# *IN VITRO* AND *IN VIVO* EVALUATION OF SIAM WEED (*Chromolaena odorata*) LEAF MEAL AS ADDITIVE IN THE DIET OF WEST AFRICAN DWARF BUCKS

**BY**

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**NOVEMBER, 2018**

## DECLARATION

I hereby declare that this Dissertation was written by me and is a correct record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of references.

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Ogadu, Chukwuemeka Bobby-Joe

Date _____

## CERTIFICATION

This Dissertation entitled "*in vitro* and *in vivo* Evaluation of Siam Weed (*Chromolaena odorata*) Leaf Meal as Additive in West African Dwarf Buck Diet" by Ogadu, Chukwuemeka Bobby-Joe meets the regulation governing the award of the Degree of Master of Agriculture Development and Sustainable Environment of the Federal University of Agriculture, Abeokuta and is approved for its contribution to scientific knowledge and literary presentation.

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**ABSTRACT**

Improved rumen and feed efficiency with reduced methane gas emission plays a key role in the sustainability and productivity of ruminants. The study which composed of two experiments evaluated the potential of Siam weed (*Chromolaena odorata*) as a modifier of rumen fermentation in West African Dwarf Bucks. Experiment 1 evaluated the chemical properties of *C. odorata* and the effect of dietary inclusion at varying levels on *in vitro* gas production, methane gas production, *in vitro* dry matter and organic matter digestibilities and post incubation parameters. Rumen fluids was collected from 25 West African Dwarf (WAD) bucks randomly allocated to 4 treatments diets with 0, 2, 4 and 6% *C. odorata* inclusion in a Completely Randomized Design. In Experiment 2, performance characteristics, nutrient digestibility, nitrogen utilization, rumen microbial count and blood chemistry of (WAD) bucks fed diets containing varying levels of *C. odorata* were investigated. Twenty five (25) WAD bucks with an average weight of 10±2kg were randomly allocated by weight to four treatment diets with 0, 2, 4 and 6% *C. odorata* inclusion for a period of 3 months. Five replicates per treatment were used for the study. Data obtained was analysed using a One-way Analysis of Variance and means compared using Duncan Multiple Range Test. Results showed that the addition of *C. odorata* to the diets significantly ($p<0.05$) increased *invitro* gas production while methane gas estimate was unaffected. Diets containing 4% *C. odorata* addition had the highest total gas output (30.67ml/200mg) and net gas output (30.37ml/200mg). *In vitro* organic and dry matter digestibilities, total digestible substrates and short chain fatty acids increased ($p<0.05$) with *C. odorata* addition to the diets and diet with 4% inclusion had highest values (31.99%, 77.08%, 154.17g and 0.15lμmol/g DM respectively). Nutrient intake, apparent digestibility of nutrients, and nitrogen utilization by WAD goats fed experimental diets were not ($p>0.05$) affected by dietary inclusion of *C.*

*odorata*. However, weight gain of experimental goats increased ($p<0.05$) with the inclusion of *C. odorata* to the diets with goats on diets with 4% inclusion having the highest value (6.20kg). Haematological parameters were not affected ($p>0.05$) by the dietary inclusion of *C. odorata*. However, serum glucose and aspartate aminotransferase increased ($p<0.05$) with the inclusion of *C. odorata*. Total anaerobic bacteria count (TABC) increased ($p<0.05$) with the inclusion of *C. odorata*. However, total protozoa and fungi counts were unaffected ($p>0.05$) by the experimental diets. This study concluded that the use of *C. odorata* as an additive at 4% inclusion can efficiently increase total gas output whilst not affecting methane emission, increased post-incubation parameters and weight gain of goats.

## DEDICATION

This Dissertation is dedicated to Almighty God who by Him this research became a reality and to my loving parents Mr. and Mrs. G.E. Ogadu.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Global warming has become a major issue for the society, agriculturist, politicians and scientists (FAO, 2009). One of the reasons to global warming are emission of greenhouse gases (GHG) such as carbon dioxide ($CO_2$), nitrous oxide ($N_2O$) and methane ($CH_4$) which agriculture largely contributes (Weiske, 2005). Animal husbandry, especially ruminants, accounts for the largest part of GHG emissions within the agricultural sector, approximately 80 % of greenhouse gas from animal husbandry is contributed by ruminants (FAO, 2008). In developed countries a lot of research has been made on feed together with long-term breeding strategy to improve the animal performance when it comes to milk production and growth (Idris *et al*., 2011). Ruminants in developing countries produce more methane per kilogram milk or meat than ruminants in developed countries. The amount of methane emission per kilogram of milk or meat produced should be reduced by increasing the animal's efficiency (Carlsson-Kanyama, 1998; Garnet 2009). A feed supplement with a high nutritional value for ruminants affecting the animal performance positively and at the same time reducing GHG emission will in the long run help people with nutritious food, increased living standard and improved economy (Goodland, 1997; Schils *et al*., 2007).

Methane is a greenhouse gas which has a global warming potential 23 times than that of carbon dioxide (Bhatta *et al*., 2007; Loh *et al*., 2008).Ruminant animals are one of the largest sources of methane emission with 81–92 million tons produced per year globally which is equivalent to 23–27% of total anthropogenic methane (IPCC, 2007). Methane produced from ruminants are through two major process; rumen fermentation and anaerobic decomposition of livestock waste. Enteric methane is produced by

methanogenic archaea and symbiotic associations of protozoa to dispose metabolic hydrogen during fermentation (Leng, 2008).

Rumen fermentation play a key role in ruminant nutrition, as it is this distinctive symbiotic feature between the host and the rumen microflora that lends the ruminant several advantages in digestive and metabolic processes over non ruminants (Nagaraja *et al*., 1997). However, products from ruminal fermentation such as ammonia and methane represent a loss in energy and nitrogen respectively. Methane produced during rumen fermentation represents a loss of 2–15% of gross energy intake and thus decreases the potential conversion of digesta to metabolisable energy. The efficiency of energy and protein utilisation in the rumen may be improved through the manipulation of microbial population and their activity (Casamiglia *et al*., 2007).

With the increase in intensive and semi-intensive animal fattening and dairy operation, there is problem of waste management with the attendant increase in manure piling thus resulting in anaerobic methane gas production. In targeting methane reduction, it is crucial to develop a strategy that decrease methane producing microbiota activities and proliferation without limiting rumen function (Gemeda and Hassen 2015).

As the world population is increasing, there is a growing increase in the demand for animal products such as meat and milk. Thus, small ruminants, like other livestock, are expected to increase in numbers. Much of the increase in sheep and goat numbers will come from developing countries and from hot and/or arid-semiarid areas (Herrero *et al*., 2008; Thornton *et al*., 2009). In Nigeria, livestock production plays an important role in the economy and in meeting protein need of rural dwellers; as such it is a common place for majority of rural families to possess goats and sheep. The world population of goats was estimated at 1.011 billion (FAOSTAT, 2014), with 96% of these being kept in developing countries.

The population of goats in West Africa was estimated at 149 million (FAOSTAT, 2014). Nigeria has a goat population of 72.4 million as at 2014 which is about 48%, 19% and 7% of West Africa, Africa and World goat population respectively (FAOSTAT, 2014). Thus, goats are an important livestock component in all agro-ecological zones especially Nigeria. The majority of these goats are kept in the rural households where they serve multiple purposes (Jahnke *et al*., 1998). West African dwarf goat is one of the popular goat breeds kept by families in Nigeria with a population of about 11 million in the South- Eastern part of Nigeria alone (Chiejine *et al*., 2015). However, the accompanying problem of methane and nitrous oxide emission are a serious threat to sustainability and as such efforts should be made to reduce their emission. The large greenhouse gas (GHG) emission and especially methane contributes to a climate change that will affect things such as rapid weather changes, increased temperature and worldwide water supplies (Moss *et al*., 2000). The weather and temperature will affect desert areas and wetland grounds, which in turn will increase the number of pests. That can be a threat to health of both humans and animals.

It has been demonstrated that tropical plants containing certain phytochemicals are a possible source of feed to ruminants in the tropics (Puchala *et al*, 2005). When tropical browse species were used as feed for ruminants, $CH_4$ emission was reduced compared to a diet containing mainly grasses. This is believed to be because of a mechanism the phytochemicals perform in the rumen. Studies made on some plants both *in vitro* and *in vivo* have shown that $CH_4$ production can be reduced by tannin and saponin rich foliage and therefore the interest of using such plants has significantly increased (Kamra *et al*., 2006).

Saponins or saponin-like substances have been reported to suppress methane production, reduce rumen protozoa counts and modulate fermentation pattern (Makkar and Becker

1997; Wang *et al.,* 1996; Hristov *et al.,* 1999). Tannins have also been reported to reduce methanogenesis, waste nitrogen in the manure of animals and thus reduce the availability of nitrogen that can be converted to nitrous oxide in the manure of animals. These bioactive compounds, otherwise known as phytochemicals have been proven through numerous researches to be beneficial in small amounts and when used appropriately. Phytochemicals are chemicals derived from plant sources such as phenolics, flavonoids, alkaloids, saponins, tannins and lignin which possess biological activities (Okwu, 2005; Oskoueian *et al.*, 2011).

*Chromolaena odorata* commonly known as Siam weed is one of those plants fairly rich in these phytochemicals. *Chromolaena odorata* is a perennial flowering herb plant in the family *Asteraceae*, it is a fast-growing invasive weed native to South and Central America. *Chromolaena odorata* is also very high in protein which could make it an unconventional source of protein for ruminants. It has been introduced into the tropical areas of Asia, Africa and other parts of the world. It is an aggressive competitor that occupies different types of lands where it forms dense strands and prevent the establishment of other flora (Afolabi, *et al.,* 2007).

## 1.1 Justification

Ruminants contribute about 23% to global methane output (IPCC, 2007). Livestock production is estimated to contribute about 18% to total nitrous oxide emission (IPCC, 2007). Both gases possess high global warming potentials.

For the sustainability of the environment and agricultural activities, research into strategies of reducing methanogenesis, increasing rumen efficiency is very important. While lots of approaches have been employed in mitigating methane and nitrous oxide emission, such approaches include; dietary approach that is, feeding mainly concentrate

diets (Sauvant and Giger-Reverdin, 2007), use of ionophores (Guan *et al.*, 2006,) use of bromoethanesulphonate (BSE) and bromopropanesulponate (BPS) (Ungerfeld *et al.*, 2004), use of lipids (Beauchemin *et al.,* 2008), use of vaccines and antibiotics **(**Wright *et al.,* 2004), have been largely inapplicable due to the high cost of implementing them in large scale ruminant production and the difficulty of managing some of these techniques especially for rural dwellers who rear majority of the goats in Sub-Sahara Africa (Jahnke *et al*., 1998). .

However, the use of plants with some secondary metabolites in the nutrition of ruminants is a promising option that has not been fully researched. The ethanol and methanol extract of *Emblica officinalis* fruit inhibited methanogenesis significantly. Supplementation of coconut oil with garlic powder improved ruminal fluid fermentation of volatile fatty acids and reduces methane emission along with protozoa population (Kongmun *et al.,* 2010). Cieslak *et al.* (2012) showed that *Vaccinium vitis* tannin had antimicrobial activity potential to indirectly mitigate methane and thereby ammonia.

*Chromolaena odorata* contains phytochemicals and it is fairly high in protein. Due to the ubiquitous nature of this plant, there have been numerous researches into its use for livestock nutrition. It has been used in poultry nutrition (Ekeyem, *et al.,* 2010) and it has also been incorporated in limited quantity in the diet of small ruminants (Apori, 2000). Although *C. odorata* has been used in livestock research; studies on its potential to reduce methanogenesis with consequent improvement on the nutritional performance of goats is limited. Hence the need for this study.

## 1.2 Objectives of the study

### 1.2.1 Broad objective

This study seeks to evaluate the effect of *Chromolaena odorata* leaf meal inclusion in the diet of West African dwarf bucks using *in vitro* and *in vivo* methods.

