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Nutrient Composition, Anti-Nutritional Factors, and In Vitro Gas Production Assessment of Some Selected Legume Crops and Stover Hay

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Abstract: Crop residues is cheapest way of making feed available to ruminant animals during dry season when there is non-availability of pastures. Consequently, a study was conducted to evaluate the nutritive value, anti-nutritional factors and *in vitro* gas production evaluation of five different legumes stovers (Bambaranut stover (T_1), Groundnut stover (T_2), Cowpea stover (T_3), Soybean stover (T_4) and Pigeon pea stover (T_5)). The stovers were harvested at the University Teaching and Research Farms of Federal University Wukari between October and November. The harvested legumes stovers were chopped into 3–5cm length and air-dried under shed for two weeks on concrete floor. The chemical composition, phytochemical and *in vitro* gas production assessment were determined using standard procedure. The dry matter (DM) ranged from 91.83- 93.55%, crude protein (CP) 11.42-22.46%, Ash content (4.00 - 14.14%), crude fibre (CF) 19.30-31.05% while fibre fractions: Neutral detergent fibre (NDF) ranged from 52.50 – 59.80% and acid detergent fibre (ADF) 27.60–33.55%. All the parameters showed significant ($P<0.05$) differences across the treatments. The anti-nutritional factors such as Phenol (0.68-1.13%), Phytate (0.03- 0.06%), Oxalate (0.013- 0.020%), Saponins (0.09-0.18%), Trypsin (0.31-0.35%) and Tannin (0.56–0.82) varied significantly ($P<0.05$) except the saponins that was similar across the treatments. The results of *in vitro* gas production assessment indicate that all the parameter such as methane (CH_4ml) 6-18ml, gas volume production (GVml) 9-28ml, metabolisable energy (ME MKJ/DM) 4.76-6.78, short chain fatty acids (SCFA µml) 0.16-0.61µml, organic matter digestibility (OMD %) 35.60 – 51.98%, dry matter degradability (DMD%) 57.65-86.63%, and fermentation efficiency (FE) 2.67-6.21 varied significantly($P<0.05$) across the treatments.The results obtained from this study indicate that crude protein content of all the selected legumes stover hay is above 10-12% recommended for ruminant animals and anti-nutrition composition is within tolerance level. Furthermore, *in vitro* gas production assessment revealed that DMD, ME and SCFA of legumes stover hay is degradable and can supply energy to the ruminants. Thus, ruminant animals can utilise them if well processed and preserved for dry season feeding.

Keywords: Legumes stover hay, chemical composition, anti-nutritional factors, *in vitro* gas evaluation

INTRODUCTION

Inadequate feeding is a major limiting factor to ruminant production in tropical Africa. Fodder is of poor nutritional value for most of the year due to the rainfall pattern. In the arid and semi-arid zones, rainfall is less than 600 mm and between 600 to 1000 mm per year, respectively (Motubatse *et al*., 2008). The management system is nomadism and transhumance pastoralism. Animals have to trek long distances in search of fodder and water. The rapid buildup of cell-wall materials and decline in crude protein (CP) content with maturity reduces the nutritional value of the forages. Little is known about the nutritional value, distribution, palatability, seedling vigour and seasonal production of the forage species that characterize the natural grassland. Besides the use of browse, other strategies can be employed to improve the feeding of ruminant animals.

During the dry season, the quality of available herbage is very low, unless the ruminant animals have access to supplementary feeds, they lose weight. Protein is especially important in growing animals since protein can be likened to bricks, building blocks used to build tissue and bone. Ruminants in rural areas of Africa and Sub-Sahara especially Nigeria, subsist under poor nutritional conditions, utilizing feedstuff from poor natural pastures and crop residues (Lanyasunya *et al*., 2006). During the dry season live weight losses do occur because the forages are generally deficient in nutrients such as protein, minerals and vitamins (Kawas *et al*., 2010). The feeding value of these feedstuffs need to be improved

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to achieve high productive performance of the animals and growth yield. Hence, supplementation with on-farm produced forage legumes or with locally available ones is being examined in relation to the ability to overcome nutritional deficiencies in the rumen and on their possible contribution of undegradable but otherwise digestible nutrients, particularly protein.

