.33 | 46.67±3.84 | 41.67±0.88 | 42.67±0.67 |
| Alkaline phosphate (u/l) | 73.00±2.00$^{ab}$ | 80.00±4.04$^{ab}$ | 70.00±2.65$^{b}$ | 69.67±1.45$^{b}$ | 83.33±4.63$^{a}$ | 75.67±3.38$^{ab}$ |
| Calcium (mg/dl) | 6.73±0.09$^{c}$ | 6.33±0.12$^{c}$ | 7.00±0.55$^{c}$ | 7.20±0.15$^{bc}$ | 8.17±0.30$^{b}$ | 10.10±0.42$^{a}$ |
| Phosphorus(mg/dl) | 9.60±1.89 | 10.10±2.00 | 5.27±1.34 | 6.33±2.85 | 7.43±1.11 | 7.99±1.33 |

$^{a,b,c}$ Means on the same row with different superscripts are significantly($P<0.05$) different.

The livers of rabbits in control group showed normal architecture of hepatic lobule with normal arrangement of hepatocytes but there was gradual disruption of hepatic lobules from those administered 10 ml aqueous FALE to the group administered 20 ml aqueous FALE and they appeared atrophied and shrunken as compared to control group (Figures 1-3). Focal periportal coagulative necrosis was observed in animals administered 10 ml and 20 ml aqueous FALE (Figures2 and 3). The necrotic areas showed cellular lysis with collapsed stroma, pyknotic nuclei and lymphocytic infiltration. It was absent in hepatocytes for animals of control group.

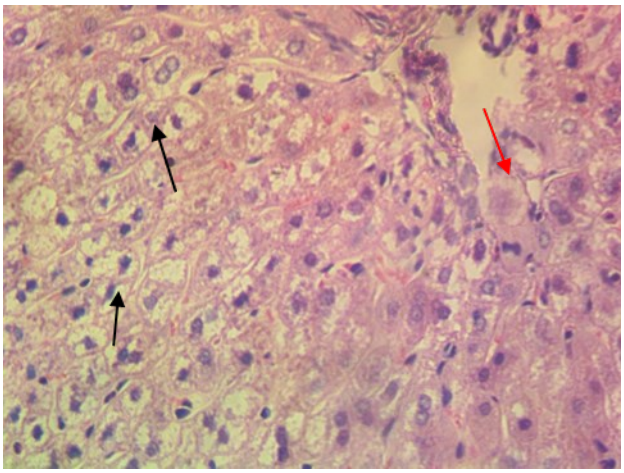

Fig. 1: Photomicrograph of liver of rabbit buck administered 0 ml of *Ficus asperifolia* leaf extract.

The hepatic cords are closely-packed. There is marked vacuolar degeneration (black arrows) of the hepatocytes with the periportal hepatocytes (red arrow) appearing spared. There is no remarkable vascular change.

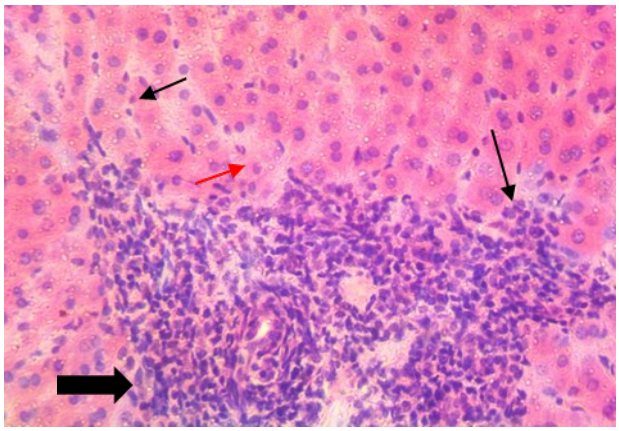

**Fig. 2: Photomicrograph of liver of rabbit buck administered 10 ml of *Ficus asperifolia* leaf extract**

Hepatic plates are closely packed. There are a few random foci of single-cell hepatocellular necrosis (black arrows). There are dense aggregates of mononuclear inflammatory cells (thick arrow). There is moderate Kupffer cell hyperplasia (red arrows). There is no remarkable vascular change.

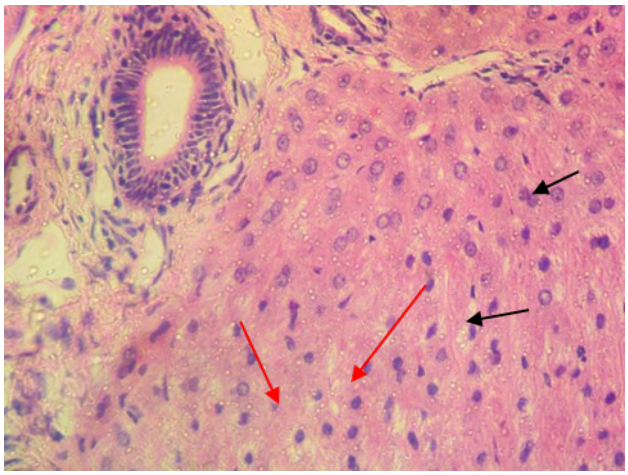

**Fig. 3: Photomicrograph of liver of rabbit buck administered 20 ml of Ficus asperifolia leaf extract.**

The hepatic plates are closely packed. There are multiple foci of necrotic hepatocytes with pyknotic, angulated nuclei (black arrows). There is no remarkable vascular change. There are a few foci of mild vacuolar change of hepatocytes (red arrows).

## DISCUSSION

Aqueous extract of *Ficus asperifolia* leaf (FALE) exhibited significant anti-hyperglycemic activity which resulted in the significant weight loss observed in the treated rabbits as compared to those in the control group. This study suggests that sex may be an important factor for consideration when selecting animals to be used in ethnobotanical studies as does performed better than bucks. The differences in bodyweight of bucks and does in this study agreed with earlier submissions on sexual dimorphism in livestock species (Mahgoub *et al.*, 2005) and disagreed with the investigations of some other researchers (Lukefahr and Ozimba, 1991; Ozimba and Lukefahr, 1991) who reported that unlike in most domestic livestock species, sex does not strongly influence growth in rabbits.

Higher total feed intake recorded in the does in this study is similar to the result obtained by Lazzaroni *et al.* (2012). They reported a slight effect of gender on productive performance, with females showing higher feed intake than males. Lazzaroni and Biagini (2002) also obtained higher feed intake by female Carmagnola Grey rabbits than males.
The non-significant values recorded for haematological parameters of rabbits administered queous *Ficus asperifolia* leaf extract in this study are an indication that the experimental animals were not affected by the different levels of administration of *Ficus asperi-*

*folia* leaf extract and were within the normal ranges (RAR, 2009) reported for healthy rabbit. This non-significant difference could be an indication of the wellness of the animals throughout the experimental period as normal haematological parameters of an animal are direct indication of absence of disease (Olafadehan *et al*., 2010). Packed cell volume is an indication of total red blood cell in whole blood compared to proportion of plasma and buffy coat/white blood cell portion. It gives certain indication of the health status of the animal. In this study, administration of *Ficus asperifolia* leaf extract at 10% inclusion level gave the highest packed cell volume although not significantly different from others. This result is an indication that *Ficus asperifolia* leaf extract has substance(s) which promotes erythrocyte production although it is contrary to the report of Dangarembizi *et al.* (2013), who reported reduced packed cell volume in rats administered aqueous *Ficus thonningii* leaf extract. Sandberg (2002) stated that reduced PCV could result from reduced bioavailability of dietary iron due to the action of anti-nutritional factors such as tannins, phytate and other polyphenolic phytochemicals. The numerical increase observed in the values of haemoglobin, red blood cell and monocytes with increasing aqueous *Ficus asperifolia* leaf extract could be attributed to inclusion at different doses to the rabbits. This could have led to a more efficient erythropoiesis in the rabbit bucks administered the extract by increasing the bone marrow capacity to produce red blood cells thereby increasing and improving the blood level condition hence, preventing anaemia (Togun *et al*., 2007; Chineke *et al*., 2006; Etim, 2010 and Etim *et al*., 2014). It is also an indication that rabbits given aqueous *Ficus asperifolia* leaf extract had the ability to transport higher volume of oxygen in their system which may enhance their health status. White blood cells play a prominent role in disease resistance especially with respect to the production of antibodies and the process of phygocytosis (Soetan *et al*., 2013). The significant increase observed in white blood cell mean values obtained from the rabbit bucks in this study was still within the reported physiological range of 4.50-11.00 x109/L for normal rabbits (RAR, 2009). This result implies that the animals' ability to combat infection or illness was not negatively affected since white blood cells are known to be among the body's defence mechanisms that fight against non-self or pathogenic organisms. Elevated values of white blood cell differentials are indicators of stress, tissue damage, chronic inflammation and presence of both parasitic and non-parasitic infection in the system of animals (Douglas *et al*., 2010; Martinez-Silvestre *et al*., 2013; Pendl, 2013; Wolfensohn and Lloyd, 2013). This may explain longevity as reported by Mbanasor *et al.* (2003) and Etim (2010) for haematological parameters of rabbit does fed *Aspilia africana* leaves. This also agrees with the report by Reilly (1993) and Etim (2010) that normal range of values for WBC indicated that the animals were healthy; resulting in high degree of resistance to disease (Soetan *et al*., 2013, Etim, *et al*., 2014) and enhanced adaptability to local environment and disease -prevalent conditions (Kabir *et al*., 2011, Okunlola *et al*., 2012, Iwuji and Herbert 2012, Isaac *et al*., 2013 and Etim *et al*.,2014). It is also consistent with the observation of Bello and Tsado (2013) that WBC values within the normal range is an indication that there were no microbial infections or presence of foreign bodies or parasites in the circulatory system of the experimental animals. Ologbobo *et al.* (1986) observed that an increase in WBC count above normal is an indication of the presence of exogenous sub-

stances and foreign bodies in the body. In this study, there was no case of such abnormal rise in values of WBC. Lymphocytes are important in forming barriers against local disease conditions and may be involved in antibody formation (Frandson, 1981). The available results also agree with the report of Ameen *et al.* (2007) that when the values for lymphocytes, leucocytes and neutrophils fall within the normal ranges as observed in this study, it implies that the feeding pattern, in this case the experimental extract, did not affect the immune system.

The analysis of the serum biochemical characteristics of the rabbits recorded significant increase in total protein and globulin values of the rabbits administered aqueous extracts of *Ficus asperifolia*. Serum biochemical analysis is used to determine the level of liver damage and to evaluate protein quality and amino acid requirements in animals (Harper *et al*., 1979). Significant increase observed in the serum protein albumin and globulin of rabbits administered aqueous *Ficus asperifolia* leaf extract might be because the rabbits were able to utilise the available protein in the extract better, leading to non-compromise of the immune system of the animals since globulins are serum proteins involved in the immune system (Charles, 2001). Also, the significant increase observed in the results of the alkaline phosphatase (ALP) among the groups administered 10ml aqueous *Ficus asperifolia* leaf extract could be attributed to a number of factors including the homeostatic mechanisms of the animals and the active ingredients in the aqueous extracts of *Ficus asperifolia* leaves being functionally relative to each other in respect of quantities available (Noboru, 2001). Alkaline phosphatase is present in tissues throughout the entire body of the animal but is particularly concentrated in the liver, bile duct, kidneys, bones and the placenta (Kim and Wycoff, 1991).

Serum biochemical parameters are useful markers for the evaluation of health status of animals. It also shows alterations in organs and tissues of animals fed with unconventional feed sources (Kudair and Al-Hussary, 2010). The results obtained show that at the doses used, *Ficus asperifolia* extract did not seem to cause any parenchymal cell lesions, necrosis or drug induced-hepatitis. It was notable that ALP levels were significantly elevated in all of the rabbit does that received *Ficus asperifolia* leaf extract. Alkaline phosphatase (ALP) is also associated with osteoblastic activity and hence its levels are elevated in rapidly growing animals (Alhassan *et al*., 2009). Serum albumin, total bilirubin and total protein concentrations were also used as clinical tools in assessing the hepatosynthetic function. The concentrations of these biochemical markers of liver function in the blood were unaltered by administration of *Ficus asperifolia* extract at the doses used; confirming that *Ficus asperifolia* was not hepatotoxic in the short term. This result is in line with the previous work done by Omoniwa *et al*., (2013) which reported *Ficus asperifolia* phytochemical screening to have detected flavonoids, saponins, alkanoids, tannins, steroids and many others which increase the level of calcium and alkaline phosphatase in the experimented animals.

According to Harper *et al.* (1979), in serum enzymology, tissue-soluble enzymes are a very important adjunct to clinical diagnosis of tissue damage or disease. Both transaminases in conjunction with other enzymes are used as indicators of liver and heart damage. Aspartate aminotransferase (AST) is in-

volved in the inter-conversion of aspartate to glutamate while alanine transferase is involved in cellular metabolism and energy processes of the cell via the citric acid cycle. The detailed histological study of liver revealed atrophy of hepatocytes in focal areas. The treated animals showed mild focal coagulative type of necrosis in hepatocytes. The probable mechanism may be the inhibition of mitochondrial function by dual effect on both beta-oxidation energy productions by inhibiting the synthesis of nicotinamide, flavin adenine dinucleotide and depletion in glutathione that result in decreased ATP production and development of cell necrosis (Drabo and Khatry, 2012).

## CONCLUSION

This study indicated that aqueous *Ficus asperifolia* leaves extract is anti-hyperglycemic and support rabbit production without any negative influence on the immune status of the rabbit.

## RECOMMENDATION

This study suggests that sex may be an important factor for consideration when selecting animals to be used in ethnobotanical studies as does performed better than bucks.

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# ORGANIC FERTILIZER RATE ON GROWTH AND YIELD OF CUCUMBER VARIETIES

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## ABSTRACT

Appropriate crop variety and organic fertilizer rate are factors that impact growth and yield of cucumber (Cucumis sativus L). Two field trials were conducted between April and December, 2015 at the Federal University of Agriculture, Abeokuta, Nigeria in the tropical rainforest-savannah transition zone of south-western Nigeria, to determine optimum rate of an organic fertilizer on three varieties of cucumber. There were four organic manure rate treatments comprising 0, 5, 10, and 15 $t.ha^{-1}$ of Gateway Organic Fertilizer brand. The three cucumber varieties investigated were: Marketmore, Poinsett and Marketer. Treatments were arranged in a split - plot arrangement fitted into Randomized Complete Block Design (RCBD) with 4 replicates. Data collected on growth and yield parameters were subjected to analysis of variance using GENSTAT discovery. Gateway Organic Fertilizer application had positive influence on growth and fruit yield of cucumber. Number of leaves, number of branches and vine length increased with increasing organic fertilizer rate. Yield was increased up to 53.63 %, 64.74 % and 51.65 % with Marketmore, Poinsett and Marketer varieties, respectively. Among the varieties, Poinsett variety was best for optimum growth and yield. Application of 10 $t.ha^{-1}$ Gateway Organic Fertilizer was optimum for fruit yield of cucumber.

**Keywords**: Gateway organic fertilizer, Poinsett, Marketmore, Marketer

## INTRODUCTION

Cucumber (*Cucumis sativus* L) is a monoecious annual crop in the Cucurbitaceae family that has been cultivated by man for over 3,000 years (Adetula and Denton, 2003; Okonmah, 2011).

It is usually propagated by seed. Economically, it ranks fourth after tomatoes, cabbage and onion in Asia and second after tomato in Western Europe (Eifediyi and Remison, 2010). However, its place is yet to be ranked in tropical Africa because of limited use. Cucumber fruits can be used as a low income source of vitamins and minerals that are lacking in most diets. It can be eaten in salads or sliced into stew, as in the tropical regions. The ascorbic acid and caffeic acid

contained in cucumber helps in reducing skin irritation (Okonmah, 2011). Cultivar selection is one of the most important decisions made during the crop production process. Desirable traits required for local cultivars include high productivity, high fruit crispness and firmness, as well as resistance to pests and diseases. Cucumber varieties are majorly three types: slicing, pickling, and burpless. Within each variety, several different cultivars have emerged with varying shapes, sizes, skin colour and carotene content (Tahir *et al*., 2019). Cucumber yields in the tropics are generally often low due to low soil fertility. The soils are poor in organic matter content and available nutrients; hence productivity and sustainability decline over time (Zingore *et al*., 2003). This is due to continuous cultivation of the tropical soils leading to mining of the soil nutrients which need to be replenished in order to reverse loss of nutrients and maintain productivity. Replenishment of nutrients and enhanced quality of tropical soil may be achieved either from organic sources, inorganic fertilizers or from both (Shangakkara *et al*., 2004). Use of chemical fertilizers alone to sustain high crop yields has not been successful, due to enhancement of soil acidity, nutrient leaching and degradation of soil physical and organic matter status (Nottidge *et al*., 2005). Adediran *et al*., (1999) reported that when organic fertilizers are applied at a rate that supplies equivalent amounts of N, P and K in mineral fertilizer, it has same effects as inorganic fertilizer. There is the need to study the effects of manure rates on cucumber. This experiment was conducted to determine the optimum rate of an organic fertilizer (Gateway Organic Fertilizer) on three cucumber varieties (Marketmore, Poinsett and Marketer).

## MATERIALS AND METHODS

The experiment was conducted at the research farm of the Federal University of Agriculture Abeokuta in the tropical rainforest-savannah transitional zone of south-western Nigeria, latitude 7°15'N; longitude 3°25'E. Pre-planting soil samples were taken randomly up to 15 cm depth from 5 locations, using a soil auger and bulked to have a composite sample that was air dried and analyzed to determine soil nutrient status. The first season (Early season) field trial was conducted from April to July, while the second season (Late season) field trial was conducted from September to December, 2015.

The Oxic Paleudulf soil (Adetunji, 1991) was mechanically ploughed and harrowed after 2 weeks. The entire field was demarcated into 48 plots of 3.0 x 2.0 m each, separated by 1.0 m path-ways. Main plot was cucumber variety (Marketmore, Poinsett and Marketer) while sub plot treatment was organic fertilizer rate at 0, 5, 10 and 15 $t.ha^{-1}$ in a split - plot arrangement fitted into Randomized Complete Block Design (RCBD) with 4 replicates.

Gateway Organic Fertilizer (a commercial brand of organic fertilizer produced in Ogun State, Nigeria**)** was applied in a single dose at 2 weeks before planting at the rate of 5, 10 and 15 $t.ha^{-1}$ while unfertilized plots served as control. Three cultivars of cucumber used for the experiment were: 'Marketmore', (open pollinated variety that is resistant to cucumber scab and cucumber mosaic virus); Poinsett (open pollinated variety that is resistant to angular leaf spot, anthracnose, downy mildew and powdery mildew) and Marketer (open pollinated variety that is resistant to downy and powdery mildew) variety. Planting was done at two weeks after organic fertilizer application. Two seeds of cu-

cumber were planted on flat beds at a spacing of 1.0 x 0.5 m. Manual weeding was at 3 weeks interval prior to flowering and fruiting. Pest control was done organically using a bio-pesticide formulation that composed of 200 g of neem shoot biomass, 100 g of pawpaw leaves, 100 g of jatropha leaves, 100 g of lemon grass, boiled in 10 litres of water. The extract was allowed to cool and foliar application was done using hand sprayer at 2 and 4 weeks after planting.

At each sampling, four cucumber plants were sampled from middle rows to determine vine length, number of leaves/plant, and number of branches. Vine length was measured from the root collar to the growing tip of the main vine. Fully-expanded leaves and branches were counted. Number of days to first and 50% flowering were determined. Healthy and mature fruits were harvested; number of fruits per plant; average fruit weight, and fruit yield were estimated.

Data were subjected to analysis of variance using GENSTAT discovery (12th ed., VSN International, Hemel Hempstead, UK), with cucumber variety and organic fertilizer rates as factors. The interactions were majorly significant and so were used to explain results, and were separated using DMRT at $P \leq 0.05$.

## RESULTS

Soil characters of the sandy loam soil varied between seasons (Table1). pH values in both seasons were near neutral. Organic matter composition was higher in the early season cropping (4.3%) which translated to higher nitrogen content; values in late season cropping were higher for available phosphorus, magnesium, calcium and zinc than in early season cropping (Table 1).

**Table 1: Pre-cropping Soil and Organic Fertilizer Analysis**

| Parameter | Early season | Late season | Organic fertilizer |
|---|---|---|---|
| **Chemical Composition** | | | |
| pH | 7.20 | 7.30 | 6.50 |
| O.M (%) | 4.32 | 1.55 | 12.32 |
| Total N (%) | 0.15 | 0.08 | 2.14 |
| Avail. P ($mg.kg^{-1}$) | 12.58 | 18.2 | 43.12 |
| Exch. Bases | | | |
| Mg ($cmol.kg^{-1}$) | 0.37 | 2.51 | 2.86 |
| K ($cmol.kg^{-1}$) | 0.61 | 0.5 | 1.50 |
| Na ($cmol.kg^{-1}$) | 0.52 | 0.39 | 1.48 |
| Ca ($cmol.kg^{-1}$) | 0.21 | 0.63 | 6.10 |
| Zn($mg.kg^{-1}$) | 0.10 | 0.11 | 2.11 |
| Fe ($mg.kg^{-1}$) | 0.41 | 0.32 | 2.02 |
| Physical | | | |
| Sand % | 52.67 | 70.2 | |
| Slit % | 31.00 | 14.00 | |
| Clay % | 16.00 | 15.8 | |
| Soil Textural Class | sandy loam | sandy loam | |

Total rainfall during the early cropping season with sunshine hour was greater than in the late cropping season (Table 2). During the experimental period, total rainfall and rainfall during the vegetative phase were higher in the late season than in the early season. However, during the reproductive phase, rainfall was higher in the early season than in the late season. Mean temperatures were higher during the vegetative phase but lower during the reproductive phase in early cropping season while in the late cropping season, mean temperatures were lower during the vegetative phase but higher during the reproductive phase (Table 2).

**Table 2: Meteorological data of the experimental site in 2015**

| | Rainfall (mm) | Sunshine (hr) | Maximum temperature ($^oC$) | Minimum temperature ($^oC$) |
|---|---|---|---|---|
| **January** | 0.00 | 6.10 | 35.40 | 20.50 |
| **February** | 17.10 | 2.10 | 33.10 | 24.60 |
| **March** | 149.00 | 5.60 | 35.30 | 25.10 |
| **April** | 87.20 | 6.10 | 33.80 | 24.10 |
| **May** | 113.80 | 6.70 | 33.10 | 23.80 |
| **June** | 116.50 | 4.20 | 31.00 | 22.80 |
| **July** | 90.70 | 3.60 | 23.50 | 28.30 |
| **August** | 92.70 | 2.40 | 22.90 | 22.90 |
| **September** | 160.00 | 2.80 | 30.40 | 22.50 |
| **October** | 205.90 | 5.90 | 31.60 | 23.00 |
| **November** | 17.60 | 6.30 | 33.50 | 23.80 |
| **December** | 0.00 | 5.90 | 33.50 | 19.30 |

There were significant differences in the number of leaves of cucumber as influenced by interaction of variety and organic fertilizer rate (Table 3). In the early season cropping, Marketmore variety cultivated with 10 $t.ha^{-1}$ organic fertilizer produced more leaves significantly higher when compared with other interactions at 4 WAP. At 6 WAP, Marketmore variety cultivated with 10 and 15 $t.ha^{-1}$ organic fertilizer produced more leaves but similar when compared with Poinsett cultivated with 5, 10 and 15 $t.ha^{-1}$ and Marketer at 10 and 15 $t.ha^{-1}$ organic fertilizer. At 8 WAP, Marketmore cultivated with 5, 10 and 15 $t.ha^{-1}$ organic fertilizer produced more leaves which were significantly higher when compared with other interactions (Table 3).

At 4, 6 and 8 WAP, during the late cropping season, Marketer variety cultivated with 15 $t.ha^{-1}$ organic fertilizer produced more leaves per plant (Table 3).

**Table 3: Effects of variety and fertilizer rate interactions on number of cucumber leaves /plant in 2015**

| Variety | Rate (t.ha$^{-1}$) | 4 | 6 | 8 | 4 | 6 | 8 |
|---|---|---|---|---|---|---|---|
| | | Weeks after planting | | | | | |
| | | Early season | | | Late season | | |
| Marketmore | 0 | 10.75cde | 18.00de | 17.00bc | 6.000h | 15.00h | 6.00c |
| | 5 | 18.00b | 34.00abc | 30.00a | 10.00ef | 31.00de | 13.00b |
| | 10 | 27.75a | 41.00a | 26.00a | 12.00 cde | 31.00de | 12.00b |
| | 15 | 19.75b | 34.00abc | 30.00a | 15.75 ab | 34.00c | 12.00 b |
| Poinsett | 0 | 7.25e | 21.00cde | 10.00cd | 7.00 gh | 18.00g | 10.00 b |
| | 5 | 9.75de | 33.00abc | 18.00b | 11.00def | 34.00c | 18.00a |
| | 10 | 10.75cde | 32.00abcd | 17.00bc | 12.00cde | 34.00c | 17.00a |
| | 15 | 10.25de | 30.00abcd | 17.00bc | 15.00ab | 37.00b | 17.00a |
| Marketer | 0 | 6.88e | 14.00e | 10.00d | 9.00fg | 23.00f | 11.00b |
| | 5 | 9.63de | 22.00bcde | 10.00d | 14.00bc | 39.00b | 11.00b |
| | 10 | 14.88bcd | 28.00abcd | 14.00bcd | 14.00bc | 39.00b | 17.00a |
| | 15 | 17 .1bc | 36.00ab | 17.00b | 16.00a | 42.00a | 18.00a |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$) using Duncan Multiple Range Test (DMRT).

Number of cucumber branches was significantly influenced by interaction of variety and organic fertilizer rate (Table 4). In the early season cropping, Marketmore variety cultivated with 10 and 15 t.ha$^{-1}$ organic fertilizer produced more branches which were significantly higher when compared with other interactions at 4 WAP. At 6 WAP, Marketer cultivated with 15 t.ha$^{-1}$ organic fertilizer produced more branches that were similar when compared with Marketmore and Poinsett cultivated with 5, 10 and 15 t.ha$^{-1}$ organic fertilizer but significantly higher when compared with other interactions. At 8 WAP, Poinsett cultivated with 5, 10 and 15 t.ha$^{-1}$ organic fertilizer produced more branches which was similar compared with Marketmore and Marketer cultivated with 15 t.ha$^{-1}$ organic fertilizer but significantly higher compared with other interactions (Table 4). During the late cropping season, Marketer variety cultivated with 15 t.ha$^{-1}$ organic fertilizer had plants with more branches across the weeks but similar when compared with Marketer variety cultivated with 10 t.ha$^{-1}$ organic fertilizer (Table 4).

**Table 4: Effects of variety and fertilizer rate interaction on number of branches / plant in 2015**

| Variety | Rate (t.ha$^{-1}$) | 4 | 6 | 8 | 4 | 6 | 8 |
|---|---|---|---|---|---|---|---|
| | | Weeks after planting | | | | | |
| | | Early season | | | Late season | | |
| Marketmore | 0 | 0.75de | 3.25bc | 2.75gh | 1.00 g | 3.50h | 3.63f |
| | 5 | 3.25ab | 4.87ab | 4.13defg | 1.00g | 5.00efg | 5.38de |
| | 10 | 4.25a | 4 25abc | 5.63bcde | 1.70f | 6.00ed | 6.63 bc |
| | 15 | 3.62ab | 5 .37ab | 5.70abcd | 1.85ef | 6.75bcd | 6.75abc |
| Poinsett | 0 | 2.75abc | 3.25bc | 3.50fg | 2.00def | 4.25gh | 4.25ef |
| | 5 | 2.75abc | 5.75ab | 7.50a | 2.25cde | 5.75def | 7.50ab |
| | 10 | 2.50bc | 5.00ab | 7.50a | 2.60abc | 6.75bcd | 7.25abc |
| | 15 | 2.50bc | 5.00ab | 7.25ab | 2.85ab | 7.50ab | 7.50ab |
| Marketer | 0 | 0. 25e | 2.25bc | 2.25h | 2.00def | 4.75gh | 5.38de |
| | 5 | 0.75de | 3.87bc | 3.87defg | 2.45bcd | 6.25cd | 6.25cd |
| | 10 | 1.50cde | 5.00ab | 5.00cdef | 2.90ab | 7.25abc | 7.25abc |
| | 15 | 2.00bcd | 6.75a | 6.75abc | 3.00a | 8.00a | 8.00a |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$) using Duncan Multiple Range Test (DMRT)

There were significant differences in the vine length of cucumber plants as affected by the interaction of variety and level of Gateway Organic fertilizer (Table 5). In the early season cropping, Marketmore variety cultivated with 5 and 10 t.ha$^{-1}$ organic fertilizer produced vines significantly longer when compared with other interactions at 4 WAP. At 6 and 8 WAP, Marketmore cultivated with 5, 10 and 15 t.ha$^{-1}$ organic fertilizer produced vines significantly longer when compared with other interactions (Table 5).

During the late cropping season, At 4 WAP, Marketer variety cultivated with 10 and 15 t.ha$^{-1}$ organic fertilizer produced vines significantly longer when compared with other interactions. At 6 and 8 WAP, Marketer variety cultivated with 15 t.ha$^{-1}$ organic fertilizer produced vines significantly longer when compared with other interactions (Table 5).

