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# Nutrient Composition, Phytochemical Properties and *In Vitro* Gas Fermentation Assessment of Some Selected Legume Forage Seeds that can be utilized by Ruminants

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**Abstract**
A total number of seven legume seeds (*Senna hirsuta*, *Senna obtusifolia*, *Senna occidentalis*, *Pueraria phaseoloides*, *Tephrosia bracteolate*, *Centrosema pubescence and Mucuna pruriens)* that could be utilised in ruminants feed were investigated for their nutrient composition, phytochemical properties and *in vitro* gas fermentation assessment study. The seeds were collected within the University environment and each one served as a treatment ($T_{1,}$ $T_{2,}$ $T_3$, $T_4$, $T_5$, $T_6$ and $T_7$). Then samples were dried and milled for laboratory analysis to determine nutrient composition, phytochemical properties and *in vitro* gas fermentation assessment using standard technique. The results of chemical composition (proximate, fibre fractions, minerals and phytochemicals) varied significantly across the treatments. Crude protein (CP), Ash and crude fibre (CF) varied significantly ($P<0.05$) from 16.06% to 35.77%, 9.35% to 13.66% and 15.47% to 22.59% respectively. Similarly, fibre fractions such as Neutral Detergent Fibre (NDF, Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), hemicellulose and cellulose also differed significantly ($P<0.05$) across the board. Minerals composition such as calcium (Ca), phosphorous (P), magnesium (Mg) copper (Cu), potassium (K), sodium (Na), sulphur, (S) manganese (Mn), zinc (Zn), and iron (Fe) and phytochemicals (tannin, phytate, phenol, saponin, oxalate alkaloids and lectin) were also varied significantly ($P<0.05$). *In vitro* gas fermentation parameters methane ($CH_4$) production, Total Gas Volume (TGV), Short Chain Fatty Acids (SCFA), Metabolisable Energy (ME), Dry Matter Digestibility (DMD %), Organic Matter Digestibility (OMD %) and Fermentation Efficiency (FE) were significantly ($P<0.05$) different across the treatments. The results of the chemical composition of selected legume seeds showed that crude protein and ash content were on the high side while crude fibre was relatively low. Also, the protein contents of all the selected legume seeds were enough to support and enhance growth, reproduction and milk production in dairy animals if properly processed. *In vitro* gas fermentation assessment revealed that ME and SCFA were relatively high an indication that the energy would be available to the animals when fed. Moreover, $CH_4$ production of legume seeds was relatively low which indicated that the seed was environmentally friendly in terms of global warming and climate change due to $CH_4$ emission and other contributory factors and more energy would be available to ruminants to utilize efficiently.

***Keywords: Legumes, In vitro, proximate composition, fibre fraction, phytochemicals***

## Introduction
Leguminous plants comprise many species, whose seeds differ considerably in their chemical composition and nutritive value. In the tropics, particularly in Nigeria, a number of them are grossly under-utilized. Some of these are *Tomentosa nilotica* (Acacia), *Dioclea reflex* a (marble plant), and *Monodora myristica* (African nutmeg). These plants impact the environment through soil reclamation, soil enrichment, protection against fire and wind, and as a haven for biodiversity and ornament. The medicinal properties include improved digestibility, antimicrobial, anti-inflammatory, antioxidant and immuno-stimulant (Eugenius *et al.*, 2017). They are being neglected in most Nigeria homes for consumption because of the long hours of cooking and tedious manual removal of skin coats and at the end of the planting season, the foliages are set on fire. They are high in nutritional value, consequently, the plants and their seeds are widely used as sources of protein. Apart from protein, the seeds provide many other important components, such as minerals and vitamins B-complex and have other vital health-protective compounds phenolic, inositol phosphates and oligosaccharides. Meanwhile, minerals are known to play important metabolic and physiological roles in living cells and are needed by the body to trigger the

