Morphological Identification of Rumen Microbial Isolate and Rumen Metabolites of West African dwarf sheep supplemented with forage-based Multi-nutrient Blocks

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Abstract

*This study investigated the rumen fermentation parameters and rumen microbial identification of West African dwarf (WAD) sheep supplemented with forage – based multi-nutrient blocks (MNB). Twenty-five male WAD sheep, 9 – 12 months of age were used. The animals were randomly assigned to five formulated experimental multi-nutrient blocks having five animals per treatment and were fed with *Panicum maximum* ad libitum as basal diet for 84 days. The multi-nutrient blocks were *Ipomoea triloba*-based multi-nutrient block (IMNB), *Merremia aegyptia*-based multi-nutrient block (MMNB), *Tithonia diversifolia*-based multi-nutrient block (TMNB), *Chromolaena-odorata* based multi-nutrient block (CMNB) and *Azadirachta indica*-based multi-nutrient block (AMNB). The chemical composition, nutrient intake and concentration of rumen metabolites were statistically compared. Also, rumen microbes were isolated and identified. Results revealed that CMNB had highest ($P < 0.05$) dry matter and crude protein contents (87.48 and 22.75% respectively) and sheep fed this block recorded highest dry matter and crude protein intakes (756.08 and 85.26 g/day respectively). Total volatile fatty acid, acetate, propionate and ammonia nitrogen concentrations as well as total viable bacteria count were higher ($P < 0.05$) in sheep fed AMNB. *Ruminococcus* spp and *Lactobacillus* spp were among the group of bacteria isolated from the rumen of sheep fed forage based multi-nutrient blocks. It was concluded that feeding of forage based multi-nutrient blocks especially CMNB and AMNB to ruminant could improve rumen microbial count and fermentation products which can also improve the efficiency of the animals towards better performance.*

Keywords: Urea-molasses blocks, ruminant microbiota, fermentation indices

Introduction

The complex rumen microbiome of polygastric animals plays essential roles in digesting feeds and supplying nutrients to host animals. To positively affect rumen functions, dietary interventions have been attempted to modulate this microbiome (Calsamiglia *et al.*, 2007). The rumen microbiome as a whole and its individual populations are often analyzed to unveil the underpinning of dietary interventions. There is paucity of information on identification of rumen microbiota of West African dwarf breed of sheep prevalent in South Western part of Nigeria and their

activities towards volatile fatty acid production. In developing countries like Nigeria, livestock are fed mainly on low quality roughages, including natural grazing and agro-industrial by-products, all of which contain large quantities of ligno-cellulosic material. These feeds are deficient in protein, energy, minerals and vitamins. In addition, at certain times of the year, the quality of grazing and browse deteriorates substantially due to seasonal influences, and livestock productivity consequently declines. Addition of foliage from tree leaves or supplementation with seed meals, or urea in the form of urea-

molasses multi-nutrient blocks to ruminant, can improve the utilization of low quality roughages. Formulation of multi-nutrient blocks (MNB) from low cost and locally available feed materials that do not compete with human food has been described as very promising (Makkar, 2007). The objective of this study therefore was to identify microbes present, and to evaluate the volatile fatty acid production, in the rumen content of West African dwarf sheep fed forage based multi – nutrient blocks as supplement to grass.

Materials and methods

Experimental site, ingredients and preparation of multi-nutrient blocks

This study was carried out at the Small Ruminant Unit of the Teaching and Research Farms Directorate of the Federal University of Agriculture, Abeokuta. Five different forage based multi-nutrient blocks (MNB) were made following the protocol of Molina-Alcaide *et al.* (2008) with little modification. *Ipomoea triloba*, *Merremia aegyptia*, *Tithonia diversifolia*, *Chromolaena odorata* or *Azadirachta indica* leaf meal with urea, sugarcane molasses, wheat offal, palm kernel meal, salt, vitamin and limestone were used to prepare the blocks (Table 1). Forage leaf meal was prepared from leaves of *Ipomoea triloba*, *Merremia aegyptia*, *Tithonia diversifolia*, *Chromolaena odorata* and *Azadirachta indica* harvested before flowering stage at the premises of Federal University of Agriculture, Abeokuta. The leaves were sun-dried separately for 2-3 days, and all the stalks were separated and discarded. The dried leaves were subsequently crumbled by hand to form leave meal. Other ingredients were

purchased from reputable feed mill in Abeokuta.

The mixing of various experimental forage based multi-nutrient blocks was done by measuring known quantity (Table 1) of individual forage leaf meal in separate containers, wheat offal, premix and palm kernel cake were added and mixed thoroughly with hand. Limestone powder was dissolved in water (400mL/100g). Known quantity of urea and salt were also dissolved in another 400 mL of water after which molasses was added and thoroughly mixed; the resulting mixture (water, salt, urea and molasses) was added to the first mixture (forage leaf meal, wheat offal, palm kernel meal and premix) and stirred thorough by hand. Limestone/water mixture was also added and mixed thoroughly. Another 200 mL of water was added to the mixture and mixed. Plastic moulds were used in casting the multi-nutrient blocks. Each separate mixture was heavily compressed separately into moulds and the blocks were made on the floor under a roofed open sided building. The moulds were removed and blocks were air-dried at room temperature for 3 – 4 days and stored until used. About 50g of each multi-nutrient block was taken, pulverised separately and used for proximate composition analysis according to AOAC (2005). Fibre fractions; neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991). Energy content was analysed using bomb calorimeter.

