Vernonia amygdalina (bitter leaf) as a phytogenic additive in the diet of West African Dwarf goats

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A 98-day feeding trial was carried out to evaluate the effect of *Vernonia amygdalina* (bitter leaf) on growth performance, faecal worm egg count and rumen microbial count of West African Dwarf goats. Using a completely randomised design, 24 West African Dwarf goats were divided into four treatment groups of six animals. Four concentrate diets were formulated to contain *V. amygdalina* at 0, 5, 10 and 15 g/kg DM. *Panicum maximum* was the basal diet. Feed intake was monitored daily and weights were taken every 2 weeks. Faecal samples were collected monthly and analysed. Rumen fluid was analysed for microbial composition at the start and at the end of the experiment. Results showed that feed intake of the goats increased as the levels of *V. amygdalina* in the diets increased. Total and daily weight gains were highest ($P \leq 0.05$) in goats fed diets containing 15g/kg *V. amygdalina*. Faecal worm egg count reduced as the level of *V. amygdalina* in the diets increased. The lowest counts, 46.7 and 46.6 eggs/gram (which were statistically similar), were obtained in goats fed diets containing 10 and 15g/kg *V. amygdalina* respectively. Bacteria, fungi and protozoa count were not significantly ($P > 0.05$) influenced by *V. amygdalina* inclusion in the diets. However goats fed diets containing *V. amygdalina* had their counts reduced by the end of the study while that of the control increased. It can be concluded that for increased feed intake and weight gain, and reduced faecal worm egg count, *V. amygdalina* could be included in the diet of West African Dwarf goats at 15g/kg DM.

Keywords: *Vernonia amygdalina*, feed additives, faecal worm egg, microbial count

Antibiotics have been used for decades as growth promoters or production enhancers in ruminants and have been confirmed to be effective in rumen manipulation (McDougall et al. 2004; Gallardo et al. 2005; Aderinboye et al. 2011). Antibiotics usage in animal feeds has been restricted, and in some countries banned, due to safety concerns (Adebayo et al. 2021). However, consumers expect good quality animal products that are safe to human health (Jouany and Morgavi 2007). In a bid to evade the use of antibiotics in animal diets due to the controversies surrounding them, some natural products such as probiotics, dicarboxylic acids, plants and plant extracts and exogenous enzymes have been identified as alternatives to antibiotic feed additives (Jouany and Morgavi 2007). These alternative additives are used now to reduce or eliminate pathogens and improve animal growth. Plants and plant extracts used as alternative to antibiotics in livestock feed are refered to as phytogenics and have been commonly used in this century.

Phytogenic feed additives are substances derived from plants; they are also referred to as phytobiotics or botanicals. They include herbs and spices and their extracts which can be added to animal feed to promote growth and improve the health status of the animals. They are being strongly considered as additions to the set of non-antibiotic growth promoters, such as organic acids and probiotics which are already well established in animal nutrition (Windisch et al. 2008). Their usefulness lies in some chemical substances that produce definite physiological actions in the body of the animals (Oko and Agiang 2009). Some of the useful herbs and spices are indigenous to Africa and include ginger (*Zingiber officinale*), garlic (*Allium sativium*), scent leaf (*Ocimum gratissimum*) and bitter leaf (*Vernonia amygdalina)* (Muhmmad et al. 2009). *V. amygdalina* is a shrub or small tree that grows throughout tropical Africa. It is popularly called bitter leaf because of its abundant bitter principles (Ekpo et al. 2007). The bitter leaf plant contains *vernonine*, *vernodalin* and