**Table 5: Effects of variety and fertilizer level interactions on vine length (cm) /plant of cucumber**

| Variety (V) | Rate (t.ha$^{-1}$) | 4 | 6 | 8 | 4 | 6 | 8 |
|---|---|---|---|---|---|---|---|
| | | Weeks after planting | | | | | |
| | | Early season | | | Late season | | |
| Marketmore | 0 | 34.46e | 112.29bc | 155.19b | 23.00h | 49.00h | 61.35i |
| | 5 | 100.00ab | 175.75a | 212.06a | 37.38efg | 108.75e | 131.53g |
| | 10 | 111.38a | 183.79a | 208.16a | 44.63cde | 114.15de | 135.32fg |
| | 15 | 79.44bc | 188.35a | 220.75a | 42.75def | 125.9bc | 143.95cd |
| Poinsett | 0 | 26.06e | 63.70e | 70.01e | 31.2gh | 54.08h | 65.60i |
| | 5 | 38.25e | 116.30bc | 144.6bc | 51.88 bc | 114de | 136.53ef |
| | 10 | 36.25e | 104.00cd | 131.58bc | 45.28 bcde | 119.15cd | 140.32de |
| | 15 | 37.75e | 112.18bc | 122.60bcd | 53.78b | 130.65b | 149.78b |
| Marketer | 0 | 31.88e | 71.88e | 71.88e | 23.00g | 65.63g | 78.10h |
| | 5 | 44.63de | 99.08cd | 99.08ed | 48.75bcd | 97.83f | 144.03cd |
| | 10 | 66.38cd | 120.60bc | 120.60cd | 63.50a | 127.00bc | 146.57bc |
| | 15 | 77.75bc | 143.58b | 143.58bc | 68.38a | 148.33a | 163.45a |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$) using Duncan Multiple Range test (DMRT).

Leaf area of cucumber was significantly influenced by the interaction of variety and organic fertilizer rate (Table 6). In the early season cropping, Marketmore variety cultivated with 5, 10 and 15 t.ha$^{-1}$ and Marketer cultivated with 10 and 15 t.ha$^{-1}$ organic fertilizer produced leaves significantly wider when compared with other interactions at 4 WAP. At 6 WAP, Poinsett cultivated with 5 t.ha$^{-1}$ organic fertilizer produced wider leaves which were similar compared with Marketmore cultivated with 5 and 10 t.ha$^{-1}$ , Poinsett at 10 and 15 t.ha$^{-1}$ and Marketer at 15 t.ha$^{-1}$ organic fertilizer. At 8 WAP, Marketmore cultivated with 10 and 15 t.ha$^{-1}$ organic fertilizer produced significantly wider leaves when compared with other interactions (Table 6).

**Table 6: Effects of variety and fertilizer rate interactions on leaf area /plant**

| Variety | Rate (t.ha$^{-1}$) | 4 | 6 | 8 | 4 | 6 | 8 |
|---|---|---|---|---|---|---|---|
| | | Weeks after planting | | | | | |
| | | Early season | | | Late season | | |
| Marketmore | 0 | 703.10de | 1418.00e | 984.50d | 353.50ef | 1227.90d | 199.80 cd |
| | 5 | 1744.40abc | 5021.00abcd | 2451.60bc | 984.40cde | 4399.80c | 681.30bcd |
| | 10 | 2577.60a | 5942.00ab | 2834.60ab | 1359.60 bc | 4571.80c | 816.70ab |
| | 15 | 2325.40ab | 4600.00bcd | 3226.80a | 1860.80 ab | 4668.70c | 706.50abc |
| Poinsett | 0 | 599.10e | 2983.00de | 636.30d | 161.50 f | 2093.40 d | 160.30d |
| | 5 | 1078.20cde | 7720.00a | 1977.90e | 959.40 cde | 7786.70a | 623.70bcd |
| | 10 | 1562.50bcd | 7046.00ab | 2224.20bc | 1029.90 cd | 7455.20a | 434.60bcd |
| | 15 | 1216,00cde | 6252.00ab | 2127.50bc | 1310.40bc | 7183.2ab | 532.30bcd |
| Marketer | 0 | 757.50de | 1443.00e | 757.50d | 663.70def | 2407.6d | 468.7bcd |
| | 5 | 933.50cde | 2929.00de | 933.50d | 1417.50bc | 4999.5c | 900.20ab |
| | 10 | 1784.20ab | 4215.00cd | 1784.50c | 1679.5 ab | 5795.2bc | 935.40ab |
| | 15 | 2430.80ab | 5905.00abc | 2430.80bc | 2280.60a | 6927.4ab | 1231.80a |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$) using Duncan Multiple Range test (DMRT).

During the late cropping season, Marketer variety cultivated with 10 and 15 t.ha$^{-1}$ organic fertilizer at 4, 6 and 8 WAP, produced wider leaves (Table 6).

The number of days to first flower, 50% flowering and days to first harvest was significantly influenced by the interaction of variety and organic fertilizer rate (Table 7). During the early cropping season, Marketmore variety not fertilized flowered earlier when compared with other interactions and also produced 50% of its flower earlier which was similar compared with Marketer at 0. 5, 10 and 15 t.ha$^{-1}$ organic fertilizer. Unfertilized Marketmore, Poinsett and Marketer varieties produced fruits which were ready for harvest earlier when compared with the fertilized (Table 7). In the late cropping season, there were no significant differences in the days to first flowering while the days to 50% flowering and first harvest were significantly different (Table 7). Fertilized Marketmore variety attained 50% flowering earlier, similar with Poinsett and Marketer cultivated with organic fertilizer. Fertilized Marketmore, Poinsett and Marketer varieties produced fruits ready for harvest earlier when compared with unfertilized plants (Table 7).

The number of fruits harvested across the weeks as influenced by the interaction of variety and organic fertilizer rates was signifi-

cantly different (Table 8). During the early cropping season, Poinsett cultivated with 10 and 15 t.ha$^{-1}$ organic fertilizer produced more fruits significantly higher compared with other interactions at 1st harvest. However, at 2nd and 3rd harvests, Poinsett and Marketer cultivated with 15 t.ha$^{-1}$ had more fruits compared with other interactions while at 4th harvest, Marketer variety cultivated with 15 t.ha$^{-1}$ produced more fruits which were significantly higher compared with other interactions (Table 8).

**Table 7: Effect of variety and organic fertilizer rate interactions on number of days to first flowering, 50% flowering and first harvest of cucumber variety**

| | | Early season | | | Late season | | |
|---|---|---|---|---|---|---|---|
| **Variety** | **Rate (t.ha$^{-1}$)** | **1st flowering** | **50% flowering** | **1st Harvest** | **1st flowering** | **50% flowering** | **1st Harvest** |
| Marketmore | 0 | 32. 00a | 38.00ab | 50.00a | 34.00a | 44.00a | 53.00a |
| | 5 | 28.00de | 36.00bc | 45.00bc | 31.00a | 42.00bc | 51.00b |
| | 10 | 28.00de | 35.00c | 45.00bc | 30.00a | 41.00cd | 49.00cd |
| | 15 | 30.00bc | 36.00bc | 45.00bc | 31.00a | 41.00cd | 49.00cd |
| Poinsett | 0 | 30.00bc | 38.00ab | 50.00a | 33.00a | 43.00a | 52.75a |
| | 5 | 27.00e | 35.00c | 45.00bc | 30.00a | 39.00ef | 50.00bc |
| | 10 | 28.00de | 36.00bc | 44.00c | 29.00a | 38.00f | 48.00d |
| | 15 | 27.00e | 36.00bc | 44.00c | 29.00a | 38.00f | 48.00d |
| Marketer | 0 | 31.00ab | 39.50a | 50.00a | 34.00a | 44.00a | 54.00a |
| | 5 | 28.00de | 39.00a | 46.00b | 32.00a | 41.00cd | 50.00bc |
| | 10 | 29.00cd | 38.00ab | 46.00b | 31.00a | 40.00de | 48.00d |
| | 15 | 29.00cd | 38.00ab | 46.00b | 31.00a | 40.00de | 48.00d |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$) using Duncan Multiple Range test (DMRT)

**Table 8: Effect of variety and organic fertilizer rate interactions on number of fruits produced by 3 cucumber varieties in 2015 early cropping season**

| | | 1st Harvest | 2nd harvest | 3rd Harvest | 4th Harvest |
|---|---|---|---|---|---|
| **Variety x rate (**t.ha$^{-1}$**)** | | | | | |
| Marketmore | 0 | 0.00d | 1.75de | 1.62f | 0.38ef |
| | 5 | 2.50bc | 3.75bc | 2.88e | 1.12def |
| | 10 | 1.75c | 4.50bc | 3.00e | 1.00def |
| | 15 | 2.25bc | 4.12bc | 2.75ef | 0.25f |
| Poinsett | 0 | 0.25d | 1.75de | 2.75ef | 1.25de |
| | 5 | 2.75bc | 3.75bc | 4.50cd | 1.50d |
| | 10 | 4.00a | 3.50bc | 4.50cd | 1.75d |
| | 15 | 3.12ab | 6.50a | 6.00ab | 2.75d |
| Marketer | 0 | 0.00d | 0.75e | 2.75ef | 2.75c |
| | 5 | 0.00d | 1.75de | 3.50de | 3.50c |
| | 10 | 0.00d | 3.75bc | 5.13bc | 5.13b |
| | 15 | 0.00d | 5.50ab | 7.00a | 7.00a |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$) using Duncan Multiple Range test (DMRT)

During the late cropping season, Poinsett cultivated with 10 and15 t.ha$^{-1}$ organic fertilizer produced more fruits which were significantly higher compared with other interactions at 1st harvest while at 2nd harvest, Poinsett cultivated with 10 organic fertilizer produced more fruits which were significantly higher compared with other interactions. At 3rd harvest, Poinsett and Marketer cultivated with 10 t.ha$^{-1}$ had significantly more fruits while at 4th harvests, marketer cultivated with 10 t.ha$^{-1}$ produced more fruits which were similar compared with Marketmore (Table 9).

**Table 9: Effect of variety and organic fertilizer rate interactions on number of fruits per plant in 2015 late cropping season**

| | | 1st harvest | 2nd harvest | 3rd harvest | 4th harvest |
|---|---|---|---|---|---|
| **Variety x rate (**t.ha$^{-1}$**)** | | | | | |
| Marketmore | 0 | 0.00f | 1.00h | 1.15j | 3.50bc |
| | 5 | 1.00f | 2.75g | 4.50h | 4.50bc |
| | 10 | 2.00cd | 5.00d | 6.45g | 4.75abc |
| | 15 | 1.50de | 4.25e | 6.80f | 3.00c |
| Poinsett | 0 | 0.00f | 1.00h | 2.75i | 3.25c |
| | 5 | 3.50b | 5.50cd | 9.75d | 4.75abc |
| | 10 | 4.00ab | 8.35a | 11.50a | 5.62ab |
| | 15 | 4.50a | 7.43b | 11.45b | 3.87bc |
| Marketer | 0 | 0.00f | 1.25h | 1.14k | 3.500bc |
| | 5 | 1.50de | 3.50cd | 6.86e | 4.75abc |
| | 10 | 2.50c | 5.30cd | 9.75a | 6.75a |
| | 15 | 2.50c | 5.76c | 10.14c | 3.00c |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$) using Duncan Multiple Range test (DMRT).

There were significant differences in the fruit yield of cucumber as influenced by interaction of variety and organic fertilizer rate in the early season cropping (Table 10). Marketmore and Poinsett cultivated with 10 and 15 t.ha$^{-1}$ organic fertilizer produced yields significantly higher when compared with other interactions (Table 10). During the late cropping season, Poinsett variety cultivated with 10 t.ha$^{-1}$ organic fertilizer produced higher yield when compared with other interactions (Table 10).

**Table 10: Effect of variety and organic fertilizer rate interaction on total fruit yield (t.ha$^{-1}$)**

| | | Early season | Late seasons |
|---|---|---|---|
| **Variety x rate (**t.ha$^{-1}$**)** | | | |
| Marketmore | 0 | 27.72gh | 20.16j |
| | 5 | 40.32e | 49.56h |
| | 10 | 69.72a | 83.16f |
| | 15 | 73.92ab | 72.24g |
| Poinsett | 0 | 29.40g | 25.20i |
| | 5 | 73.00b | 99.96d |
| | 10 | 73.92ab | 111.72a |
| | 15 | 75.60a | 108.36b |
| Marketer | 0 | 27.72gh | 21.00j |
| | 5 | 37.80f | 72.24g |
| | 10 | 39.48ef | 94.92e |
| | 15 | 40.32e | 101.64c |

Means with the same letter(s) under the same column are not significantly different ($P < 0.05$)
using Duncan Multiple Range Test (DMRT)

## DISCUSSION

The soil used for the experiment was sandy loam in texture and low in fertility. This could adversely affect the performance and productivity of cucumber plant due to the low water and nutrient retention ability of such soil. Hence, soil amendment using organic fertilizer could improve such soil.

The results of this study showed that there were significant differences among varieties in vegetative growth in both experiments, namely: Vine length, number of branches, leaf area, number of leaves and yield characters such as days to 1$^{st}$ flowering and 50 % flowering, number of fruits per plant, fruit weight per plant and total yield. Marketmore variety produced significantly longer vines,

more leaves, larger leaves and more branches than other varieties, showing greater genetic potentials for vegetative growth. The excessive vegetative growth in Marketmore variety in the early cropping season and similar trend observed in the late cropping season resulted in lower yield while less vegetative growth observed in Poinsett variety resulted in higher yield. This is an indication of vegetative growth at the expense of fruit yields. This contradicts the general belief that larger leaf area increases the photosynthetic activity and result in higher yield. It also negates the findings of Reddy et al., (2018) that vine growth and tuber yield are directly related. The consistent significant yield response exhibited by Poinsett variety in both trials could be attributed to innate quality of the variety and this agrees with the findings of Eifediyi and Remison (2010) that the differences in vegetative and yield characters could also be attributed to genetic composition of the varieties used.

Significant increase observed in vegetative growth and reproductive development with application of 15 t.ha$^{-1}$ organic fertilizer could be as a result of organic fertilizer application that increased the water holding capacity and thereby making more nutrients available and was similar to the findings of Ewulo *et al.,* (2008) and Enujeke (2013) that higher rate of manure improved moisture availability and enhanced release of more nutrients for plant growth.

Earliness in days to 1$^{st}$ and 50 % flowering, as well as days to maturity that was observed in fertilized plants, than unfertilized plant is in line with the findings of Aiyelaagbe *et al (*2007) that application of 20 t.ha$^{-1}$ or 4.2 kg NPK +Mg enhanced early flowering better than other rates of fertilizer application and no fertilizer in cucumber production.

Higher yield was observed in fertilized plants, irrespective of the rates of fertilizer application than unfertilized plants in the early cropping season, while in the late cropping season, effects of 10 and 15 t.ha$^{-1}$ applications were similar, probably because the nutrient stocks of the 5 t.ha$^{-1}$ application had been utilized in the early season. This is consistent with Soretire *et al*., 2013 that 15 t.ha$^{-1}$ Gateway Organic Fertilizer application resulted in higher growth and grain yield of soyabean.

Higher yield that was observed with application of 10 and 15 t.ha$^{-1}$ Gateway Organic fertilizer than other rates in both seasons could be due to higher rates of manure improving the soil conditions for crop establishment and also release of adequate nutrient elements for yield enhancement. This is in harmony with the reports of Aliyu (2000), Mangila *et al.,* (2007), and Agbede *et al.,* (2008) that higher rates of manure increase crop yield.

The yield response of cucumber to organic fertilizer that was higher in the late season than the early season trial observed could be due to the slow nutrient release pattern of organic fertilizer which made the period of its nutrient release coincide with that of the plants' demand for fruit formation. Similar observation has been reported on Egusi melon (Makinde et al, 2021).

The relatively good performance of Poinsett variety fertilized with 10 t.ha$^{-1}$ organic fertilizer in both early and late cropping seasons compared to other treatment combinations, confirms the findings that varieties of crops can differ in response to fertilizer, as reported by Anderson *et al* (2007) and Nedunchezhiyan *et al* (2010) that potato varieties are differently - fertilizer responsive. It could also imply that cucumber uses nutri-

ents efficiently when sourced from organic fertilizers.

In this study, it was observed that Gateway Organic Fertilizer increased growth and yield of cucumber fruits. This could be as a result of organic fertilizer that established and maintained soil physical condition for plant growth. This corroborates the studies of Agbede *et al.,* (2008), and Ewulo *et al.,* (2008) that poultry manure is not only a cheap and an effective source of N for sustainable crop production, but improves soil physical properties by reducing temperature, bulk, density, and increasing total porosity, if higher rates are applied.

## CONCLUSION

This study has shown that Gateway Organic Fertilizer application had positive influence on the growth and fruit yield of cucumber.

Among the varieties, Poinsett variety is the best for optimum growth and yield.

Application of 10 $t.ha^{-1}$ Gateway Organic Fertilizer was optimum for fresh growth and fruit yield of cucumber.

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# CARCASS CHARACTERISTICS AND REPRODUCTIVE ORGAN DEVELOPMENT OF EGG-TYPE CHICKENS FED DIETS CONTAINING *Aspilia Africana*

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## ABSTRACT

Ameliorating high feed cost in poultry production using alternative feedstuff cannot be overemphasized. Hence, this experiment was conducted to determine the effects of *Aspilia africana* leaf meal on carcass characteristics and reproductive organs of 192 twelve weeks old pullets for three months in a battery cage system erected in a pen. Dried *A. africana* leaves were milled and used to replace part of soybean meal at 0%, 10%, 20%, and 30% to form four different diets treatments $A_0$, $A_1$, $A_2$ and $A_3$, respectively. Allotted to each treatment were 48 birds divided into four replicates of 12 birds each. Data obtained were subjected to analysis of variance (ANOVA) in a Completely Randomized Design. Higher ($p<0.05$) values of live weight (1543.75 g, 1487.50 g and 1475.00 g) were recorded for birds fed diets containing up to 30% *A. africana* ($A_1$, $A_2$, $A_3$, respectively). The dressing percentage was higher ($p<0.05$) in birds fed 30% replacement when compared with birds on control diet. The cut-up-parts showed a significant difference ($p<0.05$) in the values of head, shank and back. Higher number of matured yolks was recorded for all groups of birds fed diets containing *Aspilia africana.* It was concluded that –Aspilia Africana-leaf meal can meal can replace soyabean meal up to 30% without any adverse effect of reproductive organ and carcass evaluation of egg type chicken.

**Keywords:** *Aspilia africana,* cut-up parts, egg-type chickens, live weight, organ weights, reproductive organs.

## INTRODUCTION

Poultry production is usually geared towards provision of food for man and profit for the farmer. For poultry production to be profitable, feed resources must be accessible and relatively affordable. Most conventional feed ingredients get expensive and scarce when not in season. Soybean, groundnut and maize are suitable case studies. The exploitation of locally available and cheap feed resources helps to forestall the threat poses to the future of poultry production *(*Runjaic-Antic *et al.,* 2010*)*. A number of locally sourced feed ingredients have been considered in the past. These include leaf meals of some tropical legumes and browse plants capable of supplying proteins, minerals, antibiotics and so on (Oko and Agiang, 2011). Some plant materials are fortified with medicinal characteristics showing a wide

range of pharmacological effects such as anti-inflammatory agent, astringent, anti-diarrheal, digestion-stimulating, laxative, sedative, spasmolytic and choleretic properties (Hashemi *et al.,* 2008; Ranjaic-Antic *et al.,* 2010). Plant parts are also pigmented, containing oxy-carotenoids and xanthophylls (Zakynthinos and Varzakas, 2016).

Although, layers are basically raised for the production of eggs, the carcass of old layers is also useful for human consumption as meat which gives room for improving the carcass quality alongside improving its laying performance. The effect of different types of feed introduced to birds on body weights and other development indices cannot be overemphasized. It determines the quality and quantity of the breast muscle which is considered the most valuable products in the chicken industry (Simeneh, 2019). Therefore, quality feed is required to be administered to the birds for the establishment of a quality body frame to enhance the development of the bird's reproductive system and carcass characteristics and composition (Simeneh, 2019).

Considering the reduction in cost that can be made possible by the use of non-conventional poultry feeds, not undermining the nutritional needs of the birds, *Aspilia africana* could be reckoned with. It is a plant that contains a wide range of biological activity such as antiviral, fungicide and antibacterial due to the presence of thiarubrines, a derivative of 1,2-dithiocyclohexa-3,5-diene (Masato and Wu, 1994). The plant's crude protein is estimated at 20.65% according Adedeji *et al. (*2015), having the ability to enhance growth and improve the health of an animal at a reduced cost.

# MATERIALS AND METHODS

## *Experimental site*

The experiment was carried out at the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm lies within Latitude $7^0$ $10^0$ N longitude $3^0 2^{11}$ E and altitude 76 mm located within the derived savannah zone of south western Nigeria (Google earth, 2019). It has a humid climate with mean annual rainfall of 1037 mm and temperature of about $34^0$C.

## *Experimental birds and management*

The experiment was conducted on 192 pullets for 6 weeks. The pullets, sourced at point of cage (12 weeks old), were kept in battery cages and fed commercial feed for 2 weeks acclimatization period. Adequate fresh and clean water was supplied. The birds were administered anti-stress for three days, after which they were administered with anti-coccidiosis and dewormed for another three days. At $15^{th}$ and $16^{th}$ weeks of age, the birds were vaccinated against Newcastle disease and Infectious bursa disease, respectively. Four treatments of 48 birds each with a different diet were formed and each treatment was further divided into four replicates, each replicate having 12 birds.

## *Experimental diet*

*Aspilia africana* shoot was collected and sun dried until it was crispy. The dried *A. africana* shoot was milled to powder and used to replace soybean meal at 0%, 10%, 20%, and 30% to form four different dietary treatments $A_0$, $A_1$, $A_2$ and $A_3$, respectively. The feeds were compounded under close moni-

**Table 1:** Composition of Diet used at Pullet stage

| Feed Ingredients | $A_0$ | $A_1$ | $A_2$ | $A_3$ |
|---|---|---|---|---|
| Maize | 42 | 42 | 42 | 42 |
| Wheat bran | 23 | 23 | 23 | 23 |
| Soybean meal | 15.1 | 13.59 | 12.08 | 10.57 |
| *Aspilia africana* | 0 | 1.51 | 3.02 | 4.53 |
| Bone meal | 2 | 2 | 2 | 2 |
| Limestone | 2 | 2 | 2 | 2 |
| Lysine | 0.2 | 0.2 | 0.2 | 0.2 |
| Methionine | 0.2 | 0.2 | 0.2 | 0.2 |
| *Grower Premix | 0.25 | 0.25 | 0.25 | 0.25 |
| Palm kernel cake | 15 | 15 | 15 | 15 |
| Salt | 0.32 | 0.32 | 0.32 | 0.32 |
| **Determined analysis** | | | | |
| Metabolisable energy, KCal/kg | 2416.99 | 2416.94 | 2416.9 | 2416.85 |
| Available phosphorus | 0.43 | 0.43 | 0.44 | 0.44 |
| Crude protein | 15.93 | 15.53 | 15.12 | 14.69 |
| Calcium | 1.34 | 1.36 | 1.38 | 1.39 |
| Crude fibre | 5.89 | 5.88 | 5.87 | 5.86 |
| Methionine | 0.45 | 0.45 | 0.45 | 0.45 |
| Lysine | 0.9 | 0.88 | 0.85 | 0.82 |

*premix per kg: vitamin A, 8,000,000 iu; vitamin D3, 2,000,000 iu; vitamin E, 14,000iu; vitamin K, 1,600 mg; thiamine, 600 mg; riboflavin, 2,000 mg; niacin, 7,200 mg; pantothenic, 2,800 mg; vitamin B6, 800 mg; vitamin B12, 5meg; folic acid, 600mg; biotin, 2.8meg; choline chloride, 100,000 mg; cobalt, 200 mg; copper, 2,400mg; iodine, 440mg; iron, 8,000 mg; manganese, 32,000 mg; selenium, 80 mg; zinc, 20,000; antioxidant, 50,000 mg.

***Carcass and reproduction evaluation***

At 20 weeks of age, two birds of similar mean weight were picked per replicate for carcass evaluation. Feed was withdrawn from the birds 12 hours before slaughtering to ensure emptying of the digestive tract. The birds were slaughtered, defeathered and eviscerated. The weight was determined using sensitive scale and recorded. The cut-up parts (head, neck, breast, back, thighs, drumsticks, wings and shanks) and organs (gizzard, proventriculus, intestine, heart, liver, spleen and lung) were measured and calculated as a percentage of the live weight of the birds. Reproductive parts (oviduct length, oviduct weight, number of yellow yolks, number of white yolks, ovary weight, and abdominal fat) weight were also measured and recorded.

**Table 2:** Composition of Diet Used at Laying Stage

| **Feed Ingredients** | **$A_0$** | **$A_1$** | **$A_2$** | **$A_3$** |
|---|---|---|---|---|
| Maize | 50 | 50 | 50 | 50 |
| Wheat bran | 15 | 15 | 15 | 15 |
| Soybean meal | 17 | 15.3 | 13.6 | 11.9 |
| *Aspilia africana* | 0 | 1.7 | 3.4 | 5.1 |
| Bone meal | 2.5 | 2.5 | 2.5 | 2.5 |
| Oyster shell | 7.25 | 7.25 | 7.25 | 7.25 |
| Lysine | 0.2 | 0.2 | 0.2 | 0.2 |
| Methionine | 0.36 | 0.36 | 0.36 | 0.36 |
| *Layer Premix | 0.25 | 0.25 | 0.25 | 0.25 |
| Palm kernel cake | 7.19 | 7.19 | 7.19 | 7.19 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| **Determined analysis** | | | | |
| Metabolisable energy, kCal/kg | 2469.5 | 2470.4 | 2471.3 | 2472.2 |
| Available phosphorus | 0.46 | 0.46 | 0.47 | 0.47 |
| Crude protein | 14.87 | 14.4 | 13.92 | 13.42 |
| Calcium | 3.41 | 3.47 | 3.53 | 3.58 |
| Crude fibre | 4.49 | 4.46 | 4.42 | 4.38 |
| Methionine | 0.6 | 0.6 | 0.6 | 0.6 |
| Lysine | 0.87 | 0.84 | 0.81 | 0.78 |

*premix per kg: vitamin a, 4000000iu; vitamin d3, 800000iu; vitamin e, 4800mg; vitamin k3, 800mg; vitamin b1, 600mg; vitamin b2, 2000mg; vitamin b6, 600mg; vitamin b12, 4mg; niacin, 6400mg; folic acid, 240mg; biotin, 8mg; anti-oxidant, 40000mg; choline chloride, 60000mg; manganese, 32000mg; iron, 16000; zinc, 24000; copper, 3200mg; iodine, 400mg; cobalt, 100mg; selenium, 60mg.

***Proximate analysis of* experimental diets *and Aspilia africana***

The moisture content was lower in $A_1$ and $A_3$ relative to the control (Table 3). Dry matter content related inversely to moisture content. Fat contents were higher with $A_1$ and $A_3$ but lower in $A_2$ relative to $A_0$. Similar trend was observed for ash. The ash content in $A_1$ and $A_3$ were higher but lower in $A_2$, relative to $A_0$. The crude fibre, crude protein and carbohydrate contents were higher in $A_1$, $A_2$ and $A_3$ relative to the control diets. The energy content however was higher with $A_1$ and $A_3$ but lower in $A_2$ relative to $A_0$.

**Table 3:** Proximate analysis of *Aspilia africana*

| Parameters | $A_0$ | $A_1$ | $A_2$ | $A_3$ | *Aspilia* leaf |
|---|---|---|---|---|---|
| Moisture Content (%) | 15.89 | 12.55 | 16.13 | 12.86 | 18.27 |
| Dry Matter Content (%) | 84.11 | 87.45 | 83.87 | 87.14 | 81.73 |
| Fat Content (%) | 8.73 | 9.21 | 8.58 | 9.12 | 1.16 |
| Ash Content (%) | 4.61 | 4.96 | 4.24 | 4.89 | 2.67 |
| Crude Fibre Content (%) | 5.92 | 6.78 | 6.08 | 6.52 | 9.78 |
| Crude Protein Content (%) | 18.87 | 19.35 | 18.94 | 20.08 | 10.67 |
| Carbohydrate Content (%) | 45.98 | 47.15 | 46.03 | 46.53 | 57.25 |
| Energy Content (Kcal/100g) | 331.48 | 344.51 | 329.60 | 344.51 | 278.36 |

***Statistical analysis***

The data obtained were subjected to analysis of variance (ANOVA), in a Completely Randomized Design (CRD). Significant means at $p<0.05$ were separated using Duncan's Multiple Range Test as contained in SAS (2012) software package.