### 1.2.2 Specific objectives

- To determine the chemical composition of *Chromolaena odorata* leave and nutritional composition of the experimental diets.
- To evaluate the effect of *Chromolaena odorata* leaf meal on total *in vitro* gas and methane production.
- To investigate the effect of dietary inclusion of *Chromolaena odorata* leaf meal on rumen fermentation, *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, metabolizable energy, short chain fatty acid.
- To determine the effect of *Chromolaena odorata* leaf meal on the growth performance characteristics of WAD bucks.
- To determine the effect of *Chromolaena odorata* leaf meal on nutrient digestibility and nitrogen balance in WAD bucks.
- To investigate the effect of *Chromolaena odorata* leaf meal on rumen microbial population in WAD bucks
- To determine the effect of *Chromolaena odorata* leaf meal on blood chemistry of WAD bucks.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1. West African dwarf goats

The domestic goat (*Capra negragus hircus)* is a sub-specie of goat domesticated roughly 10,000 years ago from the wild goat of Southwest Asia and Eastern Europe (Hirst, 1997). The goat is a member of the Bovidae family and is closely related to the sheep both being in the goat antelope sub-family Caprinae. Domestic goats are one of the oldest domesticated species. For thousands of years, goats have been used for their milk, meat, hair and skins in the world (Coffey *et al*, 2008). In the last century, they have also gained some popularity as pets (Mclead, 2008). Hirst (1997) reported that goat was domesticated in Western Asia 8000 BC. Goats seem to have been first domesticated roughly 10,000 years ago in the Zagros Mountains of Iran. The world population of goats was estimated at 746 million (FAOSTAT, 2014), with 96% of these being kept in developing countries. However, the arid zone holds 12% more goats than the semiarid, which contains 26% of the goats. The humid and the high land zones account for about equal proportions of the population of goats (9 and 10%, respectively) (Lebbie, 2004).

The sub-humid zone accounts for 17% of the Sub-Sahara Africa goat population (Harris, 1962, Epstein, 1971). Thus, goats are an important livestock component in all agro-ecological zones. The majority of these goats are kept in the rural households where they serve multiple purposes (Jahnke *et al*., 1998).

Goat is a multipurpose animal, providing meat, milk, clothing, fertilizer, offering loyalty and companionship; alert, intelligent and socially inclined. This animal forms an important economic and ecological niche in agricultural systems throughout the developing countries (Aina, 2012).

The West African Dwarf goat has been internationally recognized as dairy and companion animal since 1854 (Aina, 2012). They can be raised easily in all climates and do not need elaborate housing. The does and kids are odourless, but the mature bucks do have a musk-like odour, especially during the breeding season; but with proper management the scent is not overly offensive.

Although the West African Dwarf (WAD) goat is found in 'many local types' (Ngere *et al.,* 1984), no published account differentiates them. Although they are stereotypically said to be native to the forest belts, their presence in Borno State and in adjacent Republics of Cameroon and Chad suggests that they were far more widespread until recently. They correspond the West African Grassland Dwarf described for Cameroon by Ndamukong *et al.* (1989). Indeed, like muturu cattle, they may once have been the main race of goat over most of Nigeria. Just as the zebu has replaced the muturu, so WAD goats have been driven to remoter areas in the savannahs. There is a strong association between the diffusion of the Red Sokoto goat and Islam, so for example, in southern Sokoto State, the non-Islamised populations still retain WAD goats while most Hausa villages have exclusively Sokoto Red.

Goats are not native to West Africa, so the WAD goat must originally have evolved from a longlegged type, probably ancestral to today's Sahel goat. The WAD is usually black, although patched, pied, and occasionally all-white animals can be seen, even on the coast. Although Chang and Landauer (1950) argued that the WAD is a proportionate dwarf, Epstein (1971) points out that the distorted forms and extremely short legs do suggest achondroplasy. This small size is probably an adaptation to the goats' environment though the nature of the selective force is unknown. The WAD goats in the semi-arid zone resemble Sokoto Red goats in their body proportions. Paradoxically, physiological experiments have shown that the WAD goat is not particularly adapted to

high ambient temperatures (Montsma *et al.,* 1985). High temperatures and relative humidities, e.g. 30$^{o}$C and 60% relative humidity, cause a reduction in food intake. The WAD goat is believed to be trypanotolerant because it thrives in tsetse areas, Chiejina *et al*. (2015) reported that WAD goats are trypanotolerant and has a high resistance to *Haemonchus contortus.*

## 2.2. Siam weed (*Chromolaena odorata*)

*Chromolaena odorata*(L) King and Robinson Asteraceae commonly known as Siam weed, is a fast-growing perennial and invasive weed native to South and Central America. It has been introduced into the tropical regions of Asia, Africa and other parts of the world. It is an aggressive competitor that occupies different types of lands where it forms dense strands that prevents the establishment of other flora. It is a menace in plantations and other ecosystems. It suppresses young plantations, agricultural crops and smothers vegetation as it possesses allelopathic potentialities (Ambica and Jayachandra, 1980; Ambica and Jayachandra, 1982, Muniappan and Marutani, 1988). The economic value of *Chromolaena odorata* is low. Consequently, there is a relative paucity of research works on it. It is a perennial shrub native of South and Central America. In recent decades, it has become a serious pest in the humid tropics of South-East Asia, Africa and Pacific Islands. It spreads rapidly inlands used for forestry, pasture and plantation crops such as rubber, coffee, coconut, cocoa and cashew. In Nigeria, it is called“Independence” leaf or “Awolowo” weed as it was introduced into the country during the independence period. In English, it is Siam or Sapysa weed (Inyang and Adegoke, 2008).

Recently, there has been increasing research on the use of *Chromolaena odorata* in livestock nutrition. Ekeyem *et al.* (2010) reported inclusion of up to 5% with positive

results in the diet of broilers. Fasuyi *et al.* (2005) also reported positive impact on the performance of layers up to 5% inclusion. Aro *et al.* (2009) stated that *Chromolaena odorata* can also be used in the diet of ruminants at a limited quantity and if dried to reduce the presence of anti-nutritional factors.

Conclusively, since *Chromolaena odorata* is a ubiquitous plant, it can be put into use in the nutrition of goats and thus reduce feeding cost. The presence of saponins in the plant and its effect on rumen microbiota, methane production and nitrogen utilization is an area that calls for research.

### 2.2.1. Distribution of *Chromolaena odorata*

*Chromolaena odorata* is widely distributed in many tropical countries frequently well-known as native to Central and South America but it has been introduced in many other tropical countries. The geographical distribution of *Chromolaena odorata* is known to be limited to regions within 30° N and 30° S latitudes in areas with a rainfall of 500 – 1500 mm and temperature ranges from 20°C to 37°C (Zachariades *et al.,* 2009). The weed is present in every part of Nigeria. The weed invasiveness attributes include its ability to thrive in a wide variety of soils, short juvenile stage and flowering in dry season, prolific seed production and strong ability to re-sprout after burning during land preparation (Lina and Ephrime, 2011). The allelopathic properties of the weed aid it in gaining dominance in vegetation and in replacing other plants species (Zachariades *et al.*, 2009)

**Table 1: Chemical composition of *Chromolaena odorata* leaf meal**

| Nutrient | Wet Weight | Dry Weight |
|---|---|---|
| Moisture (%) | 59.50 | - |
| Crude protein (%) | 6.56 | 16.20 |
| Crude fibre (%) | 10.78 | 26.57 |
| Ether extract (%) | 0.10 | 0.25 |
| Ash (%) | 2.50 | 6.17 |
| Nitrogen free extractives (%) | 20.58 | 50.82 |
| Total metabolizable energy (Kcal/100g) | 109.46 | 270.27 |
| Cyanogenic glycosides | 0.05 | 0.13 |
| Phytates | 0.22 | 0.54 |
| Saponins | 0.80 | 1.98 |
| Tannins | 0.15 | 0.37 |

Source: Igboh, *et al*., 2009

## 2.3. *Chromolaena odorata* in livestock feeding

Information on the use of *Chromolaena odorata* in livestock nutrition isvery scanty. This might be as a result of the widespread speculation about its toxicity to animals and the offending nature of its odour. Reports of Madrid (1974) of the consequent death that occurred in cattle following ingestion of fresh *Chromolaena odorata* (C.O) leaves attested to the toxic nature of C.O. to livestock. Nwokolo (1987) delved elaborately on the mineral and amino acid composition of both cassava and C.O. leaf meals. This author assayed the availability (true digestibility) of minerals by using three week old broiler chicks and reported that the average availability of these minerals was 53.70 and 49.90%for cassava leaf meal and C.O. leaf meal, respectively. He also reported on the amino acid composition and availability of these leaf meals in his broiler chicks' bioassay. He concluded that the values obtained for both mineral and amino acid availability could be attributed to the presence of antinutrient factors in both leaves, especially of tannins sincethey occur in high concentration in plant materials and are associated with toxicity and poor growth rate and depressed dietary nutrient utilization in monogastric animals.

However, there have been reports on the successful use of dried *Chromolaena odorata* in the diet of poultry bird, and even ruminants. Aro (1990) reported that there was no significant reduction in egg production of layers fed *Chromolaena odorata* supplemented diet upto 7.5%. Aro and Fajemilehin (2005) also reported improved digestibility values of C.O supplemented diet upto 5% inclusion level.

### 2.3.1. *Chromolaena odorata* and ruminant nutrition

The general apathy towards the use of C.O. in ruminant nutrition following the reports of its toxicity (Sajise *et al*., 1974) and the death of some cattle in the Philippines after its ingestion was proven wrong by the work of Fadiyimu *et al*. (2005), who fed six species of weed (*Tridax procumbens, Chromolaena odorata, Aspilia africana, Boerhavia diffusa, Ageratum conyzoides and Sida acuta*) to a group of West African dwarf goats in a bid to determine their nutrient composition and acceptability. Their study revealed a proximate composition of C.O. as: 97.5% dry matter, 18.9% crude protein, 11.5% crude fibre, 9.1% ash, 12.8% ether extract and 47.6% nitrogen free extract. The mineral composition and antinutritional factors of C.O. in this study were: Calcium 11.7%, Sodium 7.8%, Chloride 7.0%, Magnesium 8.0%, Iron 0.9%, Zinc 5.3%, tannins 2.3% and phytin 15.7mg/kg. C.O. ranked fourth in the mean preference index among the six weeds that were analysed and the authors recommended C.O. for inclusion in ruminants' diets among the four selected weeds based on their high preference scores. Kawed (2016) reported successful inclusion of C.O in the diet of Small East African (SEA) goats upto 10% without adverse effect on performance characteristics.

## 2.4. Saponins and effect on animals

Saponins may have significant effects on all phases of animal metabolism from ingestion of feed to the excretion of wastes. Ingested saponins have been observed to influence animal performance and metabolism in a number of ways. The biological activity of saponins depends not only on the structure of the lipophilic aglycon but also on the sugar composition. The three-dimensional spatial orientations of the saponins also play an important role in its bioactivity. The influence of legume saponins on animals has been widely reviewed by Cheeke (1983).

### 2.4.1. Effects of saponins on ruminants

Saponins have influence on rumen fermentation. Lu and Jorgensen (1987) showed that saponins, isolated by ethanol extraction, hydrolyzed partially, and administered to sheep intra-ruminally, reduced microbial fermentation. Total protozoal count was also significantly reduced. Apparent digestion coefficient of organic matter, hemicellulose, and cellulose in the total digestive tract were increased by saponins in sheep fed concentrate diets.

Saponin significantly reduced methane production and the relative abundance of protozoa and anaerobic fungal populations was also significantly decreased (Guo *et al.*, 2008).

## 2.5. Tannins and their role in methanogenesis and nitrogen balance

Recently, there are numerous reports that have shown the reduction of enteric methane due to inclusion of tannin rich browses because the tannins have anti-methanogenic activity, either by direct inhibition of methanogen or indirectly through inhibition of protozoa (Animut *et al*,. 2008). Tannins are polyphenolic compounds which bind to proteins and can be used as chemical additives for protecting and decreasing ruminal fermentation of proteins in ruminant feeds (Makkar, 2003). They are complex polymers with various linkages and bonds that vary among browse species and within parts of plants (Patra and Saxena, 2011).

The huge diversity in tannin structures may explain their vast variable effects on methanogenesis and rumen function depending on source, type and level of tannin (Mueller-Harvey, 2006).

Tannins additionally, have the ability to increase protein retention and thus reduce waste nitrogen content in the feces of animals and subsequently reduce nitrous oxide emission

from manure. Nsahlai *et al.* (1999) reported increased nitrogen retention and live weight gain in ruminants fed tannin-containing feed. Byeng *et al.* (2015) also reported increased nitrogen retention of eighteen Kiko breed of goats fed tannins-containing ground pine bark diet.

## 2.6. Rumen microbiota

Ruminants are mainly fed by lignocellulosic based by-products which are rich in complex carbohydrate, hence the active microbial populations present are a derivative of the feed. The rumen epithelial or epimural bacteria community performs a vast diversity of functions necessary for host health including the hydrolysis of urea, scavenging of oxygen and the recycling of epithelial tissues (Petri *et al.*, 2013).