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venomygdin. It is known to be endowed with valuable biochemical properties, fixed oil, alkaloids, saponins, tannins and other glycosides (Bonsi et al. 1995). According to Nwanjo (2005) *V. Amygdalina* contains alkaloids, carbohydrates, tannins, saponins, flavonoids and non-cyanogenic glycosides. Tannins and phenolic monomers have been found to be toxic to some of the rumen microbes (especially ciliate protozoa), fibre degrading bacteria and methanogenic archaea and as a result methanogenesis in the rumen can also be reduced (Carlos and Edgar 2010). Mahady (2002) reported that the genus *Vernonia* was included in plants with active antimicrobial activity. Studies have been carried on the benefits of *V. Amygdalina* in livestock nutrition (Onwuka et al. 1989; Aregheore et al. 1998; Mohammed and Zakariya'u 2012). The leaf extract has been used to treat coccidiosis (Dakpogan 2006) and baciliary white diarrhoea and bronchitis (Gbolade 2009). Huffman et al. (1996) reported that the bitterness of *V. Amygdalina* might enhance the gastro intestinal enzymes especially chymotrypsin production which may enhance the digestion of sporozoites. According to Erasto et al. (2007) *V. Amygdalina* may also provide antioxidant benefits. However, more research needs to be carried out on the activity of *V. Amygdalina* as a growth promoter and as a medicinal plant especially in the diets of ruminants. Hence, this study was carried out to determine the effect of *V. Amygdalina* on growth performance, faecal worm egg count and rumen microbial population of West African Dwarf goats

Materials and methods

Location and climate of the experimental site

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

Processing of test materials and formulation of diets

V. amygdalina leaves were harvested from villages around the University and air-dried for 5 – 7 days after which they were ground to pass through a 1 mm sieve and used in diet formulation. The dietary treatments consisted of a basal diet (*Panicum maximum*) and concentrate supplement formulated to contain *V. Amygdalina* as an additive at levels 0, 5, 10 and 15g/kg DM (Table 1). The diets; basal and concentrate supplement were offered to the animals at 5% of their body weight in a ratio of 60:40 respectively.

Experimental animals and management

A total of 24 West African Dwarf bucks (male goats) with an average live weight of 7.5 ± 0.3 kg were used for the experiment. Pens were cleaned and disinfected with Izal solution prior to the arrival of the animals. The goats were kept in the quarantine section for 2 weeks for proper prophylactic treatment which consisted of intramuscular application of oxytetracycline LA and vitamin B complex at the dosage of 1 mL/10 kg body weight of the animal. The animals were maintained on *P. maximum* grass and concentrate and clean water was supplied *ad libitum.* They were thereafter divided into four treatment groups of six animals each on a weight equalisation basis and housed in individual pens. The study comprised 14 days of adaptation followed by a 12 week feeding trial and 2 weeks nutrient digestibility study. The diets were offered at 8:00 am in the morning and 2.00 pm in the afternoon. The concentrate diets were offered first and then the basal diet in separate feeding troughs. Left over of the feed offered was weighed and recorded every morning to compute feed intake on dry matter basis. Initial weights of the animals were taken on the first day of the experiment and thereafter every 2 weeks throughout the experiment.

Chemical analyses

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

Faecal sample collection and analysis

Faecal samples of about 4 g were collected directly from the rectum of each goat at the beginning of the experiment and at 2-week intervals for identification of helminth eggs using floatation techniques (Ameen et al. 2010); 3 g of each faecal sample was ground and mixed with 42 mL of water. A saturated solution was poured into the mixture of faeces and water to float the eggs following the modified McMaster method (Miller et al. 1998). Samples obtained from this were collected and put into both compartments of a McMaster counting chamber/slide and then viewed under the microscope. The number of eggs obtained within each viewed area was multiplied by 100 to get the actual number of eggs per gram.

Rumen fluid collection and analysis

Samples of unstrained rumen fluid collected from each animal were fixed with 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin solution) for the determination of microbial population by total direct count of bacteria, protozoa and fungi (Galyean 1989). This was carried out at the beginning and at the end of the study.

Statistical analysis

All data collected were subjected to one-way analysis of variance for a completely randomised design using version 9.1 of SAS software (SAS, 2003) with the following model:

$Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} = observed variation,

μ = population mean,

T_i = effect of i^{th} diet (i = 1 - 4),

e_{ij} = error term.

Significant means were separated using Duncan's procedure. Mean differences were considered significant at $P \leq 0.05$.

Results

Table 1 shows the chemical composition of the concentrate diet, *P. maximum* and *V. amygdalina*. The dry matter of the concentrate diet was 83.5% while those of *P. maximum* and *V. amygdalina* were 65.5% and 84.0% respectively. Crude protein values were 11.9%, 8.2% and 14.5% for the concentrate diet, *P. maximum* and *V. amygdalina* respectively. The neutral detergent fibre obtained for the concentrate diet was 64%, that of *P. maximum* was 65% and that of *V. amygdalina* was 64%.