Experimental Animals and Management

For this study, twenty five (25) male West African dwarf (WAD) sheep of about 9-12

Table 1: Ingredient composition (%) of the experimental multi-nutrient blocks

Ingredient	IMNB	MMNB	TMNB	CMNB	AMNB
Molasses	20.00	20.00	20.00	20.00	20.00
Urea	5.00	5.00	5.00	5.00	5.00
Wheat offal	30.00	30.00	30.00	30.00	30.00
Palm kernel	20.00	20.00	20.00	20.00	20.00
Limestone	10.00	10.00	10.00	10.00	10.00
Common salt	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Oyster shell	4.00	4.00	4.00	4.00	4.00
<i>Ipomea triloba</i> leaf meal	10.00	-	-	-	-
<i>Merremia aegyptia</i> leaf meal	-	10.00	-	-	-
<i>Tithonia diversifolia</i> leaf meal	-	-	10.00	-	-
<i>Chromolaena odorata</i> leaf meal	-	-	-	10.00	-
<i>Azadirachta indica</i> leaf meal	-	-	-	-	10.00
Total	100.00	100.00	100.00	100.00	100.00

IMNB = *Ipomoea triloba* -based Multi-nutrient Block, MMNB – *Merremia aegyptia* -based Multi-nutrient block, TMNB – *Tithonia diversifolia* -based Multi-nutrient block, CMNB – *Chromolaena odorata* -based Multi-nutrient block, AMNB – *Azadirachta indica* -based Multi-nutrient block

months of age, with an average weight of 13.5 - 18.0 kg were used. The animals were housed in individual pens and had free access to clean water. The animals were randomly assigned to the five formulated experimental multi-nutrient blocks (200g/animal/day) having five animals per treatment in a completely randomized design and were fed with *Panicum maximum* (*ad libitum*) as basal diet. The multi-nutrient blocks were *Ipomoea triloba* - based multi-nutrient block (IMNB), *Merremia aegyptia* - based multi-nutrient block (MMNB), *Tithonia diversifolia* - based multi-nutrient block (TMNB), *Chromolaena odorata* - based multi-nutrient block (CMNB) and *Azadirachta indica* - based multi-nutrient block (AMNB). During this period, leftovers were weighed and discarded the following morning to determine voluntary intake. The feeding trial lasted 84 days. At the termination of feeding trial, a 21-day

digestibility trial was conducted where three animals per treatment were transferred into individual metabolic cage and experimental feed and clean water were provided. Total faeces and urine were collected from individual animals on the last 7 days of the study.

Rumen studies

At the end of the digestibility study, 50 mL of rumen content was collected from each animal 4- hour after feeding through the use of suction tube and was immediately measured for temperature and pH using pH meter ((pH meter CT-6020) designed for measuring both pH and temperature simultaneously. The rumen content was strained through 4 layers of cheesecloth and pooled for isolation of solid-associated microbes (SAM) and liquid-associated microbes (LAM). Volatile fatty acid concentration and its fractions were analysed from both the liquid and solid portions by steam distillation process as

described by Kayol and Borilek, (1995) and ammonia-nitrogen concentration according to Lanyansunya *et al.* (2007). Total viable bacteria count was determined in roll tubes on a complete agar medium (Hobson, 1965) Also, Agar media and anaerobic jars were used for the incubation of anaerobic bacteria after which the agar plateaus were examined for bacteria colonies, the microorganisms were identified according to their colonial and cellular morphology, gram reaction, spore formation and aerobic growth (Kumagai *et al.*, 2004).

Statistical Analysis

All data collected were subjected to one way Analysis of Variance (ANOVA), using SAS (1999) procedure. Significance differences among means were compared using Duncan's New Multiple Range Test of the same software.

Results

Nutrient composition of forage-based multi-nutrient block

The proximate composition of the various forage –based multi – nutrient blocks (MNBs) used for the experiment is shown

in Table 2. *Chromolaena odorata* - based multi-nutrient block (CMNB) had significantly higher ($P < 0.05$) dry matter and crude protein contents (87.48% and 22.75% respectively) while other blocks had similar values. There was no significant difference ($P > 0.05$) in the ash content values obtained in *Tithonia diversifolia* - based multi-nutrient block (TMNB), CMNB and *Azadirachta indica* - based multi-nutrient block (AMNB) (23.10%, 22.78% and 21.80% respectively) but these values were significantly higher ($P < 0.05$) than those recorded for *Ipomoea triloba* - based multi-nutrient block (IMNB) and *Merremia aegyptia* - based multi-nutrient block (MMNB). Ether extract content was highest in MMNB (3.83%) while TMNB (2.75%) and IMNB (2.77%) recorded lowest values. The energy content of the blocks was similar ($P < 0.05$) and the values ranged from 8.50 MJ/kg in TMNB to 9.30 MJ/kg in MMNB and AMNB.

Differences in nutrient intake by animals fed various MNB as supplement to grass were significant ($P < 0.05$) (Table 3). Animals fed CMNB had highest intakes of dry matter from MNB, grass and consequently total intake (199.06, 557.02 and 756.08 g/day respectively) but least dry matter intake was observed in grass and total intake by animals fed TMNB (511.68

Table 2: Chemical composition (%) and nutrient intakes (g/day) of different forage-based multi-nutrient block

Nutrients (%)	IMNB	MMNB	TMNB	CMNB	AMNB	SEM
Dry matter	80.76 ^b	82.04 ^b	81.53 ^b	87.48 ^a	82.37 ^b	0.67
Crude protein	21.47 ^b	21.24 ^b	21.22 ^b	22.75 ^a	21.08 ^b	0.21
Ash	19.77 ^b	18.30 ^b	23.10 ^a	22.78 ^a	21.80 ^a	0.52
Ether extract	2.77 ^c	3.83 ^a	2.75 ^c	3.36 ^b	3.74 ^{ab}	0.13
Energy (MJ/kg)	8.60	9.30	8.50	8.70	9.30	0.22
NDF	52.70 ^a	52.12 ^{ab}	51.48 ^b	51.87 ^{ab}	51.12 ^b	0.19
ADF	36.32	36.09	36.13	36.20	35.29	0.22
ADL	19.27 ^{ab}	19.56 ^a	18.62 ^b	18.98 ^{ab}	18.97 ^{ab}	0.13