## RESULTS

There were significant differences in the values of live weight and dressed percentage of the birds at the end of the experiment (Table 4). Higher values of live weight (1543.75 g, 1487.50 g and 1475 g) were recorded for birds fed diets supplemented with 10, 20, and 30% A. *africana*, respectively. These values were significantly higher than the value from control ($A_0$) with 1387.5g birds. The highest dressing percentage of 70.76% was recorded in birds fed 30% which was not significantly different from other treatments except control. The cut-up parts in percentage of live weight showed significant differences for head, shank and back. The percentage heads from control and 30% replacement were higher when compared with that of 20% replacement birds, but similar to value recorded for birds fed with 10% supplement. Neck, thigh, drumstick, breast and wing percentages were not significantly affected by diets supplemented with 10, 20, and 30% A. *africana*. There were no significant differences in all the weights of internal organs measured (Table 5). The heart values ranged from 0.40 to 0.49% while that of liver ranged from 1.71 to 1.79%. The numerical differences noted in lung ranged from 0.54 to 0.60%. Least gizzard value of 3.39% was obtained in the control birds while the highest value of 3.57% was in birds fed with 20% supplement. The intestine value ranged from 5.32 - 6.48%, proventriculus ranged from 0.52 to 0.60% while spleen ranged from 0.14 to 0.21% (Table 5)

All reproductive organs determined were not significantly affected except in the number of matured yolks (Table 6). The highest number of matured yolks was from birds fed 20% replacement while the least was recorded in birds fed control diet. The range of oviduct weight was 45.38 - 54.50g while number of follicles ranged from 12.13 to 14.00. The numerical least abdominal fat value of 5.25g was obtained in the control birds while the birds on 30% replacement recorded the highest value of 14.00g (Table 6).

**Table 4: Effects of *Aspilia africana* shoot meal inclusion in the diet of egg-type chicken, on the cut-up parts**

| Parameters | $A_0$ | $A_1$ | $A_2$ | $A_3$ | SEM |
|---|---|---|---|---|---|
| Live weight (g) | 1387.50$^{b}$ | 1543.75$^{a}$ | 1487.50$^{a}$ | 1475.00$^{a}$ | 15.55 |
| Plucked weight (%) | 0.09 | 0.09 | 0.08 | 0.09 | 0.001 |
| Eviscerated weight (%) | 64.17 | 77.89 | 71.61 | 73.49 | 2.43 |
| Dressing percentage | 63.86$^{b}$ | 66.37$^{ab}$ | 67.13$^{ab}$ | 70.76$^{a}$ | 0.99 |
| **Cut up parts (% of live weight)** | | | | | |
| Head | 2.77$^{a}$ | 2.70$^{ab}$ | 2.50$^{b}$ | 2.82$^{a}$ | 0.04 |
| Shank | 3.34$^{a}$ | 3.17$^{a}$ | 3.13$^{ab}$ | 2.92$^{b}$ | 0.05 |
| Back | 15.85$^{ab}$ | 17.94$^{a}$ | 14.52$^{b}$ | 15.72$^{ab}$ | 0.55 |
| Neck | 5.66 | 4.92 | 4.44 | 9.74 | 1.28 |
| Thigh | 9.51 | 9.90 | 9.72 | 9.79 | 0.14 |
| Drum stick | 9.39 | 8.81 | 8.77 | 8.52 | 0.17 |
| Breast | 13.54 | 13.94 | 13.95 | 14.32 | 0.21 |
| Wing | 7.33 | 7.70 | 7.04 | 7.15 | 0.15 |

a,b means with different superscripts on the same row differ significantly ($p < 0.05$)

**Table 5*:* Effect of *Aspilia africana* shoot meal inclusion in the diet of egg-type chicken, on organ weights**

| Organs (% live weight) | $A_0$ | $A_1$ | $A_2$ | $A_3$ | SEM |
|---|---|---|---|---|---|
| Heart | 0.40 | 0.49 | 0.41 | 0.43 | 0.02 |
| Liver | 1.77 | 1.79 | 1.76 | 1.71 | 0.06 |
| Lungs | 0.58 | 0.56 | 0.54 | 0.60 | 0.02 |
| Gizzard | 3.39 | 3.40 | 3.57 | 3.52 | 0.08 |
| Intestine | 5.32 | 5.96 | 6.24 | 6.48 | 0.25 |
| Proventriculus | 0.60 | 0.59 | 0.54 | 0.52 | 0.02 |
| Spleen | 0.21 | 0.20 | 0.15 | 0.14 | 0.02 |

**Table 6*.* *Aspilia africana* shoot meal inclusion in the diet of egg-type chicken, effects on the reproductive organs**

| Parameters | $A_0$ | $A_1$ | $A_2$ | $A_3$ | SEM |
|---|---|---|---|---|---|
| Oviduct length (cm) | 56.93 | 59.78 | 57.75 | 61.59 | 1.43 |
| Oviduct weight (g) | 47.63 | 52.13 | 54.50 | 45.38 | 2.33 |
| No of matured yolk | 4.00$^{b}$ | 5.00$^{ab}$ | 5.50$^{a}$ | 4.38$^{ab}$ | 0.24 |
| No of follicle | 12.13 | 12.25 | 14.00 | 12.25 | 0.88 |
| Ovary weight (g) | 25.63 | 30.38 | 32.00 | 24.75 | 1.48 |
| Abdominal fat (g) | 5.25 | 8.13 | 10.00 | 14.00 | 1.87 |

a,b means with different superscripts on the same row differ significantly ($p < 0.05$)

## DISCUSSION

There were significant effects of *Aspilia africana* shoot meal on the average live weight of the birds compared to birds in the control group. This is similar to the report of Agiang *et al.* (2011) who fed quails with aqueous extracts of *Aspilia africana* leaf meal supplements at different concentrations. The dressing percentage recorded in this study significantly increased from birds on control diet to birds on 30% inclusion level. In his research on *A. africana* as feed supplements to quail, Agiang *et al.* (2011) found that the dressed weight of quails significantly improved at increased levels of *A. africana* leaf meal supplementation. This is at variance with earlier reports that plant products had no significant effects on the body weight of birds (D'Mellow and Devandra, 1995; Alcicek *et al.*, 2004). Though the spleen weight observed in this study decreased from control birds to birds fed 30% replacement, there was no significant differences in the values among the treatments. This is in agreement with the work of El-Katcha *et al.* (2016), who reported a non-significant increase in spleen weight of broilers fed with garlic extract supplemented diets. One of the indicators of hens health and vigour is the proportional shape of the head, condition of comb and wattle and brightness of the eyes. Intensity of laying is determined by lack of fat under the shank scales and pigment loss over the entire shank. As more soybean, which is richer in crude protein is been replaced with Aspilia, which is low in crude protein, the crude protein level of feed decreased. This may be responsible for instances where low values were recorded for $A_3$ birds like in shank and some reproductive organ weight which numerically low. However, the crude protein level are still within the range required for layers. A significant decrease was observed in the value of shank weight with the increase in inclusion level of *A. africana*. Aderemi *et al.* (2017) also reported a significant decrease in shank weight of broiler chickens when fermented locust bean meal (*Parkia biglobosa*) was used to replace soybean meal in the diet.

The percentage of live weight for visceral organs (heart, liver, lungs, gizzard, intestine, proventriculus and spleen) of the laying birds were statistically similar. This means that *A. africana* has no anti-nutritional factors which could be harmful to the laying birds. Ismail *et al.* (2008), reported that the presence of trypsin inhibitor in legume seeds used in animal feeds and human foods causes growth depression, and relative smaller size of organ weights to live weight in broilers fed mucuna seed meal. Aderemi *et al.* (2017) also observed smaller size for visceral organs at 100% replacement of soybean meal with fermented locust bean meal in broiler chickens. Moreover, Ayssiwede *et al.* (2011) who fed Senegal birds with graded levels of Leuceana leaves meal also observed no adverse effect on dressing carcass, liver weight, heart, lungs and spleen weight. He reported that they were all increased in birds fed Leuceana leaves based diets with no significant difference among treatments compared to the control group. Although, the percentage of live weights for visceral organs were not significant, the values of heart, liver and gizzard were similar to the reported ranges of 0.3 - 0.4, 1.8 - 2.0 and 2.8 – 3.9 respectively by Osonu *et al.* (2007).

This study observed a numerical increment in oviduct weight from control to $A_2$ but a decline in $A_3$. Yang *et al.* (2016) also reported an increase in oviduct weight in brown Leghorn laying hens fed diets supplemented with 0 and 8.5 mg/kg of L-arginine but the

oviduct weight reduced as the inclusion of L-arginine increased to 17 mg/kg. This may be connected to the insignificant increment observed in groups of birds fed *Aspilia africana* over the control group. Obuzor and Ntui (2011) identifies 3 essential amino acids which include histidine, phenylalanine and arginine in the essential oil of *Aspilia africana* leaves.

## CONCLUSION

This study revealed that inclusion of *Aspilia africana* in the diet of egg-type chicken up to 30% significantly affected the number of matured yolks. This study also discovered that *A. africana* leaf meal can substitute soybean meal in pullets' diets up 30% without any adverse effect on the cut-up parts, visceral organs and reproductive organs.

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# ASSESSMENT OF MAIZE (*Zea mays* L.) VARIETIES FOR TOLERANCE TO CONTRASTING SOIL-NITROGEN ENVIRONMENTS IN OGBOMOSO, NIGERIA

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## ABSTRACT

Millions of resource-limited farmers cultivate maize under low-soil nitrogen (N), which is a major constraint to maize production in Nigeria. Therefore, the objectives of this study were to: (i) assess the existence of genetic variation among some maize varieties for grain yield and other agronomic traits under varying N conditions, (ii) identify maize varieties with favourable alleles for tolerance to low-soil N and superior performance for grain yield across N environments. Eight maize varieties were evaluated under four (0, 30, 90 and 150 kg N $ha^{-1}$) N environments at the Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso, in 2021. The experiment was laid down in a randomized complete block design with six replicates. Data obtained were subjected to analysis of variance for each N level. Rank summation index was used to select superior variety. Significant ($P < 0.01$) mean squares were observed for grain yield and other agronomic traits of the maize varieties, across the (N) environments. Mean grain yields under low and optimal N environments were 2.8 t $ha^{-1}$ and 3.8 t $ha^{-1}$, respectively. Outstanding varieties (Pioneer KMK (30Y87); Kapam 10 and Sammaz 52) were identified by rank summation index and low-N tolerant base index, indicating that the varieties possess favourable alleles for tolerance to soil-nitrogen stress.

**Keywords**: Agronomic traits, Grain yield, Low-N tolerance, N-environments, Rank summation index, Superior variety.

## INTRODUCTION

Maize (*Zea mays* L.) is an important annual cereal crop grown for grain and forage in sub-Saharan Africa (SSA). It serves as staple food for more than 300 million people in the developing countries (Raheem *et al*., 2021) and a major source of income to farmers in the sub-region (Tandzi and Mutengwa, 2019). Maize is grown throughout the world, although with huge yield discrepancies (Tigchelaar *et al*., 2018). In West Africa, production of maize has been soaring over the years with the average total maize production of 10.2, 13.7 and 17.6 million tonnes in 2001-2005, 2006-2010 and 2011-2015, respectively (FAOSTAT, 2016). Considering the climatic and edaphic requirements of maize, there is a greater potential for its production and productivity in the savanna belt of SSA due to the low night temperatures, low pests and diseases occurrence and high influx of solar radiation (Anbessa *et al*., 2010). However, maize grain yield on farmers' fields in SSA has been con-

sistently low, averaging 1.7 t $ha^{-1}$ compared to 10.7 t $ha^{-1}$ obtainable in other parts of the world (FAOSTAT, 2016). The low yields have been attributed mainly to low soil fertility and acidity constraints. Several studies (Kamara *et al*., 2005; Badu-Apraku *et al*., 2016; Talabi *et al.,* 2017) have pointed out low-soil nitrogen (N) as an important abiotic stress reducing maize production in SSA.

Nitrogen (N) is essential for maize production and it is vital in the utilization of phosphorus and potassium, which are principal plant nutrients (Adediran and Banjoko, 1995). However, N is easily lost from the soil through leaching beneath the plant root zone during rainfall periods and volatilization (Ige *et al*., 2021). In SSA, maize production occurs mostly on soils with inherent low N due to poor weed control, total removal of crop residues after harvest and continuous cropping with little or no use of N fertilizer (Oikeh and Horst, 2001). In spite of the awareness on the importance of application of N fertilizer to maize plant, many resource-limited farmers' still apply nitrogen fertilizer at sub-optimum rates. This may be attributed to high cost of fertilizers, which makes it uneconomical, lack of technical knowledge about its application and non-availability/scarcity of fertilizers when needed (Mi *et al*., 2012). Several approaches at mitigating the low-soil N problem have been uneconomical. Low-N thus remains a great challenge to maize production on farmers' fields (Weber *et al*., 2012). Therefore, the identification and promotion of superior maize hybrids with low-N tolerance is crucial for an increased maize production and productivity in SSA.

In Nigeria, maize varieties that are tolerant to N stress are constantly being released for different agro-ecological zones. However, the varieties being cultivated by farmers' in the derived savanna agro-ecology are mostly those released for either the rain forest zones or the northern savanna zones. The derived savanna is a zone extending southwards from the Southern Guinea savanna zone into the rainforest zone of Nigeria with an estimated 10% of the country's land area (Adebayo *et al*., 2017; Kolawole *et al*., 2021). Thus, the zone combines the characteristics of both the rain forest and savanna zones. Hence, maize genotypes that are tolerant to N stress must be selected for the zone. It is therefore important that efforts be intensified towards the identification of potential maize genotypes that are adapted to low-N in the derived savanna agro-ecology in a bid to abate the impact of high nitrogenous fertilizer costs and the endemic low nitrogen nature of the savanna soil on maize production. This study aimed at assessing the existence of genetic variation among some maize varieties for grain yield and other agronomic traits under varying nitrogen conditions, (ii) identify maize varieties with favourable alleles for tolerance to low-soil N and superior performance for grain yield across N environments in the derived savanna agro-ecology of Nigeria.

## MATERIALS AND METHODS

### *Planting material, experimental design and cultural practices*

Seeds of seven maize varieties sourced from the major maize producing agro-ecologies in Nigeria and one locally cultivated maize variety in Ogbomoso (Table 1), were evaluated at the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso ($8^{o}$ 10'N, $4^{o}$ 10'E and altitude 341 m above sea level). The site is characterized by annual rainfall ranging between 1,000 and 1,200 mm and daily temperature ranges be-

tween 28 and 30°C. Soils of the experimental site are generally low in N and were classified as Alfisols (Adebayo *et al.,* 2017).

The experimental site used for this study had been under continuous maize cultivation over the years with little or no N fertilizer application. After each harvest, the residuals were completely removed from the field in preparation for the next planting season thereby depleting the soil of N incessantly. Before the establishment of this trial, soil samples were taken at the experimental site and the nutrient composition of the soil was determined at the Soil laboratory of the Department of Agronomy, University of Ibadan, Ibadan, Nigeria. The land was mechanically prepared using a tractor mounted plough and the field was subsequently partitioned into four N environments (0, 30, 90 and 150 kg N $ha^{-1}$). Each environment was separated by a 3 m alley and a gutter was used to break the lateral movement of nitrogen in the soil. The trial was a split-plot, with the four N environments as main plot factor while the eight maize varieties were considered as sub-plot factor with six replications. An experimental unit consisted of a single-row plot, 5m long spaced at 0.75m apart with 0.50m spacing between hills within a row. Three seeds were sown per hole to ascertain that at least two seeds germinate and where the three seeds were viable they were thinned to two plant stands per hill two weeks after sowing to obtain a plant density of 53,333 plants per hectare. Basal fertilizer application of P in the form of single super phosphate and K in the form of Muriate of potash were applied at the rate of 60 kg $ha^{-1}$ each at the 0 and 30 kg N $ha^{-1}$. No N was applied under 0 kg N environments. For the other environments, the nitrogen was applied in two split doses for the efficient use of nitrogen; the first application was done at two weeks after sowing and the second dose was applied 2 weeks later. A mixture of Gramoxone and Primextra were applied as pre- and post-emergence herbicides at the rate of 5.0 l $ha^{-1}$ at sowing and manual weeding was subsequently done to keep the plot weed-free.

***Data collection and analysis***

Data were recorded on the following traits on plot basis: number of days to 50% anthesis and silking was estimated as the numbers of days from planting to the day that 50% of plants had tassels shedding pollen and silk, respectively. The anthesis-silking interval was calculated as the difference between the number of days to 50% anthesis and silking. Plant and ear height were measured from the base of the plant to the first tassel branch and the node bearing the uppermost ear, respectively.

**Table: 1 List and characteristics of maize varieties evaluated in this study**

| Variety | Type of genotype | Ecology | Breeding emphasis | Year of release |
|---|---|---|---|---|
| Sammaz 52 | Open pollinated variety | Northern guinea and Sudan Savanna | Intermediate maturity, PVA content (9.8 g/g) | 2007 |
| Sammaz 27 | Open pollinated variety | Lowland tropics | Drought and *Striga* resistant | 2009 |
| SC 719 | Open pollinated variety | Southern and Northern Guinea Savanna | Drought tolerant, high yield potential and good husk cover | 2014 |
| Oba 98 | Hybrid | Forest and Savanna | Quality protein maize | 2001 |
| Oba Super 6 (Check) | Single-cross hybrid | Forest and Savanna | Nitrogen use efficiency | 2018 |
| Kapam 6 | Open pollinated variety | Savanna | Drought tolerant and Pro-vitamin *A* | 2018 |
| Kapam 10 | Open pollinated variety | Savanna | Drought tolerant and Pro-vitamin *A* | 2019 |
| Pioneer KMK (30Y87) | Hybrid | Forest, transition, Southern and Guinea Savannah | Stay green characteristics | 2014 |

Plant aspect scores were obtained using a scale of 1-9, where 1 denoted excellent overall phenotypic appearance of plants and 9 extremely poor overall appearance of plant. Ear aspect was also rated on a 1-9 scale, where 1 indicated well-filled ears with no insect and disease damages and 9 represented plots with ears having only one or no kernel. Root and stalk lodging was estimated as the proportion of plants that fell from the root or with stalk bending more than 45$^{0}$ from the vertical position and broken stalk below the upper ear, respectively. Husk cover was rated on a scale of 1 – 5; where, 1 = very tight husk extending beyond the tip and 5 = exposed ear tip. Stay-green scores were recorded on low-N plots (0 and 30 kg N $ha^{-1}$ environments) on a scale of 1 to 9; where 1 = almost all leaves below the ear were green and 9 = virtually all leaves below the ear were dead (Kamara *et al.*, 2005). The number of ears per plant was calculated as the ratio of harvested cobs per plot to the number plants at harvest. Grain yield was measured in kilograms per hectare (kg $ha^{-1}$) and adjusted to 15 % moisture content, from grain weight and percent moisture as described by Kolawole *et al.* (2018) using the following equation:

$$GY\ (\text{kg ha}^{-1}) = GWT(\text{kg plot}^{-1}) \times \frac{100 - MC}{100 - 15} \times \frac{10{,}000\ m^2}{plot\ size\ m^2}$$

Where: GWT = grain weight of harvested area, MC = moisture content of grains at harvest, moisture content for storage = 15 %, 1 hectare = 10,000m² and plot size = 3.75 m².

Combined ANOVA was conducted across the nitrogen environments using the Procedures for General Linear Model (PROC GLM) in SAS (SAS Institute, 2011). The means for each trait was computed and the specific differences between pairs of means were estimated with the Duncan's Multiple Range test (DMRT) at 0.05 probability level (Duncan, 1955). Performance of the maize varieties across low nitrogen environments was determined using the low-N base index as described by Badu-Apraku *et al.* (2011a) as follows:

Low nitrogen base index = 2YIELD + EPP – ASI – PASP – EASP – SG

Where: YIELD = Grain yield (kg ha$^{-1}$), EPP = number of ears per plant, ASI = anthesis-silking interval, PASP = plant aspect, EASP = ear aspect, SG = stay-green characteristic.

Least square mean for each trait was standardized to reduce the effects of the different scales used to measure them. The standardized values were used in the base index; a positive base index value for any maize variety indicated that the variety was tolerant to low-soil N while a negative value revealed the susceptibility of the variety to the stress (Badu-Apraku *et al.*, 2011b). To select superior variety, the rank summation index (RSI) was constructed by ranking five traits for each variety in order of preference (Mulumba and Mock, 1978; Kolawole and Olayinka, 2022). For grain yield, the higher the values, the better, while for other traits, the lower the values, the better. The ranks for each entry for the five traits were then summed up to obtain an index as:

$$RSI_1 = \sum_j^m = n^{ij}$$

Where $n^{ij}$ is the rank of variety *i* in relation to trait *j*

Pearson correlation coefficients (r) between every pair of measured trait were calculated to determine the degree of association among traits.

## RESULTS AND DISCUSSION

The combined analysis of variance revealed that the mean squares for nitrogen (N) environment and the variety were significantly ($P < 0.01$) different for all traits measured except r the number of days to silking and plant aspect for the N environment as well as stalk lodging and husk cover for the variety (Table 2). However, the variety × environment interaction was not significant for most of the traits except for root lodging.

The observed significant mean squares for most measured traits of the varieties indicated the existence of variability. Hence, there is a potential for selection of a variety suitable for production in the test environment (Obeng-Bio *et al.*, 2020). The highly significant N environment implied that selection for a suitable variety for specific N environment is feasible. The non-significant interactions between the variety × environments for all traits measured except root lodging indicated that the environmental variation did not affect the expression of most traits and their expression would be consistent in varying N environment. On the other hand, the significant mean square of variety × environments detected for only root lodging indicated that environmental variations controlled its expression. Consequently, more evaluations across multiple N environments may be needed to validate the standability of the maize varieties. Earlier studies have based genotype selection on the absence of significant interaction between genotype and environment (Derera *et al.*, 2008; Adebayo, 2014; Badu-Apraku *et al.*, 2016).

Across the low-N environments (0 and 30kg N $ha^{-1}$), the mean grain yield was 2.8 t $ha^{-1}$ and ranged between 2.1 t $ha^{-1}$ for Kapam 6 to 4.0 t $ha^{-1}$ for Pioneer KMK (30Y87), which also had the significantly highest grain yield (Table 3). Across the optimal N-environments (90 and 150 kg N $ha^{-1}$) the mean grain yield was 3.8 t $ha^{-1}$. The lowest yield was 2.6 t/$ha^{-1}$ for Sc 719 and Pioneer KMK (30Y87) had the significantly highest (5.7 t $ha^{-1}$) grain yield (Table 4). Comparing the grain yield under low-N input and optimal N conditions revealed yield reduction ranging from 7% for Sc 719 to 39% for Kapam 6, with a mean of 21%. The decline in grain yield observed may be as a result of reduction in the photosynthesis capacity of the plant (Settinni and Maranville, 1998) and kernel abortion due to nitrogen stress (Amegbor *et al.*, 2017). Thus, the increased grain yield under optimal N conditions was a response to increase in N fertilizer which is in consonance with previous report of Adu *et al.* (2018).

Among the top performing varieties under low-N conditions, Pioneer KMK (30Y87), Kapam 10 and Sammaz 52 had higher grain yield than the commercial check (Oba Super 6). However, only two maize varieties Pioneer KMK (30Y87) and Kapam 10 were superior than the commercial check for grain yield under optimal N conditions. The consistency in performance of these varieties across N environments implies the possession of some desirable genes for N-stress tolerance and further emphasizes their potential in all growing conditions.

**Table 2: Mean squares of grain yield and other agronomic traits of maize varieties evaluated across four nitrogen environments**

| Source | df | Grain yield (kg ha$^{-1}$) | Numbers of ears per plant | Days to anthesis | Days to silking | Anthesis -silking interval (days) | Plant height (cm) | Ear height (cm) | Plant as-pect (1-9) | Ear as-pect (1-9) | Root lodg-ing (%) | Stalk lodging (%) | Husk cover (1-5) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate (R) | 5 | 1369232.6 | 0.0 | 22.7$^{**}$ | 62.8 | 24.1 | 353.1 | 176.3 | 1.4 | 1.1 | 19.7$^{*}$ | 56.5 | 0.9$^{*}$ |
| Environment (E) | 3 | 42076503.7$^{***}$ | 0.2$^{***}$ | 119.5$^{***}$ | 54.4 | 186.7$^{***}$ | 3614.2$^{***}$ | 791.6$^{***}$ | 0.2 | 5.1$^{**}$ | 67.4$^{***}$ | 211.9$^{***}$ | 0.7 |
| R×E | 15 | 1473434.1 | 0.0 | 9.3$^{*}$ | 60.5$^{*}$ | 40.1 | 413.7 | 149.7 | 1.1 | 1.2 | 13.9$^{*}$ | 52.3$^{*}$ | 0.8$^{**}$ |
| Variety (V) | 7 | 14129227.5$^{***}$ | 0.1$^{***}$ | 194.9$^{***}$ | 365.9$^{***}$ | 67.5$^{**}$ | 1872.4$^{***}$ | 529.3$^{***}$ | 10.8$^{***}$ | 10.6$^{***}$ | 27.1$^{**}$ | 27.9 | 0.5 |
| V×E | 21 | 1344510.8 | 0.0 | 6.6 | 27.3 | 24.2 | 142.1 | 90.9 | 0.9 | 1.2 | 12.31$^{*}$ | 23.6 | 0.4 |
| Error | 140 | 1083105.2 | 0.0 | 5.2 | 28.6 | 23.6 | 275.1 | 106.9 | 0.7 | 1.1 | 7.3 | 26.5 | 0.3 |
| CV (%) | | 31.7 | 16.5 | 3.8 | 8.3 | 120.7 | 10.1 | 14.6 | 15.4 | 20.3 | 98.5 | 152.5 | 20.6 |

$^{*}$, $^{**}$, $^{***}$ Significant at 0.05, 0.01 and 0.001 probability levels, respectively

**Table 3: Means of grain yield and other selected agronomic traits of maize varieties evaluated in low nitrogen environments**

| Variety | Grain yield (kg ha$^{-1}$) | Numbers of ears per plant | Days to anthesis | Days to silking | Anthesis-silking interval | Plant height (cm) | Ear height (cm) | Plant aspect (1-9) | Ear aspect (1-9) | Root lodging (%) | Stalk lodging (%) | Husk cover (1-5) | Stay green charac-teristics (1 - 9) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pioneer KMK (30Y87) | 4035.56a | 0.86ab | 58.75c | 62.92c | 4.17bc | 150.75b | 69.33b | 4.50d | 4.25d | 4.69b | 2.37ab | 2.50ab | 3.83b |
| Kapam 10 | 3431.11ab | 0.91a | 58.92c | 62.08c | 3.17bc | 158.08b | 71.33ab | 5.17bcd | 4.58cd | 21.00a | 5.30ab | 2.33b | 4.58ab |
| Sammaz 52 | 3066.67bc | 0.85ab | 57.92c | 61.92c | 4.00cd | 165.17b | 72.00ab | 5.08cd | 4.83bcd | 16.91a | 6.66a | 2.67ab | 4.75a |
| Sammaz 27 | 2862.22bcd | 0.81abc | 56.83c | 63.33c | 6.50abc | 165.67b | 72.50ab | 5.83ab | 5.58abc | 16.09a | 4.64ab | 2.42ab | 5.25a |
| Oba 98 | 2640.00bcd | 0.78abc | 57.33c | 62.33c | 5.00bcd | 161.00b | 66.92b | 5.58abc | 5.58abc | 20.85a | 2.11ab | 2.92a | 4.50ab |
| Sc 719 | 2453.33bcd | 0.64d | 64.67a | 72.42a | 7.75a | 180.50a | 80.67a | 5.92a | 6.33a | 10.61ab | 2.24ab | 2.67ab | 3.83b |
| Kapam 6 | 2124.44cd | 0.76bc | 58.00c | 64.58c | 6.58abc | 157.08b | 65.83b | 5.58abc | 5.75ab | 15.75a | 3.49ab | 2.67ab | 5.25a |
| Oba Super 6 (Check) | 1893.33d | 0.72cd | 61.25b | 68.25b | 7.00ab | 152.75b | 68.08b | 6.17a | 6.25a | 15.85a | 0.00b | 2.92a | 4.58ab |
| Mean | 2813.33 | 0.79 | 59.20 | 64.70 | 5.50 | 161.40 | 70.80 | 5.50 | 5.40 | 15.20 | 3.40 | 2.60 | 4.60 |

Means with the same letter within the column are not significantly different at P = 0.05 by DMRT