*Fibrobacter succinogenes, Ruminococcus flavofaciens, Ruminococcus albus, Clostridium cellobioparum, Clostridium longisporum, Clostridium lochheadii, Eubacteria cellolusolvens* are the active cellulose degrading microbes in the rumen (Satyanagalakshmi *et al.,* 2016). *Butyrivibrio fibrisolvens, Prevotella ruminocola, Eubacterium xylanophilum and Eubacterium uniformis* greatly participate in hemicellulose degradation (Cotta, 1992). *Streptococcus bovis, Ruminobacter amylophilus* and *Prevotella ruminocola* are the dominating group of starch degrading microbes (Satyanagalakshmi *et al.,* 2016).

## 2.7. Population of methanogen in the rumen

Methanogens belong to the domain Archaea and the phylum Euryarchaeota (Murray *et al.,* 1976). Unlike bacteria, methanogens lack peptidoglgan in their cell wall (Balch and Wolfe, 1979). The methanogen population in the rumen may differ depending on the ruminant species being examined (Yan *et al*., 2010).

Methanogens are very important for the functioning of the rumen and to control hydrogen pressure maintenance. Archaea can be found in the lamb rumen 30 hours after birth (Morvan *et al*., 1994). About 113 species have been identified in the ecosystem, however only a few species of methanogens are found in the rumen (Janssen and Kirs, 2008). *Methanobrevibacter spp* were initially colonized methanogens in the lamb rumen and less population of *Methanobacterium spp. Methanobacterium formicicum, Methanobrevibacter ruminantium, Methanosaricina barkeri, Methanosaricina mazei* and *Methanomicrobium mobile* are the predominant methanogen in the rumen (St-Pierre and Wright, 2012).

## 2.8. Methanogenesis in the rumen

When ruminants consume food, feed components like carbohydrates, protein and other organic substances are degraded into monomer components by fibrolytic or primary anaerobes. These smaller units are then converted to volatile fatty acids, carbon dioxide and hydrogen. The volatile fatty acids are absorbed by the epithelium. Methanogens utilise the free carbon dioxide and hydrogen as a substrate produced from the fermentation of feed stuffs; these are the main electron acceptor and donor and produce methane. Methanogens such as *Methanosarcina* utilise methanol and methyleamines more effectively in methane production (Patterson and Hespell, 1979). Formate which is formed in the production of acetate can also be used as a substrate for methanogenesis, although it is often converted quickly to hydrogen and carbon dioxide (Barnes and Mead, 1986).

The synthesis of methane contributes to the efficiency of the rumen in that it maintains the partial pressure of hydrogen to levels that may inhibit normal functioning of microbial enzymes involved in electron transfer reactions, particularly NADH

dehydrogenase. This results in NADH accumulation and ultimately reduces rumen fermentation (Mongavi *et al* 2012). The capturing of hydrogen produced by species involved in fermentation by species involved in methanogenesis is termed hydrogen transfer (Wollin *et al.,* 1997).

Methanogens have also been reported to associate with protozoa by attaching to their external pellicle (Stumm, *et al.,* 1983). Studies have also shown that there is a reduced methanogen population and thus methane output following defaunation (Morgavi *et al.,* 2012).

## 2.9. Strategies employed in methane reduction

Methane mitigation depends on the relationship methanogens have with other organisms in the rumen. Methanogenesis can be reduced by reducing the substrates (hydrogen) available for methanogenesis or by attacking the methanogens themselves (Hook *et al.,* 2010).

Some of the strategies for reducing methanogenesis are discussed below in detail.

### 2.9.1. Nutritional manipulation

The increase in the amount of starch concentrates in the diet causes a decrease in methane production per kg dry matter intake. This is probably the most widely known approach to reduce methane emissions in ruminants. In high-starch diets the percentage of methane corresponding to the gross energy intake can be as low as 3% as compared to 6 to 8% in diets with forages as the main feed component. However, this marked decrease is only observed at levels of intake that are equal or above 2.5 times the intake required for maintenance and when the concentrate represents more than 50% of the ration (Sauvant and Giger-Reverdin, 2007).

The type of diet composition and the carbohydrate rate in the diet are very important in methane synthesis. The type of diet fed to ruminants have the capability to alter the rumen pH and subsequently the microbial pool of the rumen (Johnson and Johnson, 1995). Concentrate diet of corn and soybean has been reported to reduce methane production (Benchaar and Greathead, 2011). When concentrate diets are fed, rumen pH is reduced to a level that reduces the activities of methanogens and thus methane output is reduced (Yan *et al.,* 2010).

The shortcoming of this mitigation approach is that it requires feeding the ruminants with concentrate which is quite expensive and not economical, feasible and sustainable in ruminant production. Additionally, ruminants are designed to feed on roughages and as a result feeding them with mostly concentrates will affect their performance adversely and even the rumen microbiota.

### 2.9.2. Ionophores

Ionophore are highly lipophilic ion carriers, they pass through the permeable peptidoglygcan layer of gram-positive bacteria and penetrate into the lipid membrane (Satyanagalakshmi *et al.,* 2016). Ionophones mitigate methanogenesis by causing the deaths of methanogens (Tedeschi *et al.,* 2003). Microbiota which produces hydrogen and formate is gram negative and sensitive to ionophone, thereby preventing the formation of the necessary substrates required for methanogenesis. This leads to an effective dramatic reduction in methanogen population in the rumen. Use of ionophores have been associated with increased energy utilization in feeds (Guan *et al.,* 2006).

However, ionophores are expensive and thus not within the reach of the rural/ household farmer.

### 2.9.3. Bromoethanesulphonate (BSE) and bromopropanesulponate (BPS)

Methanogens can be inhibited by the addition of methane analogues such as BES and BPS. Some inhibitors, however, are more effective against certain species of methanogens than others and only offer short-term protection (Ungerfeld*et al*., 2004).

### 2.9.4. Effect of lipids on methane emission

Lipids such as fatty acids and oils also show some effect on the rumen methanogens. Fatty acids inhibit methanogens by binding to their cell membrane and disturbing their membrane transport (Dohme 2001). In the meta-analysis of methane, lipid supplemented in the diet of lactating dairy cows showed a 2.2% decrease in methane per 1% of supplemented lipid in the diet (Eugene *et al.*, 2008). 5.6% methane reduction per percentage unit of lipid added to the diet was observed in cattle and sheep (Beauchemin *et al.,* 2008).

Oil extracted from plants have also been shown to reduce methanogenesis (Soliva *et al.*, 2004). Refined soy oil based diet fed to beef bulls reduced methane by 39% (Jordan, 2006). Sunflower oil also has good impact on methane production, it reduced it by 22% (McGinn *et al*., 2004). Garlic, Eucalyptus, Neem oils were tested in vitro for methane production, garlic oil reduced methane production by as far as 55% (Sirohi *et al*., 2012)

However, the use of lipids and oil is not economical in a large scale ruminant production.

### 2.9.5. Use of secondary metabolites

Metabolites such as saponins, tannins and oils have anti-microbial activity which can be used as additive to reduce methanogen population in the rumen (Kamra, 2008). Herbal plant extracted products have a prominent effect on rumen microbiota either directly

changing methanogens or indirectly affecting protozoa. Saponins mitigate methane by reducing the protozoa population; tannins and essential oil have toxic effect on methanogens (Cieslak *et al*., 2009). Methanol extract of *Terminalia chebula* reduced 95% methane and double level of the extract, methane was inhibited completely. Phenolic acids such as ferulic acid, cinnamic acid and phloretic acids and some monomeric phenolics have shown to decrease methane, acetate and propionate production (Asiegbu *et al.,* 1995). The ethanol extract of *Emblica officinalis* fruit and methanol extract of the fruit inhibited methanogenesis significantly. Supplementation of coconut oil with garlic powder improves ruminal fluid fermentation of volatile fatty acids and reduces methane emission along with protozoa population (Kongmun *et al.,* 2010). Cieslak *et al.* (2012) showed that *Vaccinium vitis* tannin had antimicrobial activity potential to indirectly mitigate methane and thereby ammonia.

Using of plants rich in these metabolites in ruminant nutrition to reduce methanogenesis and waste nitrogen is a promising option especially when these plants are not in competition for human needs. *Chromolaena odorata* is fairly rich in both saponins and tannins.

### 2.9.6. Vaccines and antibiotics

Vaccines are used to prevent or control disease for a particular period. The use of vaccines targeting methanogens to reduce methanogenesis and increase productivity is a current topic. The anti-methanogen vaccine triggers the immune system of ruminants and produces antibodies against methanogens in the ruminants. A vaccine against three selected methanogens have been developed in Australia. Immunization in sheep lowered methane production by 8%, while further testing failed to confirm its efficacy in other geographical regions (Wright *et al.,* 2004).

The use of antibiotics such as monensin to reduce methanogenesis is also being investigated. Monensin inhibits gram-positive bacteria, which is responsible for supplying substrates needed for methanogenesis. Monensin acts on the cell wall of gram positive bacteria; it interferes with ion flux and decreases the acetate-propionate ratio in the rumen, effectively decreasing methane production. The use of monensin in methanogenesis is dose dependent and at lower doses it is ineffective (Soliva, *et al.* 2004). The use of vaccines in mitigating methanogenesis is still under investigation and it is not economical on a large-scale production likewise the use of antibiotics. These are the major limitations to the adoption of this mitigation strategies.

### 2.9.7. Genetic approach

Methanogenic Archaea which emits a large population of methane in the rumen is a very small population. Genetic engineering could be employed to alter the gene responsible for methanogenesis in these microorganisms. A practical approach is to stop/ reduce the production of the enzyme; methyl coenzyme-M reductase which is responsible for methane formation. Geneticist estimates that 11 to 26% methane mitigation in 10 years is attainable through genetic selection program (Soliva, *et al.* 2004). This approach is still under research and it could be very expensive to implement.

## 2.10. Importance of haematological studies in nutrition

Haematology is the discipline concerned with the study of blood, its various components and its various disease.

For nutrition to be complete, nutrient component of food must be fully digested and absorbed into the body cells where they are needed for effective functioning of the system which are ; cell metabolism, repair and maintenance (Momoh, 2005). These

nutrients are made into absorbable form in the blood where they are transported to the various body parts of the body.

Blood is a good indicator to determine the health status of an organism. It also acts as pathological reflector of the whole body; hence haematological parameters are important in diagnosing the functional status of exposed animals to toxicants (Joshi *et al*., 2002). The study of animal haematology is important so as to determine the rate at which nutrients are absorbed into the system.

Nutrient level in blood and body fluids may not be a valid indicator of nutrient functions at a cellular level, but they are considered to be proximate measures of long term nutritional status of the animal (Kerr, 1989).

Therefore a change from the usual haematological values need functional interpretation in respect to how nutrition may play a role in such changes and what such changes signify.

Some haematological parameters are discussed below.

## 2.11. Red blood cell (erythrocytes)

Erythrocytes are highly specialized cells found in animals, they are characteristically red in colour due to the presence of oxidized iron. These cells have the shape of biconcave disk, thicker at the edge than in the middle like a doughnut (Vander *et al.*, 1985).

The outstanding characteristics of erythrocytes are the presence of iron containing protein haemoglobin which mainly carries out oxygen transportation throughout the system of the animal. It is important to study the erythrocyte content of animals so as to determine their oxygen carrying capacity, which is very important. Several indices are used in erythrocyte evaluation; these include, mean corpuscular haemoglobin concentration (MCHC), and corpuscular volume (MCV).

## 2.12. Haemoglobin

This is the respiratory pigment of blood and performs an important function as oxygen-carrier, it also takes part in carbon dioxide transport. It is a major component of the red blood cell, it constitutes approximately one-third of the total weight of the red blood cell (Georgieva, 1989). Haemoglobin also acts as a buffer and plays an important role in acid-base balance of the blood (Sembulingam and Prema, 2010).

Jain (1986) reported that low haemoglobin was obtained in animals with inadequate protein intake.

## 2.13. White blood cells (leucocytes)

The leucocytes are the mobile unit of the body's protective system, they are formed partially in the bone marrow and partially in the lymph tissue. After formation, they are transported in the blood to various parts of the body where they are to be used (Guyton, 1991).

White blood cells are divided into two groups based on their functions, that is; phagocytes and immunocytes (Hoffbrand *et al.,* 2006). They are also classified based on the presence of granules in their protoplasm as granulocytes and agranulocytes. Basophils, neutrophils, eosinophils and monocytes are phagocytes as well as granulocytes while lymphocytes are immunocytes as well as agranulocytes (Georgieva, 1989).