^{abc} Means along the same row with same superscript are not significantly different ($P > 0.05$), IMNB = *Ipomoea triloba* - based Multi-nutrient Block, MMNB – *Merremia aegyptia* -based Multi-nutrient block, TMNB – *Tithonia diversifolia* -based Multi-nutrient block, CMNB – *Chromolaena odorata* -based Multi-nutrient block, AMNB – *Azadirachta indica* -based Multi-nutrient block

Table 3: Nutrient intakes (g/day) of WAD Sheep fed different forage-based multi-nutrient block supplemented with Grass

Nutrient Intake	IMNB	MMNB	TMNB	CMNB	AMNB	SEM
DM block	192.34 ^b	193.62 ^b	193.11 ^b	199.06 ^a	193.94 ^b	0.75
DM Grass	525.93 ^c	520.95 ^d	511.68 ^e	557.02 ^a	540.21 ^b	4.26
DM total	718.27 ^c	714.57 ^c	704.79 ^d	756.08 ^a	734.15 ^b	4.85
CP block	41.29 ^b	41.12 ^b	40.99 ^b	45.29 ^a	40.88 ^b	0.46
CP grass	45.49 ^c	45.06 ^d	44.26 ^c	48.18 ^a	46.73 ^b	0.37
CP total	86.79 ^{bc}	86.19 ^c	85.26 ^d	93.47 ^a	87.61 ^b	0.78
NDF block	101.31 ^b	101.91 ^{bc}	99.41 ^c	103.25 ^a	99.14 ^c	0.44
NDF grass	354.22 ^c	350.86 ^d	344.62 ^e	375.15 ^a	363.83 ^b	2.87
NDF total	455.52 ^c	451.78 ^d	444.03 ^e	478.40 ^a	462.97 ^b	3.14
ADF block	69.86 ^b	69.88 ^b	69.77 ^b	72.04 ^a	69.05 ^b	0.32
ADF grass	228.94 ^c	226.77 ^d	222.73 ^e	242.47 ^a	235.15 ^b	1.86
ADF total	298.80 ^c	296.65 ^c	292.51 ^d	341.51 ^a	304.20 ^b	2.05

^{abc} Means along the same row with same superscript are not significantly different ($P > 0.05$), IMNB - *Ipomoea triloba* -based Multi-nutrient Block, MMNB – *Merremia aegyptia* -based Multi-nutrient block, TMNB – *Tithonia diversifolia* -based Multi-nutrient block, CMNB – *Chromolaena odorata* -based Multi-nutrient block, AMNB – *Azadirachta indica* -based Multi-nutrient block, DM- dry matter, CP- crude protein, NDF- acid detergent fibre, ADF- acid detergent fibre.

and 704.79g/day respectively). Similar trend was observed in crude protein, neutral detergent fibre and acid detergent fibre consumption of experimental animals.

The rumen fermentation parameters and bacteria count in the fluid portion and solid portion of rumen content of WAD sheep fed different forage based multi-nutrient blocks are presented in Tables 4 and 5. There were significant differences ($P < 0.05$) across the treatments in all the parameters measured except in the butyric acid concentration and acetic to propionic acid ratio in the fluid portion (Table 4) of the rumen content. Furthermore, in the fluid portion of the rumen content, animals supplemented with AMNB had highest ($P < 0.05$) concentration of total volatile fatty acid (252.75mmol/100mL) and acetic acid (69.00mmol/100mL) which were not different ($P > 0.05$) from that obtained in sheep fed CMNB and TMNB. Butyric acid concentration was higher ($P > 0.05$) in sheep fed AMNB and CMNB (11.80 and

11.35mmol/100mL respectively) and least in animals fed IMNB (10.15mmol/100mL) and MMNB (10.33 mmol/100mL). Sheep fed AMNB and CMNB had higher ($P < 0.05$) total viable bacteria count (5.35 and 5.11cfu/g rumen content respectively) (liquid associated microbes) compared with other treatments. IMNB had least concentration of ammonia nitrogen (12.55mg/dL). The value of acetic to propionic ratio ranged from 1.02 in MMNB to 1.13 in CMNB. Rumen pH was higher in animals fed CMNB, AMNB and IMNB (7.02, 6.98 and 6.82 respectively) than those fed other treatment diet. In the case of solid portion (Table 5) of rumen content, sheep fed TMNB, CMNB and AMNB had higher ($P < 0.05$) but similar ($P > 0.05$) values of total volatile fatty acid and acetic acid concentration, while sheep fed IMNB recorded lowest ($P < 0.05$) value for total volatile fatty acid concentration. Propionic acid concentration was higher ($P < 0.05$) in

Table 4: Rumen fermentation parameters and bacteria count in fluid portion of rumen content of WAD fed different forage-based multi-nutrient block

PARAMETERS	IMNB	MMNB	TMNB	CMNB	AMNB	SEM
Total volatile fatty acids (mmol/100mL)	221.25 ^c	240.25 ^b	249.00 ^{ab}	243.75 ^{ab}	252.75 ^a	2.88
Acetic acid (mmol/100mL)	52.25 ^b	54.25 ^b	58.25 ^{ab}	63.75 ^{ab}	69.00 ^a	2.12
Propionic acid (mmol/100mL)	47.25 ^c	53.50 ^{bc}	54.25 ^{bc}	56.25 ^b	65.75 ^a	1.73
Butyric acid (mmol/100mL)	10.15	10.33	10.78	11.35	11.80	1.91
Acetic : propionic	1.11	1.02	1.07	1.13	1.05	0.03
Ammonia nitrogen (mg/dL)	12.55 ^c	13.66 ^b	14.79 ^a	15.24 ^a	15.36 ^a	2.58
pH	6.82 ^{ab}	6.57 ^b	6.69 ^b	7.02 ^a	6.98 ^a	0.55
Total viable bacteria (x10 ⁸ cfu/g rumen content)	3.95 ^b	3.86 ^b	4.46 ^b	5.11 ^a	5.35 ^a	0.16

