SECONDARY PLANT METABOLITES UTILIZATION IN WEST AFRICAN DWARF DOES FED COMBINED LEVELS OF *ANDROPOGON GAYANUS* (KUNTH) AND *GLIRICIDIA SEPIUM* (JACQ) WITH CASSAVA OFFAL BASED CONCENTRATE

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ABSTRACT

Twenty West African Dwarf Does of average weight of 5±0.58 kg aged between 3 and 6 months were used to determine the effect of level of Andropogon gayanus and Gliricidia sepium with cassava offal based diets on secondary plant metabolites digestibility. The five (5) treatments were I (Gs0) 100% Ag + 0% Gs; II (Gs25) 75% Ag +25%Gs; III (Gs50) 50%Ag +50% Gs; IV (Gs 75) 25% Ag+ 75% Gs; V (Gs100) 0% Ag + Gs100. Lowest and highest Saponin, Tannin, Phytate, Oxalate and hydrocyanide (HCN) digestibilities were observed in Gs100 and Gs0 , Gs100 and Gs25, Gs75 and Gs50 , Gs100 and Gs0 as well as Gs100 with Gs75 and Gs0 respectively. No definite pattern of digestibility was established in relation to levels of supplementation but it was evident that goats (does) can accommodate, through degradation of all the allelo chemicals.

Keywords: *Grass-legume mixture, cassava offal W.A.D.-Does, metabolites-digestibility,*

INTRODUCTION

Plant (herbages/forages) have coevolved with predator populations of bacteria,fungi insects and grazing animals and have developed defense mechanisms which assist their survival. Many plants also produce chemicals which are not directly involve in the process of plant growth but act as deterrents to insect fungal attack. These are called secondary metabolites (steriods, alkaloids terpenoids, coumarins, flavonoids lignins, tannins and toxic amino acids) and also primary metabolites (lipid, proteins, polysaccharides, and sugar) that have specific and general metabolites functions within plant cells (Howe and Westley, 1988). The secondary metabolites act as deterrents to insects with fungi, affect animal and man as well as the nutritive value of forages. Their deleterous effects vary with animal species in that while monogasters are more susceptible, polygasters especially ruminants have potential to denature them in their rumen (Norton, 1994). Andropogon gayanus, depending on habitat is perennial species which grow naturally in Africa with vertical root that grows up to 80cm down to give drought tolerance ability. It is highly palatable to ruminants, especially the young

cattle (Skerman and Rivervos, 1990). Gliricidia forage has been identified as one of the fodder legumes that promotes rumen ammonia production and live weight gain of animal (Ajayi D., Adeneyi and Ajayi F., 2005). There is dearth of information on the utilization of secondary metabolites in these herbages by goats. It is against this backdrop that this experiment is set out to look at effect of levels of Andropogon gayanus and Gliricidia sepium on the secondary metabolites utilization in West African Dwarf does fed cassava offal based diets.

MATERIALS AND METHODS

This experimentation was conducted at the Goat Multiplication and Research Unit of the Teaching and Research Farm of the Directorate of Farms of College of Agricultural Sciences, Olabisi Onabanjo University, Ago-Iwoye, Yewa Campus, Ayetoro, Ogun State. It is located between $7^0$15N $3^0$3E, 90-120 masl, T^0 28.9^0C/t and has its annual Rainfall between 1945mm, R.H.I., 72.81; Evaporation, 1806.9m, soil type oxic, paleustalf; soil texture, sandy loam and vegetation, forest mosaic type. Twenty (20) West African Dwarf goat of ages three (3) to six (6) months, with weight of 5 to 58kg were used for the experiment.