Table 2 shows the phytochemicals and mineral composition of *V. amygdalina*. The plant contains tannins (1.9%), saponins (2.1%), oxalate (3.9%), phytate (2.3%), alkaloids (3.2%), phenol (0.3%) and trypsin inhibitor (33.7 TUI/mg). The mineral content of *V. amygdalina* includes Na, K, Ca, P, Mg, Fe, Cu and Zn.

Concentrate intake, average initial weight and average final weight were not significantly ($P > 0.05$) influenced by the experimental diets (Table 3). Grass intake (269 g/d) and total feed intake (435 g/d) were highest ($P \leq 0.05$) in goats fed the diet containing 15 g/kg *V. amygdalina* and lowest in goats fed the control diet (containing no *V. amygdalina*). Total weight gain (3.7 kg) and daily weight gain (52.9 g/d) were also highest ($P \leq 0.05$) in goats fed the diet containing 15 g/kg *V. amygdalina* and lowest in goats fed the control and 5 g/kg *V. amygdalina* diets. The feed conversion ratio was lowest ($P \leq 0.05$) in goats fed diets containing 10 and 15 g/kg *V. amygdalina* and highest in goats fed diets containing 0 and 5 g/kg *V. amygdalina*.

Table 4 shows the faecal worm egg counts of the experimental goats. No significant ($P > 0.05$) difference was observed in the initial count. However the final count was higher ($P \leq 0.05$) in goats fed the control diet (0 g/kg *V. amygdalina*) and lower in goats fed diets containing varying levels of *V. amygdalina*. The percentage reduction in faecal worm egg count was highest ($P \leq 0.05$) in goats fed diets containing 15 g/kg *V. amygdalina* with the value of 65.0% followed by those on 10 g/kg *V. amygdalina* with 50.0 % and goats on 5 g/kg *V. amygdalina* with 43.3%. The faecal egg count of goats fed control diet increased by 25.0%.

Bacteria, fungi and protozoa counts of the experimental goats were not significantly ($P > 0.05$) affected by the experimental diets (Table 5). However, by the end of the study the bacteria count increased in goats fed the control diet and the diet containing 5 g/kg *V. amygdalina* but reduced in those fed diets containing 10 and 15 g/kg *V. amygdalina*. The fungi count also increased in the control diet but reduced in goats fed varying levels of *V. amygdalina*. The protozoa count also decreased in goats fed diets containing varying levels of *V. amygdalina*.

Table 1: Ingredients and chemical composition of experimental diets and test ingredient

	Concentrate	***Panicum maximum***	***Vernonia amygdalina***
Ingredient composition g/kg			
Cassava peel	400	-	-
Maize cob	200	-	-
Dried brewers grain	200	-	-
Wheat offal	170	-	-
Bone meal	20	-	-
Salt	10	-	-
Total	**1000**	-	-
Chemical composition %			
Dry matter	83.5	65.5	84.0
Crude protein	11.9	8.2	14.5
Ether extract	5.4	5.5	8.3
Ash	7.3	11.5	4.3
Neutral detergent fibre	64.0	65.0	64.0
Acid detergent fibre	32.0	47.6	43.0
Acid detergent lignin	15.0	20.0	38.0

Table 2: Phytochemical and mineral composition of *Vernonia amygdalina*

Parameters	**Composition**
Phytochemicals	**%**
Tannins	1.9
Saponins	2.1
Oxalate	3.9
Phytate	2.3
Alkaloids	3.2
Phenol	0.32
Trypsin inhibitor (TUI/mg)	33.7
Minerals	**mg/100g**
Na	3.3
K	7.1
Ca	10.8
Mg	13.7
P	22.1
Fe	15.7
Cu	0.6
Zn	1.8