The major function of white blood cells is exerted not within the blood vessels but the intestinal fluids (Vander *et al.,* 1985). Their major function is the defense of the body against foreign cells and invaders.

A sharp increase in the number of circulating white blood cells can be caused by mobilization and stimulation of production of these cells in a characteristic response to

infections. The formation of white blood cells is called leucopoiesis while leucopenia is a drastic reduction in the formation of white blood cells, this is a disease condition and it will make the body more vulnerable to infections (Guyton, 1991).

The study of the white blood cell of goats is to determine its count so as to have an idea of the defense status of the animal.

Neutrophils are neutral to dyes, eosinophiles retain red dyes and basophils have affinity for basic dyes.

Lymphocytes are immunologically competent cells that assist the phagocytes in defense of the body against infections and foreign invasions. There are two main types of lymphocytes based on their dependence; thymus dependent cells (they mature in the thymus, T-cells) and B-cells which are not thymus dependent. B-cells are responsible for the production of antibodies (Marshall and Hughes, 1967).

Nutrition that boost lymphocyte production will help in maintaining good health since lymphocytes are key players in body immunity.

### 2.14. Platelet (thrombocytes)

These are the final solid constituent of the blood and are about one-quarter the size of the red capsule (Georgieva, 1989). Platelets are usually cell fragments and lack a nuclei, they are formed by the disintegration of large cells, the megakaryocytes of the redbone marrow (Marshall and Hughes, 1967).

Platelets are mainly concerned with blood clothing which is very necessary for wound healing and part of the body defense mechanism. Their defense and clothing function depends on the production of insoluble protein called fibrin in the blood in which the corpuscles get entangled.

# CHAPTER THREE

## 3.0. MATERIALS AND METHODS

### 3.1. Experimental site

The study was carried out at the Laboratory of Pasture and Range Management Department and the Goat Unit of the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) of the Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. It is located on latitude 7°13,35.48"N, longitude 3025$^0$39.01"E with an elevation of 415 feet and altitude of 700 feet (Google Earth, 2017). Being located in the rainforest vegetation zone, it has humid climatic condition with a mean annual rainfall of 1,037mm and mean temperature and humidity of 34.7$^0$C and 83% respectively. The study comprises of two experiment highlighted below and was carried out between the period of October and January.

### 3.2. Experiment 1: *In vitro* evaluation of *Chromolaena odorata* leaf meal on gas production, methane gas estimate and post incubation parameters

#### 3.2.1. Preparation of *Chromolaena odorata* leaf meal

Fresh *Chromolaena odorata* leaves were sourced from College of Animal Science farm and the University environment before inflorescence so as to harvest more leaf. The leaves were air dried for 3 weeks and then milled to 2mm sieve size.

The phytochemical screening of leaf meal for tannin, saponnin, flavonoids, alkaloids and phytates of *Chromolaena odorata* leaf was carried out using the method described by Sofowora (2008).

### 3.2.2. Experimental diet

Maize stover was harvested 20cm above ground level and chopped into small size. A complete concentrate diet was formulated to contain 17% crude protein (Table 2) and *Chromolaena odorata* leaf meal was included as additive at four (4) levels of 0, 2, 4 and 6% of 100kg concentrate feed. Samples of the concentrate diets and maize stover were oven dried at $65^0$C constant weight for dry matter determination. Oven dried samples were milled and sieved through a 2mm sieve for chemical analysis and *in vitro* gas production study. Maize stover and the concentrate diets with the various inclusion levels of *Chromolaena odorata* was combined at a ratio of 60:40. The experiment was aid out in a completely randomized design. There were four treatments with 12 replicates per treatment in each run and the incubation process was run once.

**Table 2: Gross composition (%) of experimental concentrate diets**

| **Feed Ingredients** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** |
|---|---|---|---|---|
| Maize | 16.00 | 16.00 | 16.00 | 16.00 |
| Soybean | 6.00 | 6.00 | 6.00 | 6.00 |
| Wheat offal | 52.00 | 52.00 | 52.00 | 52.00 |
| Palm kernel cake | 24.00 | 24.00 | 24.00 | 24.00 |
| Bone meal | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 1.00 | 1.00 | 1.00 | 1.00 |
| **C. odorata* | 0.00 | 2.00 | 4.00 | 6.00 |
| **Total** | **100** | **102** | **104** | **106** |
| **Calculated Nutrient** | | | | |
| Crude protein | 17.44 | 17.44 | 17.44 | 17.44 |
| Crude Fat | 4.39 | 4.39 | 4.39 | 4.39 |
| Crude Fibre | 9.98 | 9.98 | 9.98 | 9.98 |
| Ash | 4.79 | 4.79 | 4.79 | 4.79 |

*Calculated nutrient did not consider *C. odorata*

### 3.2.3. *In vitro* gas production measurement

At the end of the feeding trial, rumen fluid was collected with the aid of stomach tube into a pre-warmed thermo-flask and taken to the laboratory for the preparation of incubation inoculum. Oven dried and milled samples of maize stover and concentrate diets according to the different treatments was incubated *in vitro* with the rumen fluid in 100ml calibrated transparent syringes fitted with silicon tube following the procedure of Menke and Steingass (1988). The substrate, maize stover and the experimental concentrate diets was in the ratio 6:4 respectively. Approximately 200mg of each sample was weighed into 48 fibre bags, 12 bags per treatment. The bags were carefully placed in the syringes and thereafter filled with 40ml inoculums containing cheese-cloth strained rumen liquor and buffer. The rumen liquor and buffer solution was mixed in ratio 1:2 (v/v) respectively. The syringes were tapped and pushed upward by the piston to eliminate air completely in the inoculums. The silicon tube in the syringes were then tightened by a metallic clip so as to prevent escape of gas. Incubation was carried out at 39ºC and the volume of gas was measured at 3 hours interval from 0 to 96 hours in a single run. Four blanks containing 40ml inoculum only was included so as to balance for net gas output.The aim of this procedure was to determine the total volume of gas produced.

The data obtained was fitted to the non-linear model of France *et al*. (2002):

$$A = b(1\text{-}e^{-c(t-L)})$$

Where; $A$ is the volume of gas produced at time $t$, $b$ is the potential/asymptotic gas production (ml/g DM) from the fermentable fraction of forage, $c$ is the fractional rate of gas production (/h) from the slowly fermentable fraction and $L$ is the discrete lag time prior to gas production

*in vitro* organic matter digestibility (IVOMD) and metabolisable energy (ME) of forage were calculated according to the procedure of Menke *et al.* (1979) using the formula below.

$$OMD\ \% = 14.88 + 0.889GP + 0.45CP + 0.651A$$

$$ME\ (MJ/Kg\ DM) = 2.20 + 0.136GP + 0.0574CP + 0.029CP^2$$

Short chain fatty acid SCFA (µmole/g DM) = 0.0239GPT – 0.0601 (Getachew *et al.* 1999)

Where GP = 24 hours net gas production (mL/200mg DM)

CP = crude protein content of substrate

A = ash content of substrate

*In vitro* dry matter digestibility was determined by using the formula below

$$IVDMD = \frac{\text{initial weight of sample} - \text{weight after digestion}}{\text{initial weight of sample}} \times 10$$

### 3.2.4. Determination of methane output estimate

At post incubation period, 4ml of Sodium hydroxide (NaOH, 10M) was introduced to estimate the methane production as reported by Fievez *et al.* (2005). The average of the volume of gas and methane produced from the blanks was deducted from the total volume of gas and methane produced per sample to determine net gas and methane produced respectively.

### 3.2.5. Statistical analysis

Data collected during the experimental period were subjected to one way analysis of variance (ANOVA) in completely randomised design using SAS (1999) and the means

separated using Duncan multiple range test of the same software at 5% level of significance. The model for the study is given below,

$Y_{ijk} = \mu + T_j + \xi_{ij}$

Where, $Y_{ijk}$= individual observation

$\mu$ = population mean

$T_j$= the effect of *Chromolaena odorata*

$\xi_{ij}$ = random residual error

## 3.3. Experiment 2: *In vivo* evaluation of *Chromolaena odorata* addition on growth performance characteristics, nutrient digestibility, nitrogen utilisation, rumen microbial count and blood chemistry of WAD bucks

### 3.3.1. Source of experimental animals

Twenty (20) West African dwarf bucks weighing 10±2kg, between 1-1.5 years of age was gotten from the Goat Unit of IFSERAR and used for the experiment.

### 3.3.2. Experimental animals and management

The animal house was cleaned and disinfected two weeks before the arrival of the animals together with the equipment to be used. The floor was covered with wood shavings and pens and feeders kept in place. Clean water and feed was placed in each pen before the arrival of the animals. The goats were weighed, counted, recorded and allotted to their various treatments randomly. The pens were 20 in number, 5 for each treatment

Twenty (20) West African dwarf bucks was used for the research. They were allocated randomly based on body weight into 4 treatment groups comprising of 5 goats per

treatment. Daily routine practices of cleaning and feeding was carried out throughout the duration of the experiment and every animal was closely monitored.

### 3.3.3. Feeding trial

The animals were fed the treatments for a period of 12 weeks. Concentrate diets with the test ingredient was given to the animals in the morning before they are given maize stover. Concentrate diet was fed at 3% body weight while maize stover was given *ad libitum*. The weight of the individual animals was taken weekly before feeding throughout the experimental period.

### 3.3.4. Data collection

The final weight of the animals was taken at the end of the experiment before samples were collected. Samples collected for analysis were; rumen fluids and blood samples. Data collected included the following;

#### 3.3.4.1. Performance parameters

- **Initial weight of bucks:** this was the weight of experimental bucks taken before the commencement of the experiment, it was done using Avery Weigh-Tronix Electronic scale- 1000kg capacity;
- **Final weight of bucks:** this was the weight of the bucks taken at the end of the experimental period of 12 weeks usingAvery Weigh-Tronix Electronic scale- 1000kg capacity;
- **Weight gain of bucks:** this was determined by subtracting the initial weight of bucks from their final weight;
- **Feed consumption:** this was determined by the difference between total weight of total dietary concentrate and maize stover fed to bucks daily and weight of

concentrate and maize stover refused each day by the bucks throughout the experimental period.

### 3.3.5. Digestibility trial

At the 10th through 12th week of the feeding trial, four (4) goats were taken from each treatment to the metabolic cage for digestibility trial. The first 7 days of the digestibility trial was for the adaptation of the goats to the metabolic cages and the next 7 days was for the collection of faecal and urine samples. The samples were bulked together and 10% aliquot was collected. The bottles for urine collection were rinsed with 10% sulphuric acid ($H_2SO_4$) to prevent ammonia volatilization.

Samples of the feed given to the experimental goats,quantityof faeces voided were collected and subjected to chemical analysis (dry matter, crude protein, crude fibre, ash, ether extract and nitrogen free extract).

### 3.3.6. Chemical analysis

Samples taken from the concentrate diet and *Chromolaena odorata* leaf meal were used for chemical analysis (crude protein, crude fat, nitrogen free extract, ash and moisture content) following standard procedure of AOAC (2005) and fibre fraction (neutral detergent fibre, acid detergent fibre) according to the method of Van Soest *et al.,* (1991). Samples taken from the diets was after the inclusion of the test ingredient.

### 3.3.7. Experimental formulae

#### 3.3.7.1. Feed composition

Organic matter (%) = 100- %Ash

Hemicellulose (%) = %NDF- %ADF

Cellulose (%) = %ADF- %ADL

### 3.3.7.2. Performance characteristics

Weight gain = (final weight – initial weight)kg

Feed intake (g/day) = feed given (g/day) – feed refused (g/day)

Feed conversion ratio = $\frac{\text{Total feed consumed (g)}}{\text{Weight gained (g)}}$

### 3.3.7.3. Intakes

Dry matter intake (g/day) = daily feed consumption (on wet basis) x (DM of feed/100)

Nutrient intake (g/day) = DM intake x (% nutrient in feed/100)

Apparent Nutrient digestibility % = $\frac{\text{total nutrient intake} - \text{faecal nutrient}}{\text{total nutrient intake}}$ x 100

### 3.3.7.4. Nitrogen utilisation and retention

The quantity of nitrogen retained by the goats was also studied. This was calculated using the formula below

Nitrogen Absorbed (g/day) = total nitrogen intake (g/day) – faecal nitrogen (g/day)

Nitrogen Balance (g/day)=total N intake (g/day)–(faecal N + urinary N (g/day)

Nitrogen Retention (%) = $\frac{\text{nitrogen balance (gday}-1)}{\text{total nitrogen intake (gday}-1)}$ x 100

Nitrogen intake (g/day) = feed intake on DM basis (g/day) x ((feed CP/6.25)/100)

Faecal nitrogen (g/day) = faecal output on DM basis (g/day) x ((faecal CP/6.25)/100)

Urinary nitrogen (g/day) = daily urine quantity (mL) x (urinary nitrogen/100).