Pen Management: The pens and metabolism cages (1.5mxe l 1mx 1.2m) were swept and dusted. They were later fumigated with Dettol® (chloroxylenol, strong antiseptic disinfectant produced by Reckit Benkister, Ogun State at 27ml/1 litre of water) and Diazintol® (Diazinon a strong and broad spectrum acaricides and larvicide made by Alfasan International B.V. Holland at 2ml/1 litre of water). A mixture of used automobile engine oil (1 litre) and sieved wooden ash (250grm) was basically applied on the floor of pens (adaptation, spare and experimental) to repel soldier ants (Dorylus spp) while wood shaven were later spread on the floor. Pens and the oil-ash mixture with shaven as well as disinfectant were fortnightly applied till the end of the trial. Each pen was equipped with feed (Grass/Legume and concentrate) and water containers seperately.

Feed Materials: Gliricidia sepium leaves (leaves plus fine sterm up to 6mm in diameter) (Tarawali, Tarawal, Larbe and Harrison, 1995), were harvested from pasture and range section of the farm and spread under a shade to allow for wilting overnight before feeding the next morning. Likewise Andropogon leaves were harvested 15cm from the ground (Tarawali, Tarawal, Larbe and Harrison, 1995). Cassava peels used were collected from cassava processing centers in Ayetoro, sundried to above 12% MC and stored while cassava offal were also sourced from cassava processing centers in Ayetoro sundried, store and bagged for onward concentrate compounding as shown on table 1.

Animals Management: Twenty (20) West African Dwarf does were sourced from goat units, homestead and local markets 15km radius of the campus. On arrival, they were lairaged in the adaptation pen, prior to the commencement of the experiment. They were wormed with levaject® (levamisole, a product of SKM pharma vet Ltd,

Bangolere India, 1ml/20kg i/m) injected with Ivomec® (Ivermectin, also a product of SKM pharma vet; 1ml/40kg sc) and Terroxy L/A (Oxytetracylin, Long - acting also produced by SKM PVT. L.T.D., India: 1m/10kg). They were also dipped in Diazinon solution and finally vaccinated against Peste-des-petite-ruminante (P.P.R) (Reynolds, Attahkrah and Francis, 1988) after which Tanvit® (Multivitamin and anti-stress: SKM pharma) was administered intra muscularly at 3ml/head. During an initial fourteen (14) days, they were adapted in the adaptation pen with *ad libitum* supply of the test material and well nourished concentrate. After which they transferred into their respective experimental pens.

Experimental Design and treatments: The animals were divided into 5 groups (4 each) after balancing for age and weight. Each group was randomly assigned to one of the five treatments and individual animal was completely randomised within the pens. Each animal was fed twice daily at 0800hrsGMT with both forages (4 % body weight of forage allowance) and at 1600 hours with concentrate (1% body weight of concentrate allowance). Both allowances constitute the feed allowance which was 5% body weight of the animal as shown on table 2. This feed allowance was constantly adjusted as animal weight changes. Each component was served in separate containers and fresh drinking water was available daily *ad libitum*.

The live weights of goats were measured at the beginning of the trial and subsequently at weekly interval early in the morning before feed were offered. Records of performance and criteria for this include feed intake weight change and mortality. To calculate daily feed intake, amount of a gayanus, G. sepium and concentrated offered to, and refused by each animal were recorded daily and samples of feed offered collected three times per week. Samples for storing were even dry at 65^0C for 48 hours while that for DM determination was oven dried at between 100-105^0C for 48hours in forced draught oven at the beginning of the trial and subsequently at weekly interval early in the morning before feed was offered.

Record of performance and criteria for this include feed intake, weight change and mortality. To calculate daily feed intake, amount of A. gaynus, G. sepium and concentrate offered to, refused by each animal were recorded daily and samples of feed offered were collected three times per week. After the growth trial (1st 72days) the goats were transferred to metabolism cages in the last 12days. This was made of welded wire mesh fitted with removable feeders and arranged for quantitative collection of faeces and urine separately, but feeding and management remained the same as during the growth trial. The animals were left to adjust" in the cages for 5 days after which total faeces and urine produced by individual animals were collected for 7days after. The amount of fed offered and refused were recorded daily and samples bulked separately for each animal for the entire collection period.