^{abc} Means along the same row with same superscript are not significantly different (P> 0.05), MNB =

Multi - nutrient Block

IMNB = *Ipomoea triloba* -based Multi-nutrient Block, MMNB – *Merremia aegyptia* -based Multi-nutrient block, TMNB – *Tithonia diversifolia* -based Multi-nutrient block, CMNB – *Chromolaena odorata* -based Multi-nutrient block, AMNB – *Azadirachta indica* -based Multi-nutrient block

sheep fed TMNB (5.65 mmol/100mL) and AMNB (5.35 mmol/100mL), but least in IMNB (2.95 mmol/100mL). Sheep fed CMNB and AMNB had higher (P < 0.05) but similar values (3.54 and 4.00 x10⁸cfu/g rumen content) of total viable bacteria count (solid associated microbes) while animals on IMNB recorded the least value (1.44 x10⁸cfu/g rumen content).

Isolated rumen microbes in the liquid and solid portions (solid and liquid associated microbes) of rumen content of West African dwarf sheep are shown in Tables 6 and 7. The results showed that the liquid and solid associated rumen bacteria were made up of gram positive and negative micro-organisms which comprises of cellulolytic, proteolytic, amylolytic and lactic acid bacteria. The isolates detected in the liquid

portion (Table 6) of the rumen content of animals fed IMNB and AMNB includes *Selenomonas sp.* *Ruminococcus spp* was isolated from animals fed IMNB, MMNB and AMNB, *Methanobrevibacter spp*; a methanogen was isolated from all experimental animals, *Streptococcus spp* was found present in all animals, and *Succinomonas spp* was also found in all animals except those fed CMNB. Solid associated isolate (Table 7) includes *Staphylococcus spp* (IMNB only), *Pseudomonas spp* (MMNB, TMNB and AMNB), *Bacteroides spp* (TMNB only) and *Lactobacillus spp* (all experimental animals)

Discussion

The proximate composition of the forage

Table 5: Rumen fermentation parameters and bacteria count in solid portion of rumen content of WAD sheep fed with different forage-based multi-nutrient block

PARAMETERS	IMNB	MMNB	TMNB	CMNB	AMNB	SEM
Total volatile fatty acids (mmol/100mL)	11.05 ^c	12.50 ^b	14.85 ^a	14.05 ^a	15.45 ^a	0.46
Acetic acid (mmol/100mL)	3.45 ^b	3.35 ^b	3.75 ^{ab}	3.60 ^{ab}	4.35 ^a	0.13
Propionic acid (mmol/100mL)	2.95 ^c	4.10 ^b	5.65 ^a	4.30 ^b	5.35 ^a	0.27
Butyric acid (mmol/100mL)	3.50 ^b	3.70 ^{ab}	4.35 ^{ab}	4.35 ^{ab}	4.65 ^a	0.17
Total viable bacterial count(x10 ⁸ cfu/g rumen content)	1.44 ^d	2.25 ^c	3.20 ^b	3.54 ^{ab}	4.00 ^a	0.25

^{abc} Means along the same row with same superscript are not significantly different (P> 0.05),

IMNB = *Ipomoea triloba* -based Multi-nutrient Block, MMNB – *Merremia aegyptia* -based Multi-nutrient block, TMNB – *Tithonia diversifolia* -based Multi-nutrient block, CMNB – *Chromolaena odorata* -based Multi-nutrient block, AMNB – *Azadirachta indica* -based Multi-nutrient block

Table 6: Morphological identification of isolated rumen microbiota (liquid adherent microbes) of West African dwarf sheep fed with forage-based multi-nutrient Block

Treatments	Liquid Adherent Bacteria (LAB) Isolate
<i>Ipomoea triloba</i> based multi-nutrient block	<i>Selenomonas ruminatum</i> , <i>Streptococcus faecium</i> , <i>Ruminococcus albus</i> , <i>Streptococcus bovis</i> , <i>Megashaera elsdenii</i> , <i>Ruminococcus flavefaciens</i> , <i>Clostridium lockheadii</i> , <i>Bacteroides succinogens</i> , <i>Methanobrevibacter ruminatum</i> , <i>Bacteroides amylophilus</i> , <i>Succinomonas amylolytica</i> , <i>Pseudomonas aureginosa</i> .
<i>Merremia aegyptia</i> - based multi-nutrient block	<i>Bacteroides ruminicola</i> , <i>Clostridium lockheadii</i> , <i>Methanobrevibacter ruminatum</i> , <i>Succinomonas amylolytica</i> , <i>Staphylococcus aureus</i> , <i>Lactobacillus acidophilus</i> , <i>Ruminococcus albus</i> , <i>Ruminococcus flavefaciens</i> , <i>Streptococcus faecium</i> , <i>Megasphaera elsdenii</i> , <i>Bacteroides amylophilus</i> , <i>Streptococcus bovis</i> .
<i>Tithonia diversifolia</i> - based multi-nutrient block	<i>Clostridium lockheadii</i> , <i>Ruminococcus flavefaciens</i> , <i>Lactobacillus acidophilus</i> , <i>Succinomonas amylolytica</i> , <i>Megasphaera elsdenii</i> , <i>Bacteroides succinogens</i> , <i>Methanobrevibacter ruminatum</i> , <i>Streptococcus faecium</i> .
<i>Chromolaena odorata</i> - based multi-nutrient block	<i>Lactobacillus acidophilus</i> , <i>Streptococcus bovis</i> , <i>Methanobrevibacter ruminatum</i> , <i>Megasphaera elsdenii</i> , <i>Ruminococcus flavefaciens</i> , <i>Streptococcus faecium</i> , <i>Bacteroides ruminicola</i> , <i>Clostridium lockheadii</i> , <i>Megasphaera elsdenii</i> , <i>Bacteroides amylophilus</i> .
<i>Azadirachta indica</i> - based multi-nutrient block	<i>Megasphaera elsdenii</i> , <i>Ruminococcus albus</i> , <i>Streptococcus faecium</i> , <i>Succinomonas amylolytica</i> , <i>Lactobacillus acidophilus</i> , <i>Ruminococcus flavefaciens</i> , <i>Bacteroides succinogens</i> , <i>Bacteroides amylophilus</i> , <i>Methanobrevibacter ruminatum</i> , <i>Selenomonas ruminatum</i> .