thousands of chemical reactions necessary to maintain good health (Vaclavic and Christian 2008). However, leguminous seeds contain undesirable biologically active substances, including Anti-Nutritional Factors (ANFs) tannins, alkaloids, saponin, protease inhibitors and others. In addition, feeds differ in their methane production potentiality depending on chemical composition, and plant metabolites present in them (Patra 2012; Eugenius *et al.*, 2017). Trypsin inhibitors, which are metabolites for monogastric animals, do not exert adverse effects on ruminants because they are degraded in the rumen. Generally, tannin induces negative responses such as astringency, bitterness or unpleasant taste when consumed (Huang *et al*., 2018; Ramos *et al.*, 2020). Tannins and phytates have been reported to negatively affect the bioavailability of minerals such as iron when consumed in large quantities (Clement, 2014) by forming insoluble anti-nutritional mineral complexes before absorption (Gorczyca *et al.*, 2013). Meanwhile, in ruminants, methane production in the rumen represents a 2–12% loss of feed energy (Wanapat *et al.*, 2015) decreasing the metabolisable energy content of feeds. In addition, the production of greenhouse gases from animals and their impact on climate change are major concerns worldwide (Gerber *et al.*, 2013). Methane is the second highest anthropogenic greenhouse gas after carbon dioxide, which contributes to the problems of global warming and climate change (Tubiello *et al.*, 2011). Enteric methane is normally produced during the fermentation of feeds mostly in the rumen by hydrogen trophic methanogenic archaea, which results in the inefficient conversion of the potential energy of feeds into methane that is not utilized by ruminants (Pal *et al.*, 2015). Reduction in enteric methane emission enhances the efficiency of nutrient utilization augments productivity and also reduces methane impact on global warming. Ruminant livestock productivity in many African countries is low in terms of milk and carcass yield compared to what is obtained in developed countries. However, feed constitutes 55-80% of the cost of production of livestock products (Carew *et al*., 1998). The feed resources that are abundant to provide the bulk of ruminant feeds are biomass from grasslands and crop residues which cannot sustain the animal's productivity, especially during the dry season. This poor nutrition leads to economic losses to the farmers because of loss in weight and condition of animals, poor reproductive performance and increased rates of mortality especially the young animals. Improved feeding systems based on the supplementation of grass with forage legumes will enhance milk yield and meat production. To increase the consumption of animal protein in developing countries, it is necessary to reduce the cost of meat, milk and eggs, thereby making them accessible and affordable to the majority of the people. There is a need to supplement legumes in the diets of livestock species to enhance their productivity. This study is designed to determine the chemical composition and in vitro assessment of some legume seeds that can be incorporated into feeds utilized by ruminants. There is a need to supplement legumes in the diets of livestock species to enhance their productivity. Hence, the crux of this study is to determine the chemical composition, phytochemical properties and *in vitro* assessment of some selected legume seeds utilized by ruminants.

## Materials and Methods

### *Experimental Site*

The study was carried out in the 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 rainy 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).

### *Sample Collection, Processing and Chemical Analysis*

The sample of selected seven legume seeds (*Senna hirsuta*, *Senna obtusifolia*, *Senna occidentalis*, *Pueraria phaseoloides*, *Tephrosia bracteolate*, *Centrosema pubescence* and *Mucuna pruriens*) were collected within the University environment. The samples were ground in the laboratory by an 'MG' 123 mixer grinder and subjected to chemical analysis for determination of a dry matter, organic matter, crude protein and nitrogen-free extract as described by AOAC (2006). 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). Non-fibre fraction was determined according to NRC. (2001). Minerals such as Ca, P, Mg, K, Na, S, Cu, Mn, Zn and Fe were determined as described by AOAC (2006) while phytochemicals (tannins, phytate, phenol, saponin, oxalate alkaloids and lectin) screening were done according to Sofowora (1993).

### *In vitro* gas fermentation assessment study

The triplicate per sample collected from seven different legume seeds was subjected to *in vitro* gas fermentation assessment. The rumen fluid was collected through the suction method using the hose from three West African Dwarf (WAD) goats under the same feeding regime. The animals were 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% Guinea grass. The fluid was collected into a thermos flask and taken to the laboratory. Rumen liquor and buffer (g/litre9.8$NaHCO_3$ + 2.77$Na_2HPO_4$ + 0.57KCl + 0.47NaCl + 2.16$MgSO_4.7H_2O$ + 0.16$CaCl_2.2H_2O$) solution was mixed in the ratio 1: 4 (v/v) under continuous flushing with $CO_2$and incubation was done as described by Babayemi *et al.* (2004). At the end of the 24-hour incubation period, 4ml of the 10M of NaOH solution was introduced into the syringe to absorb the $CO_2$ gas contained in the syringe and the methane value was determined according to Fievez *et al*.

