INTRODUCTION

Spondias mombin, commonly called yellow mombin, belongs to the Anacardiaceae family. It is well distributed across Mexico, South America and widely cultivated in Paleotropics and native range in West Indies. It is a multipurpose shrub of enormous economic values as food (i.e. the fruits), fence (i.e. boundary demarcation), environmental ornament and antitimour agent (Idu et al., 2002; Wiersema and Leon, 1999). Yet, there is insufficient reports on its possible utilization as fodder in ruminant nutrition particularly in Nigeria, where it grows everywhere in the rainforest belt. Although it is a deciduous shrub, it has a very high aftermath capability indicating high biomass fodder availability at all times. Since grasses and agricultural waste have been speculated to be inadequate for optimum ruminant productivcity, shrubs and trees with potential feeding values should be evaluated for diet supplementation, especially during off season when grasses and agro byproducts may not be readily available.

Several researches have shown that feed resource evaluation is an important tool that provides useful information necessary for diet formulation in order to optimize performance. In series of experimental studies, it was established that rumen microorganisms are responsible for feedstuff degradability, digestibility and utilization by the host ruminant. But the earliest research frontiers however dwelled so much on feedstuff quality testing using chemical constituent technique (Midkiff, 1984), gas production technique (Menke et al., 1979) and cell-free fungal cellulose technique (De-Boever et al., 1986). Also, nylon bags technique and feed resource digestion technique with unidentified/mixed rumen microorganisms were used to determine the nutritional quality of feedstuff meant for ruminants (Mehrez and Orskov, 1977; Tilley and Terry, 1963).

Unfortunately, some of these techniques were somewhat not adopted in animal agriculture probably due to some forms of complexities. Above all, feed resource quality evaluation using a mixed culture of rumen microorganisms may not have actually indicated which rumen microorganism was actively involved in the gas production or the fermentation processes (Makkar, 2004; Sullivan and Martin, 1999). Consequently in vitro exposure of pure culture rumen microorganisms to feedstuff sample could be a novel and better technique of feed quality evaluation.

Hence, the present study examined the nutritional profile and susceptibility of rumen microorganisms to extract of S. mombin foliage.

MATERIALS AND METHODS

Samples preparation

Fresh foliage of S. mombin was collected from the University of Ibadan Campus and authenticated at the Herbarium, Department of Botany and Microbiology at the same University. Two hundred and fifty grams (250g) of the foliage without leaf stalk was weighed and processed as fresh, air-dried and sun-dried samples. The fresh sample (which served as the control) was crushed in a mortar (Pyrex®) and stored in the freezer. The sample for air-drying (i.e. indoor drying) was spread on the bench in a well-ventilated laboratory at the Institute of Agricultural Research and Training, Ibadan, at a mean room temperature of 28.5°C for 48hrs. Sample for sun-drying (i.e. outdoor drying) was spread on a special drying platform at a mean temperature of 33.2°C for 2 days between 08.00 and 16.00hr GMT each day. The air-dried and sun-dried samples were crispy dried yet retained the green colour. Thereafter, they were ground using hammer mill (Arthur Thomas Co. USA) to a mesh size of 2mm and stored in a cool dry shelf in the laboratory. In a cold extraction, 1g of each sample was thoroughly mixed with 40ml of ethanol (80%). The mixture was left overnight and was filtered using Whiteman No. 1 filter paper®. Although concentration of the bioactive ingredients of the filtrate was not determined, it was heated for 2mins to get rid of the ethanol while the pure extract which was not also quantified was stored in McCartney bottles kept in the refrigerator.

Nutrients and antinutrients determination

While the fresh Spondias mombin foliage samples were analyzed as wet basis (control treatment), the dried samples were analyzed on dry matter basis for proximate compositions according to AOAC (2000). The fibre constituents were determined following the description of van Soest et al. (1991). The mineral and vitamin contents were estimated adopting the methods of Wiseman and Cole.
(1990) while the gross energy was measured with an adiabatic bomb calorimeter (IKA C7000, Staufen - Germany) standardised with benzoic acid as prescribed by Witt (1987).

Haemoglobinin was estimated as described by Liener (1955), mimosine (Megarrity, 1978), cyanide (Bradbury, 1999), trypsin inhibitor (Siegert et al., 1980), Oxalic acid (Bateman and Beer, 1965) while the alkaloid content was evaluated according to Henry (1973). The phytic acid was determined as demonstrated by Vaintraub and Lapteva (1988) and the total saponin and tannin contents were analyzed using the methods of Makkar et al. (1993).