IMNB = *Ipomoea triloba* based Multi-nutrient Block,MMNB – *Merremia aegyptia* based Multi-nutrient block, TMNB – *Tithonia diversifolia* based Multi-nutrient block, CMNB– *Chromolaena odorata* based Multi-nutrient block, AMNB – *Azadirachta indica* based Multi-nutrient block.

based multi-nutrient blocks in this study revealed their potentials as sources of feed supplement for ruminants. The dry matter content of the MNBs (80.76 – 87.84%) obtained in this study fell within the range (71.10 – 95.0%) reported for urea-molasses MNB made with locally available materials such as *Leucaena*, *Gliricidia* and *Moringa* (Onwuka, 1999; Aye and Adegun, 2010 and Mubi *et al.*, 2011). The neutral detergent fibre content of the MNBs which ranged from 51.12% in *A. indica* based MNB (AMNB) to 52.70% in *I. triloba* based MNB (IMNB) were similar to the range reported by Omoniyi *et al.* (2013) but higher than the reported values (19.0% and 29.7%) by Ramchurn *et al.* (2000) and Tekeba *et al.* (2013) respectively. Isah and Okufi, (2014) reported lower variation. This variation could be attributed to the inclusion of forage in the composition of the MNB used in this study since forage was the only source of variation in the ingredient

composition of the MNB. Crude protein content of *Tithonia diversifolia* leaves (9.62%) Essiett and Akpan, (2013), *Ipomoea triloba* (10.8%), Essiett and Obioboho, (2014), *Chromolaena odorata* (18.67%) Aro *et al.* (2009), *Azadirachta indica* (8.93%) Muhammed Kashif and Shafqat Ullah, (2013) were reported. The NDF and ADF contents of MNB observed in this study were indicative of the potential of the MNBs to support rumen microbial fibre requirement. The analysed crude protein range (21.08 – 22.75%) observed in all the forage based MNBs was in line with the reported value of 18.62% -21.70% reported by Isah (2012) for MNB made with different binders and 22% crude protein reported by Asaolu and Okewoye, (2013) for *Moringa* - based MNB but lower than 27.4% and 26.12% crude protein reported by Tekeba *et al.* (2013) and Aye and Adegun (2010) for urea molasses MNB and *Gliricidia* based MNB respectively. However, the crude protein content

Table 7: Morphological identification of isolated rumen microbiota (solid adherent microbes) in West African dwarf sheep supplemented with forage-based multi-nutrient block

Treatments	Solid Adherent Microbial Isolate
<i>Ipomoea triloba</i> based multi-nutrient block	<i>Streptococcus faecium</i> , <i>Succinomonas amylolytica</i> , <i>Bacillus subtilis</i> , <i>Ruminococcus flevefaciens</i> , <i>Clostridium lockhaedi</i> , <i>Lactobacillus acidophilus</i>
<i>Merremia aegyptia</i> based multi-nutrient block	<i>Pseudomonas aureginosa</i> , <i>Bacillus subtilis</i> , <i>Lactobacillus acidophilus</i> , <i>Streptococcus bovis</i> , <i>Selenomonas ruminatum</i> , <i>Bacillus aureus</i> , <i>Bacteroides rumenicola</i>
<i>Tithonia diversifolia</i> based multi-nutrient block	<i>Lactobacillus acidophilus</i> , <i>Bacteroides amylophilus</i> , <i>Pseudomonas aureginosa</i> , <i>Bacteroides succinogens</i> , <i>Ruminococcus albus</i> .
<i>Chromolaena odorata</i> based multi-nutrient block	<i>Bacillus aureus</i> , <i>Bacteroides succinogens</i> , <i>Lactobacillus acidophilus</i> , <i>Bacteroides rummicola</i> , <i>Succinomonas amylolytica</i> , <i>Ruminococcus albus</i>
<i>Azadirachta indica</i> based multi-nutrient block	<i>Succinomonas amylolytica</i> , <i>Streptococcus faecium</i> , <i>Bacteroides succinogens</i> , <i>Pseudomonas aureginosa</i> , <i>Lactobacillus acidophilus</i> ,

IMNB *Ipomoea triloba* based Multi-nutrient Block, MMNB – *Merremia aegyptia* based Multi-nutrient block, TMNB – *Tithonia diversifolia* based Multi-nutrient block, CMNB – *Chromolaena odorata* based Multi-nutrient block, AMNB – *Azadirachta indica* based Multi-nutrient block

obtained across the MNBs surpassed 8% crude protein reported as limit required by rumen microbes for optimum activity (Norton, 1994).

The high nutrient intake observed in sheep fed CMNB could be ascribed to the high crude protein content of the MNB. It is known that high crude protein content of feed stimulates more feed intake (Oldham and Alderson, 1980). The low voluntary total dry matter intake of animals fed IMNB and MMNB compared to those fed CMNB could be linked to the high neutral detergent fibre and acid detergent fibre and low crude protein content of the MNB consumed by the experimental animals. Neutral detergent fibre concentration is used as an index of gut fill to predict voluntary feed intake (Mupangwa *et al.*, 2000). Crude protein intake is a major determinant of ruminant performance due to increase

availability of fermentable protein and other nutrient required by rumen bacteria as well as a greater opportunity for dietary protein to escape rumen fermentation (Anele *et al.*, 2010).