Legumes forage have become popular among farmers, not only for use in reinforcement of planted grass pastures but also as protein banks to supplement other poor-quality roughages like cereals and grasses. Legumes are known to have high protein contents usually in the range of 120 to 230 g kg^{-1} DM (Ajayi *et al*., 2009). There is increasing interest in making use of legumes forages as sources of protein-rich supplements to improve the productivity of ruminants given low quality feeds (Moalafi *et al*., 2010). In Nigeria, ruminants usually suffer from feed shortage and nutrition both in quantity and quality during the dry season. Crop residues (mainly from cereal crops) and natural pastures are the major feed resources. Ruminant are largely made to graze/browse on natural pastures. However, the natural pastures are markedly declined in nutritional value during dry season. Similarly, the residues from cereal crops are of low nutritive value and also less consumable (Singh *et al.,* 2011). Supplementation with concentrate feeds or improved forage legumes can improved the feeding value of such feed resources but concentrates are costly and not be easily accessible to smallholder farmers (Tolera *et al.,* 2000). On the other hand, improved forages are not widely utilized as feed source in Nigeria (Gebremariam and Belay, 2016). Consequently, providing a research looking at alternative practically applicable, available and sustainable cheap supplemental feed options for ruminants particularly during the dry season is important. Therefore a study was carried out to investigate the nutrients composition, anti-nutritional factors and *in vitro* gas production assessment of some common cultivated legumes crop stovers hay in North-East part of Nigeria.

MATERIALS AND METHODS

Experimental Site

The study was carried out in Teaching and Research Farm of Federal University Wukari Taraba state. Wukari lies between latitude 7˚51'N to 7˚85'N and longitude 9˚46'E to 9˚78'E of the Greenwich meridian. The mean annual rainfall value ranges from 1000 - 1500 mm. The unset of the raining season is usually around April while the offset period is October. The mean maximum temperature is experienced around April at about 40˚C while the mean minimum temperature occurs between the period of December and February at about 20˚C (Oyatayo *et al* 2015).

Harvesting and Hay Making

Freshly harvested green legumes stovers (Bambara nut, cowpea, groundnut, pigeon pea and soya bean) were collected from Crop Production Teaching and Research Farm of Federal University Wukari. The harvesting was done in the month of October and November and the samples were collected in batches. The harvested legumes stovers were chopped into 3 - 5cm pieces size (for easy drying) and cutlass was used for the chopping exercise. Thereafter, the chopped materials were air-dried under shed for two weeks on concrete floor. The chopped legumes stover were constantly turned-over to promote thorough drying of all parts of the hay and minimize loss of nutrients. The low relative humidity (RH) couple with harmattan facilitate the dryness of the prepared hay. The chopped legumes stovers were then weighed and divided into equal portions (1kg) and each of the legumes stover serve as experimental treatment making five treatments. T_1 = Bambaranut Stover (BS), T_2 = Groundnut Stover (GS), T_3 = Cowpea Stover (CS), T_4 = Soya Bean Stover (SBS) and T_5 = Pigeon Pea Stover (PPS). All were replicated three times in a completely randomised design. However, the samples for laboratory analysis were oven dried at 65ºC and milled by 'MG 123' grinder.

Chemical Analysis

The grinded samples were subjected to chemical analysis for determination of percentage dry matter (DM%), organic matter (OM%), crude protein(CP%), crude fibre(CF%) and nitrogen free extract (NFE%) as described by AOAC (2005) while non-fibre carbohydrates (NFC) of the samples was determined according to NRC (2001). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were assayed by the method of Van Soest *et al*. (1991). Hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADL and ADF (Rinne *et al*., 1997). Phytate, Tannin, Oxalate and Saponin was determined according AOAC

(2005), while phenol and trypsin were analysed according to Sofowora (1993). Data obtained were subjected to one way analysis of variance (ANOVA) using SPSS (Version 23.0.2018). The treatments means were compared using the Duncan Multiple Range Test (Duncan, 1955).

In vitro gas production study

Rumen fluid was collected from three West African dwarf female goats. The method for collection was as described by Babayemi and Bamikole (2006) using suction tube from goats that were previously fed with 40% concentrate feed (40%corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) and 60% *Panicum maximum* at 5% body weight. The rumen fluid was collected into the thermos flask that had been pre-warmed to a temperature of 39^0C from the goats before they were offered the morning feed. Incubation procedure was as reported by Menke and Steingass (1988) using 120ml calibrated transparent plastic syringes with fitted with silicon tube. The sample weighing 200mg (n-3) was carefully inserted into the syringes and thereafter, 30ml inoculums containing cheese cloth strained rumen liquor and buffer (g/litre)of $9.8NaHCO_3+2.77Na_2HPO_4+0.57KCl+0.47NaCl+2.16MgSO_4.7H_2O+ 0.16CaCl_2.2H_2O$ in the ratio 1: 4 (v/v) under continuous flushing with CO_2 was dispensed using another 50ml plastic calibrated syringe was. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The silicon tube in the syringe was then tightened by a metal clip in order to prevent escape of gas. Incubation was carried out at 39 ± 1^0C and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24h. At post incubation period, 4ml of NaOH (10M) was introduced to estimate methane production as reported by Fievez *et al.* (2005). At post incubation, the content of the syringes were transferred into centrifuge tubes and placed immediately in cold water at 4^0C to stop fermentation. The tubes were centrifuged at 15,000 x g for 25 minutes.