### 3.3.8. Rumen microbial population and identification

Rumen content was collected with the use of a stomach tube before and end of the feeding trial and samples taken to Microbiology laboratory of the Federal University of Agriculture, Abeokuta to determine rumen microbial population and to identify

microbes present in the rumen. Portion of the rumen fluid was fixed with 10% formalin solution in sterilized 0.9% saline solution. The total direct count of anaerobic bacteria, protozoa and fungal zoospores was made by the methods of Gaylean (1989).

### 3.3.9. Blood sample collection and analysis

Blood samples were collected at the start and end of the experiments via jugular vein punctured with new hypodermic needle fitted on a new 10ml calibrated syringe in the morning before feeding`.

Haematological parameters such as white blood cell (WBC), lymphocyte, monocyte, neutrophils, eosinophil, basophils, red blood cell (RBC), haemoglobin, pack cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration were determined.

Packed cell volume (PCV and red blood cell (RBC) were determined using the method described by (Shah and Altindag, 2004a). Haemoglobin (Hb) concentration was obtained by measuring the amount of oxygen which can combine with haemoglobin, using Van Slyke apparatus and applying Hufner's factor (1.36ml oxygen per 1g of Hb) for its calculation. White blood cell (WBC) count was determined by visual counting method with the aid of Neubaer count chamber. For the differential WBC count, the test was carried out by making a thin smear on a slide and observing the different leukocytes under the microscope. The different white cell count which varies in shape and size was counted with the aid of leukocyte difference machine to determine the lymphocyte, basophil, neutrophils and eosinophils count.

### 3.3.10. Serum biochemistry analysis

Blood samples were collected in plain bottles from the goats and taken to the Department of Veterinary Physiology and Pharmacology Laboratory, FUNAAB for serum biochemistry analysis. Parameters analysed included; blood glucose, serum total protein, serum albumin, serum globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) andalkaline phosphatase (ALP).

Total protein was determined spectrophotometrically according to the method of Tietz (1995), serum albumin was measured spectrophotometrically according to the method of Donmas *et al*. (1971) while serum globulin was determined by deducting serum albumin values from total protein values. Serum glucose was assayed with the aid of a photospectrometer according to the method of Barham and Trinder (1972).

ALT, AST and ALP were determined by following the procedure described by Randox (2012).

### 3.3.11. Statistical analysis

Data collected during the experimental period were subjected to one way analysis of variance (ANOVA) in completely randomised design using SAS (1999) and the means separated using Duncan multiple range test of the same software at 5% level of significance.

### 3.3.12. Statistical model

$Y_{ijk} = \mu + T_j + \xi_{ij}$

Where, $Y_{ijk}$= individual observation

$\mu$ = population mean

$T_j$= the effect of *Chromolaena odorata*

$\xi_{ij}$ = random residual error

# CHAPTER FOUR

## 4.0. RESULTS

### 4.1. Chemical properties of *Chromolaena odorata*

The chemical properties of *Chromolaena odorata* is presented in Table 3. The values for chemical composition are; dry matter (DM) 96.90%, crude protein (CP) 17.51%, crude fibre (CF) 20.43%, ether extract (EE) 1.39%, Ash 8.52, nitrogen free extract (NFE) 52.16%, organic matter (OM) 91.48%, neutral detergent fibre (NDF) 62.65%, acid detergent fibre (ADF) 37.77%, acid detergent lignin (ADL) 10.72%,hemicellulose 24.88%, cellulose 27.05%. For the phytochemicals investigated, the values are; saponin 1.99%, tannin 2.57%, flavonoid 1.08% and alkaloid 1.26%. All parameters are on dry matter basis.

### 4.2. Proximate and fibre composition of experimental concentrate diet and maize stover

The proximate and fibre composition of the experimental diets is presented in Table 4. The four experimental diets varied significantly ($P<0.05$) in all the proximate parameters and fibre fractions. Diet containing 0% inclusion of *Chromolaena odorata* had the highest ($P<0.05$) dry matter (DM) content (93.32%) while the lowest (92.05%) was obtained in the diet with 6% *C. odorata* inclusion. The highest ($P<0.05$) crude protein (CP) value (14.97%) was observed in the diet with 6% *C. odorata* inclusion while the lowest (13.35%) was recorded in the diet with 0% *C. odorata* inclusion. The highest ($P<0.05$) values of 5.00% and 9.44% for ash and ether extract (EE) respectively was observed in the diets with 4% *C. odorata* and 6% *C. odorata* inclusion while the least values 3.85 and 8.13% for EE and ash in diets with 2% and 0% *C. odorata* inclusion for ash and ether extract respectively. For crude fibre, there was significant difference ($P<0.05$) in the percentage. Values ranged from 6.21%- 6.52% with increase in *C.*

*odorata* inclusion. The values for NFE and OM decreased significantly progressively ($P<0.05$) from 61.00% to 56.73% and 91.87% to 90.56% from 0% to 6% *C. odorata* inclusion respectively. There was significant difference ($P<0.05$) in the means for NDF and Hemicellulose. Values ranged from 58.14% - 66.14% for NDF and 18.57% - 33.38% for hemicellulose. The values decreased with 0% to 6% *C. odorata* inclusion. ADF and cellulose increased significantly ($P>0.05$). their values ranged from 32.23% - 31.69% for ADF and 28.40% - 35.23% for cellulose. The highest ($P<0.05$) value (4.38%) for ADL was recorded in the diet with 6% *C. odorata* inclusion while the lowest value (3.58%) was recorded in the diet with 4% *C. odorata* inclusion. The proximate composition of maize stover determined in this study was; DM 92.95%, CP 4.38%, CF 30.80%, EE 0.70%, ash 10.02%, NFE 54.21%, OM 89.99%, NDF 79.61%, ADF 19.95%, ADL 5.53%, hemicellulose 59.66% and cellulose 14.42%

**Table 3: Chemical composition of *Chromolaena odorata* (%DM)**

| **Parameters (%)** | |
|---|---|
| Dry Matter | 96.90 |
| Crude Protein | 17.51 |
| Crude Fibre | 20.43 |
| Ether Extract | 1.39 |
| Ash | 8.52 |
| Nitrogen Free Extract | 52.16 |
| Organic Matter | 91.48 |
| Neutral Detergent Fibre | 62.65 |
| Acid Detergent Fibre | 37.77 |
| Acid Detergent Lignin | 10.72 |
| Hemicellulose | 24.88 |
| Cellulose | 27.05 |
| Saponin | 1.99 |
| Tannin | 2.57 |
| Flavonoid | 1.08 |
| Alkaloid | 1.26 |
| Phytate | 1.33 |

**Table 4: Nutritional composition of experimental concentrate diet and maize stover fed to West African Dwarf bucks**

| Parameters (%) | M S | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Dry matter | 92.95 | $93.32^a$ | $92.72^b$ | $92.69^b$ | $92.05^c$ | 0.10 | 0.01 |
| Crude protein | 4.28 | $13.35^d$ | $13.94^c$ | $14.13^b$ | $14.97^a$ | 0.13 | 0.01 |
| Crude fibre | 30.80 | $6.21^b$ | $6.30^b$ | $6.45^a$ | $6.52^a$ | 0.03 | 0.05 |
| Ether extract | 0.70 | $4.63^b$ | $3.85^d$ | $5.00^a$ | $4.39^c$ | 0.10 | 0.01 |
| Ash | 10.02 | $8.13^d$ | $9.13^c$ | $9.30^b$ | $9.44^a$ | 0.12 | 0.01 |
| Nitrogen free extract | 54.21 | $61.00^a$ | $59.50^b$ | $57.81^c$ | $56.73^d$ | 0.33 | 0.01 |
| Organic matter | 89.99 | $91.87^a$ | $90.87^b$ | $90.70^b$ | $90.56^c$ | 0.12 | 0.01 |
| Neutral detergent fibre | 76.61 | $66.14^a$ | $62.48^b$ | $59.19^c$ | $58.14^d$ | 0.72 | 0.01 |
| Acid detergent fibre | 19.95 | $32.70^a$ | $35.63^b$ | $38.81^c$ | $39.61^d$ | 0.62 | 0.01 |
| Acid detergent lignin | 5.53 | $4.36^a$ | $3.66^b$ | $3.58^c$ | $4.38^a$ | 0.86 | 0.01 |
| Hemicelluloses | 59.66 | $33.38^a$ | $26.85^b$ | $20.38^c$ | $18.53^d$ | 1.34 | 0.01 |
| Cellulose | 14.42 | $28.40^c$ | $31.97^b$ | $35.23^a$ | $35.23^a$ | 0.65 | 0.01 |
| *ME (MJ/Kg DM) | 14.14 | $14.10^a$ | $13.66^c$ | $13.91^b$ | $13.66^c$ | 0.04 | 0.01 |

$^{abcd}$ Means on the same row having different superscripts are significantly different ($P<0.05$)

* Calculated using MAFF 1984 equation

M.S; Maize stover. $T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*

### 4.3. *In vitro* gas production and fermentation kinetics of West African dwarf bucks rumen fluid with *Chromolaena odorata* as additive

The result of the effect of the experimental diets on *in vitro* gas production (ml/200mg) is presented in Table 5. The result showed a significant difference (P<0.05) in the volumes of gas produced by the various experimental diets at 3, 6, 9, 12, 18, 24, 30, 42, 48, 60, 72, 84 and 96 hours of incubation. Treatment with 4% *C. odorata* inclusion recorded the highest (P<0.05) gas volumes; 1.50ml, 3.25ml, 4.42ml, 5.58ml, 7.17ml, 8.75ml, 10.75ml, 14.17ml, 17.50ml, 20.17ml, 27.75ml, 30.67ml, 30.67ml and 30.67ml, while treatment with 0% *C. odorata* inclusion recorded the lowest (P<0.05) values; for 3, 6, 9, 12, 18, 24, 30, 42, 48, 60, 72, 84 and 96 hours respectively. Generally, gas volumes increased from treatment 1 (0% *C. odorata* inclusion) and peaked at treatment 3 (4% *C. odorata* inclusion).

Potential gas production (*b*), fractional rate of gas production (*c*) and lag time (*L*) were significantly (P<0.05) different.

### 4.4. Post incubation parameters of West African dwarf bucks rumen fluid with *Chromolaena odorata* as additive

Table 6 shows the result of the effect of *C. odorata* additve on post incubation parameters of WAD bucks. Parameters investigated such as total gas volume (TGV), net gas volume (NGV), net methane proportion (NMP), *in vitro* organic matter digestibility (IVOMD), *in vitro* dry matter digestibility (IVDMD), short chain fatty acid (SCFA) and metabolizable energy (ME) were significantly influenced (P<0.05) by the experimental diets.

The highest value for TGV, NGV, SCFA, and IVOMD were observed in the treatment with 4% *C. odorata* inclusion while the lowest were obtained in the treatment with 0% *C. odorata* inclusion. The highest values were; 30.67ml, 30.37ml, 0.15µmol/g DM,

31.99% while the lowest values were 21.42ml, 21.12ml, 0.09µmol/g DM, and 29.30% for TGV, NGV, SCFA and IVOMD respectively.

The highest value for IVDMD was 77.08% (2% *C. odorata* inclusion) while the lowest was 66.25% (0% *C. odorata* inclusion). For ME, treatment with 6% *C. odorata* inclusion had the highest value (8.06MJ/kg DM), the lowest was recorded in the treatment with 0% *C. odorata* inclusion (7.48MJ/kg DM). Means for methane gas output was not significantly different ($P>0.05$). Net methane proportion declined significantly progressively ($P<0.05$) from 0.20 (0% *C. odorata* inclusion) to 0.13(4% *C. odorata* inclusion).