The trend observed in total volatile fatty acid (VFA) concentration obtained in the rumen fluid of the animals fed the various MNB supplement was relatively directly proportional to the total viable bacteria count. This implied that the observed variation in VFA production might be due to activities of viable bacteria transforming ingested feed in the rumen to microbial protein and energy – yielding VFAs. Supplementation of forage diet with readily fermentable carbohydrate, such as molasses used to compound the MNB, usually results in increased total ruminal VFA concentration (Perez *et al.*, 1996; Nocek, 1997). Contrary to this view, Ramos

et al., (2009) opined that VFA concentrations do not necessarily reflect VFA production and absorption rate. The highest dry matter and crude protein intake observed in animals fed CMNB supplement did not translate to highest propionic acid concentration in the rumen. The pattern observed in ammonia nitrogen concentration in the fluid portion of rumen content of experimental animals did not reflect the trend observed in dietary crude protein intake. This might be due to the fact that there are other endogenous sources of ammonia nitrogen to the rumen such as urea in saliva. Urea is broken down rapidly in the rumen to ammonia by urease enzyme. This activity enables ruminants to utilize urea entering the rumen either with the feed or in salivary secretion. The amount of blood urea recycled to the rumen depends on the ammonia concentration in the rumen and plasma urea concentration. Microbes adhering to the ruminal epithelium have the ability to produce urease. The enzyme hydrolyzes urea as it passes through the rumen to ammonia and CO₂. High ruminal ammonia levels reduce recycling by inhibiting urease activity. However, Nguyen Va Thu, (2005) is of a contrary view that rumen ammonia nitrogen concentration reflected the crude protein levels in the diets. The microbial yield is an important index to measure the amount of microbial protein made available to the animals each day (Creevey *et al.*, 2014). Total viable bacteria count was higher in sheep fed CMNB and AMNB. This could be as a result of higher ammonia – nitrogen concentration in the rumen of experimental animals observed in these treatments as rumen ammonia nitrogen encourages proliferation of bacteria (Nguyen Va Thu, 2005).

The pH values of rumen content of various experimental animals observed did not drop

below 5.8, which were optimal for efficient fibrolytic activities in the rumen (Ramos *et al.*, 2009).

The morphological, physiological and behavioural characteristics that evolved along with the varied feeding strategies in ruminants have strong effect on the rumen microbial community structure (Hoffman, 1989). From the result of morphological tests carried out, several anaerobic bacteria were isolated from the rumen content of the West African Dwarf sheep fed the experimental diets which is an indication of improved rumen environment as a result of synergy between the host community and the dietary treatments. Anaerobic bacteria are involved in the degradation process that occurs within the rumen environment. They are the principal agents of cell wall fermentation process in the rumen (Preston and Leng, 1987). Among the millions of bacteria involved in the degradation process, *Ruminococcus spp* which is a gram positive motile cellulolytic acid – producing bacterium was one of the bacteria isolated in the rumen of these animals and this was in line with the report of Akinbode (2011) who identified this bacterium in the rumen of WAD sheep fed different roughages. For many bacteria, diet was the major factor determining relative abundance. Bacterial communities from forage-fed animals were similar to each other, those from concentrate-fed animals were similar to each other but distinct from those in forage-fed animals, and those from animals that received mixed diets were intermediate between these (Henderson *et al.*, 2015). Another important bacteria isolated was *Selenomonas spp.* which is a gram-positive anaerobe, it was originally isolated from the rumen (Lessel and Breed, 1954) and this bacterium can make up to 51% of the total bacterial counts in the rumen (Caldwell and Bryant, 1966; Latham

et al., 1979). The genus *Selenomonas* constitutes a group of motile crescent-shaped bacteria within the *Veillonellaceae* family and includes species living in the gastrointestinal tracts of the animals, particularly, ruminants (Jeynes, 1956). *Succinimonas amylolytica* found in both liquid and solid portion of the rumen content of experimental animals is a gram positive, rod – shaped, acid producing, and anaerobic bacterium. Considering the solid portion, it was observed that the concentration of various VFAs as well as viable bacteria count was very low compared with the liquid portion of the rumen content of the various animals. This might be due to the fact that majority of the metabolites are soluble and were strained along with the liquid portion while fewer population of the viable bacteria adhere to the solid portion of the rumen content. Smaller values of solid associated bacteria isolated from the rumen of steers in comparison with liquid associated bacteria has been reported (Cecava *et al.*, 1990; Caro and Miller, 2002; Ipharraguerre *et al.*, 2007). According to Uden *et al.* (1982), there is a flux of both liquid and solid content through the rumen, microbes other than transient must be actively metabolizing to gain energy and growing to counteract washout and so persist in the rumen (van Soest, 1994). *Bacillus subtilis*, a gram positive, rod – shaped bacteria found in the solid portion of the rumen content of some of the experimental animals were completely absent in the liquid portion, while *Methanobrevibacter ruminantium* isolated from the liquid portion was completely absent in the solid portion. *Lactobacillus acidophilus*, an acid forming gram negative anaerobe was isolated from the liquid and solid portion of the rumen content of all animals; this revealed its prevalence and activity in the degradation

process in the rumen.

Conclusion

This study revealed that all the forage based multi-nutrient blocks had good nutrient profile in terms of dry matter, crude protein, energy and neutral detergent fibre which could support good animal performance. Feeding of forage-based multi-nutrient blocks especially *Azadirachta indica* and *Chromolaena odorata* - based multi-nutrient blocks as supplement to ruminant could improve rumen microbial content and fermentation pattern which would consequently improve the efficiency of the animals towards better performance.