**Table 4: Means of grain yield and other selected agronomic traits of maize varieties evaluated in optimal nitrogen environment**

| Variety | Grain yield (kg/ha$^{-1}$) | Numbers of ears per plant | Days to anthesis | Days to silking | Anthesis-silking interval | Plant height (cm) | Ear height (cm) | Plant aspect (1-9) | Ear aspect (1-9) | Root lodging (%) | Stalk lodging (%) | Husk cover (1-5) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pioneer KMK (30Y87) | 5706.67a | 0.98a | 60.00c | 57.17d | -2.83b | 154.08c | 66.83b | 3.92c | 3.67c | 7.98b | 5.09a | 2.83a |
| Kapam 10 | 4320.00b | 0.92a | 61.33c | 64.33bc | 3.00a | 160.42bc | 64.92b | 5.25b | 4.83b | 8.51b | 5.76a | 2.58a |
| Sammaz 52 | 3617.78bc | 0.88a | 60.83c | 63.58bc | 2.75a | 170.42b | 72.00b | 5.33b | 5.00b | 19.09ab | 10.83a | 2.75a |
| Oba 98 | 3591.11bc | 0.88a | 61.00c | 64.17bc | 3.17a | 163.83bc | 66.83b | 5.50b | 5.00b | 22.64a | 9.20a | 2.75a |
| Kapam 6 | 3511.11bcd | 0.92a | 60.50c | 63.58bc | 3.08a | 166.92bc | 71.42b | 5.25b | 5.00b | 23.48a | 11.98a | 2.75a |
| Sammaz 27 | 3253.33cd | 0.86a | 57.75d | 60.50cd | 2.75a | 162.83bc | 70.00b | 5.75b | 5.50ab | 15.45ab | 12.95a | 2.67a |
| Sc 719 | 2648.89d | 0.71b | 68.25a | 71.50a | 3.25a | 183.58a | 82.50a | 7.00a | 6.00a | 10.44ab | 7.30a | 2.58a |
| Oba Super 6 (Check) | 3386.67cd | 0.91a | 64.25b | 69.25ab | 5.00a | 161.75bc | 70.83b | 5.92b | 4.92c | 15.09ab | 9.56a | 2.92a |
| Mean | 3754.40 | 0.88 | 61.70 | 64.30 | 2.50 | 165.50 | 70.70 | 5.50 | 5.00 | 15.30 | 9.10 | 2.70 |

Means with the same letter within the column are not significantly different at P = 0.05 by DMRT

**Table 5: Traits means of the maize varieties evaluated across four nitrogen environments**

| Variety | Grain yield (kg ha$^{-1}$) | Numbers of ears per plant | Days to anthesis | Days to silking | Anthesis-silking interval (days) | Plant height (cm) | Ear height (cm) | Plant aspect (1-9) | Ear aspect (1-9) | Root lodging (%) | Stalk lodging (%) | Husk cover (1-5) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pioneer KMK (30Y87) | 4871.1a | 0.91a | 59.4c | 60.0d | 0.7b | 152.4c | 68.1b | 4.2e | 4.0d | 5.4c | 2.1a | 2.7abc |
| Kapam 10 | 3875.6b | 0.91a | 60.1c | 63.2cd | 3.1ab | 159.3bc | 68.1b | 5.2d | 4.7c | 12.1abc | 2.5a | 2.5c |
| Sammaz 52 | 3342.2bc | 0.86ab | 59.4c | 62.8cd | 3.4ab | 167.8b | 72.0b | 5.2d | 4.9bc | 15.8ab | 4.5a | 2.7abc |
| Oba 98 | 3115.6cd | 0.83ab | 59.2c | 63.3cd | 4.1a | 162.4bc | 66.9b | 5.5bcd | 5.3bc | 19.8a | 3.1a | 2.8ab |
| Sammaz 27 | 3057.8cd | 0.84ab | 57.3d | 61.9cd | 4.6a | 164.3b | 71.3b | 5.8bc | 5.5ab | 13.4abc | 4.9a | 2.5bc |
| Kapam 6 | 2817.8cd | 0.84ab | 59.3c | 64.1c | 4.8a | 162.0bc | 68.6b | 5.4cd | 5.4b | 17.9a | 4.5a | 2.7abc |
| Sc 719 | 2551.1d | 0.68c | 66.5a | 72.0a | 5.5a | 182.0a | 81.6a | 6.5a | 6.2a | 8.5bc | 2.8a | 2.6abc |
| Oba Super 6 (Check) | 2640.0d | 0.82b | 62.8b | 68.8b | 6.0a | 157.3bc | 69.5b | 6.0ab | 5.6ab | 12.9abc | 2.6a | 2.9a |
| Mean | 3283.9 | 0.8 | 60.5 | 64.5 | 4.0 | 163.4 | 70.8 | 5.5 | 5.2 | 13.3 | 3.4 | 2.7 |

Means with the same letter within the column are not significantly different at P = 0.05 by DMRT

Table 6: Grain yield and other agronomic traits of maize varieties evaluated across low-N input and variety mean performance ranking across N-environments.

| | Low-N index | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variety** | **Grain yield (kg ha$^{-1}$)** | **Ears per plant** | **Anthesis-silking interval (day)** | **Plant aspect (1-9)** | **Stay-green characteristic (1-9)** | **Ear aspect (1-9)** | **Low-N index** |
| Pioneer KMK (30Y87) | 4035.6 | 0.9 | 4 | 4.5 | 3.8 | 4.3 | 10.4 |
| Kapam 10 | 3431.1 | 0.9 | 3 | 5.2 | 4.6 | 4.6 | 6.6 |
| Sammaz 52 | 3066.7 | 0.9 | 4 | 5.1 | 4.8 | 4.8 | 3.8 |
| Oba 98 | 2640.0 | 0.8 | 5 | 5.6 | 4.5 | 5.6 | -0.7 |
| Sammaz 27 | 2862.2 | 0.8 | 7 | 5.8 | 5.3 | 5.6 | -2.6 |
| Sc 719 | 2453.3 | 0.6 | 8 | 5.9 | 3.8 | 6.3 | -5.2 |
| Kapam 6 | 2124.4 | 0.8 | 7 | 5.6 | 5.3 | 5.8 | -5.2 |
| Oba Super 6 (Check) | 1893.3 | 0.7 | 7 | 6.2 | 4.6 | 6.3 | -7.2 |
| Mean | 2813.3 | 0.8 | 6 | 5.5 | 4.6 | 5.4 | |

| | Rank Summation Index | | | | | |
|---|---|---|---|---|---|---|
| Variety | Grain yield (kg ha$^{-1}$) | Anthesis-silking interval (days) | Plant aspect (1-9) | Ear aspect (1-9) | Root lodging (%) | Rank sum |
| Pioneer KMK (30Y87) | 4871.1 | 1 | 4.2 | 4 | 5.4 | 5 |
| Kapam 10 | 3875.6 | 3 | 5.2 | 4.7 | 12.1 | 11 |
| Sammaz 52 | 3342.2 | 3 | 5.2 | 4.9 | 15.8 | 18 |
| Oba 98 | 3115.6 | 4 | 5.5 | 5.3 | 19.8 | 25 |
| Sammaz 27 | 3057.8 | 5 | 5.8 | 5.5 | 13.4 | 27 |
| Kapam 6 | 2817.8 | 5 | 5.4 | 5.4 | 17.9 | 28 |
| SC 719 | 2551.1 | 6 | 6.5 | 6.2 | 8.5 | 32 |
| Mean | 3375.9 | 4 | 5.4 | 5.1 | 13.3 | |
| Standard Error | 294.7 | 1 | 0.3 | 0.3 | 1.9 | |
| Minimum | 2551.1 | 1 | 4.2 | 4.0 | 5.4 | |
| Maximum | 4871.1 | 6 | 6.5 | 6.2 | 19.8 | |
| Oba Super 6 (Check) | 2640.0 | 6 | 6.0 | 5.6 | 12.9 | 34 |

**Table 7: Correlation coefficient (r) between grain yield and other agronomic traits of the maize varieties evaluated**

| Parameter | Grain yield (kg ha$^{-1}$) |
|---|---|
| Numbers of ears per plant | 0.53*** |
| Days to anthesis | -0.18* |
| Days to silking | -0.39*** |
| Anthesis-silking interval (days) | -0.35*** |
| Plant height (cm) | 0.41*** |
| Ear height (cm) | 0.40*** |
| Plant aspect (1-9) | -0.60*** |
| Ear Aspect (1-9) | -0.76*** |
| Root lodging (%) | 0.07 |
| Stalk lodging (%) | 0.15* |
| Husk cover (1-5) | -0.18* |

As a result of the morphological and physiological responses of the maize varieties to soil N, the increase in grain yield under optimal N conditions was accompanied with higher numbers of ears per plant, shorter anthesis-silking interval and lower ear aspect score which is in consonance with the report of Matusso and Materusse (2016).

Across the nitrogen environments, number of days to anthesis ranged from 57 to 66 days while the number of days to silking was between 60 and 72 days with an average anthesis-silking interval of 4 days (Table 5). The short anthesis-silking interval which depicts early synchronization of the pollen and silk exhibited by Pioneer KMK (30Y87), Kapam 10 and Sammaz 52 indicates tolerance to stress (Edmeades *et al*., 2000; Adebayo, 2014). The varieties displayed sizeable tolerance to stalk and root lodging (mean of 3.4 and 13.3%, respectively). Plant height ranged from 152.4 to 182.0 cm, with an average height of 157.3 cm and having ears placed at an average of 69.5 cm above ground level. The plants produced an average of 1 ear per plant across the nitrogen environments. Plant and ear aspect ratings were averagely 6.0 and 5.6, respectively. The overall maize grain yield (3.3 t.ha$^{-1}$) across N environments, was comparable to the report of Adu *et al.* (2018) and higher than the average yield of 1.8 to 2.0 t.ha$^{-1}$ obtained by farmers in the derived savanna agro-ecology (Tofa *et al*., 2021). All the evaluated maize varieties, except for SC 719 out-yielded the commercial check (2640 kg ha$^{-1}$). However, only two varieties (Pioneer KMK (30Y87) and Kapam 10) had a yield advantage of > 25% over the commercial check. This indicates that some of the varieties can adapt in the derived savanna and produce sustainable yields compared to the locally cultivated Oba super 6.

The low-N base index identified Pioneer KMK (30Y87), as outstanding and it out-yielded the commercial check (Oba Super 6) by 53 % under low-N environments (Table 6). Other two varieties (Kapam 10 and Sammaz 52) were also identified as exhibit-

ing tolerance to low soil nitrogen. The results obtained from ranking the performance of the varieties using RSI and low-N base index were similar. For RSI, the varieties with lower ranks had higher grain yield coupled with other desirable agronomic traits whereas, a positive base index value for any maize variety indicate that the variety was tolerant to low soil nitrogen. In general, the two approaches identified Pioneer KMK (30Y87), Kapam 10 and Sammaz 52 as superior varieties. Pioneer KMK (30Y87) had the maximum yield performance across N- environments irrespective of the selection method employed. It is therefore a promising variety that can be exploited in low-input agricultural systems.

The association between traits observed from Pearson's correlation coefficient (r) revealed that the number of ears per plant had strong positive and significant ($P < 0.01$) correlation with grain yield (r = 0.53**) whereas strong negative and significant ($P < 0.01$) correlation existed between grain yield and each of ear and plant aspect (r = -0.76** and -0.60**)Table 7. Overall, grain yield had either positive (number of ears per plant, stalk lodging, plant and ear heights) or negative (number of days to anthesis and silking, anthesis-silking interval husk cover, plant and ear aspect) significant correlations with all measured traits except for percent root lodging, implying that grain yield was associated with many agronomic traits. In the selection for improved grain yield which is quantitative in nature, other agronomic traits related to yield and growth are equally important for adaptability.

However, breeding maize for height in the derived savanna agro-ecology is not a priority, because tall plants have been reported to be susceptible to lodging due to strong winds and have been found to reduce yield (Izge *et al*., 2007).

## CONCLUSION

This study revealed exploitable genetic variation among the evaluated maize varieties across the N environments. Similar varieties (Pioneer KMK (30Y87), Kapam 10 and Sammaz 52) were outstanding in the low-N input, optimal N input and across the N environments. These maize varieties exhibited tolerance to low soil N, with high grain yields, early flowering as well as desirable phenotypic with tolerance to stalk and root lodging. With the low rate of fertilizer use by resource-limited farmers' in Nigeria, these varieties can be recommended to farmers' in the derived savanna agro-ecology to boost maize production.

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# AGRO-MORPHOLOGICAL VARIATION AND GENETIC POTENTIAL IN *Vigna unguiculata* subssp. *unguiculata* var. *spontanea*

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## ABSTRACT

Exploring the genetic potentials of wild relatives of crop varieties plays a critical role in broaden the narrow genetic base and introducing novel genetic diversity into the domesticated crop plants. Genetic diversity in 90 accessions of *Vigna unguiculata* subspp *unguiculata* var. *spontanea* and 3 cultivars of *V. unguiculata* subspp *unguiculata* var. *unguiculata* were investigated. Field trials were carried out at Abeokuta (2014 and 2015) and Ibadan (2014), Nigeria in a randomized complete block design with three replicates. Data collected on qualitative and quantitative traits varied among the accessions which indicated unique phenotypic features in the accessions. Early flowering accessions (NGB1140, NGB1083, NGB1136 and NGB1170) and accessions with low leaf defoliation (NGB1089, NGB1108, NGB1142, NGB1150, NGB1171, NGB1085 and NGB1177) among the cultivars were identified. Genetic diversity analysis revealed nineteen (19) homogenous groups among the accessions. Divergence among the groups was attributed more to seed yield ($R^2$ = 0.90), number of pods/plant ($R^2$ = 0.86) and days to flowering ($R^2$ = 0.86). Promising genetic potential in the *V. unguiculata* subspp *unguiculata* var *spontanea* for desirable traits, and their effective use for further improvement of cultivated cowpea through hybridization programme were revealed.

**Keyword**: cowpea, crop wild-relative (CWR), diversity, insect tolerance, multivariate analysis

## INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a common food legume cultivated in the sub-Saharan Africa and parts of Asia, Europe and, Central and South America. It is a cheap, rich source of protein and valuable food supply in Africa. The grain contains about 32% proteins on dry weight basis (José *et al*., 2014; Ddamulira and Santos, 2015) and high in essential amino acids such as lysine, leucine, phenylalanine, tryptophan and valine (Ukpene and Imade, 2015; Gonçalves *et al*., 2016). The protein content is close to certain meat type (El-Niely Hanina, 2007). Cowpea also has human health promoting components such as antioxidants, soluble and insoluble dietary fibre and polyphenols (Liyanage *et al*., 2014; Da Silva *et al*., 2018). The fodder and shelled pods are also good source of nutritious hay for livestock (Singh *et al*., 2010; Anele *et al*., 2012). Cowpea is usually intercropped with other crops to supply their nitrogen requirement due to its nitrogen-fixing ability. The plant is drought-tolerant and used to maintain soil fertility when cultivated as green manure and

cover crop (Bationo and Ntare, 2000; Alvey *et al*., 2001).

Many of the cowpea accessions (over 15,000) including the wild germplasm are maintained in the gene bank of the International Institute of Tropical Agriculture (IITA), and they represent valuable gene pool to identify new and adaptable trait for cowpea breeding (Mahalakshmi *et al*., 2007). However, the genetic base of cowpea for diverse characters still remains narrow. This can be attributed to continuous cultivation of improved varieties while discarding less superior varieties and consistent use of elite lines in hybridization programs (Fang *et al*., 2007; Meyer *et al*., 2012; Olsen and Wendel, 2013). In addition, cowpea is a self-pollinating plant with low gene flow between cultivated and wild accessions (Fatokun, 2007; Asiwe, 2009).

Attempts to broaden the genetic base may require exploring the wild relative for new and diverse traits, and their introgression into existing cultivars (Boukar *et al*., 2020). Wild relatives of crop provide repository of genes for useful adaptation such as disease resistance, abiotic stress tolerance, increased yield, improved grain quality and earliness to flowering (Fatokun *et al*., 2002; Ajeigbe *et al*., 2008; McCouch *et al*., 2013; Warschefsky *et al*., 2014). Use of wild species to introduce novel genetic diversity into elite cultivars are documented (Breithaupt, 2008; Maxted and Kel, 2009; Brumlop *et al*., 2013) but are limited usually due to many undesirable attributes in the genetic resources. Today, molecular tools can be used to eliminate the unattractive characters while exploiting the desirable traits in the wild germplasm (Boukar *et al*., 2020). *Vigna unguiculata* subspp *unguiculata* var. *spontanea*, are widely distributed in Africa (Pasquet, 1999; Coulibaly *et al*., 2002, Feleke *et al*., 2006) and researches exploring the potential benefit of this wild relative are rare. This study is a preliminary assessment with the objective to characterize some accessions of *V. unguiculata* subsp. *unguiculata* var. *spontanea* for potential phenotypic expression.

## MATERIALS AND METHODS

Ninety (90) accessions of *V. spontanea* were used in the study (Table 1). The seeds were collected from the Germplasm Units of IITA, Nigeria. The germplasm consists of accessions from different parts of Nigeria. Three cultivars of cowpea (*V. unguiculata*) were also used as check. The genetic materials were planted at the Research Farms of National Centre for Genetic Resources (NACGRAB), Ibadan and Federal University of Agriculture, Abeokuta (FUNAAB), Abeokuta. The field trials were carried out in 2014 and 2015 at Abeokuta, and 2014 at Ibadan to define three environments. Abeokuta is located at the forest-savanna transition with lat. 7°14′N and long. 3°89′E, while Ibadan is located at the derived savanna with lat. 7°37′N and long. 3°89′E**.** The rainfall was higher at Ibadan than Abeokuta during the experimental period (Table 2).

Table 1. List of cowpea accessions used, collection and their morphological characteristics

| S/No | Genotype | Species | Collection | Flower colour | Growth habit | Growth pattern | Leaf texture | Pod curvature | Twining tendency | Pod attachment |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | NGB0964 | *V. spontanea* | Abuja | Purple | Acute-erect | Indeterminate | Membraneous | Straight | Intermediate | 30° - 90° |
| 2 | NGB1068 | *V. spontanea* | Adamawa | Purple | Acute-erect | Indeterminate | Membraneous | Slightly curved | Pronounced | 30° - 90° |
| 3 | NGB1072 | *V. spontanea* | Adamawa | Purple | Semi-erect | Indeterminate | Intermediate | Straight | Slightly | 30° - 90° |
| 4 | NGB1078 | *V. spontanea* | Adamawa | Purple | Prostrate | Determinate | Membraneous | Straight | Pronounced | Erect |
| 5 | NGB1082 | *V. spontanea* | Adamawa | Purple | Climbing | Indeterminate | Membraneous | Straight | Pronounced | Erect |
| 6 | NGB1132 | *V. spontanea* | Adamawa | Purple | Climbing | Determinate | Membraneous | Straight | Slightly | Erect |
| 7 | NGB1134 | *V. spontanea* | Adamawa | Purple | Erect | Determinate | Intermediate | Slightly curved | None | 30° - 90° |
| 8 | NGB1136 | *V. spontanea* | Adamawa | Purple | Erect | Indeterminate | Intermediate | Straight | Pronounced | Erect |
| 9 | NGB1148 | *V. spontanea* | Adamawa | Purple | Erect | Indeterminate | Membraneous | Straight | Slightly | Erect |
| 10 | NGB1167 | *V. spontanea* | Adamawa | Purple | Acute-erect | Determinate | Membraneous | Straight | Slightly | Erect |
| 11 | NGB1176 | *V. spontanea* | Adamawa | Purple | Acute-erect | Indeterminate | Cariaceous | Straight | Slightly | Pendant |
| 12 | NGB1065 | *V. spontanea* | Bauch | Purple | Acute-erect | Indeterminate | Membraneous | Straight | Intermediate | Erect |
| 13 | NGB1099 | *V. spontanea* | Bauch | Purple | Erect | Indeterminate | Membraneous | Straight | Pronounced | Erect |
| 14 | NGB1152 | *V. spontanea* | Bauch | Purple | Erect | Indeterminate | Intermediate | Slightly curved | Intermediate | 30° - 90° |
| 15 | NGB0963 | *V. spontanea* | Benue | Purple | Acute-erect | Indeterminate | Intermediate | Straight | Intermediate | Pendant |
| 16 | NGB1044 | *V. spontanea* | Bornu | Purple | Erect | Determinate | Cariaceous | Straight | Intermediate | Pendant |
| 17 | NGB1047 | *V. spontanea* | Bornu | Purple | Prostrate | Indeterminate | Intermediate | Straight | Pronounced | Erect |
| 18 | NGB1058 | *V. spontanea* | Bornu | Purple | Climbing | Determinate | Membraneous | Straight | Intermediate | Erect |
| 19 | NGB1079 | *V. spontanea* | Bornu | Purple | Climbing | Determinate | Intermediate | Straight | Pronounced | Erect |
| 20 | NGB1086 | *V. spontanea* | Bornu | Purple | Semi-erect | Indeterminate | Intermediate | Straight | Slightly | Pendant |
| 21 | NGB1105 | *V. spontanea* | Bornu | Purple | Erect | Indeterminate | Membraneous | Straight | Pronounced | 30° - 90° |
| 22 | NGB1115 | *V. spontanea* | Bornu | Purple | Erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 23 | NGB1125 | *V. spontanea* | Bornu | Purple | Climbing | Determinate | Intermediate | Straight | Intermediate | 30° - 90° |
| 24 | NGB1126 | *V. spontanea* | Bornu | White | Semi-erect | Determinate | Intermediate | Straight | None | Erect |
| 25 | NGB1130 | *V. spontanea* | Bornu | Purple | Erect | Indeterminate | Membraneous | Slightly curved | Slightly | 30° - 90° |
| 26 | NGB1151 | *V. spontanea* | Bornu | Purple | Erect | Indeterminate | Intermediate | Straight | Slightly | Erect |
| 27 | NGB1090 | *V. spontanea* | Jigawa | Purple | Semi-erect | Indeterminate | Intermediate | Straight | Slightly | Erect |
| 28 | NGB1100 | *V. spontanea* | Jigawa | Purple | Semi-erect | Indeterminate | Intermediate | Curved | Intermediate | Erect |
| 29 | NGB1109 | *V. spontanea* | Jigawa | Purple | Erect | Indeterminate | Membraneous | Slightly curved | None | Erect |
| 30 | NGB1111 | *V. spontanea* | Jigawa | Purple | Erect | Determinate | Intermediate | Slightly curved | Intermediate | 30° - 90° |
| 31 | NGB1133 | *V. spontanea* | Jigawa | Purple | Acute-erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 32 | NGB1150 | *V. spontanea* | Jigawa | Purple | Erect | Indeterminate | Intermediate | Straight | Pronounced | Erect |
| 33 | NGB1165 | *V. spontanea* | Jigawa | Purple | Acute-erect | Indeterminate | Intermediate | Slightly curved | Pronounced | 30° - 90° |
| 34 | NGB1027 | *V. spontanea* | Kaduna | Purple | Erect | Indeterminate | Membraneous | Straight | Intermediate | Erect |
| 35 | NGB1028 | *V. spontanea* | Kaduna | Purple | Erect | Determinate | Membraneous | Straight | Slightly | Erect |
| 36 | NGB1081 | *V. spontanea* | Kaduna | Purple | Semi-erect | Indeterminate | Intermediate | Straight | Pronounced | Erect |
| 37 | NGB1094 | *V. spontanea* | Kaduna | Purple | Erect | Determinate | Membraneous | Straight | Slightly | 30° - 90° |
| 38 | NGB1123 | *V. spontanea* | Kaduna | Purple | Erect | Determinate | Intermediate | Slightly curved | None | 30° - 90° |
| 39 | NGB1127 | *V. spontanea* | Kaduna | White | Semi-erect | Determinate | Intermediate | Straight | None | Erect |
| 40 | NGB1163 | *V. spontanea* | Kaduna | Purple | Acute-erect | Determinate | Membraneous | Slightly curved | Slightly | 30° - 90° |
| 41 | NGB1171 | *V. spontanea* | Kaduna | Purple | Acute-erect | Indeterminate | Intermediate | Straight | Pronounced | Erect |
| 42 | NGB1014 | *V. spontanea* | Kano | Purple | Acute-erect | Determinate | Intermediate | Straight | Slightly | Erect |
| 43 | NGB1022 | *V. spontanea* | Kano | White | Acute-erect | Determinate | Intermediate | Straight | Slightly | Erect |
| 44 | NGB1038 | *V. spontanea* | Kano | Purple | Erect | Indeterminate | Membraneous | Straight | Pronounced | Erect |
| 45 | NGB1053 | *V. spontanea* | Kano | White | Acute-erect | Indeterminate | Membraneous | Straight | Slightly | Pendant |
| 46 | NGB1089 | *V. spontanea* | Kano | Purple | Acute-erect | Indeterminate | Membraneous | Straight | Slight | 30° - 90° |
| 47 | NGB1113 | *V. spontanea* | Kano | Purple | Erect | Indeterminate | Membraneous | Straight | Intermediate | Erect |

Source: National Centre for Biotechnology and Genetic Resources (NACGRAB)

Table 1 (continued)

| S/No | Genotype | Species | Collection | Flower colour | Growth habit | Growth pattern | Leaf texture | Pod curvature | Twining tendency | Pod attachment |
|---|---|---|---|---|---|---|---|---|---|---|
| 48 | NGB1118 | *V. spontanea* | Kano | Purple | Prostrate | Determinate | Membraneous | Slightly curved | Intermediate | 30° - 90° |
| 49 | NGB1140 | *V. spontanea* | Kano | Purple | Climbing | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 50 | NGB1158 | *V. spontanea* | Kano | Purple | Acute-erect | Determinate | Membraneous | Straight | Slightly | Erect |
| 51 | NGB1166 | *V. spontanea* | Kano | Purple | Erect | Determinate | Membraneous | Slightly curved | None | 30° - 90° |
| 52 | NGB1069 | *V. spontanea* | Nasarawa | Purple | Prostrate | Determinate | Intermediate | Straight | None | Erect |
| 53 | NGB1093 | *V. spontanea* | Nasarawa | Purple | Erect | Indeterminate | Intermediate | Curved | Intermediate | Erect |
| 54 | NGB1160 | *V. spontanea* | Nasarawa | Purple | Erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 55 | NGB1162 | *V. spontanea* | Nasarawa | Purple | Acute-erect | Indeterminate | Membraneous | Slightly curved | Slightly | 30° - 90° |
| 56 | NGB0952 | *V. spontanea* | Niger | Purple | Acute-erect | Determinate | Cariaceous | Straight | Intermediate | Erect |
| 57 | NGB1006 | *V. spontanea* | Niger | Purple | Erect | Indeterminate | Intermediate | Straight | Pronounced | 30° - 90° |
| 58 | NGB1018 | *V. spontanea* | Niger | Purple | Semi-erect | Determinate | Intermediate | Straight | None | 30° - 90° |
| 59 | NGB1063 | *V. spontanea* | Niger | Purple | Erect | Indeterminate | Intermediate | Straight | Slightly | Erect |
| 60 | NGB1098 | *V. spontanea* | Niger | Purple | Erect | Indeterminate | Intermediate | Straight | Slightly | 30° - 90° |
| 61 | NGB1135 | *V. spontanea* | Niger | Purple | Erect | Indeterminate | Membraneous | Straight | Pronounced | 30° - 90° |
| 62 | NGB1170 | *V. spontanea* | Niger | Purple | Acute-erect | Determinate | Intermediate | Straight | Intermediate | Erect |
| 63 | NGB1175 | *V. spontanea* | Niger | Purple | Prostrate | Indeterminate | Intermediate | Straight | Pronounced | Erect |
| 64 | NGB1177 | *V. spontanea* | Niger | Purple | Acute-erect | Determinate | Cariaceous | Straight | Intermediate | Pendant |
| 65 | NGB0975 | *V. spontanea* | Oyo | Purple | Acute-erect | Determinate | Intermediate | Straight | Intermediate | Erect |
| 66 | NGB1054 | *V. spontanea* | Oyo | Purple | Acute-erect | Intermediate | Intermediate | Straight | Slightly | Erect |
| 67 | NGB1040 | *V. spontanea* | Sokoto | Purple | Erect | Indeterminate | Membraneous | Straight | Slightly | Erect |
| 68 | NGB1060 | *V. spontanea* | Sokoto | Yellow | Erect | Indeterminate | Cariaceous | Slightly curved | Intermediate | Erect |
| 69 | NGB1071 | *V. spontanea* | Sokoto | Purple | Semi-erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 70 | NGB1075 | *V. spontanea* | Sokoto | Purple | Semi-erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 71 | NGB1087 | *V. spontanea* | Sokoto | Yellow | Semi-erect | Determinate | Cariaceous | Slightly curved | Slightly | 30° - 90° |
| 72 | NGB1124 | *V. spontanea* | Sokoto | Purple | Erect | Indeterminate | Membraneous | Straight | Intermediate | Pendant |
| 73 | NGB1128 | *V. spontanea* | Sokoto | Purple | Prostrate | Indeterminate | Intermediate | Straight | Intermediate | 30° - 90° |
| 74 | NGB1137 | *V. spontanea* | Sokoto | Purple | Semi-erect | Determinate | Intermediate | Straight | None | 30° - 90° |
| 75 | NGB1159 | *V. spontanea* | Sokoto | Purple | Climbing | Indeterminate | Membraneous | Straight | Pronounced | Pendant |
| 76 | NGB1174 | *V. spontanea* | Sokoto | Yellow | Semi-erect | Determinate | Intermediate | Straight | None | 30° - 90° |
| 77 | NGB1106 | *V. spontanea* | Taraba | White | Acute-erect | Determinate | Membraneous | Slightly curved | Intermediate | Erect |
| 78 | NGB1108 | *V. spontanea* | Taraba | Purple | Erect | Indeterminate | Membraneous | Straight | Intermediate | Erect |
| 79 | NGB1110 | *V. spontanea* | Taraba | Purple | Acute-erect | Indeterminate | Membraneous | Straight | Pronounced | Erect |
| 80 | NGB1017 | *V. spontanea* | Taraba | Purple | Acute-erect | Indeterminate | Membraneous | Straight | Slightly | Erect |
| 81 | NGB1143 | *V. spontanea* | Taraba | Purple | Semi-erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 82 | NGB1050 | *V. spontanea* | Yobe | Purple | Acute-erect | Determinate | Intermediate | Slightly curved | Intermediate | Erect |
| 83 | NGB1083 | *V. spontanea* | Yobe | White | Erect | Indeterminate | Membraneous | Slightly curved | Slightly | Erect |
| 84 | NGB1116 | *V. spontanea* | Yobe | Purple | Erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 85 | NGB1141 | *V. spontanea* | Yobe | Purple | Semi-erect | Determinate | Intermediate | Slightly curved | Intermediate | Erect |
| 86 | NGB1142 | *V. spontanea* | Yobe | Purple | Erect | Indeterminate | Intermediate | Straight | Intermediate | Erect |
| 87 | NGB1146 | *V. spontanea* | Yobe | Purple | Erect | Indeterminate | Intermediate | Straight | Slightly | 30° - 90° |
| 88 | NGB1168 | *V. spontanea* | Yobe | Purple | Acute-erect | Indeterminate | Membraneous | Slightly curved | None | 30° - 90° |
| 89 | NGB1169 | *V. spontanea* | Yobe | Purple | Erect | Indeterminate | Membraneous | Straight | Pronounced | Erect |
| 90 | NGB1173 | *V. spontanea* | Yobe | Purple | Acute-erect | Determinate | Intermediate | Slightly curved | None | Pendant |
| 91 | IFE-BPC | *V. unguiculata* | Oyo | Yellow | Erect | Indeterminate | Intermediate | Straight | Slightly | Erect |
| 92 | IFE Brown | *V. unguiculata* | Osun | White | Erect | Determinate | Intermediate | Slightly curved | Intermediate | Pendant |
| 93 | SAM-PEA10 | *V. unguiculata* | Kano | Purple | Acute-erect | Determinate | Cariaceous | Slightly curved | Intermediate | Erect |

Source: National Centre for Biotechnology and Genetic Resources (NACGRAB)

**Table 2. Average weather conditions of the experiment from September to December during the experimental period**

| Geographical data | Abeokuta | | Ibadan |
|---|---|---|---|
| | 2014 | 2015 | 2014 |
| Research station | FUNAAB | | NACGRAB |
| Ecological zone | Forest-savanna transition | | Derived savanna |
| Longitude | 03°26′E | | 3°89'E |
| Latitude | 7°14′N | | 7°37'N |
| Altitude (masl) | 162 | | 184 |
| Min. temperature (°C) | 22.56 | 22.15 | 25.9 |
| Max. temperature (°C) | 31.75 | 32.25 | 35.2 |
| Mean temperature (°C) | 27.03 | 27.20 | 26.83 |
| Mean rainfall (mm) | 75.41 | 66.33 | 82.93 |
| Relative humidity | 65.09 | 59.88 | 70.3 |

Sources: Agro-meteorology stations of Federal University of Agriculture, Abeokuta (FUNAAB) and National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan

The experiment was laid out in a randomized complete block design with three replicates. The accessions were planted in single-row plots of 3 m length and inter-plot spacing of 0.75 m to minimize variation within the blocks. Two seeds were planted per hole at 0.30 m apart and emerging seedlings were thinned to one plant stand at 2 weeks after sowing. Five plants were selected randomly from the middle plants and measured for plant traits following the descriptor of Bioversity International (BT, 1983). The characters include plant height at flowering (cm), number of main branches, days to 50% flowering, pod length (cm), number of seeds per pod, number of pods per plant, 100-seed weight (g) and seed yield per plant (g). Flower colour, growth habit and pattern, leaf texture, pod curvature, twining tendency and pod attachment were scored. Insect infestation was considered as leaf defoliation based on visual examination of the cowpea leaflets. Five leaf defoliation intensity were defined: 0% insect infestation with 0 to 19% leaf damage; 25% (20 to 39%), 50% (40 to 59%), 75% (60 to 79%) and 100% (80 to 100%) (Rahman *et al.*, 2008).