**Table 5: Effect of *Chromolaena odorata* additive on *in vitro* gas production (ml/200mg) and fermentation kinetics of West African dwarf bucks**

| Incubation hours | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|
| 3 | $0.75^{b}$ | $1.42^{a}$ | $1.50^{a}$ | $1.17^{a}$ | 0.009 | 0.12 |
| 6 | $2.08^{b}$ | $3.00^{a}$ | $3.25^{a}$ | $2.25^{b}$ | 0.001 | 0.12 |
| 9 | $3.17^{b}$ | $3.92^{ab}$ | $4.42^{a}$ | $3.25^{b}$ | 0.001 | 0.15 |
| 12 | $4.08^{b}$ | $5.00^{a}$ | $5.58^{a}$ | $4.00^{b}$ | 0.001 | 0.17 |
| 18 | $5.25^{b}$ | $6.83^{a}$ | $7.17^{a}$ | $5.08^{b}$ | 0.001 | 0.22 |
| 24 | $6.17^{b}$ | $8.25^{a}$ | $8.75^{a}$ | $6.42^{b}$ | 0.001 | 0.27 |
| 30 | $7.17^{b}$ | $10.50^{a}$ | $10.75^{a}$ | $8.08^{b}$ | 0.001 | 0.34 |
| 36 | $8.42^{b}$ | $13.25^{a}$ | $14.17^{a}$ | $10.92^{b}$ | 0.001 | 0.47 |
| 42 | $11.08^{c}$ | $16.33^{a}$ | $17.50^{a}$ | $14.00^{b}$ | 0.001 | 0.52 |
| 48 | $13.92^{c}$ | $18.83^{a}$ | $20.17^{a}$ | $16.58^{b}$ | 0.001 | 0.51 |
| 60 | $18.17^{c}$ | $25.42^{a}$ | $27.75^{a}$ | $21.92^{b}$ | 0.001 | 0.76 |
| 72 | $21.42^{c}$ | $29.17^{a}$ | $30.67^{ab}$ | $26.58^{b}$ | 0.001 | 0.75 |
| 84 | $21.42^{c}$ | $29.17^{a}$ | $30.67^{ab}$ | $26.58^{b}$ | 0.001 | 0.75 |
| 96 | $21.42^{c}$ | $29.17^{a}$ | $30.67^{ab}$ | $26.58^{b}$ | 0.001 | 0.75 |
| *b* | $28.56^{c}$ | $37.51^{b}$ | $43.59^{a}$ | $35.82^{b}$ | 0.001 | 1.152 |
| *c* | $0.11^{b}$ | $0.13^{ab}$ | $0.16^{a}$ | $0.11^{b}$ | 0.008 | 0.017 |
| *L* | $1.13^{a}$ | $1.07^{b}$ | $1.05^{b}$ | $1.11^{a}$ | 0.004 | 0.008 |

$^{abc}$ Means on the same row having different superscripts are significantly different ($P<0.05$)

b: potential/ asymptotic gas production (ml/g DM), c: fractional rate of gas production (/h), *L*: lag time

$T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*

**Table 6: Effect of *Chromolaena odorata* additive on post incubation parameters of West African dwarf bucks**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|
| TGV (ml) | $21.42^{c}$ | $29.17^{ab}$ | $30.67^{a}$ | $26.58^{b}$ | 0.75 | 0.01 |
| NGV (ml) | $20.42^{c}$ | $28.87^{ab}$ | $30.67^{a}$ | $26.58^{b}$ | 0.75 | 0.01 |
| Methane (ml/200mg) | 4.17 | 4.00 | 3.92 | 4.42 | 0.22 | 0.87 |
| NMP | $0.20^{a}$ | $0.14^{b}$ | $0.13^{b}$ | $0.16^{ab}$ | 0.01 | 0.03 |
| NMP % | $19.78^{a}$ | $14.20^{b}$ | $16.35^{ab}$ | $16.35^{ab}$ | 0.90 | 0.03 |
| IVOMD % | $29.30^{b}$ | $31.60^{a}$ | $31.99^{a}$ | $30.11^{b}$ | 0.25 | 0.01 |
| IVDMD % | $66.25^{b}$ | $77.08^{a}$ | $76.25^{a}$ | $74.17^{a}$ | 1.51 | 0.03 |
| TDS (g) | $132.50^{b}$ | $154.17^{a}$ | $152.50^{a}$ | $148.33^{b}$ | 2.93 | 0.03 |
| SCFA (µmol/g DM) | $0.09^{b}$ | 0.14 | $0.15^{a}$ | $0.09^{b}$ | 0.01 | 0.01 |
| ME (MJ/kg DM) | $7.48^{b}$ | $7.94^{a}$ | $7.99^{a}$ | $8.06^{a}$ | 0.04 | 0.01 |

$^{abc}$ Means on the same row having different superscripts are significantly different ($P<0.05$)

TGV- Total gas volume, NGV- Net gas volume. NMP- Net methane proportion, IVOMD- *in vitro* organic matter digestibility, IVDMD- *in vitro* dry matter digestibility, SCFA- Short chain fatty acid, ME- Metabolizable energy, TDS: Total digestible substrate, $T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*

## 4.5. Performance characteristics of West African dwarf bucks fed *Chromolaena odorata* as additive

Table 7 shows the performance characteristics of WAD bucks fed diets with 0%, 2%, 4% and 6% *Chromolaena odorata* leaf meal additive respectively. The initial weight, final weight, daily concentrate intake, daily maize stover intake, total feed intake and feed conversion ratio were not significantly (P>0.05) different. However, parameters such as weight gain and daily weight gain were significantly (P<0.05) different. The highest value (6.20kg) for weight gain was observed in the treatment with 4% *C. odorata* inclusion and the least value (4.50kg) was observed in the diet with 6% *C. odorata* inclusion. Likewise, the highest (P>0.05) value (73.81g) for daily weight gain was recorded in the treatment with 4% *C. odorata* inclusion and the lowest value (53.87g) was observed in the diet with 6% *C. odorata* inclusion.

## 4.6. Nutrient intake of West African dwarf bucks fed *Chromolaena odorata* as additive and maize stover

Table 8 shows the nutrient intake of WAD bucks fed diet with *C. odorata* as additive and maize stover. Bucks fed diets with 4% *C. odorata* recorded the highest values for total DM, CP, EE, ash, NFE, OM and metabolizable energy (ME) intake. The values are 517.47, 57.91, 18.77, 52.79, 315.91, and 504.89g/day. Total ME intake was 78.50kj/kg DM.

Ether extract intake from concentrate was significant (P<0.05). The highest value (17.29g/day) was obtained in the diet with 4% *C. odorata* inclusion while the lowest value (12.88g/day) as observed in the diet with 2% *C. odorata* inclusion.

### 4.7. Fibre intake of West African dwarf bucks fed *C. odorata* as additive and maize stover

The fibre intake of WAD bucks fed diets with *C. odorata* additive and maize stover is shown in Table 9. Total fibre intake, NDF, ADF, ADL, hemicellulose and cellulose values were not significant ($P>0.05$). However, hemicellulose intake from concentrate diets was significantly influenced ($P<0.05$) by the experimental diets. Values decreased from 116.06g/day to 61.11g/day for diets with 0% *C. odorata* inclusion to 6% *C. odorata* inclusion.

**Table 7: Effect of *Chromolaena odorata* additive on growth performance characteristics of West African dwarf bucks**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|
| Initial weight (kg) | 10.00 | 10.01 | 9.90 | 10.00 | 1.00 | 0.62 |
| Final weight (kg) | 14.80 | 15.10 | 16.10 | 14.50 | 0.53 | 0.76 |
| Weight gain (kg) | $4.80^{b}$ | $5.00^{ab}$ | $6.20^{a}$ | $4.50^{b}$ | 0.24 | 0.05 |
| Daily weight gain ($gday^{-1}$) | $57.14^{b}$ | $59.52^{ab}$ | $73.81^{a}$ | $53.57^{b}$ | 2.86 | 0.05 |
| Concentrate intake ($gday^{-1}$) | 347.68 | 334.37 | 345.74 | 329.79 | 13.88 | 0.97 |
| Maize intake ($gday^{-1}$) | 187.78 | 187.74 | 211.95 | 193.31 | 17.88 | 0.97 |
| Total feed intake ($gday^{-1}$) | 535.46 | 522.11 | 557.69 | 523.10 | 30.63 | 0.98 |
| Feed conversion ratio | 9.37 | 8.77 | 7.56 | 9.76 | 0.74 | 0.80 |

[ab] Means on the same row having different superscripts are significantly different ($P<0.05$)

$T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*

**Table 8: Effect of *Chromolaena odorata* additive on nutrient intakes (g/day) of West African dwarf bucks**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|
| DM intake from conc. | 324.46 | 310.83 | 320.47 | 303.61 | 12.92 | 0.95 |
| DM intake from MS | 174.54 | 174.50 | 197.01 | 179.69 | 16.62 | 0.95 |
| **Total DM intake** | **499.00** | **484.53** | **517.17** | **483.29** | **28.45** | **0.98** |
| CP intake from conc. | 46.49 | 46.61 | 49.37 | 48.85 | 1.95 | 0.94 |
| CP intake from MS | 8.03 | 8.03 | 9.06 | 8.26 | 0.76 | 0.97 |
| **Total CP intake** | **54.44** | **54.64** | **57.91** | **57.63** | **2.64** | **0.95** |
| EE intake from conc. | 16.10 | 12.88 | 17.29 | 14.48 | 0.72 | 0.14 |
| EE intake from MS | 1.32 | 1.31 | 1.48 | 1.36 | 0.12 | 0.97 |
| **Total EE intake** | **17.41** | **14.19** | **18.77** | **15.83** | **0.82** | **0.23** |
| Ash intake from conc. | 28.27 | 31.56 | 31.57 | 30.67 | 1.27 | 0.80 |
| Ash intake from MS | 18.80 | 18.80 | 21.23 | 19.36 | 1.79 | 0.97 |
| **Total ash intake** | **47.07** | **50.36** | **52.79** | **50.03** | **2.95** | **0.94** |
| NFE intake from conc. | 211.29 | 198.28 | 201.01 | 187.59 | 8.37 | 0.82 |
| NFE intake from MS | 101.80 | 101.77 | 114.90 | 104.79 | 9.69 | 0.97 |
| **Total NFE intake** | **313.08** | **300.05** | **315.91** | **292.38** | **17.28** | **0.97** |
| OM intake from conc. | 319.41 | 302.80 | 314.17 | 299.12 | 12.68 | 0.95 |
| OM intake from MS | 168.98 | 168.94 | 190.72 | 173.95 | 16.08 | 0.97 |
| **Total OM intake** | **488.39** | **471.74** | **504.89** | **473.07** | **27.71** | **0.98** |
| ME intake from conc. | 49.02 | 45.67 | 48.08 | 45.03 | 1.94 | 0.89 |
| ME intake from MS | 26.55 | 26.54 | 29.97 | 27.34 | 2.52 | 0.97 |
| **Total ME intake** | **75.58** | **72.21** | **78.05** | **72.37** | **4.30** | **0.96** |

[abc] Means on the same row having different superscripts are significantly different ($P<0.05$)

DM- Dry matter, MS- Maize stover, CP- Crude protein, EE- Ether extract, NFE- Nitrogen free extract, OM- Organic matter, ME- Metabolizable enegy, $T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*

**Table 9: Effect of *Chromolaena odorata* additive on fibre intakes (g/day) of West African dwarf bucks**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|
| CF intake from conc. | 22.43 | 20.76 | 21.78 | 21.50 | 0.89 | 0.94 |
| CF intake from MS | 57.84 | 57.82 | 65.28 | 59.54 | 5.51 | 0.97 |
| **Total CF intake** | **80.26** | **78.59** | **87.06** | **81.04** | **6.28** | **0.97** |
| NDF intake from conc. | 229.94 | 208.90 | 204.64 | 191.74 | 9.15 | 0.56 |
| NDF intake from MS | 149.49 | 149.46 | 168.73 | 153.90 | 14.23 | 0.97 |
| **Total NDF intake** | **379.43** | **358.36** | **373.38** | **345.64** | **22.14** | **0.96** |
| ADF intake from conc. | 113.88 | 119.12 | 134.18 | 130.63 | 5.31 | 0.52 |
| ADF intake from MS | 37.46 | 37.45 | 42.28 | 38.57 | 3.57 | 0.97 |
| **Total ADF intake** | **151.34** | **156.57** | **176.46** | **169.19** | **8.56** | **0.75** |
| ADL intake from conc. | 15.16 | 12.24 | 12.38 | 14.45 | 0.63 | 0.26 |
| ADL intake from MS | 10.38 | 10.38 | 10.69 | 11.72 | 0.99 | 0.97 |
| **Total ADL intake** | **25.54** | **22.62** | **24.10** | **25.13** | **1.51** | **0.92** |
| Cellulose intake from conc. | 98.72 | 106.88 | 116.18 | 121.80 | 4.86 | 0.37 |
| Cellulose intake from MS | 27.08 | 27.07 | 30.56 | 27.88 | 2.58 | 0.97 |
| **Total cellulose intake** | **125.80** | **133.95** | **152.37** | **144.06** | **7.15** | **0.61** |
| Hemicellulose intake from conc. | $116.06^{a}$ | $89.78^{b}$ | $70.46^{bc}$ | $61.11^{c}$ | 6.09 | 0.01 |
| Hemicellulose intake from MS | 112.03 | 112.01 | 126.45 | 115.33 | 10.66 | 0.97 |
| **Total hemicellulose intake** | **228.09** | **201.78** | **196.91** | **176.44** | **14.39** | **0.69** |

$^{abc}$ Means on the same row having different superscripts are significantly different ($P<0.05$)

CF- Crude fibre, MS- Maize stover, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin, $T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*

## 4.8. Apparent nutrient digestibility of West African dwarf bucks fed diet with *Chromolaena odorata* leaf meal additive

Table 10 shows the apparent nutrient digestibility of WAD bucks fed diet with *C. odorata* additive. The highest values for DM, CP, EE, CF, ash, NFE and OM digestibility are; 94.90%, 84.08%, 90.84%, 84.29%, 91.99%, 71.22% and 66.59% respectively. The highest value for DM, EE, CF and OM was observed in the diets with 0% *C. odorata* these values are however not significant (P>0.05).