Table 3: Feed intakes and growth performance of goats fed experimental diets

Parameters	T1	T2	T3	T4	SEM	P value
Concentrate intake (g/d)	162.7	162.9	164.1	165.3	0.04	0.42
Grass intake (g/d)	213.4^{c}	226.1^{b}	238.4^{b}	269.3^{a}	5.64	0.03
Total dry matter intake (g/d)	376.1^{c}	389.0^{bc}	402.6^{b}	434.6^{a}	7.64	0.02
Average initial weight (kg)	7.4	7.5	7.4	7.5	0.19	0.10
Average final weight (kg)	10.2	10.2	10.8	11.2	0.28	0.48
Total weight gain (kg)	2.8^{c}	2.7^{c}	3.4^{b}	3.7^{a}	0.16	0.04
Daily weight gain (g/d)	40.2^{c}	38.7^{c}	48.7^{b}	52.9^{a}	2.38	0.03
Feed conversion ratio	9.4^{a}	10.1^{a}	8.3^{b}	8.2^{b}	0.49	0.04

Means in the same row having different superscripts are significantly different ($P \leq 0.05$)
T1, T2, T3 and T4 represent 0, 5, 10 and 15 g/kg *Vernonia amygdalina* inclusion levels

Table 4: Faecal worm egg count of West African Dwarf goats fed experimental diets

Parameters	T1	T2	T3	T4	SEM	P value
Initial count (egg/g)	133.3^{a}	100.0^{b}	133.3^{a}	133.3^{a}	4.73	0.001
Final count (egg/g)	166.7^{a}	56.7^{b}	66.7^{b}	46.7^{b}	14.73	<0.001
Reduction (egg/g)	-33.3^{d}	43.3^{c}	66.7^{b}	86.7^{a}	9.73	<0.001
Reduction %	-25.0^{d}	43.3^{c}	50.0^{b}	65.0^{a}	7.29	<0.001

Means in the same row having different superscripts are significantly different ($P \leq 0.05$)
T1, T2, T3 and T4 represent 0, 5, 10 and 15 g/kg *Vernonia amygdalina* inclusion levels

Table 5: Rumen microbial count of West African Dwarf goats fed experimental diets

Parameters	T1	T2	T3	T4	SEM	P value
Before the experiment						
Bacteria count (10^6cfu/ml)	0.30	0.33	0.43	0.53	0.04	0.077
Fungi count (10^6cfu/ml)	0.07	0.10	0.07	0.13	0.02	0.752
Protozoa count (10^3cell/ml)	0.53	0.43	0.73	0.83	0.06	0.081
After the experiment						
Bacteria count (10^6cfu/ml)	0.40	0.47	0.37	0.37	0.04	0.848
Fungi count (10^6cfu/ml)	0.10	0.07	0.03	0.01	0.02	0.330
Protozoa count (10^3cell/ml)	0.53	0.30	0.50	0.20	0.06	0.397

T1, T2, T3 and T4 represent 0, 5, 10 and 15 g/kg *Vernonia amygdalina* inclusion levels

Discussion

The crude protein of *V. amygdalina* obtained in this study was lower than 18.0% and 23.3% reported by Ezekwe and Obidoa (2001) and by Atangwho et al. (2009) respectively. A wide array of phytochemicals such as tannins saponins, oxalate, phytate, phenol, alkaloids and flavonoids have been reported (Ejoh et al. 2007; Eleyinmi et al. 2008; Njan et al. 2008; Atangwho et al. 2009; Banjoko et al. 2018) to be present in *V. Amygdalina* as observed in this study. The value obtained for saponin in this study is comparable to 2.2% reported by Atangwho et al. (2009), while the values of tannins and alkaloids in this study were higher than those reported by Atangwho et al. (2009). A good composition of macro- (Ezekwe and Obidoa 2001) and micro-minerals (Atangwho et al. 2009) have also been shown to be present in *V. Amygdalina* as observed in this study. The variations in the values between this study and some previous studies may be as a result of the processing method employed or age of the plant or season of harvest. According to Okukpe et al. (2019) and Atangwho et al. (2009) the phytochemicals and mineral elements present in *V. Amygdalina* are responsible for the bioactivities and health benefits possessed by the plant.