(2005). The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 hours respectively. Metabolisable energy (ME) and organic matter digestibility (OMD) were estimated as described by Menke and Steingass (1988), while the short-chain fatty acid (SCFA) was calculated as reported (Getachew *et al.*, 1999). Metabolable energy (ME) = 2.20 + 0.136 GV + 0.057CP + 0.0029CF (Menke and Steingass, 1988). Organic matter digestibility (OMD) = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA. Short-chain fatty acids (SCFA) = 0.0239GV – 0.0601(Getachew *et a*l., (1999), where TGV, CP, CF and XA are total gas volume (ml/200mg DM), crude protein, crude fibre and ash, respectively. Graphs of the volume of gas produced every 3-hour interval of the 3 replicates of each sample were plotted against the incubation time. From the graph, the degradation characteristics were estimated as defined in the equation: $Y = a + b\ (1\text{-}e^{ct})$ (Ørskov and McDonald, 1979) where Y = gas volume production at time (t), a = gas produced from the soluble fraction, b = gas produced from insoluble but degradable fraction, c = rate of gas production, t = incubation time. Data obtained were subjected to a one-way analysis of variance (ANOVA) and means were compared where significant differences occurred using the Duncan multiple range F-test (SPSS version 23.0, 2018).

## Results and Discussion

### *Chemical Composition of Seven Selected Forage Legumes Seeds*

Table 1 shows the proximate composition (Crude protein (CP), Ash, Ether extract (EE), Crude fibre (CF), Nitrogen free extract (NFE), Dry matter (DM), Organic matter (OM), and carbohydrates (CHO) of seven selected legume seeds. All the parameters observed varied significantly ($P<0.05$) across the treatments. *Tephrosia bracteolata* ($T_5$) and *Pueraria phaseoloid* seed ($T_4$) had the highest CP (35.77%) and the lowest value (16.06%) respectively. However, the least crude protein value (16.06%) reported for $T_4$ (*Pueraria phaseoloid* seed) was higher than the threshold value (10 – 12% CP) of crude protein reported for small ruminant animals. Also, $T_5$ (*Tephrosia bracteolata*) had the highest EE (2.38%) and OM (90.65%) while $T_6$ (*Centrosema pubescence* seed) had the lowest EE (1.34%) and highest in NFE (46.74%). Ruminant animals' protein requirements vary with the stage of production, size of the animal, and expected performance. During lactation, larger ruminants typically require more protein per day than small ruminants but as a lesser percentage of their total dry matter intake. Cattle requirements for crude protein (CP) increase with increasing lactation and rate of gain. Protein is required for milk production and reproductive tract reconditioning after calving. Young growing cattle, in particular, need relatively high levels of CP in their diets to support muscle growth. Creep feeds or forages for nursing calves should contain at least 15%CP (Jane and Brand, 2023). *Tephrosia bracteolata* seeds have been documented to contain a high percentage of crude protein which is confirmation of higher CP value (35.77%) obtained in this work. Research conducted by Jakhmola *et al*. (2018) reported that *Tephrosia bracteolata* seeds contain 35.3% CP and it can serve as a good source of protein for ruminants if processed. The percentage value (35.3%) reported by this author was similar to 35.77% reported in this study. Furthermore, the CP content (16.06%) obtained for *Pueraria phaseoloid* seeds was slightly higher than (the 15% CP) reported by Adejumo *et al.* (2015) while the value (32.10% CP) obtained for Mucuna pruriens is within the range values reported by Pugalenthi (2005). The variation observed could be attributed to soil nutrient and ecological factors. However, the lowest CP value (16.06%) reported for $T_4$ (*Pueraria phaseoloides* seed) is higher than the threshold value (10-12%CP) of crude protein reported for small ruminant animals. Generally, the percentage ranged values (16.06 – 35.77%) of crude protein (CP) obtained in this work can meet the protein requirements of all categories of ruminant animals. However, the presence of phytochemicals such as tannin and saponins that form complexes with protein which makes it indigestible and unavailable to the ruminants and other live stocks indicates that the seeds need to be processed before including the legume seeds in their diets. The ash content of the legume seeds indicates their mineral content. *Senna occidentalis* and *Senna obtusifolia* are known to contain high levels of mineral content as reported by Ibrahim *et al*. (2013). The crude fibre content of *Senna hirsuta* seeds (22.59%) is consistent with the 22.52% reported by Diarra *et al*. (2019). The ether extract (EE) obtained from selected legume seeds was relatively low. Generally, legumes have a low-fat content (Boland *et al*., 2001) except oil legume seeds such as soybean and groundnut. However, ether extract contributes to the energy levels of the feed composition. The nitrogen-free extract (NFE) percentage composition ranged from 36.38% to 46.74%, in *Mucuna pruriens* seed ($T_7$) which had the lowest value while *Centrosema pubescence* seed ($T_6$) had the highest NFE value. NFE is a soluble carbohydrate component of selected legume seeds, and this implies that NFE could support the production of volatile fatty acids (VFAs) in the rumen during fermentation and provides energy for ruminants (Blummel *et al.*, 1997).