Total faecal output and urine were collected in the morning before feeding and watering. The faeces were weighed fresh and 10% aliquots of each day's collection for each animal were taken and prepared for storage and DM determination as mentioned earlier. Feeds and faceal samples were separately and thoroughly mixed

and milled to pass through a 0.60mm sieve and stored in hermetically sealed containers prior laboratory analysis. The urine was collected in a plastic tray placed under each cage. 10ml of 10% concentrated H_2SO_4 was added to the tray daily to prevent microbial colonisation and prevent NH_4 volatilisation from the urine. The total output of urine for animal was measured (Chen and Gomez, 1992) and 10% aliquots were saved in stoppered numbered plastic bottles and stored at -5^0C until needed for chemical analysis.

Laboratory Analysis: Feed and faecal samples were analysed for their deleterious or anti nutritional factors or secondary metabolites (Howe and Westhly 1988), which were assayed thus: Saponin was done by method of Strong (1979) Tannin was determined by protein precipitation method according to Hagerman and Butter, (1983) Method of Maga (1983) was used for Phytate while Oxalate was analysed with the Rapid method of Beutler, Beeker, Michael and Walter (1980). Lastly, Cyanide content of sample was determined using an automated enzyme assay (Poonan and Hahn, 1984)

Statistical Analysis: Data obtained from these samples chemo-metric were used to calculate the metabolites digestibility. They were subjected to analyses using one way ANOVA/completely randomized design using individual goats as replicates. Model sums of square were partitioned to test the linear and quadratic trend of inclusion/supplementation using the general linear models (GLM) procedures as package due S.A.S (2002) and significantly different means were separated using least significance difference at 0.5 level of probability in the same package. The general linear model is as defined thus:

$Xy = \mu + \alpha_i + e_{ij}$

Xy = individual data generated from the fixed treatment (Gs0 - Gs100) effects

μ= Grand population mean

α_i = the fixed treatments effects

e_{ij} = the error (replicate) term within each treatment.

RESULTS AND DISCUSSION

Table 1 and 2 show the components in concentrate and dietary treatment allocation respectively. The chemo-metric of secondary plant metabolites, antinutrients for antinutritional factors, toxic factors, phytoalexins, allelochemicals, deleterious principle and undesirable factors from animal nutrition stand point are presented on table 3. They are undesirable but their presence is beneficial to the plant synthesing them in that as they are important component of plant coevolutionary and survival mechanism from predatory organisms and harsh weather (ligno-cellosic component) (Howe and Westley, 1980). In addition to this, they could also be of pharmaceutical importance (defaunative) (Mackie *et al.*, 1798), antihelminthic (Rozenthal and Janson. 1979) and antihypercholesteroleamic agent (Ernest, 1996). Of the metabolites

(Mg100gm-1) saponin ranged from 0.96 (A. gayanus) to 17.13 (concentrate). These values were different in quantity and unit of measurement (Ogungbesan, Ogunyemi and Olatifede, 2005; Ogungbesan, Akinboye, Apata and Olusanya, 2006, and 2010). Though this corroborates the findings of Rosenthal and Janzen (1979), who reported that Saponin is present in all almost all the higher plants, yet Tannin observed in G. sepium was lower than that reported by Ogungbesan (2010) and that in A .gayanus seems uncommon. Skermen and Riveros (1990) have reported brachiara radicans as being called "Tanner Grass" which implies possible presence of properties of tannin activity in this family Graminae. The phytate in G. sepium was higher than that recorded by Ogungbesan (2010) in G.sepium (101.22) but lower than G. sepium obtained by Aletor and Omodara (1994).

The unsual presence of phytate in A. gayanus which has not been reported could arise from the fact that phytate have been recorded from seeds and agro-industrial by products of Graminae (Eeckahout and De paepe 1994). Oxalate found both in A. gayanus and G.sepium were lower than that reported by Ologhobo (1989). Lastly, Cyanide (HCN) content was low in G.sepium (0.18) and was in consonance with the findings of Ologhobo (1989) while non was detected in A.gayanus which was contrary to the submission of Conn (1981) who reported that cyanogens are present Graminae. Those antiquality factors present in the concentrate must have been from individual ingredients with those factors (Conn, 1981).