The supernatant was discarded and the residues were oven dried at 55^0C for 48hrs to estimate *in vitro* dry matter digestibility (IVDMD %) and other post incubation parameters such as metabolisable energy (ME) and organic matter digestibility (OM) were calculated as reported by Menke and Steingass (1988) and short chain fatty acids (SCFA) were estimated at 24h post gas collection according to (Getachew *et al.,* 1999). The volume of gas produced every 3hours interval of the 3 replicates of each sample was plotted against the incubation time and from the graph, the gas production characteristics were estimated using the equation $Y = a + b (1-e^{ct})$ as described by Ørskov and McDonald, (1979), where Y = volume gas produced at time (t), a = intercept (gas produced from the soluble fraction), b = gas produced from insoluble but degradable fraction, c = gas production rate constant for the insoluble fraction (b), t = incubation time.

$$IVDMD = \frac{\text{Initial dry matter input} - \text{Dry matter residues}}{\text{Initial dry matter input}} \times 100 \quad (1)$$

$$\text{Fermentation efficiency (FE)} = \frac{DMD/Kg}{GVmlKg\ of\ DM} \quad (2)$$

Organic matter digestibility (OMD %) was calculated as OMD=14.88 + 0.8893GV + 0.45CP + 0.651XA, (Menke and Steingass, 1988): Metabolisable energy (ME) was calculated as ME = 2.20 + 0.136 GV + 0.057CP + 0.0029CF (Menke and Steingass, 1988) and Short chain fatty acids, (SCFA µml) as 0.0239GV – 0.0601(Getachew *et al.,* 1999) was also obtained, where GV, CP, CF and XA are gas volume, crude protein, crude fibre and ash, respectively. Data obtained were subjected to analysis and means were compared where significant using Duncan multiple range F-test (SPSS version 23.0, 2018).

RESULTS AND DISSCUSSION

Chemical composition of selected Legumes stover Hay

The Table 1 showed the summary of chemical composition of selected legumes stovers hay, dry matter (DM), Crude protein (CP), Ash content, crude fibre (CF), nitrogen free extract (NFE), ether extract, (EE), non-fibre carbohydrates (NFC), organic matter (OM) and carbohydrate (CHO). All the parameters considered were significantly different across the treatments. The dry matter (DM) ranged (91.83% to 93.55%) of selected legumes stover hay were on high side. This was expected due to the fact that forage hay are usually preserved at low moisture content of less than 10 – 15%. The nutritive value of hay is determined by the growth stage and plant species of the parent crop, field losses of nutrients and changes taking place during storage (which can be reduced by the use of chemical preservatives) McDonald *et al*., (2010). Crude protein ranged from 11.42% to 22.46%, suggesting that all the selected legumes stover hay were well-harvested, processed and preserved. Levels of crude protein are above the critical value of 7.0% or 70g/kg recommended for small ruminants by NRC (1981) and inclined to minimum protein requirement of 10 – 12% recommended by Gatenby (2002) for ruminants. Ash content that represent mineral composition of legumes stover hay ranged from 4.00% in T_5 (Pigeon pea) to 14.14% in sample T_4 (Soya bean stover). The values of ash obtained in this study are higher than the 3.05 – 7.70% reported by Anele *et al*. (2010) for fresh plant of legumes.

The variation in ash composition may be attributed to plant species and variety. However, the ash content of these selected legumes stover hay can adequately meet the mineral requirement of ruminant animal except for Pigeon pea that need mineral supplementation when feed alone.

Crude fibre ranged from 19.30% in T_5 (Pigeon pea stover) hay to 31.05% in T_2 (Groundnut stover) which showed that the CF of T_5 (Pigeon pea stover) hay was the lowest of all the treatments. This may be due to differences in harvesting period as it was the only sample harvested before the flowering time. The crude fibre values obtained in this study is slightly lower than the 36.30 – 36.65% reported by Ajayi *et al*. (2011). A report by Meale *et al*. (2012) showed that the higher the fibre content of a diet, the lower the DMI and fermentability. Generally, the crude fibre content of legumes hay is relatively low, thus ruminants can digest and utilise them effectively. Nitrogen free extract (NFE) ranged from 42.46% - 52.24%. This shows that all the legumes stover has moderate levels of soluble carbohydrate that can provides enough energy couple with sufficient rumen ammonia nitrogen (NH_3-N) for rumen microbes for effective feed degradation and digestibility.

Fibre Fractions

Table 2 above showed the fibre fractions of selected legumes stovers hay. The neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL). Hemicellulose and cellulose ranged from 52.67% – 59.80%, 27.60 – 33.55%, 13.15 – 17.70, 24.90 – 26.25 and 14.45 – 15.85%, respectively. All the fibre fractions differs significantly (P<0.05) across the treatments. The fibre fractions (NDF, ADF, and ADL) have implication on digestibility. The neutral detergent fibre (NDF), which is a measure of the plants cell wall contents and it includes portion of ADF and hemicellulose. The NDF value is important for determining forage dry matter intake. As NDF increase in forage, dry matter intake decreases. Neutral detergnt fibre (NDF) is inversely related to the plants digestibility (Gillespie, 1998). NDF is correlated with the level of dry matter intake by cows; the lower the NDF, the higher the level of intake. However, a low NDF content is indicative of higher forage degradability (Mahala *et al*., 2012). The neutral detergent fiber (NDF) range values (52.67 – 59.80%) obtained fall within 45-65% classified as medium quality forage by Sing and Oosting (1992).