Isolation and identification of rumen microorganisms

Samples of rumen liquor were collected in sterile bottles from cattle and goats just slaughtered at Bodija abattoir, Ibadan. Meanwhile, what the animals were fed prior to liquor collection was not taken into cognizance. The samples were pooled and 1g was thoroughly mixed with 9ml of distilled water in a serial dilution procedure given by Black (1986). In an asceptic condition, 1ml of the serial dilution was mixed with 5ml of sterile Eosin Methylene Blue – EMB (idg Lab M®®, UK) and Plate Count Agar – PCA (idg Lab M®®, UK) for rumen bacteria isolation. The mixture was incubated using Temperatureregl (GmbH, Germany) at 37°C for 48hrs under anaerobic condition recommended by Levet (1990). Subculture of the growth observed was done by flaming and streaking followed by incubation at 37°C overnight to obtain pure isolates.

Using flamed inoculating wire loop, samples were collected from the pure isolates in an Oxygen free atmosphere and further subcultured in a liquid substrate “Peptone Water Broth” (idg Lab M®®, UK) and stored in an incubator at 35°C. Similarly, 1ml of the serial dilution was mixed with 5ml of sterile Potato Dextrose Agar – PDA (DM215, Micro Master Laboratories®) and an antibiotic (Vancomix, Evans®) was added to prevent bacterial growth and then incubated for 4 days at 34°C according to Kudo et al. (1990). The rumen fungal isolates were purified and transferred into liquid broth (Malt Extract: Difco Laboratories, USA) stored in an incubator at 35°C. The rumen bacteria pure isolates were subjected to morphological and biochemical tests as reported by Yokoyama and Johnson (1993). Meanwhile, the procedure was modified to include antibiotics (i.e. Ionophore and Avopacín) for detailed characterization of the rumen microbial strains. Originally, Avopacín and Ionophore (monesin, lasalocid) were used to depress certain rumen microbial strains in order to improve feed utilization efficiency (Aderinoye and Onwuka, 2010; Yokoyama and Johnson, 1993).

The rumen fungi isolates were identified according to Kudo et al. (1990). The identification features were modified to include some structural characteristics like reproductive stage, sporangiophore, mycelium and rhizoid for precise characterization of the rumen fungal strains. Although concentration of the S. mombin foliage extract was not estimated, susceptibility of the rumen microorganisms was examined by subjecting each of the identified rumen bacteria and fungi to the S. mombin foliage non-reconstituted pure extract. Each of the 12-punched-well agars was filled with the non-reconstituted foliage extract, streaked with each of the identified rumen microorganisms at a time and incubated overnight in the case of rumen bacteria and 4days for rumen fungi (Levet, 1990).

Experimental design, data collection and analysis

Randomized Completely Block Design where the feedstuff processing technique was blocked was adopted and all the parameters were determined in triplicates. Data were collected on proximate compositions, fibre components, mineral and vitamin contents as well as antinutritional constituents of the fresh, air-dried and sun-dried S. mombin foliage. Also, information on the suspected rumen microorganisms following morphological examination and biochemical tests were recorded. The growth pattern around each of the wells was observed and recorded as minimum inhibition concentration after overnight and 4 days incubation periods for the identified rumen bacteria and fungi respectively (Black, 1986). The sets of data obtained were subjected to analysis of variance procedure of SAS (1999) and the means were separated as given by Duncan’s multiple range test of the same test package.

RESULTS AND DISCUSSION

Nutritional compositions

The nutrient compositions of processed S. mombin foliage are given in Table 1. All the parameters measured were significantly improved (P<0.05) by air-drying and sun-drying compared to the control treatment. Meanwhile the sun-dried values were superior in all the cases except in gross energy and cellulose, where there were no statistical differences (P>0.05) in the air-dried and sun-dried values. The crude protein value ranged from 4.9% in control to 10.3% (air-dried) and 15.1% in sun-dried. Ether extract, crude fibre and ash values were 1.3%, 2.1% and 1.5% in control compared to 13.3%, 18.4% and 13.8% in sun-dried. The energy value varied from 2.5kcal/g in air-dried to 2.9kcal/g in sun-dried compared to as low as 0.9kcal/g in control. The neutral detergent fibre, acid detergent fibre and acid detergent lignin values which were as high as 67.9%, 53.6% and 9.3% respectively in sun-dried, was slightly followed by air-dried (43.8%, 21.9% and 6.8%) with the least values (11.5%, 7.2% and 2.9%) in control.