### *Fibre fractions of seven selected legume seeds*

Presented in Table 2 are the fibre fractions (neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), Hemicellulose, and Cellulose) of selected legume seeds. All the fibre fractions varied across the board. The NDF, ADF, ADL, Hemicellulose and Cellulose ranged from 59.10 to 61.60%, 30.09 to 40.39%, 13.46 to 18.85%, 15.30 to 23.00% and 20.73 to 27.10%) respectively. The variation observed in fibre fractions of legume seeds could be attributed to different species of legume seeds used in this study. Taherzadeh *et al*. (2015) reported that high levels of fibre in feed materials can decrease ruminal digestibility, leading to reduced nutrient availability for ruminants. The ranged values of NDF (59.10 to 61.60%) and ADF (30.09 to 40.39%) obtained in this study indicate that the selected legume seeds could be classified as excellent and quality feed resources to ruminants as reported by Rusdy (2016) and Robert (2022). The Duo scholars reported that the NDF of feed that falls within (38-62%) is as excellent quality and ADF that is above 40% is of poor quality. However, the values of fibre fractions (NDF, ADF, ADL, hemicellulose and cellulose) obtained in this study for legume seeds indicate that the seeds can be digested by ruminants (owing to their stomach structure) if included in their diets.

***Minerals composition of seven selected legume seeds***

Table 3 shows the summary of some mineral composition of selected legume seeds. The minerals such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), copper (Cu), sulphur (S) manganese (Mn) and zinc varied significantly ($P<0.05$) across the treatments. The significant differences were due to the species and varieties of legume seeds examined. Calcium (Ca) and phosphorus (P) are very important due to their roles in skeletal structure, metabolism and milk. The ranged values of Ca (0.04 – 0.77%) and phosphorus (0.04 – 0.22%) levels obtained in this study were below the ranged values (0.9 – 1.2%) of dietary Ca and P (0.25 – 0.35%) on dry matter basis (DM) recommended for dry cow (Robert, 2022). However, it was reported that high dietary P (>0.5%) may promote hypocalcemia whereas some new research suggests low diet P (0.2%DM) may be protective. Magnesium is needed for several biochemical reactions and many of these transfer chemical energy in cells which indicates the importance of this mineral in carbohydrate metabolism. Mg requirement in ruminants is between 0.21% and 0.35% DM, depending on dietary K (Robert, 2022). The ranged values (0.14 – 0.50) of Mg obtained in this work fall within the reported value recommended for ruminants. Sulphur in the body is not associated with any mineral fractions but mainly with sulphur- containing amino acids. During fermentation, the microbes of the rumen can incorporate sulphur into the amino acids that form microbial protein. The values of sulphur obtained in this work would be enough to enhance the rate of protein synthesis in the rumen and supply nitrogen compounds to microbes. Copper (Cu) plays metabolic roles and associated with protein and involved in the production of haemoglobin and assists in oxidation of food within cells. The values (4.13 - 7 92mg/kgDM obtained for legume seeds can meet the ranged values (2- 6mg/kg) required by sheep but below 8-20mg/kgDM required by cattle ((John, 2006), however, high level (15-20mglkgDM of Cu was reported to cause chronic toxicity in sheep while about four or five times will produce similar symptoms in cattle. Zinc is a constituent of several important enzymes and also enhances the effective utilisation of vitamin A. The deficiencies are a reduced appetite and poor growth leading to general poor conditions. The observed values (17.31–34.96mg/kgDM) for zinc in this study can meet requirements (9-14mg/kgDM) for sheep and fall within requirements (20- 50mg/kgDM) for cattle (John, 2006).