The acid detergent fibre (ADF) encompasses the cellulose and lignin portions of the cell wall. The levels of ADF is crucial in determining forage digestibility. As ADF increases, forage digestibility decreases and vice versa. The higher the ADF the lower the plants digestible energy. High NDF could result in low intake while high ADF may engender low digestibility (Babayemi *et al*., 2010). It is the chemical component of feed that determines its rate of digestion. Forage with high ADF values is classified as low quality roughage (Rusdy, 2016). According to Kellems and Church (1998), roughage with less than 40% ADF as high quality and those that are greater than 40% as poor quality. Base on this classification the value (27.60 – 33.55%) of ADF obtained in this work indicate that the legumes stover hay is of high quality. Acid detergent lignin (ADL) of a plant is the most indigestible component of the fibre fraction (Gillespie, 1998), and its amount will also influence the plant digestibility, however, the range values (13.70- 17.70%) ADL obtained in this study showed that about 82.30 - 86.85% of legume stovers hay could be digested by ruminant animals.

Anti-Nutritional Factors of Selected Legumes Stover Hay

Table 3 above shows the Anti-Nutritional Factors of Selected Legumes Stover hay. Phenol, phytate, saponin, tannin, trypsin and oxalate ranged from 0.68 – 1.13, 0.03 – 0.06, 0.09 – 0.18, 0.56 – 0.82, 0.31 – 0.35 and 0.013 – 0.02%, respectively. The anti-nutritional factors of the selected legumes stover hay were significantly (P<0.05) different across the treatments except for saponin that was similar across the board. Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients (Cammack, 2006). They prevent bloat in cattle, reduce gastrointestinal nematode numbers. Phenolic compounds help to prevent the death of crop as phenolic compounds from plant extracts act as antimicrobial agent (Ofokansi, *et al.,* 2005). The presence of phenols (Farquer, 1996) indicates that such plants (legumes hay stovers) could act as anti-inflammatory, anti-clotting, antioxidant, immune enhancers and hormone modulators (Okwu, and Omodamiro, 2005). The results from the current study indicated that total phenols fall within a range (0.68 – 1.08) which may not have negative influence on digestibility (Abarghuei *et al*., 2014).

Phytic acid (deprotonated phytate anionin) is an anti-nutrient that interferes with the absorption of minerals from the diet. Phytic acid has a strong binding affinity to minerals such as calcium, magnesium, iron, copper, and Zinc. This make the minerals unavailable for absorption in the intestines (Ekholm, 2003). The ranged (0.03 – 0.06%) levels of phytate obtained in this study is less than 0.16% reported for *Pennisetum purpureum* and range values (0.50 – 0.66%) reported for vetiver grass by Falola *et al.,* (2013). The knowledge of phytate is very important because phytate have adverse effect on digestibility of minerals as reported by Nwokolo and Bragg (1977).

In ruminants, saponins were implicated in causing bloat, however, later studies indicate that they were not involved in the bloat syndrome since saponins may also undergo bacterial degradation in the rumen. However, Das *et al. (*2012) reported that plants rich in saponins enhance the flow of microbial protein from the rumen, increase the efficiency of feed utilisation and decrease protozoal populations. Higher DM and OM digestibility co-efficiencies have been reported in sheep supplemented with a saponin content of between 2% and 4 % (Das *et al*., 2012). In the present study, the saponin levels of selected legumes stover hay cannot have negative impact in nutrients uptake and utilisation by ruminants. Furthermore, saponin in some tropical fruits was also observed as an active compound responsible for the suppression of methanogenesis in faunated and defaunated rumen fluid (Hess, et al., 2003). Therefore, leaves of tropical plants should be incorporated into ruminant feed to reduce ruminal methanogenesis.

Trypsin inhibitors are widespread within the plant kingdom (Tacon, 1997). Their concentration is negligible in cereals and they can be highly concentrated in some legumes such as soybean (Guillaume *et al*., 1999) with trypsin inhibitors ranging between 2 to 6 mg/g (Francis *et al.,* 2001). Trypsin inhibitors, which are ANFs for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen (Cheeke and Shull, 1985). They reduce the nutritive value of poor-quality diets, but can also have substantial benefits for ruminant productivity and health when improved temperate forages are fed. Tacon, (1997) and Francis *et al.,* (2001), stated that trypsin content in the feed, reduced the digestibility of protein. However, the ranged values (0.31 – 0.35%) obtained in this study was very low thus, it cannot reduce protein digestibility in ruminants.