## 4.9. Nitrogen utilization of West African dwarf Bucks fed *Chromolaena odorata* leaf meal as additive

The result for nitrogen utilization of WAD bucks fed diet with *C. odorata* additive is shown in Table 11. The values for nitrogen intake from concentrate and maize stover, total nitrogen intake, faecal and urinary nitrogen, total nitrogen excreted, nitrogen absorbed and nitrogen retention were not significant (P>0.05).

**Table 10: Effect of *Chromolaena odorata* addition on apparent nutrient digestibility of West African Dwarf bucks**

| Parameters (%) | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|
| Dry matter | 74.90 | 73.69 | 73.86 | 72.87 | 2.39 | 0.99 |
| Crude protein | 83.65 | 83.55 | 84.08 | 83.43 | 0.98 | 0.52 |
| Ether extract | 90.84 | 88.78 | 89.71 | 88.21 | 0.75 | 0.86 |
| Ash | 89.56 | 91.99 | 89.11 | 91.27 | 0.68 | 0.92 |
| Nitrogen free extract | 70.47 | 68.93 | 71.22 | 68.65 | 1.74 | 0.95 |
| Organic matter | 66.59 | 63.70 | 65.79 | 63.32 | 3.21 | 0.98 |
| Crude fibre | 84.29 | 83.52 | 83.37 | 79.91 | 1.92 | 0.88 |

$T_1$: 0% C. odorata, $T_2$: 2% C. odorata, $T_3$: 4% C. odorata, $T_4$: 6% C. odorata

**Table 11: Effect of *Chromolaena odorata* addition on nitrogen utilization of West African Dwarf bucks**

| **Parameters (%)** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **SEM** | **P-value** |
|---|---|---|---|---|---|---|
| Nitrogen Intake (g/day): | | | | | | |
| Concentrate | 7.43 | 7.46 | 7.82 | 7.90 | 0.31 | 0.94 |
| Maize stover | 1.28 | 1.28 | 1.45 | 1.32 | 0.12 | 0.94 |
| **Total nitro. intake (g/day)** | 8.71 | 8.74 | 9.27 | 9.22 | 0.42 | 0.95 |
| Nitrogen excretion (g/day) | | | | | | |
| Faecal nitrogen | 1.30 | 1.02 | 1.35 | 1.40 | 0.02 | 0.48 |
| Urinary nitrogen | 0.23 | 0.26 | 0.25 | 0.23 | 0.01 | 1.00 |
| **Total nitro. excreted (g/day)** | 1.53 | 1.28 | 1.60 | 1.63 | 0.02 | 0.80 |
| Nitrogen absorbed (g/day) | 7.41 | 7.72 | 7.92 | 7.82 | 0.43 | 0.97 |
| Nitrogen balance (g/day) | 7.18 | 7.46 | 7.67 | 7.59 | 0.44 | 0.97 |
| Nitrogen retention % | 82.43 | 82.27 | 82.74 | 82.32 | 0.62 | 0.99 |

$T_1$: 0% C. odorata, $T_2$: 2% C. odorata, $T_3$: 4% C. odorata, $T_4$: 6% C. odorata

### 4.10. Microbial count of rumen fluid of West African dwarf bucks fed *Chromolaena odorata* as additive

The result of the effect of *C. odorata* addition on rumen microbial count of WAD bucks is presented in Table 12. Values of total anaerobic bacteria count (TABC) was significant ($P<0.05$). The highest value (2.48 x $10^6$cfu/ml) was recorded in treatment with 6% *C. odorata* inclusion while the lowest value (1.44 x $10^6$cfu/ml) was recorded in the treatment with 2% *C. odorata* inclusion. The values for total fungi count (TFC) and total protozoa count (TPC) were not significantly ($P>0.05$) influenced by the experimental diets.

### 4.11. Blood parameters of West African dwarf bucks fed *Chromolaena odorata*as additive

Table 13 shows the blood parameters at the end of the experiment of WAD bucks fed *C. odorata* as additive. Glucose and AST were significant ($P<0.05$). Bucks that received 6% *C. odorata* addition had the highest glucose value (99.00%) while the lowest value (68.00%) was obtained from bucks fed 4% *C. odorata* additive. The values for AST increased significantly ($P>0.05$) with increase in *C. odorata* inclusion. The values ranged from 59.67% for bucks on 0% *C. odorata* inclusion to 75.33% for bucks on 6% *C. odorata* inclusion. Other parameters such as red blood cell, white blood cell, haemoglobin, packed cell volume, lymphocyte, eosinophil, basophil, monocyte, neutrophil, total protein, albumin, globulin, ALT and ALP were not significantly influenced ($P>0.05$) by the dietary treatments.

**Table 12: Effect of *Chromolaena odorata* additive on microbial count of the inoculum**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|
| TABC ($x10^6$ cfu/ml) | $1.52^c$ | $1.44^c$ | $2.10^b$ | $2.48^a$ | 0.10 | 0.01 |
| TFC ($x10^6$ cfu/ml) | 0.05 | 0.06 | 0.07 | 0.08 | 0.01 | 0.34 |
| TPC ($x10^3$ cfu/ml) | 4.80 | 4.90 | 4.98 | 5.04 | 0.06 | 0.61 |

TABC: Total anaerobic bacteria count, TFC: Total fungi count, TPC: Total protozoa count, , $T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*

**Table 13: Effect of *Chromolaena odorata* additive on blood parameters of West**

| Parameters | NV | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Packed cell volume (%) | 22-38 | 31.00 | 29.33 | 29.33 | 29.00 | 0.99 | 0.92 |
| White blood cell ($x10^9$/L) | 5-12 | 5.00 | 5.23 | 5.77 | 5.83 | 0.20 | 0.92 |
| Red blood cell ($x10^{12}$/L) | 8-18 | 9.43 | 9.97 | 9.97 | 10.01 | 0.18 | 0.64 |
| Haemoglobin (g/dL) | 8-12 | 8.83 | 9.27 | 9.67 | 9.80 | 0.38 | 0.85 |
| Lymphocyte (%) | 50-70 | 68.00 | 68.33 | 68.00 | 71.00 | 0.90 | 0.65 |
| Eosinophil (%) | 1-8 | 0.33 | 0.33 | 1.00 | 1.33 | 0.25 | 0.44 |
| Basophil (%) | 0-1 | 0.67 | 1.00 | 1.33 | 0.67 | 0.19 | 0.63 |
| Neutrophil (%) | 25-48 | 29.00 | 29.33 | 27.33 | 25.00 | 1.06 | 0.51 |
| Monocyte (%) | 0-4.0 | 1.67 | 1.00 | 2.33 | 2.00 | 0.33 | 0.59 |
| Total protein (g/dL) | 6.4-7.0 | 6.27 | 6.50 | 6.80 | 6.53 | 0.11 | 0.44 |
| Albumin (g/dL) | 2.7-3.9 | 3.27 | 3.07 | 3.63 | 3.70 | 0.13 | 0.31 |
| Globulin (g/dL) | 2.7-5.7 | 3.00 | 3.43 | 3.17 | 2.83 | 0.10 | 0.18 |
| Glucose (mg/dL) | 60-100 | $90.33^{a}$ | $75.33^{b}$ | $68.00^{bc}$ | $99.00^{ab}$ | 4.37 | 0.02 |
| AST (u/l) | 60-167 | $59.67^{b}$ | $60.00^{b}$ | $65.33^{ab}$ | $75.33^{a}$ | 2.60 | 0.01 |
| ALT (u/l) | 15-52 | 20.33 | 33.67 | 23.33 | 25.33 | 2.34 | 0.22 |
| ALP (u/l) | 93-287 | 42.67 | 46.33 | 37.33 | 48.33 | 2.17 | 0.32 |

$^{abc}$ Means on the same row having different superscripts are significantly different ($P<0.05$)

$T_1$: 0% *C. odorata*, $T_2$: 2% *C. odorata*, $T_3$: 4% *C. odorata*, $T_4$: 6% *C. odorata*, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase , NV: Normal values. Normal values were according to Veterinary manual 2016.

# CHAPTER FIVE

## 5.0. DISCUSSION

The percentage dry matter recorded for (Siam weed) *Chromolaena odorata* leaf meal in this study was slightly higher than that reported by Aro *et al*. (2009), Ekeyem *et al*. (2010), and Kawed (2016) who recorded values of 87.40%, 91.44% and 90.49% respectively. This disparity in dry matter percentages may be due to the growth stages of the leaf, seasonal variations in the study areas. Flowering and matured plants tend to have less moisture and more fibre compared to emerging plants, dry matter percentage is always higher during the dry season compared to the rainy season. The crude protein values of *Chromolaena odorata* leaf meal in the present study was similar to those reported by Igboh *et al*. (2009) which was 16.17%, Aro *et al*. (2009) with 18.67% and Ekeyem *et al*, (2010) who reported 16.67%. Crude fibre value of *Chromolaena odorata* in this study is higher than those reported by several researchers, Apori (2000) reported 9.70%, Igboh *et al*. (2009) reported 10.76% and Aro *et al*, (2009) reported 11.64%. The plausible reason for the higher crude fibre value recorded in this study may be due to seasonal variation and the stage of growth at the time of harvest of these leaves. Low CP and high cell wall contents of plant materials were reported to be associated with advances in maturity of plants (McDonald *et al.* 2002). The percentage ash is within range of the values reported by Ekeyem *et al*. (2009) and Kawed (2016) with 8.56% and 10.40% respectively but higher than the values reported by Aro *et al*. (2009) and Igboh *et al*. (2009) who reported 3.63% and 6.17% respectively. Planting season and variation in soil nutrient level may be the reason for the observed variations. Other values such as nitrogen free extract (NFE) and ether extract (EE) were similar to those reported by Igboh *et al*. (2009), Aro *et al*. (2009) and Ekeyem *et al*. (2010).

The value of saponins and tannins in *Chromolaena odorata* in this study was lower than that reported by Agaba and Fawole (2016) which were 3.48% and 4.10% respectively. However, the value of tannin was higher and that of saponins similar to the report of Igboh *et al*. (2009) which were 0.37% and 1.98% respectively. The percentage of flavonoid and alkaloid were higher than that reported by Agaba and Fawole (2010) and the value of phytate was also higher than that the report of Igboh (2009) which were 0.77%, 1.55% and 0.54% for flavonoid, alkaloid and phytate respectively. These slight variations can be attributed to various factors such as; methods of extraction, stage of maturity of leaf. The dry matter percentage of maize stover in this study is similar to the reports of Biwi (1986), Tolera and Sundstol (1999), Fabian (2011) who had 93.40%, 92.50% and 93.50% DM respectively. However, Fabian (2011) reported a higher crude protein (CP) percentage of 5.60% compared to that reported in this study. Tolera and Sundstol (1999) had a similar CP percentage of 4.8% to the one reported in this study. Neutral detergent fibre (NDF) values were similar to that reported by Biwi (1986), Tolera*et al*. (1999) and Ouda and Nshalat (2007) who had 80.70%, 77.00% and 72.46% respectively. However, Fabian (2011) reported a higher NDF value of 87.80%. These observed variations in proximate parameters may be due to different planting seasons, level of maturity of the maize stover at harvest, and variation in soil nutrients.