In as much as they are phytochemical, their concentrations are bound to be influenced by factors such as plant parts assayed, age of plants, session of harvests, soil fertility, specific and varietal variations, cultivar differences, post harvest treatments, and growing conditions (water, and drought stress, photo periodicity) (Rosenthal and Janzen 1979) as well as laboratory analytical dissimilarities. Table 4 shows the intake and digestibility of these undesirable factors. Although the term digestibility is used, technically, it is ruminal degradation, attenuation or denaturation by microbal enzymes such that post ruminally negative effect would be precluded and normal bio-utilization could be facilitated.

The intake must have been determined by those present in the various combinations of feed consumed. There was virtually complete degradation of saponin among the treatments. Also, there was no significant effect of level of inclusion on the denuturation. This has been corroborated by Rosenthal and Janzen (1979), Ologhobo (1989) and Ogungbesan (2010), that its pharmaceutical property of anti-hyper cholesterolaemia can not be manifested ruminant rather in monogastric (Ernest, 1996). The same trend observed for Saponin repeated itself in Tannin digestibility. The Tannin in the feed could have been made up of the soluble hydrolysable tannin that can be completely degraded in rumen but protein its rumen by pass value would be compromised. This phenomenon has been confirmed by Preston and Leng (1987), Onwuka (1992); Ogungbesan (2005) that there was no significant effect of the inclusion on the Phytate digestion which was also similar among the treatments. There was a slight linear effect on decompositon of Oxalate, that is, the higher the

inclusion of Gliricidia,the lower the oxalate degradation albeit majority of it was degraded. No linear significant was also witnessed in Cyanide denaturation but there was similar and total decomposition among the treatments which mean that the microbes were able to secrete hydrocaynide hydrolytic enzymes. This has been confirmed by several workers (Rosenthal and Janzen, 1979 and Ogungbesan, 2010). The various degradations by animals in different levels irrespective of the inclusion level has been reported by Devendra (1990) who observed that there are specific breed and even individuality of different in tolerance and utilization of plant allelochemicals or undesirable factors by ruminants. Apart from intra ruminal degradation, there are other methods of detoxification, ruminants produce mucin in their saliva that binds tannin and release protein for digestion (Hoffinan, 1987), herbivores also have detoxification mechanism like mixed function oxidase, epoxide hydrases, reductases, hydrolytic enzymes and group transfer enzymes.

Other strategies that could be targeted at plant involve selection and breeding species, variety and accession with inherenthy low allelochemicals. Molecular manipulation of gene(s) controlling these factors can be tried but if trait is polygenic, it could affect other characters.

Dilution technique: A simple approach to reduce toxicity is by feeding the toxic plant in mixture with other plants, thus diluting the effective level of each compound.

Wilting technique: enzymes capable of degrading specific secondary compound often occur with the compound in different structures in the same plant cells an reactions occur when cell meinbraneasre disrupted.

Cutting management: One that will ensure feeding between flushies when the allechemicals are at their peak in concentration.

Fertilizer management: This will alleviate situation of nutritional stress in plant which stimulate the biosynthesis and secretion of these phyto alexins (Lowry 1989).

Zoological manipulation: This includes intraruminal fusion or inoculation of bacterial species that can degrade anti-nutrient from host rumen into rumen of other ruminants where multiplication and subsequent detoxification will continue (Allison *et al.*, 1992) or even the genetic manipulation of otherwise rumen microbes with less degrading potential into those that can secrete enzymes that can detoxitate the undersirable factors in her bages have been successful (Keith,1995).

CONCLUSION

Despite the presence of these allelochemicals in plants, it evident that goats (ruminant) can tolerate and utilize these herbages effectively. Attention should be drawn toward exploring the pharmaceutical potentials of these vegetations for ethnobotanical purposes in both men and animal.Management strategies that will rationalise land use and at same time integrate ruminant production should be embarked upon like alley forming with grasses, intensive feed garden, alley grazing; rotational system, alley grazing, permanent system, fodder tree bark, Three Strata Forage System (Tsfs) and Sloping Agricultural Land Technology (SALT).