Oxalate in the samples ranged from 0.013 in T_3 (Cowpea stover) to 0.02% in T_5 (pigeon pea) which is presumably low to cause metabolic disorder to the ruminants. Dietary oxalate plays an important role in the formation of Ca oxalate, and a high dietary intake of Ca may decrease oxalate absorption and its subsequent urinary excretion. Non-ruminants appear to be more sensitive to oxalate than ruminants because in the latter, rumen bacteria help to degrade oxalate. If ruminants are slowly exposed to a diet high in oxalate, the population of oxalate-degrading bacteria in the rumen increases sufficiently to prevent oxalate poisoning. The oxalate ranged values (0.01 – 0.02%) is less than ranged values (0.19 - 0.33) reported by Falola *et al.* (2013) which could be attributed to plant variety and specie. The levels of oxalates reported in this work is very low and consequently it cannot pose any toxicity problems, since it is below 2-5g reported by Oke (1969).

Tannins ranged from 0.56 in T_3 (cowpea stover hay) to 0.82% in T_5 (pigeon stover hay) indicates that the percentage of tannin in all the selected legumes stover hay are acceptable to ruminants without consequential effects. In ruminants, dietary condensed tannins (2-3%) have been shown to impart beneficial effects because they reduce the wasteful protein degradation in the rumen by the formation of a protein-tannin complex (Barry, 1987). Goel & Makkar, (2012) reported that low dietary tannin levels improve nitrogen utilization by ruminants. This is possible because tannins have the capability to alter the site of protein digestion from the rumen to the intestines, hence improving amino acid absorption. In the language of ruminant digestive physiology, this is referred to as rumen escape protein and is purported to lead to higher growth rates, milk yield, and fertility (Mueller-Harvey, 2005). The level of tannin that adversely affect digestibility in sheep and cattle is between 2 and 5% (Diagayette and Huss, 1981). Goats are known to have threshold capacity of about 9% dietary tannin (Natis and Malachek, 1981). The value reported here suggests that tannin levels of selected legumes stover hays cannot affect the animals (ruminants) adversely. Hence, foliage analysed in this study has the potential to enhance bypass protein and reduce blood cholesterol when included in ruminant feed.

***In vitro* production characteristics gas 200mg/DM of selected legumes stover at 24hours incubation period.**

Table 4 showed the *in vitro* gas production characteristics (a, b, a+b, c, t and Y) of selected legumes stovers. All the parameters were similar across the board except the ‘b’ (extent of gas production from insoluble but degradable fraction) and ‘a + b’ (potential extent of gas production) that were significantly different across the treatments. This might be due to the different species of legumes stovers used for hay production. The soluble fraction ‘a’ makes the attachment by rumen microorganisms to be done easily and leads to much gas production. Generally, soluble fraction ‘a’ of legumes forages usually low compared to cereal stovers that contain high levels of soluble carbohydrates of which these legumes hay stovers cannot be exception.

Somart *et al.,* (2000) reported that gas volume is a good parameter to predict digestibility, fermentation, its product and microbial protein synthesis of the substrate by microbes in the *in vitro* system. The extent of gas production ‘b’ described fermentation of the insoluble but degradable fraction of the samples which is influenced as a processor for microbial protein synthesis by crude protein content of legumes stover hay. A linear relationship has been established between high crude protein in forages and in vitro degradability (Njidda *et al*., 2010). This facilitated high rate of microbial activities by supplying the required nitrogen for their cellular protein synthesis as established by Roger *et al.,* (1977). Pigeon pea stover hay (T_5) had distinct higher degradation by the microbes compared to cowpea stover hay (T_3). The values of ’b’ The values of ‘b’ obtained in this study (7.33 –25.33) were consistent with those reported for dry matter (DM) degradation of some tropical legumes and grasses (Ajayi *et al*., 2007 and the values of 9.5 – 32.0ml/200mg DM reported for some crop residues (Babayemi *et al*., 2009). Ørskov and Ryle (1990) maintained that the rate ‘c’ determines digestion time and consequently how long a potentially digestible material would occupy space.

The potential degradability ‘a+b’ of a diet depicts the level at which the diet could be degraded if it were in the actual rumen of the animal (*in vivo*). This largely depends on how much of the fibre fractions (NDF and ADF) have been broken down for easy access of the microbes to the nutrients available in the diet. At 24hrs, there were significant variations among the treatments such that it was highest for the T_5 (pigeon pea) and lowest for the T_2 (ground nut stover hay) respectively. However, T_5 values for ‘a + b’ was similar to T_1 (Bambara nut stover hay) and T_4 (soybean stover stover) hay. The relative high values of the potential extent of gas production recorded for T_1, T_4 and T_5 was due to medium levels of carbohydrate fraction embedded in them. Getachew *et al*., (1999) stated that it is well known that gas production is basically the result of fermentation of carbohydrate to volatile fatty acid (acetate, butyrate and propionate). Menke and Steingass (1988) also reported that fermentable carbohydrate increase gas production while degradable nitrogen compound decrease gas production to some extent because of their binding of carbohydrate with ammonia. This suggests the reasons why gas production of the samples was low compare to grasses and cereal forages.