Agronomic data were subjected to analysis of variance using the GLM procedure in SAS version 9.1.1 (SAS Institute, 2002), where accession and environment were considered as fixed factors and block as a random factor. Standard error of mean difference was used to separate the means performance of the accessions. Genetic diversity among the 90 accessions of *V. spontanea* was determined based on their genetic distance using the FASTCLUS and Canonical procedures in SAS.

## RESULTS

The flower colour of the accessions varied

from purple to white and yellow. Over 60% of the accessions were indeterminate, with most ranging from acute erect to erect and semi-erect. The leaf texture and pod curvature were mostly membranous and straight, respectively. Pod attachment was erect for most of the accessions (Table 1).

Significant ($p < 0.01$) variation was revealed among the wild relative of cowpea for yield and other agronomic characters (Table 3). The influence of the environment on the expression of the characters was revealed by the significant ($p < 0.01$) effect of accession × environment interaction (GEI). Of the total source of variation, GEI contributed most to the total variation for days to 50% flowering, pod length and 100-seed weight. Accession effect also contributed considerably to the variation in these traits including plant height and number of pods/plant. The coefficient of variation (Table 4) revealed high variability among the accessions for plant height at flowering (37.91), number of pods/plant (28.51), 100-seed weight (24.65) and yield (33.60) over the environment. The variation was high for number of seeds/pod (30.63) at Ibadan. The mean of the traits, except for days to flowering, pod length and number of pods/plant, varies considerably with environment.

NGB1140 and NGB1170 had fewer days to flowering at Abeokuta (43 days) in 2014 (Table 5). Days to flowering was also low in NGB1140 (43 days), NGB1083 (43 days) and NGB1136 (42 days) at Ibadan. The least average for the trait, over the three environments was observed in NGB1140 and NGB1170 (45 days). NGB1079, NGB1130 (Bornu), NGB1118, NGB1140 (Kano), NGB1177 (Niger), NGB1087, NGB1063 (Sokoto) exhibited high pod length at Abeokuta (2014) and Ibadan. NGB1132 (Adamawa) had high pod length in the environments, and NGB1081 (Kaduna) in Ibadan and Abeokuta (2015). Accessions with high number of pods in at least two environments include NGB1167 (Adamawa), NGB1047 (Bornu), NGB1133 (Jigawa), NGB1140 (Kano), NGB1075, NGB1060 (Sokoto), NGB1108 (Taraba) and NGB1141 (Yobe).

**Table 3. Mean squares of seven agronomic characters in 90 accessions of *V. spontanea* and 3 genotypes of *V. unguiculata* over two locations**

| Source of variation | Block (envr) (df = 6) | Accession (A) (df = 92) | Environment (E) (df = 2) | A x E (df = 184) | Error (df = 552) | Proportion in Total SS (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Accession | Location | A x L |
| Plant height at flowering (cm) | 278.90 | 2814.70** | 103980.04** | 795.36** | 182.42 | 36.19 | 29.06 | 20.45 |
| Days to 50% flowering | 0.57 | 101.50** | 2304.44** | 62.44** | 0.73 | 36.14 | 17.84 | 44.46 |
| Pod length (cm) | 1.15 | 10.27** | 0.32** | 5.67** | 1.93 | 30.87 | 0.02 | 34.10 |
| Number of seeds/pod | 2.90 | 22.14** | 3746.58** | 27.55** | 2.36 | 12.79 | 47.07 | 31.84 |
| Number of pods/plant | 26.05 | 111.40** | 64.74** | 35.04** | 12.77 | 42.65 | 0.54 | 26.83 |
| 100-seed weight (g) | 0.20 | 11.59** | 548.14** | 7.03** | 0.31 | 29.40 | 30.25 | 35.67 |
| Seed yield/plant (g) | 1.99 | 141.49** | 15496.25** | 131.47** | 5.15 | 18.32 | 43.62 | 34.04 |

* significant at $p < 0.05$, ** significant at $p < 0.01$

**Table 4. Mean and Variation of Characters Evaluated in the Cowpea Accessions over three Environments**

| Line | Plant height at flowering (cm) | Days to 50% Flowering | Pod length (cm) | Number of seeds/pod | Number of pods/plant | 100-seed weight (g) | Seed yield/plant (g) |
|---|---|---|---|---|---|---|---|
| *Abeokuta 2014* | | | | | | | |
| Mean | 46.40 | 53.31 | 9.39 | 11.28 | 12.94 | 2.78 | 15.26 |
| Range | 110.34 | 25.00 | 12.50 | 8.34 | 25.17 | 6.14 | 45.81 |
| CV (%) | 32.75 | 2.30 | 13.39 | 17.31 | 31.75 | 27.14 | 11.31 |
| *Ibadan 2014* | | | | | | | |
| Mean | 47.14 | 53.32 | 9.32 | 4.52 | 12.66 | 0.67 | 1.39 |
| Range | 110.67 | 26.00 | 7.23 | 12.00 | 24.67 | 2.83 | 4.61 |
| CV (%) | 37.41 | 1.14 | 12.14 | 30.63 | 32.74 | 33.35 | 40.14 |
| *Abeokuta 2015* | | | | | | | |
| Mean | 13.34 | 48.34 | 9.34 | 10.37 | 12.00 | 3.32 | 3.61 |
| Range | 10.88 | 2.50 | 16.88 | 7.00 | 14.00 | 18.06 | 45.33 |
| CV (%) | 17.40 | 1.17 | 18.30 | 11.21 | 17.16 | 16.20 | 96.60 |
| *Pooled* | | | | | | | |
| Mean | 35.63 | 51.66 | 9.35 | 8.72 | 12.53 | 2.26 | 6.75 |
| Range | 72.31 | 16.67 | 7.45 | 6.31 | 18.02 | 8.32 | 22.71 |
| CV (%) | 37.91 | 1.65 | 14.86 | 17.61 | 28.51 | 24.65 | 33.60 |

Coefficient of variation (CV)

**Table 5. Days to flowering, pod length and number of pods/plant in accessions of *V. spontanea***

| Origin | Days to 50% Flowering | | | Pod length (cm) | | | Number of pods/plant | | |
|---|---|---|---|---|---|---|---|---|---|
| | Abk_2014 | Iba_2014 | Abk_2015 | Abk_2014 | Iba_2014 | Abk_2015 | Abk_2014 | Iba_2014 | Abk_2015 |
| Abuja | | | | | | | | | |
| Min/Max | 46.00 | 58.00 | 48.00 | 10.33 | 10.17 | 10.33 | 15.00 | 15.00 | 10.52 |
| Adamawa | | | | | | | | | |
| Min | 46.00 | 42.00 | 47.50 | 5.00 | 5.00 | 7.44 | 2.33 | 2.33 | 9.60 |
| Max | 68.00 | 68.00 | 49.08 | 10.67 | 11.00 | 10.85 | 26.67 | 26.67 | 16.50 |
| Mean | 54.90 | 56.23 | 48.36 | 8.84 | 9.15 | 9.30 | 11.00 | 11.00 | 12.02 |
| Bauchi | | | | | | | | | |
| Min | 48.00 | 45.00 | 48.92 | 8.67 | 7.53 | 8.82 | 10.67 | 9.33 | 10.00 |
| Max | 56.00 | 56.00 | 49.00 | 9.33 | 9.20 | 11.27 | 13.00 | 13.00 | 15.00 |
| Mean | 52.67 | 49.67 | 48.97 | 8.91 | 8.32 | 10.11 | 12.00 | 11.00 | 12.09 |
| Benue | | | | | | | | | |
| Min/max | 55.00 | 49.00 | 49.50 | 10.45 | 8.83 | 10.92 | 16.00 | 10.00 | 10.30 |
| Bornu | | | | | | | | | |
| Min | 46.00 | 46.00 | 47.00 | 8.43 | 7.03 | 7.17 | 9.67 | 9.67 | 9.42 |
| Max | 62.00 | 62.00 | 49.33 | 13.20 | 12.23 | 10.75 | 27.50 | 22.33 | 17.50 |
| Mean | 53.27 | 51.00 | 48.12 | 9.73 | 9.41 | 8.97 | 13.08 | 12.54 | 12.80 |
| Jigawa | | | | | | | | | |
| Min | 45.00 | 46.00 | 47.50 | 7.67 | 8.33 | 7.25 | 7.33 | 7.33 | 9.00 |
| Max | 56.00 | 57.00 | 48.92 | 10.40 | 10.87 | 10.10 | 22.33 | 22.33 | 17.00 |
| Mean | 51.67 | 53.00 | 48.44 | 9.23 | 9.71 | 8.85 | 12.71 | 12.71 | 11.75 |
| Kaduna | | | | | | | | | |
| Min | 45.00 | 45.00 | 47.50 | 9.00 | 8.33 | 8.77 | 10.33 | 9.93 | 9.55 |
| Max | 68.00 | 65.00 | 48.58 | 17.50 | 10.33 | 10.67 | 18.33 | 18.33 | 20.00 |
| Mean | 55.00 | 54.50 | 48.10 | 10.83 | 9.55 | 9.81 | 13.75 | 13.07 | 12.46 |
| Kano | | | | | | | | | |
| Min | 43.00 | 43.00 | 47.00 | 8.33 | 6.37 | 7.88 | 5.00 | 5.00 | 9.00 |
| Max | 57.00 | 57.00 | 49.17 | 11.33 | 11.00 | 10.03 | 26.67 | 26.67 | 22.00 |
| Mean | 49.20 | 51.47 | 48.23 | 9.73 | 9.60 | 9.11 | 14.13 | 14.17 | 12.09 |
| Nasarawa | | | | | | | | | |
| Min | 47.00 | 54.00 | 48.00 | 8.73 | 7.23 | 8.38 | 7.33 | 7.33 | 9.46 |
| Max | 61.00 | 59.00 | 49.50 | 10.67 | 10.90 | 10.37 | 11.00 | 13.67 | 10.02 |
| Mean | 52.50 | 57.25 | 48.38 | 9.52 | 9.34 | 9.65 | 9.75 | 10.67 | 9.75 |
| Niger | | | | | | | | | |
| Min | 43.00 | 45.00 | 47.00 | 6.57 | 7.97 | 8.06 | 8.00 | 8.00 | 8.00 |
| Max | 61.00 | 63.00 | 49.00 | 11.50 | 11.33 | 10.68 | 14.33 | 14.33 | 16.00 |
| Mean | 52.00 | 54.04 | 48.27 | 9.13 | 9.85 | 9.36 | 11.28 | 11.52 | 10.44 |
| Oyo | | | | | | | | | |
| Min | 57.00 | 57.00 | 49.00 | 8.67 | 7.53 | 8.80 | 9.33 | 9.33 | 14.00 |
| Max | 59.00 | 59.00 | 49.17 | 11.67 | 9.40 | 9.09 | 14.33 | 14.33 | 16.50 |
| Mean | 58.00 | 58.00 | 49.09 | 10.17 | 8.47 | 8.95 | 11.83 | 11.83 | 15.25 |
| Sokoto | | | | | | | | | |
| Min | 46.00 | 45.00 | 47.50 | 7.67 | 7.57 | 7.85 | 6.00 | 6.00 | 8.32 |
| Max | 68.00 | 63.00 | 49.00 | 12.67 | 11.10 | 9.50 | 27.00 | 27.00 | 20.67 |
| Mean | 57.40 | 53.37 | 48.46 | 9.10 | 9.24 | 8.61 | 15.23 | 14.42 | 12.30 |
| Taraba | | | | | | | | | |
| Min | 46.00 | 50.00 | 47.83 | 8.13 | 7.17 | 7.65 | 4.00 | 4.00 | 15.00 |
| Max | 59.00 | 59.00 | 48.58 | 9.67 | 10.47 | 9.78 | 22.33 | 22.33 | 17.50 |
| Mean | 53.20 | 55.00 | 48.20 | 8.99 | 8.49 | 8.67 | 13.87 | 13.73 | 16.10 |
| Yobe | | | | | | | | | |
| Min | 45.33 | 43.00 | 47.00 | 7.67 | 8.50 | 7.96 | 8.00 | 8.00 | 8.93 |
| Max | 62.00 | 59.00 | 49.00 | 10.00 | 10.40 | 9.55 | 23.33 | 23.33 | 13.00 |
| Mean | 54.15 | 53.44 | 48.05 | 8.87 | 9.37 | 8.92 | 12.93 | 12.93 | 10.17 |
| Check | | | | | | | | | |
| IFEBPC | 55.00 | 47.00 | 49.00 | 8.73 | 8.53 | 10.40 | 13.33 | 10.00 | 10.63 |
| IFE-BROWN | 47.33 | 45.00 | 49.00 | 8.43 | 8.73 | 10.63 | 13.00 | 11.67 | 9.52 |
| SAM-PEA10 | 46.67 | 45.00 | 48.50 | 8.00 | 8.27 | 10.28 | 13.67 | 15.33 | 10.62 |
| SED | 1.00 | 0.50 | 0.46 | 1.03 | 0.92 | 1.40 | 3.35 | 3.38 | 1.68 |

Standard error of mean difference (SED)

Considerable higher number of seeds/pod was exhibited among the *V. spontanea* (11 to 14 seeds) accessions than the checks (7/8 seeds) at Abeokuta in 2014 (Table 6). Of these accessions, only NGB1111 (Jigawa) produced higher number of seeds/pod (12) than the checks at Ibadan. The following accessions had similar number of seeds to the checks in Abeokuta (2015): NGB1109 (Jigawa), NGB1163 (Kaduna), NGB0952, NGB1135 (Niger), NGB1040, NGB1174 (Sokoto), NGB1106 (Taraba), NGB1116, NGB1173 (Yobe). Genetic potential for higher seed yield was observed in NGB1134 (Adamawa), Bornu (NGB1125), NGB1150 (Jigawa) NGB1171 (Kaduna), NGB1089 (Kano), NGB1098 (Niger), NGB1054 (Oyo), NGB1137 (Sokoto) and NGB1108 (Taraba) in Abeokuta (2014). Yield in Ibadan was low due to high rate of insect infestation compared to Abeokuta in 2014. However, NGB1158 (Kano), NGB1093 (Nasarawa) and NGB1137 (Taraba) produced significantly higher yield than the check. Insect infestation was controlled at Abeokuta in 2015, and highest yields were obtained in the checks during this period. Insect infestation ranged from 0 – 75% in Abeokuta (2014) and 50 – 100% in Ibadan. Minimum insect infestation (0%) was observed in NGB1082 (Adamawa), NGB1150 (Jigawa), NGB1171 (Kaduna), NGB1089 (Kano), NGB1128, NGB1071 (Sokoto), NGB1108 (Taraba), and NGB1142 (Yobe) at Abeokuta compared to the checks (50 – 75%). However, NGB1089, NGB1108, NGB1142, NGB1150, NGB1171 had 75% insect infestation and NGB1071, NGB1082, NGB1128 (100%) at Ibadan. NGB1085 (Bornu) maintained a 50% infestation in both environments while NGB1177 (Niger) had 25% and 50% infestation at Abeokuta and Ibadan, respectively.

The cowpea accessions were separated into nineteen homogenous groups based on pooled mean and FASTCLUS procedure of SAS (Table 7). The multivariate analysis separated IFEBROWN, NGB1078 (Adamawa), NGB1068 (Bornu), and NGB1167 (Adamawa) in clusters 5, 13, 14 and 18 respectively, and the $D^2$ distance (longest) revealed genetic distinctness of IFEBROWN and NGB1167 from the other accessions. The closest genotype to IFEBROWN were NGB1171 (Kaduna), NGB1089 (Kano) and NGB1137 (Kano), while NGB1047 (Bornu), NGB1075 (Sokoto) were closer to NGB1167. The cowpea accessions were not distributed into the clusters following the States of collection which suggested a close origin among the genetic materials. Divergence among the 19 clusters was attributed more to yield followed by number of pods and days to flowering based on the coefficient of determination (Table 8). High yield was related to the check (IFEBROWN) in Cluster 5, then NGB1171 (Kaduna), NGB1089 (Kano), NGB1137 (Sokoto) in Clusters 3 and NGB1134 (Adamawa), NGB1150 (Jigawa) and NGB1098 in Cluster 10. Accessions in Cluster 18 were revealed as high pod-bearing plants with high number of seeds/pod. NGB1047 (Bornu), NGB1075 (Sokoto), NGB1108 (Taraba) in Cluster 15 and NGB1060 (Sokoto), NGB1141 (Yobe) in Cluster 11 were also classified as plants with high number of pods/plant. Cluster 6 with NGB1014 and NGB1140 (Kano) was described as early flowering group with high number of pods/plant. Cluster 3 (NGB1171, NGB1089, NGB1137) was revealed as group with considerable high yielding plants and high number of seeds/pod.

**Table 7. Nineteen clusters among 93 accessions of cowpea and squared distance ($D^2$) to cluster**

| Cluster | Accession | Distance | |
|---|---|---|---|
| | | Shortest | Longest |
| 1 | NGB1127 (Kaduna), NGB1166, NGB1022 (Kano), NGB1162 NGB1160 (Nasarawa), NGB0952, NGB1063 (Niger), NGB1159, NGB1174 (Sokoto), NGB1142, (Yobe) | 8.40** (19) | 232.93** (5) |
| 2 | NGB1093 (Nasarawa), NGB1094 (Kaduna) | 24.28** (9) | 232.11** (5) |
| 3 | NGB1171 (Kaduna), NGB1089 (Kano), NGB1137 (Sokoto) | 18.88** (10) | 180.75** (18) |
| 4 | NGB1071 (Sokoto), NGB0975 (Oyo), NGB1065 (Bauchi), NGB1110 (Taraba), NGB1123 (Kaduna) | 14.74** (19) | 181.54** (5) |
| 5 | IFEBROWN | 44.61** (3) | 376.58** (18) |
| 6 | NGB1014, NGB1140 (Kano) | 41.59** (8) | 268.59** (5) |
| 7 | NGB1132 (Adamawa), NGB0963 (Benue), NGB1044, NGB1126, NGB1115, NGB1130, NGB1105 (Bornu), NGB1028, NGB1027, NGB1081 (Kaduna), NGB1069 (Nasarawa), NGB1018 (Niger), NGB1087 (Sokoto), NGB1017 (Taraba), NGB1169 (Yobe) | 6.98** (16) | 236.43** (5) |
| 8 | NGB1113, NGB1158 (Kano), NGB1106 (Taraba) | 10.56** (17) | 132.69** (5) |
| 9 | NGB1090, NGB1109 (Jigawa), NGB1099, NGB1152 (Bauchi), NGB1083 (Yobe), NGB1170, NGB1177 (Niger), | 9.00** (17) | 184.27** (5) |
| 10 | NGB1134 (Adamawa), NGB1150 (Jigawa), NGB1098 (Niger), | 10.54** (12) | 154.95** (18) |
| 11 | NGB1060 (Sokoto), NGB1141 (Yobe) | 32.24** (19) | 300.34** (5) |
| 12 | NGB1006 (Niger), NGB1163 (Kaduna), NGB1054 (Oyo) | 10.54** (9) | 117.70** (5) |
| 13 | NGB1078 (Adamawa) | 23.16** (4) | 278.86** (5) |
| 14 | NGB1068 (Adamawa) | 27.65** (1) | 280.42** (5) |
| 15 | NGB1047 (Bornu), NGB1075 (Sokoto), NGB1108 (Taraba) | 30.49** (12) | 199.14** (5) |
| 16 | NGB1148, NGB1136 (Adamawa), NGB1118, (Kano), NGB1050, NGB1100, NGB1175 (Niger), NGB1168 (Yobe), | 6.98** (7) | 181.17** (5) |
| 17 | IFEBPC, SAMPEA10, NGB1086, NGB1125, NGB1079 (Bornu), NGB1111, NGB1133 (Jigawa), NGB1038 (Kano), NGB1135 (Niger), NGB1128 (Sokoto), NGB1143, (Taraba) | 9.00** (9) | 147.54** (18) |
| 18 | NGB1167 (Adamawa) | 35.92** (15) | 376.58** (5) |
| 19 | NGB0964 (Abuja), NGB1072, NGB1082, NGB1176 (Adamawa), NGB1151, NGB1058 (Bornu), NGB1165 (Jigawa), NGB1040, NGB1053, NGB1124 (Sokoto), NGB1116, NGB1146, NGB1173 (Yobe), | 8.40** (1) | 218.29** (5) |

** significant at $p < 0.01$

**Table 8. Divergence Among the 19 Clusters in 93 Cowpea Accessions for Yield and Yield-Related Traits**

| Cluster | Days to 50% flowering | Pod length (cm) | Number of pods/ plant | Number of seeds/ pod | Seed yield/plant (g) |
|---|---|---|---|---|---|
| 1 | 54.95 (1.07) † | 9.40 (0.80) | 9.85 (1.83) | 9.35 (1.79) | 5.06 (1.27) |
| 2 | 51.36 (1.92) | 13.18 (1.19) | 9.28 (1.65) | 9.67 (0.86) | 5.53 (0.57) |
| 3 | 49.23 (2.03) | 8.62 (0.81) | 16.33 (1.79) | 10.00 (0.51) | 16.30 (2.63) |
| 4 | 55.33 (1.43) | 8.61 (0.78) | 13.09 (2.27) | 6.58 (0.48) | 7.87 (1.45) |
| 5 | 47.11 (0.00) | 9.27 (0.00) | 11.39 (0.00) | 8.83 (0.00) | 24.34 (0.00) |
| 6 | 45.50 (0.71) | 10.17 (0.09) | 19.27 (2.67) | 7.99 (1.43) | 2.52 (0.93) |
| 7 | 49.93 (1.23) | 9.68 (0.97) | 10.86 (1.53) | 7.61 (1.00) | 3.42 (1.22) |
| 8 | 50.57 (0.36) | 9.03 (1.12) | 16.90 (1.07) | 9.37 (1.62) | 9.24 (2.19) |
| 9 | 48.17 (1.82) | 9.53 (0.89) | 10.99 (0.67) | 10.48 (0.38) | 5.65 (0.74) |
| 10 | 52.47 (0.36) | 8.99 (0.48) | 11.96 (0.48) | 8.82 (1.61) | 14.37 (1.69) |
| 11 | 54.41 (0.69) | 8.49 (0.03) | 20.20 (2.31) | 6.06 (0.71) | 2.61 (1.17) |
| 12 | 55.66 (1.19) | 9.89 (0.29) | 13.77 (1.12) | 9.21 (1.89) | 12.29 (0.64) |
| 13 | 61.44 (0.00) | 8.69 (0.00) | 13.28 (0.00) | 8.50 (0.00) | 6.34 (0.00) |
| 14 | 53.00 (0.00) | 6.57 (0.00) | 4.76 (0.00) | 9.83 (0.00) | 3.62 (0.00) |
| 15 | 57.08 (1.51) | 8.91 (0.83) | 21.37 (1.11) | 8.71 (1.29) | 9.66 (2.33) |
| 16 | 49.97 (1.41) | 9.27 (0.88) | 8.64 (1.29) | 7.89 (1.18) | 6.40 (1.22) |
| 17 | 48.85 (1.75) | 9.33 (1.09) | 12.62 (1.09) | 8.69 (1.52) | 8.84 (1.41) |
| 18 | 61.67 (0.00) | 10.36 (0.00) | 22.78 (0.00) | 11.17 (0.00) | 4.25 (0.00) |
| 19 | 52.62 (1.33) | 9.08 (0.61) | 13.22 (1.31) | 9.42 (1.15) | 4.61 (1.48) |
| $R^2$ | 0.86 | 0.48 | 0.86 | 0.48 | 0.90 |

† standard deviation within cluster
Coefficient of determination ($R^2$)

## DISCUSSION

This study was an important step to identify valuable agronomic traits in wild-relative of cowpea; *Vigna unguiculata* subspp. *unguiculata* var. *spontanea*, and select them for further utilization in breeding programmes of cowpea. Morphological variation in growth pattern/habit and flower colour are important for the classification of the cowpea accessions. Significant differences observed among the 90 *spontanea* accessions for agronomic traits revealed presence of natural variation, and possibility of selecting parental genotypes with valuable traits. Knowledge of genetic variation is inevitable for identifying genetic potentials in breeding programmes (Undals *et al*., 2011). Significant effect of GEI demonstrated the dependence of the accession on the environment for the expression of the traits therefore, possibility to maximize the adaptive variation of the accessions in diverse environments. However, small seed size and twinning growth habit are wild and undesirable attributes common in the cowpea accessions. Days to flowering, number of pods/plant and seed yield are important agronomic traits to distinguish among the *spontanea* accessions. Early flowering accessions which include NGB1140, NGB1083, NGB1136 and NGB1170 were identified and could be selected as promising parental material for development of the trait. Another significant trait was low insect infestation. Low leaf damage observed in some of the *spontanea* accessions which include NGB1089, NGB1108, NGB1142, NGB1150, NGB1171, NGB1085 and NGB1177 could provide a good level of resistance to create tolerance to insect-pest in the cultivated cowpea. Outstanding *spontanea* accessions within clusters 6, 11, 15 and 18 (high number of pods/plant) clusters 3, 9 18 (high number of seeds/pod) and clusters 3 and 10 (high seed yield) can be selected for yield-related traits and improvement of yield in the cultivated cowpea through hybridization program. In conclusion, exploring the genetic potential in *Vigna unguiculata* subspp *unguiculata* var. *spontanea* through characterization of the agro-morphological variation present in the available genetic resource is an important step. Although, var. *spontanea* has been described as the progenitor of var. *unguiculata* (Pasquet 1999), adaptability of this wild variety for agro-morphological characters are crucial for further improvement of the cultivated cowpea, especially in the midst of present and predictable climate change.