The observed nutritional values contradicted the report of Bhikmioya and Oriakhi (2004) where fresh S. mombin leaves had 10.06% crude protein and dry leaves 6.41%. Although the crude protein contents (4.9 to 15.1%) observed in the present study were less than 18.83% reported by Mecha and Adegbola (2006), they were within the reported values 3.6 – 22.5% (ESGPPIP, 2008), 5.08 – 10.06% (Bhikmioya and Oriakhi, 2004) and recommended range value of 1.61 – 7.76g per kilogram metabolic weight (W0.735g) (NRC, 1981) for ruminant nutrition.

The ether extract, crude fibre and ash contents observed were within 0.7 – 9.3%, 7.8 – 40.7% and 1.0 – 12.5% respectively recorded in some forages (Mecha and Adegbola, 2006). Meanwhile, the energy value was less than 0.7 – 6.88Mcal recommended by NRC (1981) for optimal ruminant productivity. However, all the values were in agreement with the nutritional requirements recommended by Givens et al. (2000). The range values of neutral detergent fibre (11.5 – 67.9%), acid detergent fibre (7.2 – 53.6%) and cellulose (4.3 – 44.3%) were within 18.31 – 68.87% recorded in some other ruminant feed resources (Iidahor et al., 2012; Rosji and Ipou, 2002).

As, drying was observed to significantly improved (P<0.05) all the minerals determined. Calcium, Iron, Magnesium, Potassium and Phosphorous values were highest (0.21%, 0.003%, 0.353%, 0.463% and 0.533%) in sun-dried followed by air-dried (0.17%, 0.002%, 0.193%, 0.343% and 0.153%) and control (0.083%, 0.001%, 0.093%, 0.153% and 0.193%). Furthermore, Sodium, Zinc, Copper and Manganese values were highest (0.233%, 0.0034%, 0.0009% and 0.004% respectively) in sun-dried sample and slightly followed by air-dried sample (0.153%, 0.0029%, 0.0005% and 0.003%) with the least (0.73%, 0.002%, 0.0002% and 0.001%) in fresh sample (control). The Magnesium, Phosphorus, Sodium and Potassium values were within the ranges 0.04 – 0.25%, 0.16 – 0.4% and 0.5 – 0.8% and 0.06 – 0.18% respectively recommended for ruminant animals (NRC 1981).

However, the Calcium level was less than 0.21 – 0.58% recommended for optimal ruminant productivity (Church, 1993; NRC, 1981). In all the vitamins evaluated, it was observed that dry processing significantly depressed (P<0.05) the values compared to the control. The Ascorbic acid, Riboflavin and Niacin values varied from 9.1 to 15.4mg/100g, 0.033 to 0.053mg/100g and 0.143 to 0.213mg/100g in sun- dried and air-dried samples but were quite high (27.8mg/100g, 0.083mg/100g and 0.323mg/100g) in control. The levels of Riboflavin and Niacin observed were less than 4.5 – 32mg/100kg BW and 26 – 182mg/100kg BW respectively recommended for ruminant animals. This observation concurred with the earlier report by Wiseman and Cole (1990) that vitamins were insufficient in processed feed hence their inclusion in ruminant nutrition is essential. The observed depression in the vitamin values could be largely due to their nature as biologically active biochemical compounds that are generally sensitive to their physical and chemical environments. According to Coelho, (1999), several vitamins contain unsaturated carbon atoms or have double bonds that make them highly susceptible to oxidation. Although, feed processing tend to improve the distribution and digestibility of nutrients, it could be harmful to heat labile nutrients such as vitamins that can easily oxidized (Gadient, 1986; Schneider, 1986).