***Some phytochemical properties of seven selected legume seeds***

Table 4 shows some phytochemical properties (tannins, phytate, phenol, saponin, oxalate alkaloids and lectin) of selected legume seeds. Tannins, phytate, phenol saponin, oxalate, alkaloids and lectin ranged between 51.41 and 676.48mg/100g, 74.75 and 308.46mg/100g, 12.84 and 30.72mg/100g, 20.16 and 82.25mg/100g, 9.17 and 17.45mg/100g and 58.10 and 74.33mg/100g respectively. Significant differences ($P<0.05$) occurred among the seven selected legume seeds in all the phytochemicals screened which was expected, owing to the different species of legume seeds examined. *Mucuna pruriens* ($T_7$) had the highest values in all the phytochemicals observed in all the seven selected seeds but had the lowest value in phenol. The values obtained for tannins for all the seeds except *Mucuna pruriens* seed cannot have a negative impact on the ruminants regarding the digestion of protein, carbohydrates and minerals by forming complexes with them, thus reducing their availability in the rumen and post-rumen. Tannins in legume seeds vary by species as it's shown in this work. High levels of tannin (5-10%) were reported to have toxic effects on ruminants, leading to negative impacts on their health and well-being. However, goats are known to have a threshold capacity of about 9% dietary tannins (Natis and Malachek, 1981). The values of tannins obtained in this study could be tolerated by sheep, goats and cattle without adverse effects on their health. Phytates and oxalate secondary metabolites hindered the absorption and utilisation of minerals such as $Ca^{2+}$, $P^{2+}$, $Zn^{2+}$, $Mg^{2+}$, $Co^{2+}$, $Mn^{2+}$ and $Fe^{2+}$. The two phytochemicals exert adverse effects on animal performance mainly by binding Ca, P, Mg, Zn and some other trace minerals making them unavailable for absorption and utilisation (Diarra *et al*., 2019). However, ruminants through the action of rumen microbes can secrete the digestive enzyme phytase to unlock the stored phosphorus as phytic acid and make it available to ruminants. Furthermore, oxalate affects Ca and Mg metabolism (Onwuka, 1983), however, ruminants can utilise it considerable amounts of high oxalate without adverse effects owing mainly to microbial degradation in the rumen. Saponins are characterised by a bitter taste and foaming properties. Saponins have been shown to have both beneficial and detrimental effects on ruminant nutrition. On the one hand, saponins have been found to improve rumen fermentation, increase microbial protein synthesis, and enhance nutrient utilization in ruminants. On the other hand, saponins can also have negative effects such as reducing feed intake, altering rumen microbial populations, and causing hemolysis in ruminants due to their detergent-like properties (Min *et al*., 2003). Moreover, saponins have been implicated in reducing the uptake of certain nutrients including glucose and cholesterol. Saponin in some tropical fruits was observed as an active compound responsible for the suppression of methanogenesis in faunated and defaunated rumen fluid (Hess, *et al*., 2003). Phenols had been reported to decrease protein, carbohydrate and mineral digestibility and might also lower the activity of the digestive enzyme and damage the mucous membrane. The levels (12.84 – 30.72mg/100g) reported in this work cannot have negative effects on the ruminants. Lectins are carbohydrate-binding proteins found in plants, animals and microorganisms. Various studies have revealed that lectins can impair nutrient absorption and impaired intestinal function in ruminants, resulting in decreased performance and even death. However, some studies reported that lectins have beneficial effects on ruminant health by improving immune function and reducing inflammation. Moraes *et al.* (2016) reported that supplementing the diet of dairy cows with lectins from soybeans improved the cow's immune response and reduced inflammation. Similarly, a study carried by Haque *et al.* (2017) observed that feeding goats a diet containing lectins from jack beans had no adverse effects on nutrient utilisation and performance. The values (58.56 -74.33mg/100g) obtained in this work may not have adverse effects on ruminants. Alkaloids are group of nitrogen – compounds found in several plants used as feed for ruminants. The effects can be both beneficial and harmful depending on the types and dose of alkaloids ingested. Ruminants animal have varying tolerance levels for alkaloids in the feed, as well as the age and physiological status of the animal. Some type of alkaloid such as mimosine from *Leucaena leucocephala* was reported to cause a decrease in voluntary intake, growth rate and fertility in ruminants (Makkar *et al.*, 1995). In contrast, other alkaloids such as nicotine and caffeine have been shown to increase feed intake and improve animal performance in certain ruminant species (Kumar *et al.*, 2015).