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# MEAT QUALITY CHARACTERISTICS OF CHICKENS FED WITH DIETARY SUPPLEMENTATION OF VITAMIN E AND SELENIUM IN THE TROPICS

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## ABSTRACT

Climate change has created a pronounced heat-stress challenge to the poultry industry in the tropics with resultant effect on the quality of meat produced, therefore it becomes vital to seek methods in alleviating this problem. The objective of this research was to determine the impact of dietary vitamin E and selenium (VE+Se) on quality characteristics of meat. A total of 150-day old broiler chickens were fed diet containing VE+Se at 0,100 mg VE+ 0.05 mg Se, 200 mg VE+ 0.1 mg Se, 300 mg VE+0.15 mg Se and 400 mg VE+0.2 mg Se Kg of feed. At the 49th day of age, birds were slaughtered for meat evaluation. Data generated on meat quality: carcass characteristics, sensorial profile, water holding capacity (WHC), water absorption capacity (WAC), colour and proximate composition, were arranged in a One-way ANOVA. The highest ($p<0.05$) dressing (%) and prime cuts (thigh, drumstick and breast) were observed from the 400 mg VE + 0.2 mg Se feeding. Highest flavour and tenderness were recorded from the 400 mg VE + 0.2 mg Se feed while juiciness and overall flavour were highest in control and 400 mg VE + 0.2 mg Se diet. Dietary VE+Se had significant ($p<0.05$) effect on meat colour, highest ($p<0.05$) b*(yellowness) intensity was observed from 100 mg VE + 0.05 mg Se and 400 mg VE+0.2 mg Se diets, a*(redness) in 0 mg and lightness in the VE+Se supplemented groups. These findings showed dietary supplementation of VE+Se up to 400 mg Ve + 0.2 mg Se in poultry diets influenced prime cuts (thigh, breast and drumstick) and improved consumer perception and meat colour.

**KEYWORDS:** Meat quality attributes, antioxidants, selenium, vitamin E, sensorial response, poultry meat

## INTRODUCTION

Meat plays a significant role in diets of people because of its appealing flavour, texture and high nutritional worth. However, there are numerous factors limiting the quality and acceptability of meat and meat products to the consuming populations. Aside from microbiological hazards and probable contaminants inherent in the food industries, oxidation of lipids, muscle myoglobin and protein are major causes of deterioration of quality of muscle foods. Oxidation of lipids leads to discolouration, drip losses, off-odour and off-flavour development in meat;

likewise decreases in the nutritional quality and safety by the formation of secondary reaction products in foods after cooking and processing (Morrissey *et al.,* 1998).

Quality is defined as the sum of demands of the consumer concerning foodstuffs (Woodward and Wheelock, 1990), therefore the anticipation of every consumer of meat is that it should be healthy, rich in protein and exhibit no off-flavour.

Several management practices and dietary modifications have been employed in improving performance, health and products from animals. Inclusion of vitamin E and Selenium in diets is essential for the integrity and optimal function of the productive, muscular, circulatory, nervous, and immune systems in animals. Addition of vitamin E to bird diets improves birds' health and productivity and also provides a source of vitamin E that is useful for human nutrition and health. The use of VE+Se in diets may provide a possible option in combating quality loss in meat from the farm before been processed into other meat products (Kim et al., 2010). It is therefore conceivable to improve the antioxidant capacity by supplying antioxidants in the diet such as vitamin E and enzyme precursors (selenium) since vitamin E and selenium supplementation in poultry diets results in an increase of vitamin E concentration in the tissue, leading to an increase in the stability of meat (Grau *et al.,* 2001, Ryu et., 2005). Therefore, lowering these oxidations can enhance the shelf-life stability of meat and meat products.

The use of antioxidants has been put forward and cited as means of mitigating against stress with positive effect on animal health and its products (Surai et al., 2019a, b; Surai, 2020). . Different nutritional approaches for supplementing broiler chicken diets with vitamin E have been explored to delay the onset of lipid oxidation and improve the quality of poultry meat. The dietary supplementation with vitamin E has been reported to improve the oxidative stability of poultry meat (Goñi *et al.,* 2007) and reduction of lipid peroxidation and improving the colour stability and quality of poultry meat (Zhang *et al.,* 2020). Selenium supplementation has been opined to positively improve the antioxidant activity in plasma and tissue, growth performance, decrease lipid oxidation and improve meat quality and chemical composition of meat ([Markovic´ *et al.,* 2018). In addition, the combination of selenium with vitamin E has been revealed to have synergistic effects in reducing lipid oxidation in breast meat of broilers under stress (Habibian *et al.* (2016).

Despite the extensive information on the effect of vitamin E on broiler chicken, little information is available on extra supplementation of VE+Se on quality characteristics of broiler chicken meat in the tropics. Hence this study was conducted ~~is~~ to determine the influence of dietary supplementation of VE+Se on quality characteristics of broiler chicken meat

## MATERIALS AND METHODS

The research was conducted at the Poultry Unit of the Teaching and Research Farms Directorate (TREFAD), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (latitude 7$^{o}$ 10' N, longitude 3$^{o}$ 2' E).

One hundred and fifty (150) day-old broiler chicken (Ross308) were allotted to five dietary treatments containing 0 mg VE + 0 mg Se,100 mg VE + 0.05 mg Se, 200 mg VE + 0.1 mg Se, 300 mg VE + 0.15 mg Se and 400

mg VE + 0.2 mg Se Kg. The Vitamin E and Selenium were supplemented in the basal feed/diet (Table I) respectively in a completely randomized design. Each treatment group consisted of three replicates with ten (10) birds each. A basal diet was formulated (Table 1) and was subsequently supplemented at the varying levels of VE+Se (0 mg VE + 0 mg Se,100 mg VE + 0.05 mg Se, 200 mg VE + 0.1mg Se, 300 mg VE + 0.15mg Se and 400 mg VE + 0.2mg Se) per Kg of feed. The nutrient composition of the basal diet was determined using AOAC International (2005). The supplemented feed were given throughout the 49-day trial. Feed and water were provided without restriction to birds.

**Table 1: Gross composition and Nutrient Level (%) of Basal Diet**

| **Ingredient** | **Amount (%)** |
|---|---|
| Maize | 47.00 |
| Groundnut cake | 15.00 |
| Soybean meal | 23.00 |
| Wheat offal | 8.00 |
| Bone meal | 6.00 |
| Limestone | 3.00 |
| Lysine | 0.25 |
| Methionine | 0.25 |
| *Premix | 0.25 |
| Salt | 0.25 |
| **Total** | **100** |
| **Determined Analysis** | |
| Metabolizable Energy (kcal/kg) | 2713.00 |
| Protein (%) | 20.00 |
| Crude Fat (%) | 3.90 |
| Crude Fibre (%) | 3.70 |

*Premix provided per kilogram of diet: transretinyl acetate, 3.44 mg; cholecalciferol, 0.075 mg; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; vitamin E; 11IU, nicotinamide, 40 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamin), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg, Selenium; 0.23mg.

At the 49th day of experiment, birds nearest to the average weight of the birds from each replicate were selected and slaughtered for meat quality evaluation. The final weight in each treatment group were 2066.87, 2136.38, 2175.09, 2220.20, 2467.03 g respectively; (Table 2). Before slaughtering, broiler chickens were starved for 12 hours and slaughtered via neck slit and then allowed to bleed. Cut-up parts and organs were weighed using sensitive scale and expressed as percentage of the liveweight. Meat was excised from the breast region, placed in a polythene bag and labelled accordingly for laboratory analysis.

A sensory panel consisting of 7 assessors was set up. Samples of meat were excised from two birds from each replicate, cut into cubes and tagged for identification. The samples were put in labelled polythene bag and cooked in a water bath at 70°C for 20 minutes. The meat was allowed to cool down at room temperature. A seven (7) member judge were educated on the assessment procedure and were subsequently required to masticate on each sample from each replicate and evaluate some of the sensory characteristics such as colour, juiciness, meaty flavour, tenderness, saltiness, overall flavour and overall acceptability using a nine-point hedonic scale (Peryam and Girardot, 1952). Like extremely =9, like very much =8, like moderately =7, slightly like =6, neither like nor dislike =5, dislike slightly =4, dislike moderately =3, dislike very much =2, dislike extremely =1.

The muscle colour was determined by taking a sample of sliced meat from the posterior part of the breast muscle obtained from broiler after slaughter and viewed using a Colorimeter (chroma meter CR- 410, Japan). The meat samples were placed in a Petri dish and the colorimeter was placed over the meat and the values displayed were recorded. The values of L*, a* and b* colorimetric coordinates were determined. L*= corresponds to lightness, a*= corresponds to redness, b*= corresponds to yellowness.

The water-holding capacity (WHC) of meat samples excised from the breast were determined using a centrifugation technique (Hamm, 1960).. Triplicate 15 g samples of meat were slurred using mortar and pestle and placed in centrifuge tubes, 22.5 ml of 0.6 M saline solution was added and the contents stirred for 1 min with a glass rod. After stirring**,** the sample was refrigerator for 15 mins. The meat slurry was stirred again for 1 min and immediately centrifuged at 2000 rpm (Merlin 503, Spectral scientific Ltd, Great Britain) for 15 mins. The supernatant layer was decanted and the volume recorded. The amount of added solution retained by the meat was reported as the water holding capacity in ml per 100 g meat.

$$\textbf{Water-Holding Capacity (\%)} = \frac{Before\ centrifuge - After\ centrifuge}{Before\ centrifuge} \times 100$$

Before Centrifuge = Amount of saline solution added, After Centrifuge = Amount of solution decanted

A modified centrifugation method (Arganosa *et al.,* 1991) was used to determine the water absorption capacity (WAC) of the breast samples. Triplicate Five grams (5 g) of sample was blended (KenWood Processor) with 10 mls of distilled water for 1 min. The homogenized mixture was poured and rinsed with 10 mls of distilled water into

a pre-weighed centrifuge tube. The mixture was centrifuged (Merlin 503, Spectral scientific Ltd, Great Britain) at 2000 rpm for 25 mins. The remaining unabsorbed water was decanted after centrifugation and the water absorbed by meat was calculated.

$$\textbf{Water Absorption Capacity (\%)} = \frac{\textbf{gram of water absorbed}}{gram of meat} \times 100$$

Gram of Water Absorbed = Weight of meat with centrifuge tube before centrifugation – weight of meat with centrifuge tube after centrifugation, Gram of Meat = gram of meat sample

The proximate composition of the replicate meat samples was carried according to AOAC International (2005) to determine crude protein, ether extract and ash content.

Data were arranged in a one-way analysis of variance and analysed using the GLM procedure of the SAS/STAT module (SAS, 2003). Significant differences between means were separated using Tukey HSD test at $p>0.05$. Data are presented as means and pooled standard error of means.

## RESULTS AND DISCUSSION

All carcass characteristics parameters measured were not significantly influenced except live-weight, dressing percentage, thigh, drumstick and breast (Table 2). Live-weight and dressing percentage values were higher in the birds fed extra supplementation of VE+Se compared to the control, but was significantly highest from the 400 mg VE+ 0.2 mg Se diet. This is in contrast to the report of Tayeb and Qader (2012) who observed no differences in the in weight and dressing percentages of birds at 42 (74.70%) and 49 (75.48 %) days of age, with values obtained in the current study (76.54%) higher than the previous. This observed positive effect in the study is a direct result of the improved and better performance indices resulting in the higher live weight of birds, thereby affirming the role of vitamin E and selenium in growth and protection of the biological system (Cheng *et al.,* 2017) -as there exists a positive relationship between vitamin E and Se. Thigh, drumstick and breast values were lowest from the control (11.23, 9.80, 21.41) and 100 – 300 mg VE+Se diets, with the best prime cut-up value obtained from 400 mg VE + 0.2 mg Se group (12.26, 11.24, 25.19). This is not consistent with the reports of Habibian *et al.* (2016), Leonel *et al.* (2007) and Choct and Naylor (2004) who observed no differences in performance, carcass, breast, thigh, drumstick and abdominal fat yield percentages. The organs (liver and spleen) were statistically similar in all groups (Table 2), this is contrary to the report of Singh *et al.* (2006) who observed a synergistic effect resulting in a significantly higher bursal and spleen weight when birds were fed extra supplementation of 200 mg VE+ 0.2 mg Se but in agreement with Niu *et al.* (2009a, b) who stated no impact on weights of lymphoid organs with similar VE+Se supplementations**.** The non-significant scenario in organs, especially the spleen, reveals no extreme stressors on birds or indicative of no stress as reduced spleen weight is suggestive of physiological stress experienced by birds (Puvadolpirod and

Thaxton, 2000). Although incremental use of VE has been reported to increase relative weight of liver (Akbari *et al.,* 2008), current study with a higher supplementation in addition with Se however did not reveal any such influence on its weight as reported by Özkan et al., (2007) and Habibian *et al*., (2014).

**Table 2: Effect of Dietary Vitamin E on Carcass Characteristics of Broiler chicken**

| Parameter | Control | 100mg VE+0.05 mg Se | 200mg VE+0.1 mg Se | 300mg VE+0.15 mg Se | 400mg VE+0.2 mg Se | SEM | P-Value |
|---|---|---|---|---|---|---|---|
| *Final weight (g) | 2066.87[b] | 2136.38[b] | 2175.09[b] | 2220.20[b] | 2467.03[a] | 40.28 | 0.0010 |
| Live-weight (g) | 2071.67[b] | 2200.00[b] | 2283.33[b] | 2300.00[a] | 2416.67[a] | 36.84 | 0.0130 |
| Dressing (%) | 65.48[c] | 66.77[c] | 69.61[b] | 71.28[b] | 76.54[a] | 1.13 | <0.0001 |
| **Cut up part (%)** | | | | | | | |
| Head | 2.37 | 2.16 | 2.31 | 2.24 | 2.12 | 0.04 | 0.2720 |
| Neck | 3.72 | 3.43 | 3.16 | 3.46 | 3.43 | 0.11 | 0.7010 |
| Thigh | 11.23[b] | 10.88[b] | 11.56[b] | 11.42[b] | 12.26[a] | 0.15 | 0.0320 |
| Drumstick | 9.80[b] | 10.83[b] | 10.59[b] | 10.87[b] | 11.24[a] | 0.19 | 0.0049 |
| Shank | 4.61 | 4.32 | 4.27 | 4.52 | 4.30 | 0.18 | 0.6080 |
| Back | 11.74 | 12.51 | 13.66 | 12.23 | 12.72 | 0.26 | 0.1770 |
| Wings | 6.34 | 6.27 | 6.44 | 6.78 | 6.94 | 0.09 | 0.0730 |
| Breast | 21.41[b] | 21.40[b] | 22.80[b] | 24.43[a] | 25.19[a] | 0.55 | 0.0049 |
| **Organs (%)** | | | | | | | |
| Liver | 2.09 | 1.96 | 2.07 | 2.40 | 1.83 | 0.08 | 0.2420 |
| Spleen | 0.11 | 0.11 | 0.10 | 0.14 | 0.09 | 0.12 | 0.7730 |
| Empty gizzard | 1.66 | 1.69 | 1.52 | 1.66 | 1.67 | 0.04 | 0.7360 |
| Lungs | 0.59 | 0.61 | 0.62 | 0.69 | 0.53 | 0.03 | 0.6710 |
| Heart | 0.51 | 0.46 | 0.49 | 0.52 | 0.44 | 0.01 | 0.4230 |

[a,b,c] :values in same row not sharing a common superscript are significant different ($p<0.005$)
SEM: Standard Error of Mean *: Average final treatment weight of birds at 7 weeks of age

All sensorial parameters of meat excised from broiler chicken were significantly influenced by VE+Se supplementation except colour, saltiness and overall acceptability profile of the meat (Table 3). Juiciness was lowest in 100 mg VE + 0.05 mg Se diet with increase as the level of supplementation increased. Meaty flavour and tenderness (6.71, 7.05) was highest from meat sampled from birds fed diet containing the highest supplementation of VE+Se while overall flavour was lowest from diets of 100 mg Ve + 0.05mg Se and 200mg VE+ 0.10mg Se. The result of the current study affirms the report of Zdanowska-Sasiadek *et al.* (2016) that the addition of dietary vitamin E resulted in high pH, low cooking loss and better sensory quality of fresh breast meat. Therefore, the ability of both vitamin E and selenium in inhibiting oxidative processes will cause a reduction in the degree of oxidation products generated which is capable of

deteriorating the quality of meat by imposing negative effects on sensory attributes (Kennedy *et al*., 2005) of meat. Juiciness is an important contributor to eating-quality of meat (Lyon *et al*., 2004) and it is influenced by the WHC of such meat sample. The numerical increase in the WHC in the VE+Se fed birds may account for the perception of the meat samples by assessors. Water-holding capacity (WHC) and water absorption capacity (WAC) were not influenced by dietary supplementation of VE+Se. However, WHC values in the VE+Se supplemented groups were numerically higher compared to the control group with the highest value of WHC (35.11%) recorded in meat excised from birds fed diet supplemented with 400 mg VE+0.2 mg Se. A similar trend was observed in WAC with numerically least value observed from the control group (Table 3). Water holding capacity reveals the extent of drip loss in meat. A low water holding capacity in muscles can increase the liquid outflow and lead to loss of soluble nutrients and flavour (Otto *et al*., 2004) and inadvertently resulting in depression in quality of meat. The water holding capacity, therefore determines the quality of meat perceived by a consumer by affecting the sensory quality and influencing its usability for processing. In this study, higher water holding capacity recorded was due to the concentrations of VE+Se. The higher concentration of VE+Se in diet could result in higher deposition of VE and Se in meat, protecting the integrity of cell membranes by reducing the oxidative changes in the membrane lipids (Li *et al.,* 2009). As a result, membranes remain intact longer, reducing the leakage of sarcoplasmic fluid into the extracellular spaces and maintaining cell integrity, thereby improving water holding capacity of meat in chicken. The numerically higher WHC of the meat in VE+Se supplemented group is a good indicator that the meat has high juiciness as observed in the sensorial analysis. This research is suggestive that VE+Se supplementation has a beneficial effect on the quality of meat (Juiciness, meaty flavour, overall flavour) and in combination with selenium, may be more effective in improving the antioxidative defence system of cells and tissues, as the protective effect of *a*-tocopherol against oxidation and its concomitant influence on sensory attributes of the meat is dependent on the dietary level added into the diet.

**Table 3: Effects of VE+Se on Sensorial profile, WHC and WAC of meat**

| Parameter | Control (0mg) | 100mg VE+0.05 mg Se | 200mg VE+0.1 mg Se | 300mg VE+0.15 mg Se | 400mg VE+0.2 mg Se | SEM | P-Value |
|---|---|---|---|---|---|---|---|
| Colour | 6.05 | 5.62 | 5.90 | 6.00 | 6.14 | 0.10 | 0.5600 |
| Juiciness | 6.48$^{a}$ | 5.05$^{c}$ | 5.52$^{b}$ | 6.29$^{a}$ | 6.95$^{a}$ | 0.17 | 0.0010 |
| Meaty flavour | 6.14$^{b}$ | 5.10$^{c}$ | 5.43$^{c}$ | 6.14$^{b}$ | 6.71$^{a}$ | 0.15 | 0.0060 |
| Tenderness | 6.71$^{b}$ | 5.71$^{c}$ | 5.76$^{c}$ | 6.52$^{b}$ | 7.05$^{a}$ | 0.13 | 0.0010 |
| Saltiness | 6.05 | 5.48 | 5.90 | 5.95 | 6.29 | 0.12 | 0.2990 |
| Overall flavour | 6.52$^{a}$ | 5.67$^{b}$ | 5.95$^{b}$ | 6.52$^{a}$ | 7.10$^{a}$ | 0.15 | 0.0270 |
| Overall acceptability | 6.61 | 5.56 | 6.61 | 6.50 | 6.78 | 0.16 | 0.1150 |
| WHC | 31.78 | 34.22 | 33.11 | 30.89 | 35.11 | 0.66 | 0.2520 |
| WAC | 44.04 | 45.92 | 62.43 | 47.11 | 54.59 | 2.99 | 0.2830 |

$^{a, b, c;}$ Values in a row not sharing a common superscript are significantly different (P<0.05).
SEM: Standard Error of Mean.

The dietary supplementation of VE+Se was not significant ($p>0.05$) on all colour intensities of anterior section of meat (Table 4) except b* (yellowness). Yellow (b*) intensity was highest from 100 mg VE+0.05 mg Se and 400 mg VE+0.2 mg Se group. Although, the expression of yellowness did not follow any trend, it however agrees with Miezeliene *et al*., (2011) who noticed an upsurge in yellowness (b*) with increased supplementation of Se from 0.15 to 0.5 mg with basal VE of 40 mg, but divergent to Kim *et al* (2010). Using a lightness (L) value of 53 as a cut-off to make distinction between pale and normal poultry meat as Castromán *et al*., (2013) all samples in the current study were within normal range with values for 400 mg VE+0.2 mg Se group numerically lower compared to other groups (Table 4).

Values for L, a, b and L* intensities of breast meat were significantly influenced by the dietary supplementation of VE+Se in diets (Table 4). All significant parameters did not follow any particular pattern. Least values for L and L* (lightness) (49.38, 56.44) intensities were observed from the control group with meat from other VE+Se supplemented groups lighter in colour. A higher a (redness) intensity was observed in the control group while least and statistically similar intensities were observed in VE+Se treatment groups (Table 4). Meat colour is the foremost and primary criterion used to ascertain the quality and acceptability of meat by consumers (Mancini and Hunt, 2005). Poultry meat has been classified as white because of its pink colour, therefore the sorting of its colour parameter ratio to establish nicotinamide haemochrome (pink colour defect). Sole and combined usage of VE and Se by Kim *et al*. (2010) and Ryu *et al*. (2005) revealed no effect on meat surface colour; this is contrary to observations in the current study which resulted in increased lightness and decreased redness intensities. The observed changes in some colour intensities negates the report of Miezeliene *et al*., (2011) who detected a decreased lightness (L*), and increased redness (a*) and yellowness (b*) intensities as a direct consequence of supplementation with Se. The meat colour intensities observed may be due to the synergistic effect of both vitamin E and selenium as the supra-nutritional dietary levels of sole vitamin E has been revealed to improve and positively affect WHC and coloration of meat (Jensen *et al*., 1998). An increasing supplementation of vitamin E above recommended levels has been documented to improve the meat quality by decreasing the oxidation of lipids in the muscle resulting in the discoloration (lightness) of the meat by delaying the oxidation of myoglobin (Salami *et al.,*2015; Bellés *et al.,* 2018). The lightness range in the current study anterior (L: 49.73 - 53.65) and posterior (49.38-55.42) fell within the range (L=53-57) reported by Castromán *et al.* (2013) and lower than the Lightness limit (60) used by Van Laack *et al*., (2000) for pale poultry breast meat. Comparison between the colour intensity of the anterior to the posterior section of the meat shows a reduction in redness and yellowness in the latter in all groups, reduction in lightness in the control but with an increase in the VE+Se supplemented groups. Since most perception of appearance is based on the anterior, colour intensities observed will influence consumer preferences.

**Table 4: Effect of Dietary Supplementation of Vitamin E on Colour of Breast Meat of Broiler Chicken**

| Parameter | Control (0mg) | 100mg VE+0.05 mg Se | 200mg VE+0.1 mg Se | 300mg VE+0.15 mg Se | 400mg VE+0.2m g Se | SEM | P-Value |
|---|---|---|---|---|---|---|---|
| **Anterior Section** | | | | | | | |
| L | 50.69 | 53.65 | 50.12 | 52.29 | 49.73 | 0.65 | 0.30 |
| a | 13.42 | 13.96 | 13.47 | 10.53 | 11.26 | 0.52 | 0.12 |
| b | 7.39 | 10.02 | 8.04 | 6.96 | 9.69 | 0.43 | 0.06 |
| L* | 57.74 | 60.52 | 57.19 | 59.29 | 56.80 | 0.62 | 0.30 |
| a* | 15.28 | 15.62 | 15.39 | 11.99 | 12.99 | 0.57 | 0.14 |
| b* | 9.57$^{c}$ | 12.95$^{a}$ | 10.48$^{b}$ | 8.81$^{c}$ | 12.93$^{a}$ | 0.58 | 0.04 |
| **Posterior Section** | | | | | | | |
| L | 49.38$^{c}$ | 54.53$^{ab}$ | 50.26$^{bc}$ | 55.42$^{a}$ | 53.05$^{abc}$ | 0.71 | 0.0079 |
| a | 13.92$^{a}$ | 11.27$^{b}$ | 11.45$^{b}$ | 10.10$^{b}$ | 10.98$^{b}$ | 0.38 | 0.0080 |
| b | 6.68 | 5.87 | 5.51 | 6.97 | 5.80 | 0.25 | 0.3070 |
| L* | 56.44$^{c}$ | 61.42$^{a}$ | 57.33$^{b}$ | 62.26$^{a}$ | 60.01$^{a}$ | 0.68 | 0.0023 |
| a* | 15.92$^{a}$ | 12.66$^{b}$ | 13.16$^{b}$ | 11.33$^{b}$ | 12.44$^{b}$ | 0.57 | 0.0020 |
| b* | 8.76 | 7.22 | 6.98 | 8.39 | 7.20 | 0.34 | 0.3625 |

$^{a,\ b,c}$ Means not followed by the same superscript are significantly different ($P<0.05$) along the row
**SEM**: Standard Error of mean

The supplementation of VE+Se had no significant ($p>0.05$) effect on proximate composition of meat (Table 5). This result is consistent with Körösi-Molnar *et al.* (2004) who found no substantial impact of selenium or vitamin E on protein and ash content fractions. So also is Ševčikova *et al.*, (2006) on crude protein and fat when different selenium sources were fed in diets to broiler chickens. Tayeb and Qader (2012) reported a positive effect of combination of vitamin E and selenium on chemical composition in breast meat (protein percentage), however, the range of values obtained in the study except 300 mg VE+0.15 mg Se were within the range reported (22.18 - 23.44).

**Table 5: Proximate composition of breast meat from broiler chickens fed diets containing supplemented with VE+Se**

| Parameter (%) | Control (0mg) | 100mg VE+0.05mg Se | 200mg VE+0.1mg Se | 300mg VE+0.15mg Se | 400mg VE+0.2mg Se | SEM | P-Value |
|---|---|---|---|---|---|---|---|
| Dry Matter | 25.75 | 25.24 | 25.88 | 23.84 | 24.40 | 0.55 | 0.8240 |
| Ether extract | 2.55 | 2.44 | 2.50 | 2.28 | 2.40 | 0.06 | 0.7390 |
| Ash | 0.71 | 0.70 | 0.71 | 0.57 | 0.62 | 0.03 | 0.6940 |
| Crude Protein | 22.50 | 22.11 | 22.68 | 20.99 | 21.43 | 0.46 | 0.8400 |

**SEM** – Standard Error of Mean

## CONCLUSION

In conclusion, supplementation of extra dietary vitamin E and selenium up to 400 mg Vitamin E+ 0.2 mg Selenium in poultry diets has been shown to improve prime cuts (thigh, breast and drumstick); improve consumer perception through improved sensorial attributes (juiciness, meaty flavour, tenderness, overall flavour) of meat and also influence meat colour intensity (L, a, L*, a*, b*) However, supplementation with VE+Se did not have additional effect on meat proximate composition.

## AUTHORS CONTRIBUTION

This work was financed by all authors indicated. This study was concepted and carried out under the advice and guidance of Ekunseitan D.A. and Ekunseitan, O.F. The field-work was carried out by Alagbada I.M. Asa J.O. and Alao, O.A. (undergraduate students). Data compilation, statistical analysis and manuscript revision was done by Ekunseitan D.A., Ekunseitan O.F. and Adegun W.O. Laboratory analysis was conducted and supervised by Ekunseitan O.F. and Ekunseitan D.A. All authors read and approved the final manuscript.