It was observed that all the fresh sample values were seemingly lower, suggesting feed drying (particularly in hay form) superiority over fresh form (fodder). The observed distinct disparities in all the values recorded in the nutritional parameters, could be largely due to the moisture contents and possibly due to denaturation of heat-labile nutrients thereby enhancing the concentration of others. While, the disparities with other reported values could be due to the agro climatic differences, stage of growth, plant species and the laboratory protocols adopted.
Reed 1986, Olomu, 1989 discovered that drying drastically reduced (P<0.05) all the antinutrients except in Leucaena (Reed 1986) and 2.05% found in Gliricidia (Ahn et al., 1989) but was lower than 3. – 14.0% reported in Leucaena (D’Mello and Fraser 1981). Also, the mimosine and saponin concentrations were observed to be less than 12.0% and 11.0% respectively discovered in Leucaena (Tangendjaja et al., 1990). Since Barry and McNabb (1999) reported that tannin concentration greater than 4.0% could depress feed intake and there are several reports that ruminants can tolerate and utilize some levels of antinutrients (Hoskin et al., 1997), S. mombin foliage could be a suitable feed resource in ruminant production.

More significantly, the oxalic acid, tryptophan inhibitor, alkaloid, haemaglutinin and cyanide values were less than the threshold levels reported in livestock (Otumbo, 2011). The relatively safe levels estimated in all the antinutrients determined, could probably due to the part collected and maturity of the foliage. The observed supremacy of feedstuff drying technique could explain the need for proper feed processing prior to utilization in ruminant nutrition.

Susceptibility of the identified rumen microorganisms

The morphological and biochemical tests of the rumen microorganisms are shown in Tables 3 and 4. The identified rumen bacteria were bacteroides ruminicola, R. succinogenes, Butyrivibrio fibrisolvens and Lactobacillus ruminus. Others were Ruminococcus albus, R. flavifaciens, Selenomonas ruminantium and Streptococcus bovis. The rumen fungi strains were Neocallimastis frontalis, Orpinomyces joyontii, Saccharomyces cerevisiae, Maruc species, Cucumomyces communis and Rhiizopus species. The kinds of rumen microorganisms that were recorded in the rumen liquor could be possibly due to the nature of feed the hosts (cattle and goats) were fed and probably due to their age.
The minimum inhibition concentration of *S. mohnii* foliage extract on the rumen microorganisms is expressed in Table 5. All the treatments were observed to absolutely inhibit (2mm MIC) *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *Butyrodiestrum ruminicola*, *Streptococcus bovis* and *Lactobacillus ruminis* and mildly inhibited (1.60 – 1.62mm MIC) *Bacteroides succinogenes*. Air-dried and sun-dried samples were also observed to absolutely inhibited (2mm MIC) *Selenomonas ruminantium* and mildly inhibited (1.51 – 1.52mm MIC) *Ruminococcus albus*. Similarly, sun-dried and fresh samples absolutely inhibited (2mm MIC) *Caeccomyces communis*, *Saccharomyces cerevisiae* and *Neocallimastix frontalis* but *Oripinomyces joyonii* was mildly inhibited (0.31 – 1.31mm MIC). On the other hand, it was discovered that *Mucor* and *Rhizopus* species were absolutely insoluble (0.0mm MIC) to all the treatments.

It was shown that all the rumen microorganisms were susceptible to the processed *S. mohnii* foliage pure extract except *Mucor* and *Rhizopus* species that were absolutely not inhibited, indicating that dry processing technique may not improve rumen microbial degradation and subsequent nutrient utilization of feedstuff resource by the host as reported by some scientists. The high degree of susceptibility observed in the present study is somewhat not clear because the anti-nutrients concentrations in browse plants reported by several scientists to be a possible cause of rumen microbial toxicity vis-à-vis host rumenant were tremendously reduced by the feedstuff processing techniques evaluated in the present study. Meanwhile, it could be due to the growth media used, number of punched-well, foliage extract concentration and probably due to the medium of extraction. The absolute insusceptibility of the mould species could simply be due to their characteristics mode of growth.
microorganisms that would be made available in commercial quantity for feed resource quality rapid test anywhere in the world.

CONCLUSIONS

The results revealed that drying technique significantly improved the proximate, fibre and mineral compositions of S. mombin foliage. In contrast, all the vitamins determined were considerably reduced by drying. Similarly, the concentrations of all the antinutrients were significantly lowered by drying. Microorganisms that would be made available in commercial quantity for feed resource quality rapid test anywhere in the world.

REFERENCES


SMITH, C., VAN MEGAN, W. et al. 2010. Regulatory effect of monesin of feed resources may be necessary prior to utilization in ruminant feeding. On the all the antinutrients were significantly lowered by drying. Similarly, the concentrations of all the antinutrients were significantly lowered by drying. Microorganisms that would be made available in commercial quantity for feed resource quality rapid test anywhere in the world.