***In vitro* gas fermentation characteristics of seven selected legume forage seeds at 24hrs Incubation Period**

The fermentation of soluble fraction (a),extent of gas production from insoluble but degradable fraction (b), potential extent of gas production (a+b), rate of gas production at time (c), volume of gas produced (y) at time 't' and time of production (t) at 24hrs incubation period are presented in Table 5. The gas characteristics 'a, b, a+b, c, t and y ranged between 0.00 and 3.17, 12.00 and 24.00, and 12.00 and 26.00, 0.026 and 0.080, and 6-00 and 14.00 and 3.64 and 11.00 respectively. The *in vitro* gas production characteristics of the substrate in the liquors from the animals showed that there were significant differences in the 'a', 'b' 'a + d', 'c','t' and 'y' values. This may be due to the different types and species of legume seeds examined in this study. The values for the nitrogen-free extract (NFE) that represents the soluble carbohydrate fraction of selected legume seeds had values that were significantly different ($P < 0.05$) for all the treatments. Therefore the treatments behaved similarly in terms of 'a' 'b' 'a + b' 'c''t' and 'y'. Getachew *et al*. (1999) reported that gas production can be attributed to the nature of carbohydrate fractions contained in the substrates. The intercept value 'a' for all the legume seeds (treatments) at 24hrs ranged from 0.00 in $T_4$ (*Pueraria phaseoloides*) seed to 3.17 in $T_7$ (*Mucuna pruriens*) seed. The value for absolute 'a' used ideally reflects the fermentation of soluble fraction in this study. It makes the attachment by rumen microorganisms to be done easily and leads to much gas production. Therefore, more ruminant microorganisms worked on $T_2$ and $T_7$ and this leads to higher gas production.

The extent of gas production 'b' described fermentation of the insoluble but degradable fraction in *Senna occidentalis* ($T_2$) and *Mucuna pruriens* seeds ($T_7$) which recorded high values of 24.00 and 12.00 respectively. This could be attributed to a relatively high amount of rumen-degradable protein in the seeds. This also facilitated a high rate of microbial activities by supplying the required nitrogen for their cellular protein synthesis as established by Roger *et al*. (1977). A linear relationship has been established between high crude protein in forages and *in vitro* degradability (Njidda *et al*., 2010). The ranged values (19.00 – 24.00ml/200mgDM) 'obtained in this study were within 7.33 – 25.33ml/mgDm reported for dry matter (DM) degradation of some tropical legumes crop hay stovers (Amuda and Alagbe 2023) and the ranged values of 9.5 – 32.00ml/200mg DM reported for some crop residues (Babayemi *et al*., 2009). 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 $T_2$ *(Senna occidentalis)* and lowest for the $T_4$ (*Pueraria phaseoloides*) respectively. The high value of the potential extent of gas production recorded for $T_2$ and $T_7$ was due to relatively high levels of fermentable carbohydrate fraction embedded in them. Getachew *et al*., (1999) stated that it is well-known that gas production is basically the result of the fermentation of carbohydrates to volatile fatty acid (acetate, butyrate and propionate). Menke and Steingass (1988) also reported that fermentable carbohydrates increase gas production while degradable nitrogen compound decrease gas production to some extent because of their binding of carbohydrates with ammonia. This explains the reason why all the values of gas characteristics were very low compared. The volume of gas 'y' at a 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 gas volume 'y' of the incubated samples varied across the treatments, it means that the species had an effect on legume seeds regarding the "c".t and "y " gas characteristics. However, 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 the 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 selected legume seeds as potential feed resources. Since gas production is dependent on the relative proportion of soluble, insoluble but degradable and undegradable particles of feed; a mathematical description of gas production profiles allows the evaluation of substrate and fermentability of soluble and slowly fermentable components of feeds (Getachew *et al*., 1998). Based on the above assumption, therefore, it could be adduced that among the legume seeds studied, $T_5$, $T_4$, $T_1$ and $T_6$ would provide a minimal proportion of residue that would take up space if utilised in *in vivo* studies and also persist as indigestible residue. Ørskov and Ryle (1990) reported that the rate (c) determines digestion time and consequently how long a potentially digestible material would occupy space. Therefore the potential extent of digestion ('b') values obtained for treatments 2 and 7 demonstrated that they possess more potentially degradable carbohydrates than $T_1$, $T_3$, $T_4$, $T_5$ and $T_6$ respectively. Also, the results presented in Table 5 actually demonstrated that digestion rates ('c') and potential extent ('b') of gas production provided a more meaningful index of nutritional value than ultimate digestibility comparatively. In this work, the conversion of true fermented organic matter into gas varied with the type of legume seeds incubated.