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# MARKETING ANALYSIS OF FLUTED PUMPKIN (*TELFAIRIA OCCIDENTALIS* HOOK F.) IN ALIMOSHO LOCAL GOVERNMENT AREA, LAGOS STATE, NIGERIA

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## ABSTRACT

Fluted pumpkin is a Non-Timber Forest Product (NTFP) of high importance to the socioeconomic life of the rural and urban dwellers. This study analyzed the marketing of Fluted Pumpkin (*Telfairia occidentalis Hook*) locally known as '*ugu*' in different market locations in Alimosho Local Government Area, of Lagos State, Nigeria. Descriptive Statistics was used toestimate the socio-economic characteristics while inferential statistics was used to analyze the factors affecting the marketing of Fluted pumpkin (*Telfairia occidentalis Hook*) in the study area. Data were collected through the use of well-structured questionnaire administered to Fluted pumpkin traders in five (5) selected markets in Alimosho Local Government Area. These included Ayobo, Iyana-Ipaja, Dopemu, Ikotun and Igando markets. 200 copies of questionnaire comprising of 40 in each market were administered to the traders. Socio- economic characteristics of *Telfairia occidentalis* traders and marketing variables such as transportation cost, rent, labour cost, selling price, cost price, market tax, among others were collected and analysed. The results showed that *ugu* sellers were involved in both wholesale (47.4%) and retail (42.5%) marketing which implied that there were more wholesalers than retailers which was attributed to purchase of *ugu* in bulk. Marketing of Fluted pumpkin is gender sensitive; all 100% of the respondents were women; 56.8% had primary education andwere married. They all sourced their capital from personal savings. Profitability analysis acrossthe five markets revealed that it was highest (₦25,669.74) in Iyana Ipaja and least (₦20,785.53) in Ayobo market. Total revenue across markets revealed that it was highest (₦106, 881.58) in Iyana Ipaja market and lowest (₦94, 782.90) also in Ayobo market. The marketing efficiencyof the respondents in each market revealed that Iyana Ipaja is the most efficient (129.99%) which indicated that for every ₦100 invested in Ugu market there is about ₦30 profit. It was concluded from the study that the trade of Ugu is a profitable venture in Alimosho Local Government Area and also capable of providing employment to people. Hence, there is need for enlightenment program on how to improve the profitability of *Telfairia occidentalis* through efficient marketing.

**Keywords:** Non-Timber Forest Product (NTFP), Marketing Efficiency, Profitability.

## INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis*, Hook F.) is one of the most important vegetables cultivated in Nigeria. It is generally referred to as a leaf and seed vegetable. The leaf has highnutritional, medicinal and industrial values being rich in protein, fat, minerals and vitamins (Ndor *et al.*, 2013). It is locally called

Ugu in Igbo land, Eweroko in Yoruba, Kabewa in Hausa,and Ikong-Ubong in Efik. *Telfairia* is a greenish leafy vegetable that is found in West Africa but it is mostly grown in various parts of southern Nigeria. It is a valuable leafy vegetable, indigenous to the south eastern Nigeria. Among the important indigenous vegetables, *Telfairia occcidentalis* from the family cucurbitaceae is widely consumed in Nigeria and cultivated for its edible succulent shoots and leaves as a garden crop mainly by the Igbo tribe. With the migration of Igbos to other parts of Nigeria. *Telfairia* is now grown in almost every part of the country (Akoroda, 1990). In the middle belt region of Nigeria, *Telfairia* is now being cultivatedboth as a garden crop and also as a commercial crop during both rainy and dry seasons.

The importance of plant seeds, particularly in the diet of people in the developing countries isgrowing increasingly for various reasons. First, the seed has nutritive and calorific values, which makes them essential in diets as good sources of protein, edible oils and fat. The seeds are also a capable source of raw materials for local industries, especially in the oleo chemical and animal feed industries (Christian, 2007). *Telfairia* contains calcium, iron, potassium, andmanganese. Fluted pumpkin leaves are a good source of dietary fibre that keeps the digestive system healthy. It also offers a good amount of vitamins A, B2, C and E. These vitamins help in maintaining cells, tissues, membranes, and also the skin and treat wounds. It is also recommended for patients who are suffering from low blood production due to the important minerals that help boost the blood in the body system. Iron, being the essential mineral in the red blood cell can be so effective when there is insufficient blood circulation in the body. As aresult of this, fluted pumpkin has been used to increase the level of blood in the body system. Vegetables are generally effective for weight loss and fluted pumpkin leaves cannot be overlooked due to their high dietary fibre content which helps to lose weight and lower appetite. It also contains little or no calories which completely reduces the chances of storing calories in the body (www.finelib.com).

Vegetables are suitable in the farming system since they are generally short-duration crops, which makes them suitable for mixed cropping, association and intercropping. Fluted pumpkin germinates 10 days after planting and can be harvested two to four weeks after planting or when the stems are long. Therefore, this leads to high cropping intensity and higher income perunit area. Fluted pumpkin can also be a source of supplementary income to farmers and can begrown successfully as intercrop alongside trees, therefore, yielding more profits from forest plantation. In establishing a fluted pumpkin plantation, labour is required which could serve as a source of employment. In this case, there are involvements of people on the large-scale production of fluted pumpkin (Agropedia, 2009).

As a result of the high nutritional, medicinal and economic value of fluted pumpkin, there is aneed for information on its trade and potential to enhance the livelihood among the inhabitants of both rural and urban areas. Lack of information on the marketing and low sale of non-timberforest products make them undervalued in the agricultural commodity commodity markets (Opabode and Adeboye, 2005).

Aiyelaagbe and Kintomo (2002) reported that the major reason for the low profitability

of non- timber forest products (NTFPs); *Telfairia occidentalis* for example, is the absence of an organized information system about the importance of NTFPs which is to help individual producer and marketer organize production, distribution and marketing of their products. Also, production of *Telfairia occidentalis* has been insufficient to meet up with the demand of consumers which is probably due to a lack of information on its economic importance and marketing efficiency. *Telfairia occidentalis* is a non-timber forest product that provides food both for human and animal consumption; for medicinal purposes; for aesthetic values; and most importantly, to generate income so as to sustain the livelihood of people within and outside the forest communities.

Regardless of the importance of non-timber forest products in Nigeria, and Lagos State, in particular, markets for non-timber forest products most especially *Telfairia occidentalis*, which add value at the local level are not well informed. Despite their high degree of importance, they are still classified as minor in the forest.

The Economics of both rural and urban areas can rely on non-timber forest products to generate income, food and medicine; As a result of this, there is a need to place more emphasis on the benefits of the natural renewable earner. It becomes relevant that the study analyses the marketing of *Telfairia occidentalis* for livelihood sustenance in Alimoshso Local Government Area in Lagos State**.** The main objective of this study was to analyse the marketing of *Telfairia occidentalis* (Fluted pumpkin) in Alimosho Local Government Area, Lagos state, Nigeria. Specifically, the study aimed at:

- describing the socio-economic characteristics of the marketers of *Telfairia occidentalis*
- estimating the profitability of *Telfairia occidentalis* in the study area.
- determining the marketing efficiency of the respondents in the selected markets.
- identifying the constraints involved in the marketing of *Telfairia occident*

## METHODOLOGY

Alimosho is a Local Government Area in Lagos State, Nigeria with the largest population of about 3,082,900, according to population 2019-projection (Metro Lagos, 2022). The 2006 Census claimed the population was 1,288.714 but the Lagos State Government argued that the population as at 2006 within the LGA was more than 2 million residents (Fagbohun *et al*, 2020; Alimosho LGA, 2022). Alimosho occupies coordinates 6036`38``N 3017`45``E. It has now been subdivided into several Local Community Development Areas (LCDA). Majority of the peopleliving in these areas are predominantly Aworis and Egbados while the main occupation of its settlers is peasant farming.

Simple Random Sampling Technique was adopted to select 40 fluted pumpkin sellers from each of the five (5) purposively selected markets which gave a total of 200 respondents for thisstudy. The markets selected were Ayobo, Dopemu, Iyana-Ipaja, Ikotun and Igando. They were purposively selected because they are the major markets in Alimosho LGA of Lagos State where sales of Fluted pumpkin are predominant.

Descriptive tools such as frequency, means, mode and percentages were used to analyze the socio-economic variables. The budgetary technique was used to estimate the cost and returns of fluted pumpkin marketing in the study area.

Variable costs (VC) consist of Labour cost, Rent cost, Transportation and market tariffs. Fixed costs (FC) included the cost of bags and baskets used for storage.

$TC = TVC + TFC$---------- ----------Equation. 1

Where:

TC= Total cost

TVC= Total variable cost

TFC= Total fixed cost

$GP = TR - TVC$ ---------- Equation 2

Where:

GP = Gross Profit

TR = Total revenue given as Py*Y

Where Py is price per unit of product

Y = Product

$NP = GP - TFC$ …….Equation 3

Where:

NP = Net profit

TFC= Total fixed cost.

The multiple linear regression model was used to determine the factors that contributed to the selling price of *Telfairia occidentalis* in the study, the model specification was given as

$Y = a + bX_1 + bX_2 + bX_3 + bX_4 + e_i$ ------- Equation 4

Where:

Y= the selling price

$X_1$= Labour cost (₦)

$X_2$= Rent cost (₦)

$X_3$= Transportation cost (₦)

$X_4$ = Market tariffs (₦)

e= Error terms

b1, b2 .....Co - efficient of independent variables and are the estimated parameters.

This implied that an inverse relationship exists between transaction costs and quantity of Flutedpumpkin supplied by marketers.

**Profitability Ratio**

Rate of Returns on investment

$$(\text{RORI }\%) = \frac{TR - TC}{TC} \times \frac{100}{1} \quad \text{---------- Eq. 5}$$

**Analysis of marketing efficiency**

$$\textbf{ME} = \frac{Total\ sales}{Total\ marketing\ cost} \times \frac{100}{1} \quad \text{---------- Eq. 6}$$

## RESULTS

The socioeconomic characteristics of the respondents revealed that all the respondents are females, (Table 1). This might be attributed to the fact that selling of vegetables such as Ugu requires a little effort which makes it convenient for women. This agreed with the findings of Agbugba, (2003) that reported women are key players in the marketing of indigenous leafy vegetables. Majority (71.6%) of the women's age ranged between 30 and 50 years. This agreed with the findings of Yohanes, (2015) that the age structure of most practitioners of vegetable marketing are active and middle-aged dominated. The variation in age brackets across the markets was further illustrated in figure 1 where age group < 20 years had the lowestpercentages in all the five markets whereas Ayobo and Ikotun markets had highest percentages(42.1% and 36.8% respectively) for 31-40 years while Dopemu, Iyana-Ipaja and Igando markets had highest percentages (42.1%, 39.5% and 42.1% respectively) for age group 41-50 years. The mean household size is 7 as illustrated in figure 2 which showed that all respondentshad between 6-7 persons in their households. This is due to their understanding that a larger household size will bring about cheaper labour which they can rely on in supporting their businesses.

**Level of Education:** Averagely, more than half (56.8%) of *Ugu* sellers had primary school education (figure 3) This may be attributed to the fact that primary school education is sufficient in enabling them to read and write which can be used for their business activities. This agreed with the discovery of Agbugba *et al.*, 2017 who indicated that the majority of the respondents in the study area had primary education (37%), followed by those with no formal education (34%), respondents with secondary education (17%), and those with tertiary education (2%).

**Ethnic Group**: Majority (85.8%) of the women were Ibo due to the fact that *Ugu* is associated to be a south-eastern vegetable (Fig. 4).

The socioeconomic variables shown in Table 1 are further illustrated with the following figures:

# RESULTS AND DISCUSSIONS

## Table 1: Socioeconomic Characteristics of 'Ugu' Traders

| Variables | Ayobo | | Dopemu | | Iyana-Ipaja | | Ikotun | | Igando | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Freq. | % | Freq. | % | Freq. | % | Freq. | % | Freq. | % |
| **Age** | | | | | | | | | | |
| Less than 20 | 2 | 5.3 | 2 | 5.3 | 2 | 5.3 | 2 | 5.3 | 1 | 2.6 |
| 21-30 | 3 | 7.9 | 8 | 21.1 | 4 | 10.5 | 7 | 18.4 | 5 | 13.2 |
| 31-40 | 16 | 42.1 | 10 | 26.3 | 12 | 31.6 | 14 | 36.8 | 12 | 31.6 |
| 41-50 | 13 | 34.2 | 16 | 42.1 | 15 | 39.5 | 12 | 31.6 | 16 | 42.1 |
| Above 50 | 4 | 10.5 | 2 | 5.3 | 5 | 13.2 | 3 | 7.9 | 4 | 10.5 |
| Total | 38 | 100 | 38 | 100 | 38 | 100 | 38 | 100 | 38 | 100 |
| Mean | 43 | | 46 | | 42 | | 51 | | 49 | |
| **Sex** | | | | | | | | | | |
| Male | - | - 100 | - 38 | - 100 | - | - 100 | - | - 100 | - | - 100 |
| Female | 38 | 100 | 38 | 100 | 38 | 100 | 38 | 100 | 3 | 100 |
| Total | 38 | | | | 38 | | 38 | | 8 | |
| | | | | | | | | | 38 | |
| **Household size** | | | | | | | | | | |
| 3 - 4 | 14 | 36.8 | 20 | 52.6 | 14 | 36.8 | 18 | 47.4 | 16 | 42.1 |
| 6 - 7 | 20 | 52.5 | 16 | 42.1 | 19 | 50.0 | 17 | 44.7 | 18 | 47.4 |
| 8 and above | 4 | 10.5 | 2 | 5.3 | 5 | 13.2 | 3 | 7.9 | 4 | 10.5 |
| Total | 38 | 100 | 38 | 100 | 38 | 100 | 38 | 100 | 38 | 100 |
| Mean | 7 | | 6 | | 5 | | 5 | | 6 | |
| **Level of education** | | | | | | | | | | |
| None | 1 | 2.6 | 3 | 7.9 | 1 | 2.6 | 3 | 7.9 | 1 | 2.6 |
| Primary school Sec- | 22 | 57.9 | 20 | 52.6 | 23 | 60.5 | 22 | 57.9 | 21 | 55.3 |
| ondary schoolTertiary | 14 | 34.2 | 13 | 34.2 | 11 | 28.9 | 12 | 31.6 | 13 | 34.2 |
| school Total | 1 | 5.3 | 2 | 5.3 | 3 | 7.9 | 1 | 2.6 | 3 | 7.9 |
| | 38 | 100 | 38 | 100 | 38 | 100 | 38 | 100 | 38 | 100 |
| **Ethnic group** | | | | | | | | | | |
| Yoruba | 6 | 15.8 | 5 | 13.2 | 6 | 15.8 | 4 | 10.5 | 6 | 15.8 |
| Ibo | 32 | 84.2 | 33 | 86.8 | 32 | 84.2 | 34 | 89.5 | 32 | 84.2 |
| Hausa | - | - 100 | - 38 | - 100 | - | - 100 | - | - 100 | - | - 100 |
| Total | 38 | | | | 38 | | 38 | | 3 | |
| | | | | | | | | | 8 | |

Note: Freq. - Frequency

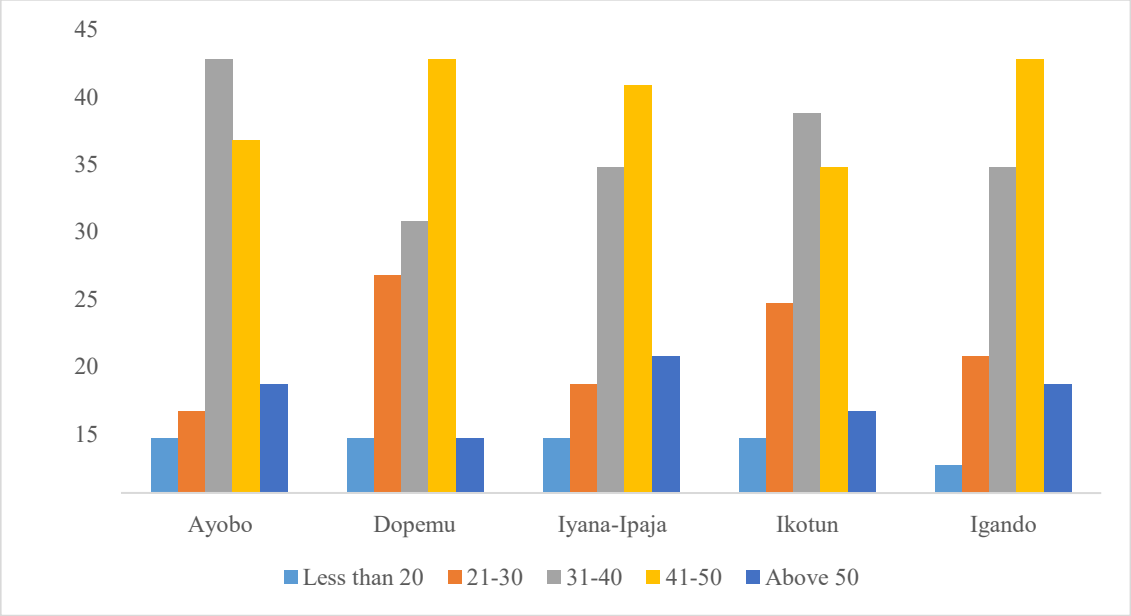


**Fig. 1: Age Distribution across the Five Markets**

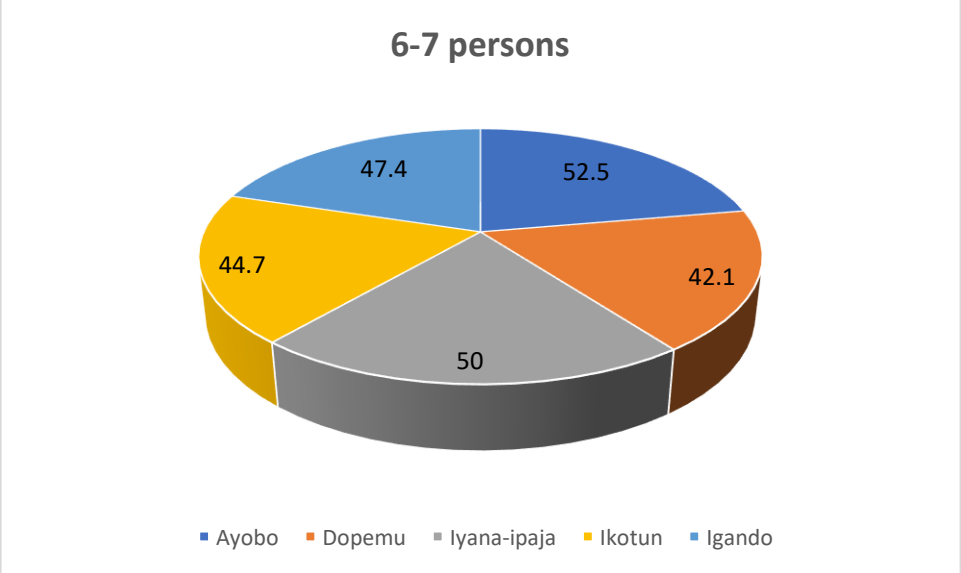


**Figure 2: Household Sizes Across the Five Markets**

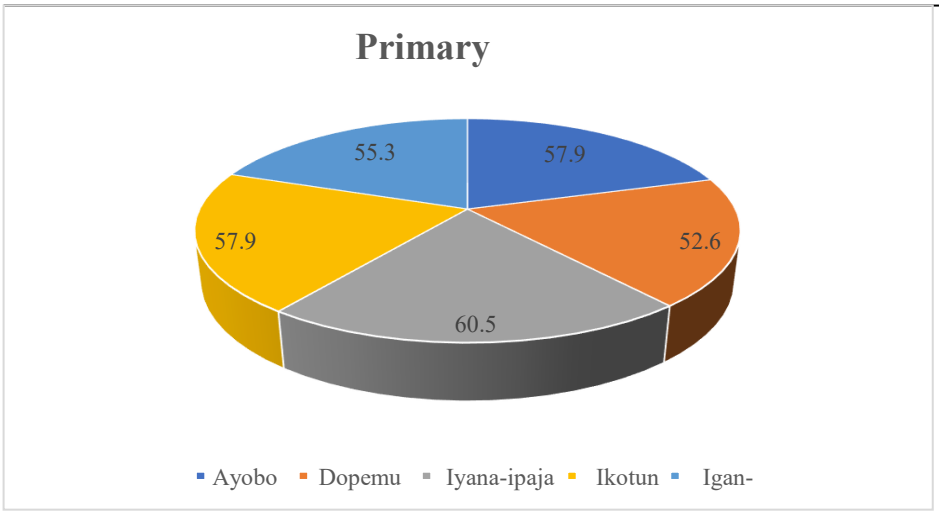


**Figure 3: Level of Education**

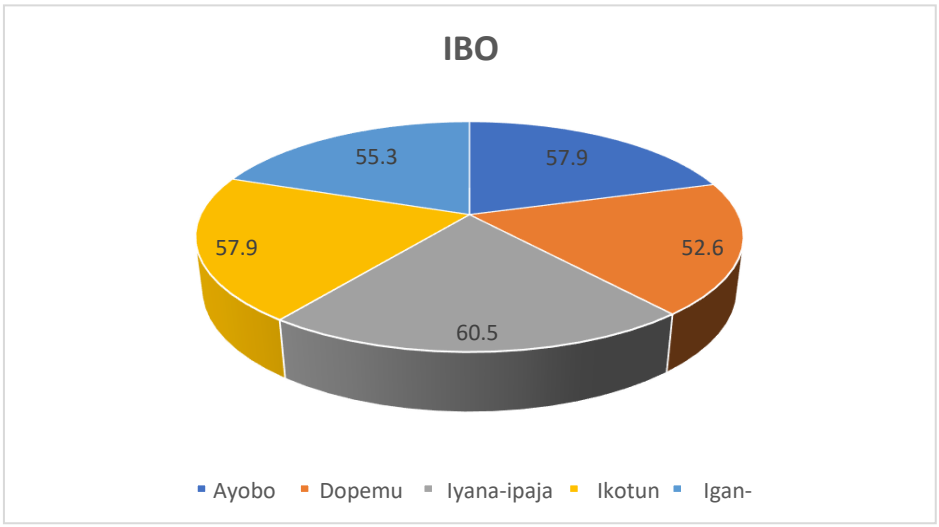


**Figure. 4: Ethnic Group**

**Table 2: Profitability of Ugu (Fluted pumpkin)**

| Variable | Ayobo | Dopemu | Iyana Ipaja | Ikotun | Igando |
|---|---|---|---|---|---|
| Total cost | 75,007.58 | 78,397.62 | 82,222.58 | 73,140.52 | 79,143.22 |
| Total Variable cost | 73,997.37 | 77,386.84 | 81,211.84 | 72,130.26 | 78,131.58 |
| Total Fixed Cost | 1,010.21 | 1,010.78 | 1,010.74 | 1,010.52 | 1,011.64 |
| Total Revenue | 94,782.90 | 100,328.95 | 106,881.58 | 95,065.79 | 101,210.53 |
| Gross profit | 20,785.53 | 22,942.11 | 25,669.74 | 22,935.53 | 23,078.95 |
| RORI | 26.36 | 27.97 | 29.99 | 29.97 | 27.88 |

The profitability of the respondents revealed that Iyana-Ipaja had the highest values of all the variables while Ayobo had the lowest (Table 2). This implies that Iyana-Ipaja has the highest number of Ugu buyers which could be as a result of their dominant population. Therefore, Ugu marketing is profitable.

**Table 3: Marketing Efficiency of the Respondents in each market**

| Market | Marketing efficiency (%) |
|---|---|
| Ayobo | 126.36 |
| Dopemu | 128.04 |
| Iyana-paja | 129.99 |
| Ikotun | 129.97 |
| Igando | 127.88 |

The result of the marketing efficiency of the respondents in each market revealed that Iyana Ipaja is the most efficient (129.99%) which indicated that for every ₦100 invested in Ugu market there is about ₦30 profit (Table 3). This suggested that Ugu market is profitable for the sellers. Afolabi, (2007) made a similar observation in his marketing of selected food items in South-western Nigeria indicating that a vegetable marketer with a ₦1000 increase in the ₦3,343.

**Table 4: Regression analysis showing the factors affecting the marketing of ugu**

| Variables | Coefficients | Standard error | p-value |
|---|---|---|---|
| Constant | 92.261 | 19.244 | 0.000 |
| Transportation cost | 0.079 | .019 | 0.000 |
| Market tax | -0.369 | .082 | 0.000 |
| Rent | 0.002 | .002 | 0.384 |
| Labour | -0.047 | .017 | 0.008 |
| R-square | 0.429 | | |
| Adjusted R- Square | 0.392 | | |
| F-Value | 11.648 | | |
| P-value | 0.000 | | |

The F-value of the tested variables was 11.648 and significant at $p < 0.001$ meaning that the model is fit (Table 4). R-Square was 0.429 which means that 42.9% of the variability in the dependent variable (selling price) was jointly explained by the specified independent variablesin the model. Most of the independent variables had a positive relationship with the dependentvariable except market tax and cost of labour which had negative relationship with the dependent variable. Three variables out of four independent variables were found to be statistically significant at acceptable levels (Table 4).

Transportation cost was positively related to the selling price of ugu and statistically significant at 1% probability level. This means thata unit increase in transportation cost by ₦1 will increase the selling price of ugu by 7.9%. However, the coefficient of market tax was found to be negatively related to the selling price but statistically significant at 1%. The coefficient being 0.369 suggested that additional increase in market tax by ₦1 brought about 36.9% reductions in selling price of ugu. This couldmean that market tax on ugu marketing may be low and hence unable to increase price of uguin the market. In addition, coefficient of cost of labour was negatively related to selling price and significant at 1% implying that additional cost of labour by ugu seller will reduce selling price by 4.7% (Table 4). This suggested that most of the ugu sellers have been using family labour for their business.

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# EFFECTS OF REVIVE® ON SPERMIOGRAM OF DOG IN THE PRESENCE OR ABSENCE OF TEASER BITCH

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## ABSTRACT

There are numerous indications for collection of semen from a male dog, yet there are various limitations that have been identified with the most common method used. Two adult male and one female local dogs with mean weight of 13.4 ± 0.7 kg were used in this study. Treatment orders ($T_1$ – $T_4$) used were 10 ml placebo administered orally 30 minutes before semen collection (SC) in the presence of teaser bitch ($T_1$); 400 mg of Revive® capsules administered orally 30 minutes before SC in the presence of teaser bitch ($T_2$); 10 ml of placebo administered orally 30 minutes before SC in the absence of teaser bitch ($T_3$) and 400 mg of Revive® capsule administered orally 30 minutes before SC in the absence of teaser bitch ($T_4$). A cross over design was used in this study in which each dog acted as his own control and participated in all the treatment groups. $T_1$– $T_4$ were assigned based on complete block design wherein each dog received each treatment thrice in a week. Each dog was ejaculated using digital manipulation method. The length of time to obtain ejaculate (Collection time, CT) was recorded. Semen volume (V), Sperm concentration (C), motility (M), and percentage live sperm (L) were determined for each ejaculate, using standard methods. The results were statistically evaluated using complete block analysis of variance (ANOVA) at level of significance of P=0.05. The mean collection time was 232.5±10.2secs for all treatments, CT was lower in $T_1$ compared to $T_2$ but the difference between dogs for CT was not significant. Mean Semen volume showed statistical difference between dogs. Mean values of V, C, M and L varied between the different groups. Results of this study showed that Revive® appeared not to have an effect on any of the ejaculate characteristics.

**Key words:** Ejaculate; Placebo; Preliminary study; Revive®; Semen; Sperm

## INTRODUCTION

The indications for collecting semen from a male dog include artificial insemination, cryopreservation or diagnostic purposes and the most common method used for collection is digital stimulation (Mason, 2018). Under ideal conditions, this procedure is performed in the presence of an estrous bitch (Concannon, 2011). Although some dogs consistently give high quality semen samples when collected for semen, other dogs are more difficult to collect and give ejaculates with low numbers of sperm and other sub-optimal semen characteristics (Mason, 2018). This necessitates the use of sexual preparation in conjunction with se-

men collection. Sperm output from males of several species can be increased by supplementary sexual preparation (SSP). The use of SSP in conjunction with semen collection has been shown to optimize the number of spermatozoa in the ejaculate of rabbit, bull and stallions (Perumal, 2015). In dogs, the use of bitch in oestrus is a major method of sexual preparation used or the use of pheromones in the form of vaginal discharge from a bitch in estrus preserved on bedding or other absorbent materials (Kolster, 2018). Unfortunately, availability of teaser bitches and pheromones is frequently limited due to their monoestrous nature and clinicians are therefore forced to collect semen from dogs without sexual preparation (Kutzler, 2018). Although semen can be collected consistently from some dogs without the presence of a teaser bitch, collections done without availability of a bitch in oestrus often result into decreased semen volume and sperm count (Barber *et al.,* 2018).

Numerous pharmacologic agents have been shown to enhance male reproductive performance in many species (Kolster, 2018). Prostaglandin F2alpha ($PGF_{2}\alpha$) and Oxytocin are smooth muscle contracting drugs that have been used. $PGF_{2}\alpha$ increased the total number of spermatozoa in the ejaculate of bulls, rabbits, rams and stallions (Ungerfeld et al., 2018). Oxytocin is recognized as having endocrine and paracrine role in male reproduction. During ejaculation, a burst of oxytocin is released from the neurohypophysis into and stimulate contraction of the reproductive tract thereby aiding sperm release (Ungerfeld et al., 2016).

Revive® is a Chinese herbal product that boosts sexual performance in men. It enhances the relaxation of the corpora cavernosa and delays the lengthen period of the tunica albuginea, thereby increasing libido and sustaining erection firm enough for sexual satisfaction. Scientifically, research has proven that this product boosts the excitability of impulses from the brain and local nerves, hence allowing blood to flow in and fill the spaces within the tissues (Kedi Healthcare, 2018). The major active ingredient in Revive® is *Radix ginseng* and other herbs like *Epimedii, Fructus tribuli, Radix polygon multiflori, Cortex eucommiae, Cordyceps militaris* (Gao et al., 2020). Ginseng extracts improved sperm production in men and may have some usefulness in treating impotence. The ginsenosides, which appear to be the active components are thought to depress blood prolactin levels, thereby increasing libido. In one clinical study, 90 patients with erectile dysfunction were treated with ginseng saponins (600 mg orally per day). Treatment improved rigidity, tumescence, and libido, but not the frequency of coitus. (Bagchi et al., 2011; Lyttleton, 2013 ).