References

- Akinbode, R.M. 2011. Comparative Studies on the effects of different roughages on the performance, digestibility and rumen ecology of West African dwarf Sheep. M. Agric Dissertation. Department of Animal Nutrition, Federal University of Agriculture, Abeokuta. Nigeria.
- Anele, U., Arigbede, O., Sdeukum, K.-H., Ike, K., Oni, A., Olanite, J., Amole, G., Dele, P. 2010. Effects of processed cowpea (*Vigna unguiculata* L. Walp) haulms as a feed supplement on voluntary intake, utilization and blood profile of West African dwarf sheep fed a basal diet of *Pennisetum purpureum* in the dry season. Anim Feed Sci. Technol 159, 10–17.
- AOAC 2005. Official Methods of Analysis, 18th Ed., Association of Official Analytical Chemists. Arlington, VA
- Aro, S. O., Osho I. B., Aletor, V. A. and Tewe, O. O. 2009. *Chromolaena odorata* in Livestock Nutrition. Journal of Medicinal Plants Research. 3(13): 1253-1257
- Asaolu, V. O. and Okewoye, A. T. 2013.

- Moringa multi-nutrient block supplementation effects on feed utilization by West African dwarf goats fed a basal diet of cassava peels. *Science Focus* 18 (1), 63 - 72
- Aye, P. A. and Adegun, M. K. 2010.** Digestibility and growth in West African dwarf (WAD) sheep fed Gliciridia based multi-nutrient block supplements. *Agricultural and Biology Journal of North America* 1 (6): 1133–1139
- Caldwell, D. R. and Bryant. M. P. 1966.** Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Applied Microbiology*. 14:794-801.
- Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L. and Ferret, A. 2007.** “Invited review: essential oils as modifiers of rumen microbial fermentation,” *Journal of Dairy Science*, 90 (6), 2580–2595
- Carro, M. D. and Miller. E. L. 2002.** Comparison of microbial markers (15N and purine bases) and bacterial isolates for the estimation of rumen microbial protein synthesis. *Animal Science*. 75:315–321.
- Cecava, M. J., Merchen, N. R. , Gay, L. C. and Berger, L. L.. 1990.** Composition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. *J. Dairy Science* 73:2480–2488.
- Creevey, C. J., Kelly, W. J., Henderson, G and Leahy, S. C. 2014.** Determining the culturability of the rumen bacterial microbiome. *Microbial Biotechnology*. 7:467-479.
- Doetsch, R.N and Robinson R.Q. 1953.** The bacteriology of the bovine rumen: a review. *J. Dairy Science*. 36:115–142.
- Duncan, D.B 1955.** Multiple Range and Multiple F-tests. *Biomedical*. 11: 1-42.
- Essienn, U. A. and Akpan, E. M. 2013.** Proximate Composition and Phytochemical Constituents of *Aspilia africana* (Pers) C. D. Adams and *Tithonia diversifolia* (Hemsl) A. Gray Stems (Asteraceae). *Bulletin of Environment, Pharmacology and Life Sciences* 12 (4): 33-37
- Essienn, U. A. and Ukpong, U. J. 2014.** **Comparative Phytochemical, Nutrient and Anti-Nutrient of Stems of *Ipomoea Involucrata* Beauv, *Ipomoea. Triloba* L. and *Ipomoea Batatas* Lam. *American Journal of Food and Nutrition* 2 (4): 71-76 .**
- G o o g l e E a r t h . 2 0 1 1 .** <http://www.google.earth>
- Hobson, P. N. 1965.** Continous culture of some anaerobic and facultatively anaerobic rumen bacteria. *J. General Microbiology*. 38:80-167.
- Henderson G., Cox F., Ganesh S., Jonker A., Young W. and Janssen H. P. 2015.** A conserved microbial community supporting ruminant herbivory. *Science PLOS Biology* (In press). Downloadable at <http://www.globalcensus.org.nz/samples.html>
- Hofmann, R. R. 1989.** Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia* 78: 443.
- Ipharraguerre, I. R., Reynal, S. M., Lineiro, M., Broderick, and Clark, J. H. 2007.** A comparison of sampling sites, digesta and microbial markers, and microbial references for assessing the post ruminal supply of nutrients in dairy cows. *J. Dairy Science*. 90:1904-1919.