***In vitro gas fermentation parameters of selected forage legume seeds at 24hrs Incubation Period***

Table 6 showed *in vitro* fermentation parameters (Methane (CH4), Gas volume (GV), Metabolisable energy (ME), Short chain fatty acid (SCFA), Fermentation efficiency (FE), Organic matter digestibility (OMD) and Dry matter degradability (DMD) ) of selected seven legumes forage seeds at 24hhrs incubation period. Gas production is an indication of microbial degradability of samples (Babayemi *et al*., 2004b, Fievez *et al*., 2005). All the parameters, observed in this study showed that the varieties and differences in legume species had significant (P<0.05) impacts on the nutritive value of selected legumes seeds. The lowest and highest $CH_4$ production and total gas volume (TGV) were obtained in *Centrosema pubescence* ($T_6$) and *Senna occidentalis* ($T_2$)respectively. In most cases, feedstuffs that showed a high capacity for gas production were also observed to be synonymous wiith high methane production. Methane ($CH_4$) production in the rumen is an energetically wasteful process and an energy loss to the ruminants, since the portion of the animal's feed converted to $CH_4$ is eructated as gas. However, levels of $CH_4$ production were relatively low which indicates that more energy would be available to ruminants. Generally, gas production is a function and reflection of degradable carbohydrate and therefore, the amounts depends on the nature of the carbohydrates (Demeyer and Van Nevel, 1975; Blummel and Becker, 1997). Gas production from protein fermentation is relatively small compare to carbohydrate fermentation which could be attributed to the presence of secondary metabolites (tannins, saponins) contained in selected legume seeds (Babayemi *et al*., 2004a) and potency of rumen liquor for incubation. Metabolisable energy (ME), short chain fatty acid (SCFA) and dry matter degradability (DMD) production all differed significantly (P<0.05) across the treatments. In all these parameters, value for the ME, SCFA, OMD and DMD ranged from 4.93 to 7.08MJ/KgDM, 0.23 to 0.56µmol, 41.01 to 57.45% and 49.50 to 56.50% respectively. A correlation between ME values measured *in vivo* and predicted from 24hr *in vitro* gas production and chemical composition of feed was reported by Menke and Steingass (1988). The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed (Getachew *et al*., 2000). Although, gas production is a nutritionally wasted product (Mauricio *et al*. 1999) but provides a useful basis from which metabolisable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) could be estimated. Short chain fatty acid (SCFA) is directly proportional to metabolisable energy (ME) (Menke *et al*., 1979) in this study. The levels of ME and SCFA reported in this work suggest that energy would be available to ruminants if the seven selected legume seeds form part of their diets. However, the ranged values obtained for OMD and DMD were relatively low which might due to suppression effects of some phytochemicals compounds on rumen microbes. Ndou *et al.* (2021) reported that different legume seeds contain varying levels of anti-nutritional factors, such as lectins and tannins, saponin, phenols which may affect the organic matter digestibility (OMD), dry matter digestibility (DMD%) and nutrient availability to the animals. Consequently, the legume seeds must be processed before including them in ruminants' diets for efficient utilisation. Furthermore, the information obtained on the chemical composition and fibre fractions can be useful in formulating ruminant diets to meet their nutritional requirements. Therefore, the results suggest that these legume seeds may require some form of processing or treatment to improve their nutrient digestibility and availability for ruminants' utilisation.