The volume of gas ‘Y’ at time” t”is the peak of gas production for each sample at 24hrs incubation period. Since rate ‘’c” of gas production at time’ ‘t” and volume of gas “Y” of the incubated samples were similar across the treatments, it means that differences in specie had no effect on hay stovers regarding the “c”.t and “Y “ characteristics of the gas. However, there are many factors that may determine the amount of gas to be produced during fermentation, depend on the nature and level of fibre, the presence of secondary metabolites (Babayemi *et al*., 2004) and potency of the rumen liquor for incubation. It is possible to attain potential gas production of a feedstuff if the donor animal from which rumen liquor for incubation was collected got the nutrient requirement met.The utilisation of roughages is largely dependent on microbial degradation therefore the rate and potential extent of gas production would provide a useful basis for the evaluation of the legumes stover hay as potential feed resources. Since gas production is dependent on the relative proportion of soluble, an insoluble but degradable and undegradable particle of feed; mathematical description of gas production profiles allows evaluation of substrate and fermentability of soluble and slowly fermentable component of feeds (Getachew *et al*., 1998). Based on the above assumption, therefore, it could be adduced that among the legumes stover hays studied, ground nut stover hay (T_2) and cowpea stover hay (T_3) would provide minimal proportion of residue that would take up space if utilised in *in vivo* studies and also persists as indigestible residue.

In vitro gas production parameters (200mg/DM) of selected legumes stovers hay at 24hrs incubation period

The *in vitro* techniques are available to evaluate the nutritive value of feed at low cost (Getachew *et al.,* 2004). Presented in table 4 is the *in vitro* gas production parameters such as methane (CH4ml), gas volume (GVml), metabolisable energy (ME MJ/KgDM), short chain fatty acid (SCFA µml), organic matter digestibility (OMD%), dry matter degradability (DMD%) and fermentation efficiency (FE) of selected legumes stover hay. All the observed parameters, in this study varied across the treatments, indicating that forage species and types had significant effects on the nutritive value of selected legumes stover hay. The lowest CH_4 and GV production was obtained from pigeon pea stover hay while the highest CH_4 and gas production (GV) were obtained from groundnut stover hay (T_2) and cowpea stover hay (T_3) respectively. In most cases, feedstuffs that showed high capacity for gas production were also observed to be synonymous for high methane production. Methane (CH_4) production in the rumen is an energetically wasteful process, since the portion of the animal's feed, which is converted to CH_4, is eructated as gas. However, the total gas volume (TGV) and methane production of legumes stover hay compared well with other legumes forages. Generally, gas production is a function and a mirror of degradable carbohydrates and therefore, the amounts depends on the nature of the carbohydrates (Blummel and Becker, 1997). The relative low volume of gas production in this study at 24hrs incubation might be adduced to low levels of fermentable carbohydrate coupled with presence of phytochemicals which could have suppressed gas production being the characteristics of legumes forages. Gas production from protein fermentation is relatively small compared to carbohydrate fermentation. Gas volumes also have shown a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993), which implies that if animal is fed legumes stover hays included in roughage diet such animal, will have better feed intake and growth rate. The ME values obtained in this study (4.76-6.78MJ/KgDM) were within the range of 4.5 to15 $MJkg^{-1}DM$ reported by Menke and Steingass (1988) and ME values of various European feeds. Metabolisable energy (ME) values are very useful and important for purposes of ration formulation and to set the economic value of feeds for trading purposes. The *in vitro* gas production method is accurate and predicts feed intake, digestibility, microbial nitrogen supply and animal performance (Blummel and Ørskov, 1993).

For the past two decades, the technique had been used in advanced countries as an instrument to determine the amount of short chain fatty acids, carbon dioxide and metabolisable energy of feed for ruminants (Blummel and Becker, 1997; Getachew *et al.,* 1999). The SCFA ranged value (0.40-0.61µml) obtained was slightly lower than 0.62-0.77µmol reported by Binuomote and Babayemi (2012). Gas production was directly proportional to SCFA (Beuvink and Spoelstra, 1992), the higher the gas produced, the higher the short chain fatty acids. Short chain fatty level indicates that the energy is available to the animal and it contributes up to 80% of animal daily energy requirement (Fellner, 2004). In this study, short chain fatty acid (SCFA) is directly proportional to metabolisable energy (ME) as reported by Menke *et al*. (1979). Moreover, short chain fatty-acids (SCFA) is very important for relating feed composition to production parameters and to net energy value of the forages, therefore production of SCFA from *in vitro* gas measurement will be increasingly important in a developing country such as Nigeria. Getachew *et al.,* (1999) stated that gas production is basically the result of fermentation of carbohydrate to volatile fatty acid (acetate, butyrate and propionate). Low methane production from this study indicate a decreased proportion of acetate and butyrate with increased propionate production as more hydrogen would be available for production against methane (CH_4) production. Acetate and butyrate are lipogenic which leads to synthesis of butter fat in milk while propionate is glucogenic which leads to production of lean meat.