The effect of *C.odorata* on the all the proximate parameters was significant. Bayssa *et al.* (2016) made a similar observation when they supplemented the concentrate diets of Arsi-Bale goats with leaf of *Acacia toritilis*. The proximate components of the diets changed with the inclusion of the leaf meal. The increase in crude protein, fibre and ash contents of diets with *Chromolaena odorata* leaf additive justifies the possible feeding value of the leaf as protein and minerals supplement to feeds with lower level of protein and minerals.

A higher gas volume with increase in *C. odorata* corresponded to a higher CP. Positive correlations between crude protein and gas production in diets have been reported (Ndlovu and Nherera, 1997, Gasmi-Boubaker *et al*., 2005, Aderinboye *et al*. 2016). Additionally, a higher crude protein diet encourages more microbial fermentation, the higher the CP in the diets, the higher the gas produced (Popova *et al*. 2012, Igbal and Hashim 2014). A similar report has noted that addition of different doses of *Leucaena leucocephala* and *Salix babylonica* extracts (0.60, 1.20, 1.80 mL extract per g of DM) increases gas volume (Jiménez-Peralta *et al*, 2011). Differences in chemical composition (i.e. CP and CF) of the substrates could be responsible. However, the result of this work is contrary to the reports of several researchers (Kalita *et al*. 1996, Wang *et al*. 1997, Hristov *et al*. 1999, Liu *et al.* 2003, Hess *et al.* 2003a,b, Hu *et al.* 2005a,b, Guo *et al.* 2008 and Silivong 2012). They all reported reduction in *in vitro* gas production on addition of saponin extract or leaf meals rich in saponin such as *Yucca schidigera, Sapindus saponaria* and *Camellia sineis.* The disparity in the results of this current research from those of the researchers above could be attributed to the levels of saponin in the test leaf meals, the crude protein contents of the leaf meal and in addition most of the authors reviewed who reported reduction in gas volume used mainly the saponin extracts of these plants at various levels and not necessarily the leaf meals.

The non-significant effect *C. odorata* on methane gas estimate is in accordance with the report of Sungchhang *et al* (2016) and Jiménez-Peralta *et al*, (2011) who did not observe any significant difference in methane output of rumen liquor of goats and growing lambs who had *Flemingia macrophylla* and *Leucaena leucocephala* leaf meal supplemented diets respectively. Gunun *et al*. (2011) also observed that methane output was not affected in goats supplemented with Mao (*Antidesma thwaitesianum*) seed meal. This result is contrary to that of Guo *et al.* (2008), Sliwinski *et al.* (2010), Hartanto *et al.*

(2017) and Li *et al.* (2018) who observed reduced methane output when tea saponin, plants rich in tannins and saponins, monensin, monensin and vegetable oils respectively were included in the diets of goats. Saponin level, type of saponins in *C. odorata* and level of inclusion could be the reason for the non-significant effect of *C. odorata* on methane gas output. However, net methane proportion (NMP) reduced significantly with increase in the inclusion *C. odorata* leaf meal, this is an indication that *C. odorata* has methane reduction potentials.

Values of the estimated parameters obtained from the fermentation kinetics of gas production model for substrates studied showed that the potential gas production (*b*), fractional rate of gas production (*c*) and lag time (*L*) were significantly affected by the various dietary treatments. This result is in agreement with the report of Olagoke (2015) who observed that variation of fibre contents and cashew nut liquid inclusion in the diet of WAD goats had significant effect on fermentation kinetics. Sirohi *et al*. (2012) observed that potential gas production increased in high fibre, medium fibre and low fibre diets with the inclusion of various oils. For all the treatments in this study, values of gas production from soluble fraction (*a*) were positive and significantly different which are associated to the significant increase in gas production rate constant and decrease in lag time.

There is a positive correlation between *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), short chain fatty acid (SCFA) and gas production, and gas production is a good predictor for the production of volatile fatty acid, which is positively related to microbial mass production (Menke and Steingass 1988, Liu *et al*. 2002). In the current study, while IVOMD and SCFA values increased with an increase in *C. odorata* inclusion from 0% to 4% inclusion and decreased with 6% inclusion, the ME values increased with increase in *C. odorata* inclusion in all the treatment. Jiménez-

Peralta *et al*, (2011) had a similar observation with growing lambs fed *Leucaena leucocephala* supplemented diet. Yan *et al.* (2007) found that garlic oil and juniper berry oil increased IVOMD, ME and SCFA of goats. However, this result is contrary to that of Olagoke (2015) who observed a decrease in IVOMD, ME and SCFA in WAD goats diet with cashew nut liquid supplementation. Higher CP value of feeds, higher gas output could be the reason for the increase values in this study.

*In vitro* dry matter digestibility (IVDMD) can give an idea of the microbial population and activity during substrate fermentation (Kongman *et al*, 2010). In the current study, IVDMD percentage increased with increase in *C. odorata* supplementation. This is contrary to the observation of Hartanto *et al*. (2017) who reported that monensin supplementation had no effect on IVDMD of female Boer goats. Kawed (2016) observed increase in IVDMD percentage with increase in the inclusion of *C. odorata* leaf meal in the diet of SEA goats. Higher *in vitro* dry matter digestibility of *C. odorata* supplemented diets was possibly due to higher level of CP of the diets, and stage of plant maturity. The provision of protein may enhance the activity of the rumen microorganisms and improve digestibility of feedstuffs (McDonald *et al.,* 2010).

Goats fed diet supplemented with 4% *C. odorata* leaf meal were most superior in terms of performance characteristics. The improved performance characteristics could be attributed to the high CP percentage of the diet, higher feed intake, higher CP intake and higher CP digestibility compared to other treatments. This suggest that *C. odorata* is a good protein source as it improved feed intake, CP digestibility and CP intake up to 4% level of inclusion. This result is supported by the observations of Apori *et al*. (2000) and Kawed (2016) who experimented on WAD and small East African (SEA) goats respectively and reported positive performance characteristics of goats fed *C. odorata* supplemented diets.

Total dry matter intake, CP intake, EE intake, ash intake, NFE intake and OM intake was not influenced by the *C. odorata* supplementation. Aro (1990) worked on the effect of *C. odorata* supplementation and there was no significant effect on dry matter and nutrient intake. Apori (2000) had a similar result in rabbits, Silivong (2012) did not observe any significant difference in the dry matter and nutrient intake of Bach Thao goats fed paper mulberry and muntingia leaf meal diet. However, Kawed (2016) observed a reduced dry matter and nutrient intake in SEA goats fed *C. odorata* supplemented diets when inclusion level exceeded 10%. He opined that the unpleasant smell of *C. odorata* leaf meal may reduce dry matter and subsequently nutrient intake at higher inclusion level. The non-significance in dry matter intake and total nutrient intake in this study suggest that prolonged sun drying of *C. odorata* leaf meal may improve its acceptability in the diets of goats.

Digestibility for DM, CP, EE, CF, ash and NFE in this study were not significantly affected by *Chromolaena odorata* inclusion in the diets. This observation is similar to the reports of Apori (2000) and Kawed (2012) who experimented on rabbit and SEA goats respectively, they did not record any significant effect on digestibility of DM, CP, EE, CF, ash and NFE of rabbits and goats fed *C. odorata* supplemented diets respectively. Silvong (2012) also did not observe any significant effect on nutrient digestibility of goats fed mulberry supplemented diets. Also Park *et al*. (2014) did not observe any effect of *Forsythia suspense* supplementation on nutrient digestibility of native Korean goats. This is an indication that *C. odorata* inclusion in the concentrate ration of WAD bucks does not have any negative effect on nutrient digestibility and utilization. On the other hand, high fiber and lignin contents in the plant are known to affect total tract apparent nutrient digestibility in ruminants. For example, Mekasha *et al*. (2002) reported that high fiber and lignin diets decrease nutrient digestibility. In this

study however, the fibre levels of the diets were not high (6.21 – 6.52%), which is another plausible reason for the non-significance observed in apparent nutrient digestibility. However, Yusuf *et al*. (2017) who fed Boer goats with *Andrographis paniculata* supplemented diets reported significant effect on nutrient digestibility, values increased with increasing inclusion of the leaf meals. He opined that the increased DM, NDF and ADF digestibilities could be due to the increase in the populations of *Ruminococcus albus, Ruminococcus flavefaciens* and *Fibrobacter succinogenes* in the rumen of goats fed *Andrographis paniculata* diets. The increase in CP digestibility in the *Andrographis paniculata* supplemented goats could be due to the tannin present in *Andrographis paniculata*, which protected the dietary protein from ruminal degradation.

No effects of *C. odorata* supplementation were observed on total nitrogen intake, discharged nitrogen through feaces and urine or nitrogen retention. Rumen ammonia concentration was reduced and nitrogen retention rate was increased by intra-ruminal supplementation of 60 g *Yucca schidigera* extract (Hristov *et al.,* 1999) or 120 ppm *Yucca schidigera* extract containing saponins (Santoso *et al.,* 2004). Santoso *et al.* (2007) also reported that triterpene saponin decreases nitrogen excreted through urine. The report of Park *et al*. (2014) did not observe any effect of *Forsythia suspense* supplementation on nitrogen utilization of native Korean goats is in accordance with the observation of the current study. *C. odorata* containing saponins did not affect nitrogen retention in this study, indicating that *C. odorata* does not have the ability to alter nitrogen metabolism in the rumen at 2, 4 and 6% levels of inclusion.

According to Ahamefule *et al,* (2005) packed cell volume (PCV) is an index of toxicity and its range varies with breeds. In the present study, PCV values were within the physiological range of 22 – 38% reported by Krammer (2000) for goats. Aikhuomobhogbe and Orheruata (2006) observed that low PCV results in anaemia. This

is attributed to reduced oxygen carrying capacity of the blood. The PCV values observed in this study is an indication that the treatment diets were nourishing, non-toxic and influenced adequate blood production and supply. The red blood cell (RBC) and white blood cell value (WBC) values were within the normal range for WAD goats reported by Daramola *et al*, (2005). The RBC values in this study suggests that the diets supports good health status of the goats. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating medium (Ahamefule, *et al*. 2005). Similar results were reported for haematology of indigenous Pedi goats fed *Vachellia karroo* leaf meal supplemented diets (Brown *et al*., 2006). Serum values such as total protein, albumin, globulin, Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were within normal range for goats reported by (Merck manual, 2016). However, aspatate aminotransferase (AST) though within the normal range reported in Merck manual (2016) for goats increased with increasing level of *Chromolaena odorata* inclusion. This could be a pointer that *C. odorata* could be toxic to the liver of goats at higher inclusions and longer duration of feeding.

## 5.1. Conclusion

The following conclusions can be made from this study;

1. Proximate composition of diets was affected due to various inclusions levels of *C. odorata*.
2. Dietary inclusion of *C. odorata* at all levels increased the *in vitro* cumulative gas production, affected rumen fermentation kinetics parameter.
3. *In vitro* organic matter digestibility, *in vitro* dry matter digestibility, short chain fatty acid, metabolisable energy all increased significantly with the addition of *C. odorata* to the diets while methane gas estimate was unaltered.
4. West African dwarf buck fed diet containing 4% of *C. odorata* as additive had the best performance in terms of weight gain and daily weight gain.
5. Apparent nutrient digestibility and nitrogen utilisation was unaffected by the addition of *C. odorata* at various levels.
6. The addition of *C. odorata* showed no adverse effect on the intake of feeds and nutrients by West African dwarf bucks.
7. Rumen microbial population increased with increase in *C. odorata* addition. However, total protozoa and fungi population was unaffected.
8. Haematological parameters were not affected by *C. odorata* addition in the diets of the bucks. However, serum values were affected with bucks on 4% *C. odorata* addition having the highest serum protein and albumin values.

## 5.2. Recommendation

Based on the results obtained from this study, the following can be recommended for West African dwarf bucks;

1. *C. odorata* at 4% inclusion could be employed as an additive in West African dwarf buck nutrition as it resulted in improved ruminal fermentation, while not increasing methane emission and improved post incubation parameters (*in vitro*).
2. On *in vivo* studies, *C. odorata* addition at 4% resulted in improved growth performance characteristics, while apparent digestibility, nitrogen utilisation, feed and nutrient intake remained unchanged and thus, could support the efficient utilisation of nutrients and performance of West African dwarf bucks.
3. Further research should be carried out on the effect of *C. odorata* on methanogenesis at higher inclusion levels and preferably with the use of gas chambers.

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