Feed intake of the goats increased as the levels of *V. Amygdalina* increased in the diets which showed the superiority of the diets containing *V. Amygdalina* over the control. The increased grass intake and total dry matter intake observed in goats fed diets containing *V. Amygdalina* was an indication that *V. Amygdalina* promoted dry matter intake. This was indicative of better degradability of the feed and hence increased intake. It also implied that the secondary metabolite in *V. Amygdalina* did not interfere with the digestion process in the rumen. According to Olafadehan and Okoye (2017) feed intake is a function of availability, palatability and fibre digestion and digesta flow rate. Fajemisin et al. (2010) also reported increased nutrient intake when graded levels of *V. Amygdalina* leaf meal were included in the diets of West African Dwarf ewes. The highest total weight gain obtained was in goats fed 15g/kg DM *V. Amygdalina*, which was associated with the highest feed intake observed at this inclusion level, suggesting that the improved feed intake translated into higher weight gain in the animals. Higher weight gain is obviously a consequence of increased feed intake and utilisation. It also means that the diet was well digested and the nutrients well utilised by the goats. Aynalem and Taye (2008) reported greater growth rates of lambs with higher levels of inclusion of *V. Amygdalina* leaf in the diet. Fajemisin et al. (2010) also reported a higher weight gain with increasing levels of *V. Amygdalina* supplementation in the diets of ewes. The better feed conversion ratios obtained in goats fed diets containing 10 and 15 g/kg DM *V. Amygdalina* suggested that diets containing 10 and 15 g/kg DM *V. Amygdalina* were well utilised by the animals and the animals were able to convert their feed to flesh better than those on other treatments. The lower the feed conversion ratio, the more efficient animals are at converting feed to flesh.

Reduction in faecal worm egg counts in goats fed diets containing varying levels of *V. Amygdalina* indicated that the concentration of the active ingredients in *V. Amygdalina* at these levels of inclusion was enough to affect the intestinal worm count. The highest reduction of 65% was obtained at 15g/kg DM inclusion level of *V. amygdalina*. Efficacy of *V. Amygdalina* in reducing faecal worm egg count could be attributed to a variety of secondary metabolites present in it such as tannins, alkaloid salt, glycoside, triterpenoid, flavonoids, anthracenoides, anthracyanins, coumarins and saponins (Agnes et al. 2013). This was also supported by Waterman (1992) who reported that parasite paralysis and/or death may have been attributed to secondary metabolites. Nfi et al. (1999) reported the anthelminthic efficacy of *V. Amygdalina* in ethnoveterinary to be 52.4%. However, Engel (2007) reported that *V. Amygdalina* contains seven different steroid glycosides as well as four sesqueterpene lactones that could be attributed to killing of the parasite. It is therefore considered that the reduction in faecal egg worm expulsion in this experiment could have been linked to these compounds. Alkaloids present in the *V. Amygdalina* could have make worms weak, unable to eat which could cause paralysis and death as a result of lack of energy, which could lead to reduction in faecal egg count. On the other hand, saponin present in *V. Amygdalina* could have caused feed refusal to the worm which means starvation of the parasite. Tannins (Butter et al. 2000) and phenolic compounds (Feireira et al 2013) have been reported to have an effect on helminths. This study affirms the report of Abosi and Raseroka (2003) that *V. Amygdalina* plant exhibits antihelmintic properties. Some phytogenic plants such as *Ocimum gtatissimum* (Adebayo et al. 2019) and *Petiveria alliacea* (Rosado-Aguilar et al. 2020) have also been implicated as anthelmintic because of the secondary compounds inherent in them. Worm reduction in small ruminants is advantageous as it will invariably improve productivity in animals and increase the profit of farmers. According to Ombasa (2012) diseases caused by helminths increase production cost and productivity losses.

Decrease in the bacteria counts at the end of the study in goats fed 10 and 15 g/kg DM was an indication that higher levels of *V. Amygdalina* could affect bacteria population in the rumen. Fungi and protozoa counts also reduced in goats fed diets containing *V. Amygdalina* by the end of the study. This implied that *V. Amygdalina* is anti-bacteria, anti-fungi and anti-protozoa and suggested that at higher levels, the secondary metabolites in *V. amygdalina* such as tannins, saponin and phenolic acid were able to affect microbial activity. The result therefore confirms the report of Mahady (2002) that the genus *Vernonia* was included in plants with active antimicrobial activity. Carlos and Edgar (2010) reported that tannins and phenolic monomers have been found to be toxic to some of the rumen microbes, especially ciliate protozoa, fibre degrading bacteria and methanogenic archaea, and as a result methanogenesis in the rumen can also be reduced.

Conclusion

V. amygdalina leaf meal improved feed intake and weight gain, and reduced faecal worm egg count up to 15g/kg DM inclusion level.

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