Research on rumen methanogenesis and its inhibition was initiated with aim of increasing feed efficiency. This means that reduced methane production will lead to greater efficiency in feed utilisation. Depending on the level of feed, composition of the diet and digestibility, 2.15% of the gross energy in the feed is lost through methane production (Holter and Young, 1992). Therefore, legumes stover hay will enhance better utilisation of other roughages diet in production of lean meat. Menke and Steingass (1988) also stated that fermentable carbohydrate increase gas production, while degradable nitrogen compound decrease gas production, to some extent because of their binding of carbohydrate with ammonia. Saponin, a secondary metabolite is might be responsible for low methane gas production which agrees with the work of Babayemi *et al.,* 2004a. There are many factors that may determine the amount of gas to be produced during fermentation, depending on the nature and level of fibre, the presence of secondary metabolites (Babayemi *et al.,* 2004a) and potency of the rumen liquor for incubation. Incubation of roughages with bicarbonate buffers produced about 50% of the total gas from buffering of the SCFAs and the rest is generated from fermentation (Blummel and Ørskov, 1993). The organic matter digestibility (OMD) which could be said to be a measure of degradability (potentials) of the microbes on the substrates especially in the presence of sufficient ammonia nitrogen (NH_3-N) which has influence on bacterial fermentation was highest in treatment 3, followed by 4 (3>4>2>1>5). This suggest that T_5 (Pigeon pea stover hay) was the best in terms of gradual release of gas because quick release of gas from fermentation processes can quickly accumulate and cause distention of the rumen which subsequently cause the diaphragm to be under pressure which may lead to suffocation as a result of difficulty in breathing especially when the animal has difficulty in expelling the gas (Bloat).

Dry matter degradability (DMD), value of 86.63% obtained for T_2 (Groundnut stover hay) was the highest while the least value of 57.65% was recorded for T_5 (Pigeon pea stover hay). The DMD value is a good measure of the amount of dry matter in the feed that can be degraded by microbes in the rumen of ruminants. The result obtained indicates that groundnut stover hay (T_2) will do better compared to other legumes stover hay. The value (2.67%) of Fermentation Efficiency (FE) obtained from T_3 and Total Gas Volume (TGV) value (28.00%) suggest that the legume hay type contained soluble carbohydrates (soluble sugar) which favours a higher gas production and rapid fermentation kinetics.

CONCLUSION

The results obtained from this study indicate that crude protein content of all the selected legumes stover hay is above 10-12% recommended for ruminant animals and anti-nutrition composition is within tolerance level, consequently feeding them out to ruminants animal will not have negative effect on their health status, nutrients digestibility, absorption and utilisation. Furthermore, *in vitro* gas production assessment revealed that DMD, ME and SCFA of legumes stover hay is degradable and can supply energy to the ruminants. Thus, ruminant animals can utilise them if well processed and preserved for dry season feeding.

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Table 1. Chemical Composition (100g/DM) of Selected Legume Crops stover hay

	Treatments					
PRTS (%)	T_1	T_2	T_3	T_4	T_5	SEM
Dry Matter	92.01^c	92.08^c	92.75^b	91.83^c	93.55^a	0.080
Crude Protein	13.05^c	11.42^e	12.08^d	13.81^b	22.46^a	0.104
Ash	10.87^c	11.58^b	10.40^c	14.14^a	4.00^d	0.086
Crude Fibre	29.90^b	31.05^a	28.90^c	27.95^d	19.30^e	0.157
Nitrogen free extract	44.78^c	44.50^c	47.03^b	42.46^d	52.24^a	0.180
Ether Extract	1.40^b	1.45^b	1.60^b	1.65^b	2.00^a	0.051
NFC	17.23^{bc}	17.95^{ab}	16.13^c	13.91^d	19.04^a	0.284
Organic Matter	89.13^b	88.42^c	89.60^b	85.86^d	96.00^a	0.086
Carbohydrate	74.68^c	75.55^a	75.93^a	70.41^d	71.54^c	0.141

a,b,c =mean on the same row with different superscripts are significant (P<0.05) different

T_1 = Bambaranut stover (BS), T_2 = Groundnut stover (GS), T_3 = Cowpea stover (CS), T_4 = Soya bean stover (SBS), T_5 = Pigeon pea stover (PPS), PRTS = Parameters and SEM = Standard error of mean

Table 2. Fibre Fractions of Selected Legume Crops stover hay.