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Table 1: Component of Concentrate (%)

Ingredients	Percentage
Cassava offal	40
Cassava peeling	15
Groundnut Cake	15
Palm kernel cake	25
Bone meal	4
Premix	0.5
Salt	0.5

Source: Experimentation, 2010

Table 2: Dietary Treatment Allocation

Materials	G50	GS25	G550	G575	GS100
Gliricidia sepium(Gs)	-	25	50	75	100
Andropogon gayanus (Ag)	100	75	50	25	-
Gs +Ag (forage allowance) 4% body weight	4	4	4	4	4
Concentrate (% body weight)	1	1	1	1	1
Forage + concentrate (feed allowance) 5% body weight	5	5	5	5	5

Source: Experimentation, 2010

Table 3: Secondary plant metabolite assay in feed components (mg 100gm-1)

Metabolism	Gliricidia sepium	Andropogon gayanus	Concentrate
Saponin	0.96	6.89	17.13
Tannin	2.10	1.06	4.11
Phytate	116.73	76.40	129.31
Hydrocyanide	0.18	0.00	0.66

Source: Experimentation, 2010

Table 4: Intake and Digestibility of secondary plant metabolites in West African Dwarf Does fed levels of Andropogon gayanus and Gliricidia sepium with cassava offal based concentrate.

Metabolites	Levels					SEM	Probability	
	G50(control)	Gs25	Gs50	Gs75	Gs100		L	Q
Saponin (g/day)								
Intake	4.84a	6.86ab	5.76ab	5.73ab	7.15a	0.30	xx	x
Faecal	0.005	0.022	0.013	0.021	0.27	0.01		
Digestible (%)	99.79a	99.71a	99.68a	99.63a	99.61a	0.07	Ns	Ns
Tannin (g/day)								
Intake	1.45bc	1.64a	1.43C	1.51b	1.47bc	0.02	Ns	Ns
Faecal	0.006	0.010	0.005	0.011	0.014	00		
Digestible(%)	99.31a	99.39a	99.30ab	98.67ab	98.63	0.11	Ns	Ns
Phytate (g/day)								
Intake	12.03b	15.12a	13.18ab	11.78b	14.07a	0.44	x	x
Faecal	0.148	0.189	0.128	0.195	0.237	0.00		
Digestible (%)	98.75a	98.74a	99.51a	98.26a	98.29a	0.15	Ns	Ns
Oxalate (g/day)								
Intake	12.90a	12.68a	11.82ab	11.46ab	10.49b	0.03	xxx	Ns
Faecal	0.103	0.247	0.191	0.264	0.238	0.01		
Digestible(%)	99.20a	98.88a	98.38ab	97.69b	\97.32b	0.19	x	x
HCN (g/day)								
Intake	27.45b	29.06b	32.78ab	34.57a	35.70a	0.74	xxx	
Faecal	0.00	0.01	0.01	0.02	0.02			
Digestible (%)	100a	99.97a	99.96a	99.94a	99.94a	6.28	Ns	Ns

P: Probability for (L) linear and (Q) quadratic trends
$^{x}P<0.05$, $^{xx}P<0.01$, $^{xxx}P<0.001$
abc: Means in same row with same superscripts are similar (P> 0.05)
G50=% Gliricidia sepium + 100% Andropogon gayanus + concentrate.
Gs25= 25% Gliricidia sepium + 75% Andropogon gayanus + Concentrate
Gs50=50% Gliricidia sepium + 50% Andropogon gayanus + concentrate
Gs75
L: Level of supplementation calculated as percentage of total feed allowance (Forage + concentrate) of 50g
DM kg^{-1} live weight
Gs75= 75% Gliricidia sepium +25% Andropogon gayanus + concentrate
Gs100= 100% Gliricidia sepium +0% Andropogon gayanus + concentrate
Source: Experimentation, 2010