- Isah, O. A. 2012.** In Vitro Fermentation and Chemical Constituents of Urea-Molasses Feed - blocks made with Different Binders for Ruminants. *Nigerian Journal of Animal Production*. 39: 142-149.
- Isah, O. A. and Okufi D. 2014.** Effect of Various Protein Sources in Urea-Molasses Feed Block on Chemical Constituents and In Vitro Fermentation in Ruminants. *Nigerian Journal of Animal Science*. 16 (2) : 272-279.
- Jeynes, M.H. 1956.** Analysis of the Genus *Selenomonas* with respect to its transfer to the Protozoa. *Int. Bulletin of Bacteriology, Nomenclature and Taxonomy*. 6 (2): 53–59.
- Kayol, L.T and Borilek, G.O.D. 1995.** Spectrophotometric determination of Volatile fatty acids. *Analytical Chemist*. 34:112-117.
- Kumagai, H., Kumagae, S., Katsunosuke, M. and Tsuyoshi, E. 2004.** Effect of supplementary probiotics to two different diets on dry matter intake, daily gain, digestibility, ruminal pH and fecal microbial populations and metabolites in female goats.
- Lanyansunya, T.P., Wang, H., Kariuku, S., Mukisira, E., Abdulrasak, S., Kibitok, N. and Ondiek, J. 2007.** The potential of *Commelina benghalensis* as forage for ruminant Animals. *Feed Science Technology*. 144: 185–195.
- Latham, M. J, Sharpe, E and Weiss, N. 1979.** Anaerobic cocci from the bovine alimentary tract, the amino acids of their cell wall peptidoglycans and those of various species of anaerobic *Streptococcus*. *Journal of Applied Bacteriology*. 47: 209–221.
- Lessel, E. R. and Breed, R. S. 1954.** *Selenomonas boskamp*, 1922-a genus that includes species showing an unusual type of flagellation. *Bacteriology Review*. 18:165-169.
- Makkar, H. P. S. 2007.** Feed supplementation block technology – past, present and future. In Editors: Makkar H P S, Sanchez M and Speedy A W.) *Feed Supplementation Blocks. Urea-molasses multivitamin blocks: simple and effective feed supplement technology for ruminant agriculture*. FAO Animal Production and Health Paper 164, 1 - 12 . <ftp://ftp.fao.org/docrep/fao/010/a0242e/a0242e00.pdf>
- Merck, 2003.** Feeding and Nutritional Management. Available at www.merckvetmanual.com.
- Molina-Alcaide, E. Pascual, M. R. Cantalapiedra-Hijar, G. Morales-García, E. Y. and Martín-García, A. I. 2008.** Effects of concentrate replacement by feed blocks on ruminal fermentation and microbial growth in goats and single-flow continuous-culture fermenters *J. Animal Science* 87:1321-1333..
- Muhammed K. and Shafqat U. 2013.** Chemical Composition and Minerals Analysis of *Hippophae rhamnoides*, *Azadirachta indica*, *Punica granatu* and *Ocimum sanctum* Leaves. *World Journal of Dairy & Food Sciences* 8 (1): 67-73
- Mubi, A. A., Kibon, A. and Mohammed, I. D. 2011.** Effects of multivitamin block supplementation on the performance of grazing Yankasa sheep in the wet season of Guinea Savanna region of Nigeria. *International Journal of Sustainable Agriculture*, 3 (3): 103 - 106.
- Mupangwa, J. F., Ngongoni, N. T., Topps, J. H. and Hamudikuwanda, H. 2000.** Effects of Supplementing a

- Basal Diet of *Chloris Gayana* Hay With one of Three Protein – Rich Legume Hays of *Cassia Rotundifolia*, *Lablab Purpureus* and *Macroptilium Atropurpureum* Forage on Some Nutritional Parameters In Goats. *Tropical Animal Health and Production*. **32**: 245–256.
- Nguyen V. T. 2005.** Effect of supplementation with sesbania (*Sesbania grandifolia*) leaves on rumen parameters, and in vivo, in sacco and in vitro digestibility in swamp buffaloes fed rice straw or elephant graa. Workshop seminar, 23 – 25 May, 2005. Cantho University Vietnam. MEKARN–CTU.
- Nocek, J. E. 1997.** Bovine acidosis: Implication of laminitis. *J. Dairy Science*. **80**:1005-1023.
- Norton, B. W. 1994.** Tree legumes as dietary supplements for ruminants. In: Gutteridge, R. C. and Shelton, H. M. (Editors) *Tropical Tree Legumes in Tropical Agriculture*. Cab International, Uk, 177-201.
- Oldham, J. D. and Alderman, G. 1980.** Recent advances in understanding protein-energy interrelationships in intermediary metabolism in ruminants. In: *Protein and Energy Supply of High Production of Milk and Meat*. Pergamon Press, Oxford, 33 pp.
- Omoniyi, L. A., Isah, O. A., Adewumi, O. O. O., Arigbede, O. M. and Onwuka, C. F. I. 2013.** Physico-chemical properties and storability of urea molasses multi-nutrient feed-block (UMMB) as dry season supplement for ruminants:.. *Journal of Applied Agricultural Research*, **5**(1): 113-121
- Onwuka, C. F. I. 1999.** Molasses blocks as supplementary feed resources for ruminants. *Arch. Zootech*. **48**: 89 - 94.
- Perez, J. F., Balcells, J., Guada, J. A., and Castrillo, C. 1996.** Determination of rumen microbial – nitrogen production in sheep: A comparison of urinary purine derivative excretion with methods using ¹⁵N and purine bases as markers of microbial – nitrogen entering the duodenum. *Br. J. Nutr*. **75**:699-709.
- Preston, T. A and Leng, R.A 1987.** Matching Ruminant Production systems with Available Resources in the Tropics and Subtropics. Penambul Books, Armidales NSW <http://www.utafoundation.org/P&L/preston&leng.htm>
- Ramchurn, R., Raggoo, R. J. and Ruggoo, A. 2000.** Digestibility and growth in the domestic rabbit using multi-nutrient blocks as feed supplement. *Livestock Research for Rural Development* **12**(1):15-19.
- Ramos, S., Tejido, M. L., Martinez, M., Ranilla, M. J. and Carro, M. D. 2009.** Microbial protein synthesis, ruminal digestion, microbial population, nitrogen balance in sheeo fed diets varying in forage – to concentrate ration and type of forage. *J. Anim. Sci*. **87**:2924–2934.
- SAS (Statistical Analysis System). 1999.** SAS/STAT User's guide, version 6 (Volume 2; Fourth Edition). Cary North Carolina, SAS Institute, Incorporated 846p.
- Tekeba, E. Wurzinger, M. Baldinger, L. and Zollitsch, W. J. 2013.** Effects of dietary supplementation with urea-molasses multi-nutrient block on performance of mid lactating, local Ethiopian and crossbred dairy cows. *Livestock Research for Rural Development* **25**(6):20-25
- Uden, P., Rounsaville, T. R., Wiggans, G.**

R., Van Soest, P. J. 1982. The measurement of liquid and solid digesta retention in ruminants, equines and rabbits given timothy (*Phleum pratense*) hay. *Br. J. Nutr.* 48:329-335.

Van Soest, P. J. 1994. *Nutritional Ecology of the Ruminant*. Second Edition, Cornell University Press, Ithaca, NY, USA. ISBN - 12: 9780801427725, p476.

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