	Treatments					
PRTS (%)	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**
NDF	57.45^b	57.60^b	59.80^a	56.50^b	52.50^c	0.194
ADF	32.55^{ab}	31.55^{bc}	33.55^a	30.90^c	27.60^d	0.260
ADL	17.15^a	16.50^{ab}	17.70^a	15.30^b	13.15^c	0.284
Hemicellulose	24.90^b	26.05^a	26.25^a	25.60^{ab}	24.90^b	0.178
Cellulose	15.40^{ab}	15.05^b	15.85^a	15.60^{ab}	14.45^c	0.108

a,b,c== means on the same row with different superscripts are significantly (P<0.05) different

T_1 = Bambaranut stover, T_2 = Groundnut stover, T_3 = Cowpea stover, T_4 = Soyabean stover, T_5 = Pigeon pea stover, NDF = Neutral Detergent Fibre, ADF = Acid Detergent Fibre, ADL = Acid Detergent Fibre, PRTS = Parameters and SEM = Standard error of means

Table 3. Anti-Nutritional Factors of Selected Legume Crops stover hay

	TRTS					
PRTS (%)	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Phenol	1.08^b	0.72^d	0.68^e	1.08^c	1.13^a	0..09
Phytate	0.06^a	0.04^b	0.03^e	0.04^b	0.06^a	0.01
Saponin	0.18	0.16	0.09	0.14	0.11	0.05
Tannin	0.74^b	0.63^d	0.56^e	0.66^c	0.82^a	0.07
Trypsin	0.34^{ab}	0.31^c	0.31^c	0.33^b	0.35^a	0.02
Oxalate	0.016^b	0.017^b	0.013^c	0.014^c	0.020^a	0.03

a,b,c = means on the same row with different superscripts are significantly (P<0.05) different

T_1 = Bambaranut stover, T_2 = Groundnut stover, T_3 = Cowpea stover, T_4 = Soya bean stover, T_5 = Pigeon pea TRTS = Treatments, PRTS = Parameters and SEM = Standard error of means

Table4. *In vitro* gas production characteristics 200mg/DM of selected legumes stover at 24hours incubation period.

	TRTS					
PRTS	T_1	T_2	T_3	T_4	T_5	SEM
a (ml^3)	2.67	2.00	2.67	1.67	2.67	0.172
b (ml^3)	21.33^{ab}	7.33^c	16.67^b	20.33^{ab}	25.33^a	1.040
a+b (ml^3)	24.00^{ab}	9.33^c	19.33^b	22.00^{ab}	28.00^a	1.142
c (mlh^{-1})	0.053	0.092	0.071	0.065	0.044	0.011
t (hr)	12.00	9.00	12.00	10.00	12.00	0.931
Y(ml3)	12.67	6.00	11.33	11.33	13.00	1.406

a,b,c = means on the same row with different superscripts are significantly (P<0.05) different

T_1 = Bambaranut stover, T_2 = Groundnut stover, T_3 = Cowpea stover, T_4 = Soya bean stover, T_5 = Pigeon pea stover, TRTS = Treatments, SEM = Standard error of means.

a = gas produced from immediately soluble fraction, b = extent of gas production from insoluble but degradable fraction, a+b = potential extent of gas production, c = rate of gas production at time (t), t = incubation time (hrs) and Y = volume of gas produce at time (ml^3)

Table 5. In vitro gas production parameters (200mg/DM) of selected legumes stovers at 24hrs incubation period

	TRMTS					
PRTS	T1	T2	T3	T4	T5	SEM
CH4 (ml)	14.00^a	18.00^a	15.00^a	13.33^a	6.00^b	0.81
GV (ml)	19.33^b	24.00^{ab}	28.00^a	22.00^{ab}	9.00^c	1.20
ME(MJ/KgDM)	5.66^{bc}	6.21^{ab}	6.78^a	6.06^{ab}	4.76^c	0.16
SCFA (µml)	0.40^b	0.51^{ab}	0.61^a	0.47^{ab}	0.16^c	0.03
OMD (%)	45.02^b	48.90^{ab}	51.98^a	49.86^{ab}	35.60^c	1.09
DMD (%)	64^{ab}	86.63^a	74.86^{ab}	68.67^{ab}	57.65^b	3.09
FE	3.53^b	3.68^b	2.67^b	3.13^b	6.21^a	0.24

a,b,c = means on the same row with different superscripts are significantly (P<0.05) different

T_1 = Bambaranut stover, T_2 = Groundnut stover, T_3 = Cowpea stover, T_4 = Soya bean stover, T_5 = Pigeon pea stover, TRTS = Treatments, SEM = Standard error of means.
CH4 = Methane, TGV=Total Gas Volume, ME = Metabolisable Energy, SCFA = Short Chain Fatty Acid, DMD = Dry Matter Degradability, OMD = Organic Matter Digestibility and FE = Fermentation Efficiency.

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