An increase in number of spermatozoa ejaculated in the dog would be of benefit when collecting semen for immediate insemination or storage. Historical work has documented an increase in the number of spermatozoa in the canine ejaculate if collection occurred in the presence of an estrous teaser bitch. However, collections done without the availability of a bitch in estrus often have decreased volumes and total number of spermatozoa (Valerie, 2009). Unfortunately, the availability of teaser bitches is frequently limited due to their monoestrous nature. There is need for a suitable pharmacologic agent as sexual preparation during collection, that could be able to increase the number of spermatozoa in the ejaculate with or without the presence of an estrous teaser bitch.

This study was carried out to determine whether the presence of an estrous teaser bitch will improve the ejaculate characteristics of dog to the same extent as administration of Revive® and whether the effects would be additive. It also sought to evaluate the effects of Revive® on the semen volume, sperm concentration, motility and percentage live sperm in the presence of an oestrous teaser bitch and the effects of Revive® on the spermiogram in the absence of an oestrous teaser bitch.

## MATERIALS AND METHODS

### *Research design*

This study was carried out in the Theriogenology unit of the Department of Veterinary Public Health and Reproduction COLVET, FUNAAB between 21st October and 21st December 2019. A cross over design was used in this study in which each dog used stood as his own control and also participated in all the treatment groups. The dogs were treated with one of two treatments 30 mins before each collection either in the presence or absence of teaser bitch. Treatments were either sterile saline( placebo) (10ml orally) or Revive® (Revive capsules,400mg orally).

### *Drug*

Revive® is in capsule form containing (*Epimedii*(80mg)*, Radix ginseng*(80mg) *Fructus tribuli*(80)*, Radix polygoni multiflora* (80mg)*, Cortex eucommiae*(40mg)*, Cordyceps militaris* (40mg)). It is supplied as 30 capsules/ bottle (Kedihealthcare, 2018). The dosage of the drug used in this study was based on the dosage recommended by the manufacturer for humans

### *Animals*

Three adult local comprising of 2 intact males and 1 intact pubertal, non-pregnant female with mean weight of 13.4 ± 0.7 kg, and age ranging between two and three years were used in this study. They were purchased from different owners who used them either for security or kept them as pet. The dogs were housed individually in concrete-floored kennels at the Veterinary Teaching Hospital, FUNAAB. They were fed once daily on household food (Spaghetti, Rice and noodles) supplemented with sufficient amount of proteins (fish) and palm oil, while water was provided *ad libitum*. They were dewormed with subcutaneous injection of 7.5% levamisole hydrochloride (Levacide®, Norbrook laboratories, Northamptonshire, United Kingdom) at the dose of 10 mg/kg, while external parasites were treated by dipping in Diazintol® (Animal Care, Nigeria) solution. Prior to onset of the study, the dogs were acclimated for 14 days in the animal containment facilities. During the acclimation period, the female dog was treated with Misoprostol (Cytotec®) by administering 200 mcg per os for one week during which vaginal smears were collected from her to stage her cycle. Thereafter, the male dogs were trained for two weeks to get them accustomed to semen collection by digital manipulation method. Two dogs that responded to training by maintaining penile erection and ejaculating were eventually used for this study (one of the male dogs though maintained penile erection but did not ejaculate). Semen was manually collected from the dogs twice weekly. Before commencement of the study, the dogs were adjudged to be clinically healthy and free of any reproductive disorders based on results of physical examinations, complete blood counts and fecal examinations.

### *Research procedure*

***Semen collection***

Ethical approval for this study was obtained from the Research Ethics Committee, College of Veterinary Medicine (FUNNAB/COLVET/CREC/2019/10/04), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The treatments given to the dogs were either sterile saline (10 ml orally) or Revive® (400 mg orally) The dogs received one of two treatments 30minutes before each collection either in the presence or absence of teaser bitch. The treatments were assigned based on complete block design such that the 2 dogs received all treatments. Four different treatment orders were designed (to ensure that treatment orders were not repeated) as stated below

- First treatment (T1) - 10 ml of sterile distilled water (placebo) administered orally 30minutes before semen collection in the presence of teaser bitch.
- Second treatment (T2) - 400 mg of Revive® capsule dissolved in 10ml of sterile distilled water) and administered orally 30 minutes before semen collection in the presence of teaser bitch.
- Third treatment (T3) - 10 ml of sterile distilled water (placebo) was administered orally 30 minutes before semen collection in the absence of teaser bitch.
- Fourth treatment (T4) - 400 mg of Revive® capsule was dissolved in 10 ml of sterile distilled water and administered orally 30 minutes before semen collection in the absence of teaser bitch.

Each dog received each of the above treatments thrice in a week (every other day of the week) before semen collection making a total of 24 collections for the two dogs. The sterile saline injections allowed each dog to stood as his own control. A period of 7 days was observed in between control and treatment. Semen was collected from each dog using digital manipulation method and length of time to obtain ejaculate from each dog was noted. The penis was vigorously massaged through the prepuce at the level of bulbus glandis until a partial erection developed, the prepuce was quickly retracted caudally past the bulbus glandis and firm constant pressure was applied to the penis behind the bulbus glandis by squeezing the penis between index finger and thumb. Digital pressure was further applied behind the bulbus glandis which constricted the penile venous return and this resulted in development of full erection displayed by maximal pelvic thrusting followed by ejaculation. The first and second (sperm rich fraction) portions of the ejaculate were collected into a sterile transparent sample bottle which was removed when the beginning of the third fraction was observed as characterized by appearance of clear prostatic fluid. Each dog was monitored following collection until the penis regressed to its ventral position and covered by the prepuce.

**Semen macroscopic evaluation**

The collected ejaculate was immediately transferred to a graduated sterile test tube. The volume, colour and composition of the ejaculate was immediately observed and recorded. The length of time to obtain ejaculate was also recorded to adjudge ease of collection for each treatment.

**Semen microscopic evaluation**

Evaluation of semen was immediately carried out following collection. This included determination of sperm concentration, percentage of motile spermatozoa, percentage of live spermatozoa.

***Sperm concentration***

Concentration was determined using modi-

fied Neubaeur haemocytometer method. Sperm suspension was diluted with cold 2.9% Na Citrate buffer in varying dilutions depending on the ejaculate. Diluted semen was mounted on the haemocytometer under the coverslip by capillary action. The spermatozoa were thereafter left for five minutes to settle before counting under light microscope. The mean number of cells counted in five squares in each counting chamber was determined; this was multiplied by dilution factor used for each ejaculate depending on the viscosity which was 1 in 20 in this study. This was then divided by volume per square multiplied by number of squares counted to obtain sperm concentration in millions per ml, this value was multiplied by volume of each ejaculate to obtain sperm concentration per ejaculate.

***Sperm motility***

Motility was assessed immediately after collection by subjective visual examination under a light microscope at 40X magnification by placing a drop of 2.9% sodium citrate (Na Citrate) buffer on a warm glass slide followed by a drop of the ejaculate covered with a cover slip. Sperm motility was expressed as percentage of total motile cells (slowly, moderately and rapidly progressive spermatozoa in the ejaculate) Albrizio et al., 2013.

***Sperm viability***

A thin smear was made from this preparation; one hundred spermatozoa were visualized in duplicate under light microscope at 1000X magnification for live spermatozoa. The average for each was calculated. Sperm viability was assessed using Eosin Nigrosin stain according to standard procedure. A drop of Eosin stain was placed on a warm glass slide in which a drop of semen was placed followed by three drops of Nigrosin stain.

***Statistical Analysis***

The results are given in terms of means and comparisons were statistically evaluated using complete block analysis of variance (ANOVA). Significant difference was acknowledged if $P \leq 0.05$. The hypothesis tested was that there was no change in the progressive motility of spermatozoa, but an increase in total number of spermatozoa, in the ejaculate, when dogs were treated with Revive® compared with placebo in the presence or absence of teaser bitch.

## RESULTS

Following all the treatments T1(treatment with placebo in the presence of teaser bitch), T2 (treatment with Revive® in the presence of teaser bitch), T3 (treatment with placebo in the absence of teaser bitch) and T4 (treatment with Revive® in the absence of teaser bitch) all the dogs ejaculated and the ejaculation processes were without any complication.

Mean collection time was 232.5±100 seconds with a range of 211.2 to 253.8 seconds (Fig.1). The mean collection time for each dog showed no significant difference between T1, T3 and T4, but the mean difference between T1 was statistically longer than T2. However, the difference between dog for collection time was not significant (Fig.2).

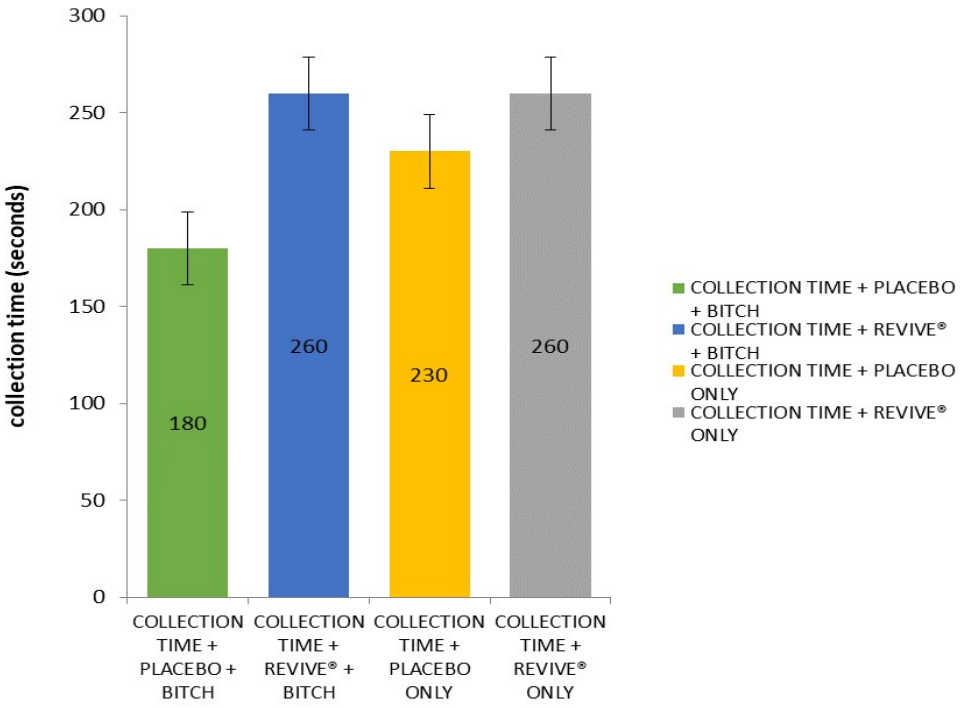


**Figure 1**: **Mean Collection Time of the Dogs**

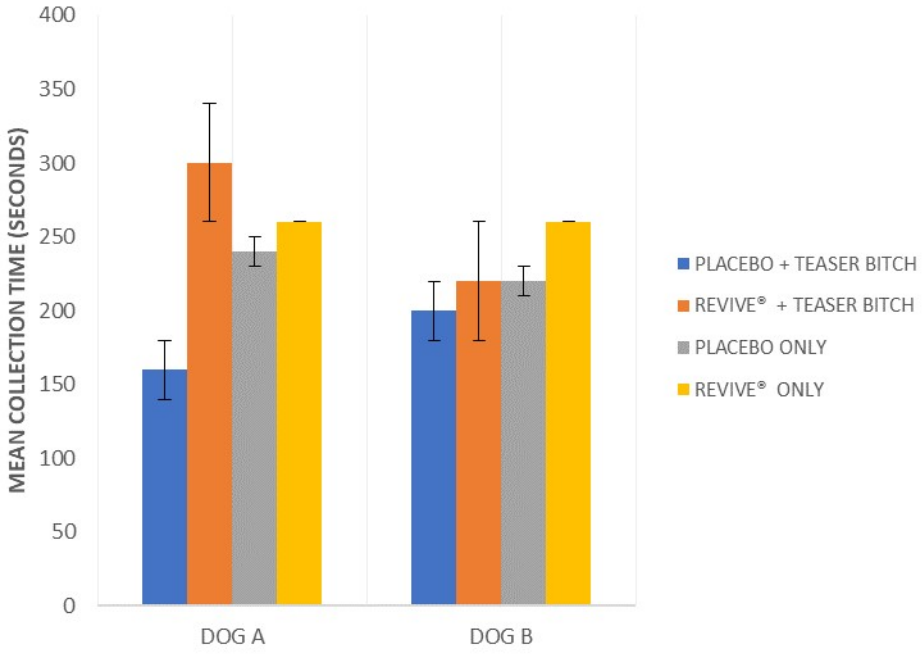


**Fig 2**: **Mean collection time for each dog**

The semen collected throughout the study was clear and did not have any debris or blood deposit. The colour of the sperm rich fraction (F2) was cloudy white, and this was the fraction that was evaluated within the first 10 minutes post-collection. Semen volume recorded for each dog showed no statistical difference between T2 and T4 compared to T1 and T3 but the difference between dogs was however significant (Fig.4). Difference between dogs was not significant (Fig.5) when measuring the mean sperm concentration in the ejaculate. There was variability in the concentration obtained for the different treatments (Fig.6) wherein T4 had the highest sperm concentration while T2 had the lowest value but they were not statistically significant.

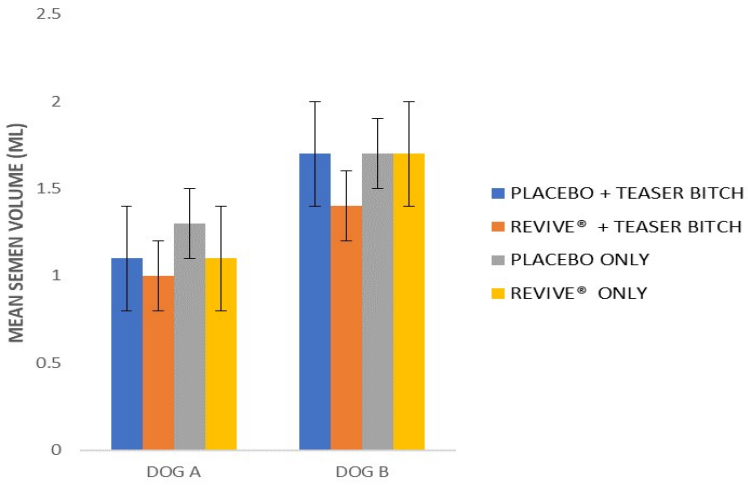


**Fig.3. Mean semen volume of the dogs**

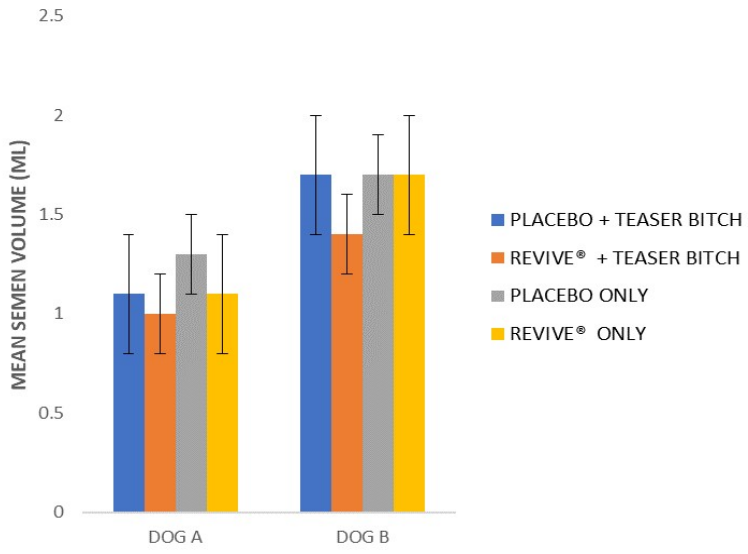


**Figure 4. Mean semen volume of each dog**

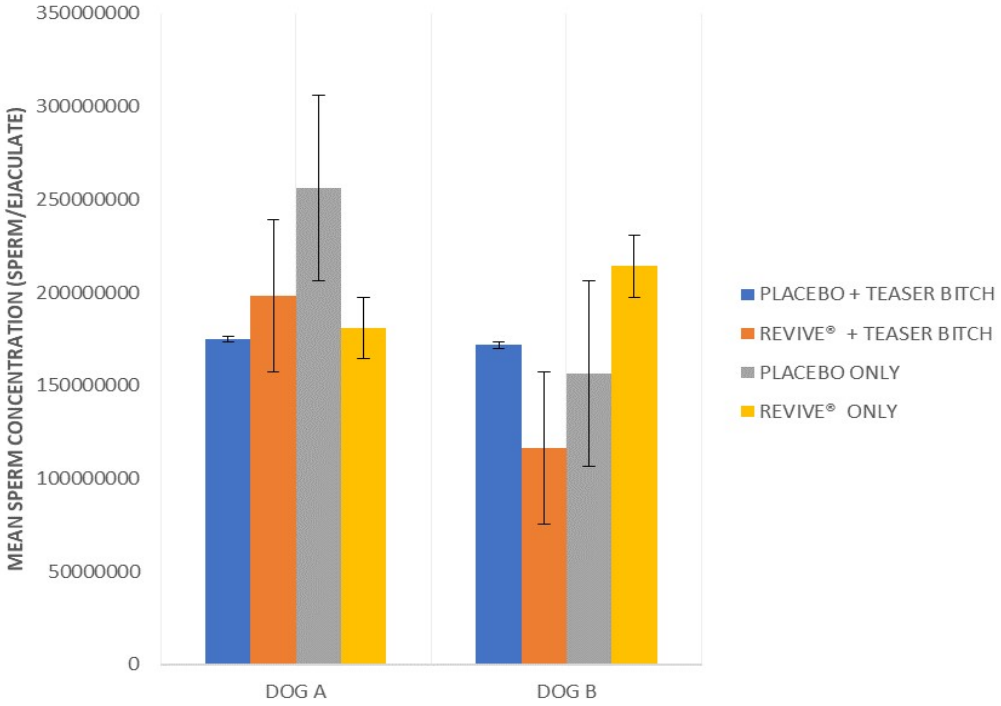


**Fig.5. Mean sperm concentration of each dog**

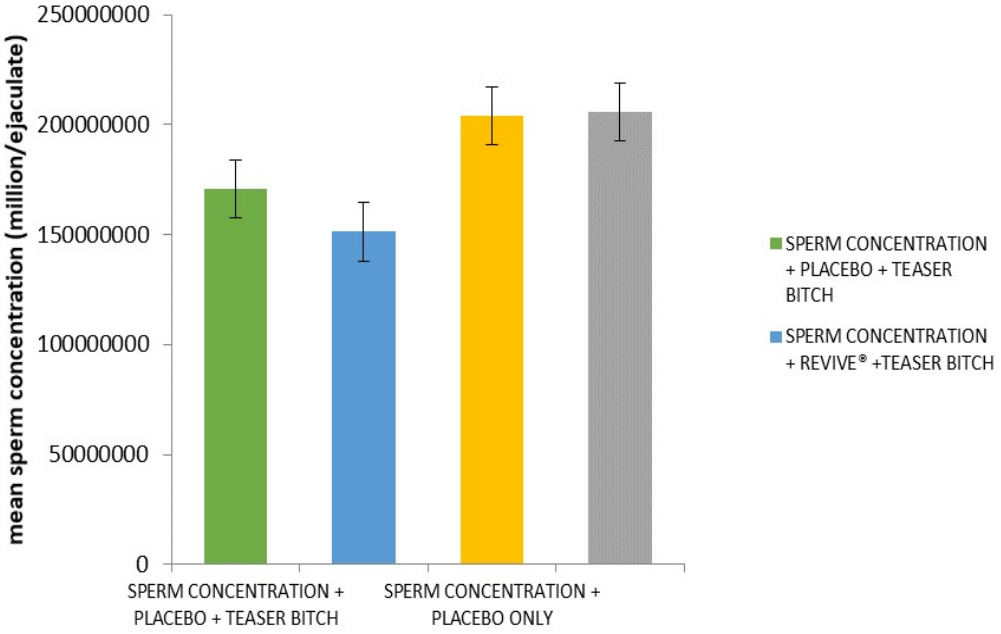


**Fig.6. Mean sperm concentration of the dogs**

The mean % live sperm was 95.3±0.8 % for all treatments in this study. (Fig.7). The treatments given did not make any significant difference on the % live sperm in the ejaculate of the dogs and the difference between dogs was also not significant (Fig.8). T2 had the highest sperm motility but there was no difference in the mean between treatments (Fig.9) and between the dogs (Fig.10).

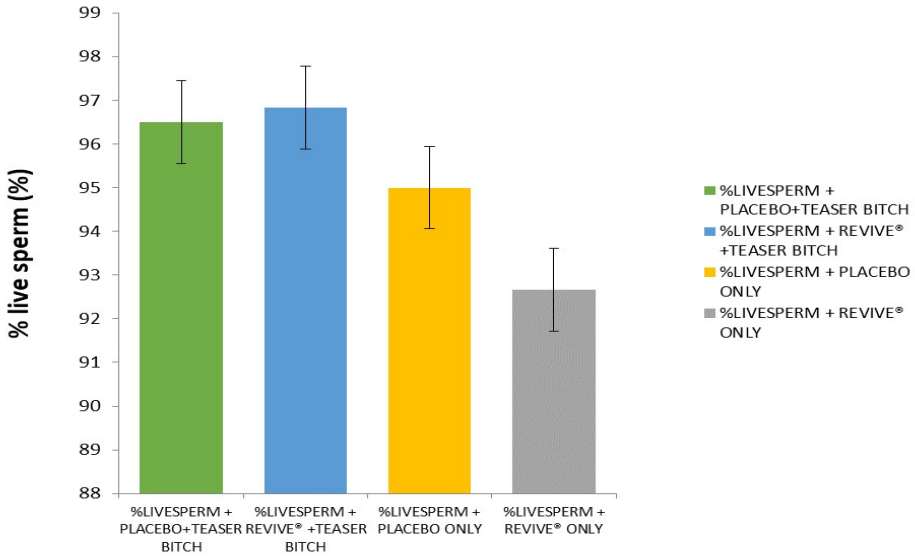


**Figure 7. Mean % live sperm of the dogs**

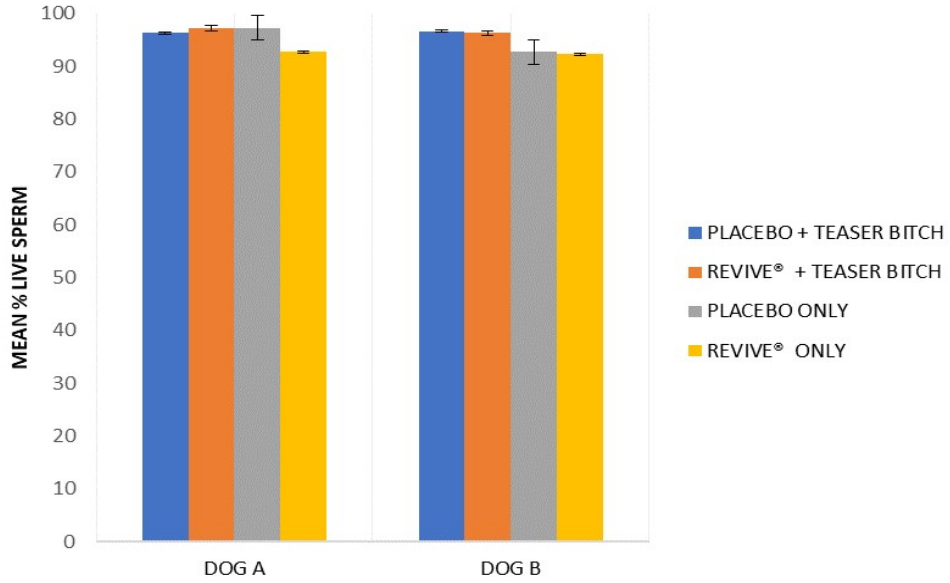


**Figure 8. Mean % live sperm of each dog**

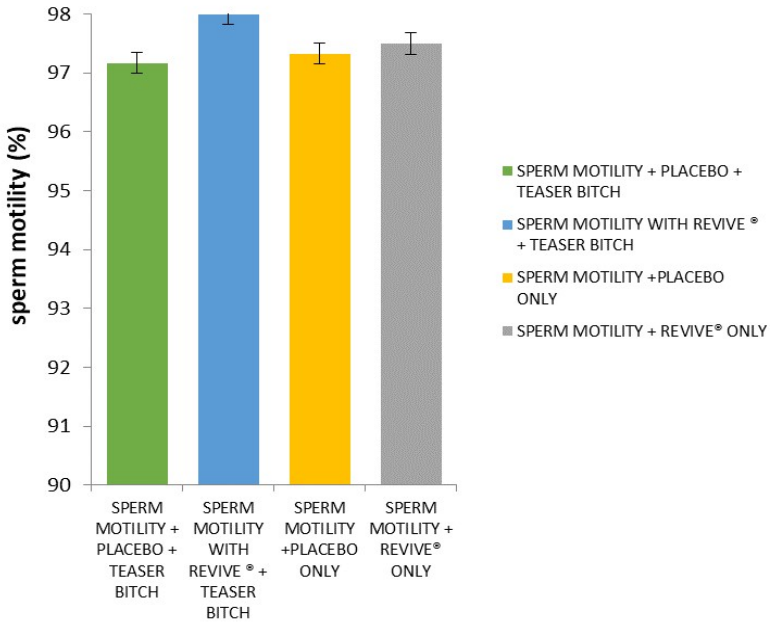


**Figure 9. Mean sperm motility of the dogs**

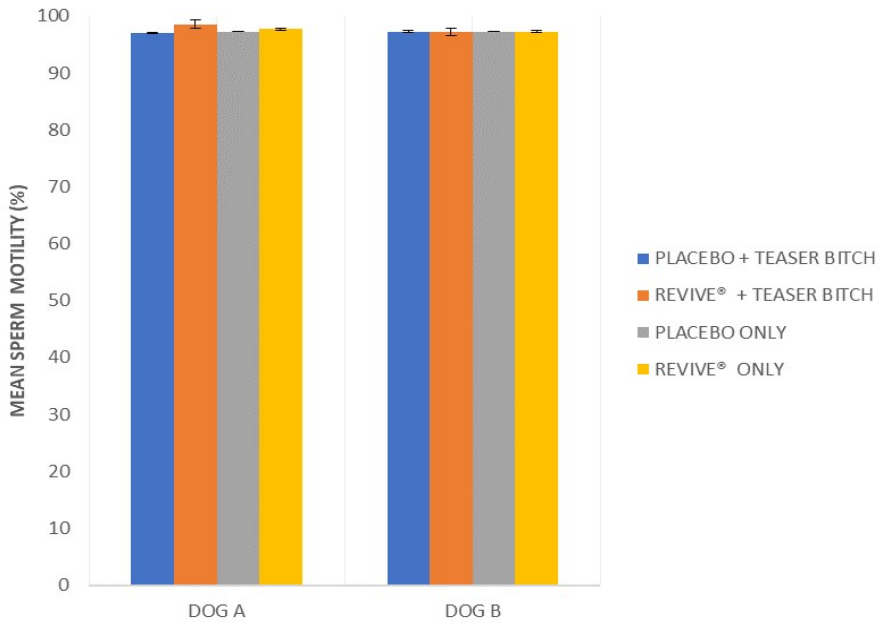


**Fig.10. Mean sperm motility for each dog**

## DISCUSSION

Results of this study showed that Revive® appeared not to have an effect on any of the ejaculate characteristics of semen when used in the manner described in this study. Revive® is a herbal product known to boost sexual performance in men (by sustaining strong and hard erection). Its constituents are mainly made up of various anti-oxidants and natural herbs known to be used for aphrodisiacs. Its mechanism of action which is by acting on impulses from blood and local nerves thus allowing blood to flow in and fill the penile tissues (Kedi HealthCare, 2018) is thought to be similar to that of smooth muscle contracting drugs (e. g. oxytocin) that have previously been used for sexual preparations before semen collection (Ungerfeld et al., 2018). The collection time that was longer in dogs treated with Revive® before semen collection whether in the presence or absence of teaser bitch may be attributed to the fact that collection was done 30 minutes following administration of Revive®. It is possible the timing was too short for exhibition of its full effect. An alternative study design in which repeated measures of collection would be done following administration of Revive® may further explain this. Multiple obstacles exist that impede a controlled clinical trial evaluating effect of neutroceutical supplementation on semen parameters in dog (Lopate, 2010). One major obstacle is difficulty finding lengthy studies and observing dogs with suitable semen quality for evaluation in a comparative trial. This was one of the major obstacles encountered in this study in which the number of dogs acquired at the onset of the study could not be used all through because one was aspermic.

## CONCLUSION

This present research was carried out as a preliminary study, further research is needed using more dogs and other techniques before the use of Revive® is considered ineffective as supplementary sexual preparation in dog semen collection.

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