**Conclusion**

Generally, all the legumes seeds considered contained high levels of protein, minerals (Ca, P, Mg, K, Na, Cu, Mn, Zn and Fe) and low levels of fibre composition couple with relative low levels of lignin which means the ruminants can digest about 80% and utilise them efficiently, therefore legumes seeds is a good source of protein for ruminants. *In vitro* digestibility results of some selected legumes seeds utilised by ruminants showed that methane ($CH_4$) production was relatively low indicating that more energy would be available to the animals and feeding out to ruminants would not contribute to global warming and climate change effect. Similarly, total gas volume (TGV), metabolisable energy (ME), short chain fatty acid (SCFA), fermentation efficiency (FE), organic matter digestibility (OMD) and dry matter digestibility (DMD) of examined legume seeds were very low considering high levels of crude protein content embedded in them. Therefore, the selected legume seeds should be processed in order to reduce their phytochemicals compound and exploit their full potential as a feed resources to ruminants and non-ruminants livestock in feed industries.

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**Table 1: Proximate Composition of Selected Legumes Seeds**

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **PRMTS (%)** | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | SEM |
| **CP** | $19.28^e$ | $23.87^c$ | $22.10^d$ | $16.06^f$ | $35.77^a$ | $19.41^e$ | $32.10^b$ | 0.32 |
| **ASH** | $10.23^b$ | $13.39^a$ | $13.34^a$ | $13.66^a$ | $9.35^c$ | $10.38^b$ | $10.28^b$ | 0.06 |
| **EE** | $1.36^c$ | $2.24^{ab}$ | $2.20^b$ | $2.24^{ab}$ | $2.38^a$ | $1.34^c$ | $2.18^b$ | 0.03 |
| **CF** | $22.59^a$ | $21.20^c$ | $21.86^{bc}$ | $21.40^c$ | $15.97^e$ | $22.13^{ab}$ | $19.05^d$ | 0.13 |
| **NFE** | $46.54^a$ | $39.30^c$ | $40.50^c$ | $46.64^a$ | $36.54^d$ | $46.74^a$ | $36.38^d$ | 0.18 |
| **DM** | $93.31^{ab}$ | $93.20^b$ | $93.95^a$ | $93.12^b$ | $92.82^b$ | $93.16^b$ | $93.30^{ab}$ | 0.18 |
| **OM** | $89.77^b$ | $86.61^c$ | $86.66^c$ | $86.34^c$ | $90.65^a$ | $89.62^b$ | $89.72^b$ | 0.06 |
| **CHO** | $69.13^a$ | $60.50^d$ | $62.36^c$ | $68.04^b$ | $52.50^f$ | $68.87^a$ | $55.43^e$ | 0.09 |

***a, b ,c , d, e =Value with different upper scripts in a column differ significantly (P<0.05).***

***$T_1$=Senna hirsuta seed, $T_2$=Senna occidentalis seed, $T_3$= Senna obtusifolia seed, $T_4$ = Pueraria phaseoloid seed, $T_5$ = Tephrosia bracteolata seed, $T_6$ = Centrosema pubescence seed, $T_7$ = Mucuna pruriens seed, DM = Dry Matter, CP = Crude Protein, EE = Ether Extract, CF = Crude Fibre, NFC = Non-Fibre Carbohydrate, OM = Organic Matter, NFE = Nitrogen Free Extract, CHO = Carbohydrates, SEM = Standard Error of Mean, PRMTS = Parameters.***

**Table 2: Fibre fractions of selected legume seeds**

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **PRMTS (%)** | T1 | T2 | T3 | T4 | T5 | T6 | T7 | SEM |
| **NDF** | $59.10^c$ | $60.61^{ab}$ | $60.88^{ab}$ | $61.60^a$ | $57.19^d$ | $59.68^{bc}$ | $61.12^a$ | 0.25 |
| **ADF** | $34.14^c$ | $35.75^b$ | $35.64^b$ | $35.89^b$ | $30.09^d$ | $33.64^c$ | $40.39^a$ | 0.17 |
| **ADL** | $14.13^{cd}$ | $17.93^b$ | $18.85^a$ | $17.78^b$ | $14.79^c$ | $13.46^d$ | $17.39^b$ | 0.16 |
| **CELLULOSE** | $20.01^b$ | $17.82^c$ | $16.79^d$ | $18.11^c$ | $15.30^e$ | $20.18^b$ | $23.00^a$ | 0.16 |
| **HEMICELL** | $24.96^c$ | $24.86^c$ | $25.24^{bc}$ | $25.71^{bc}$ | $27.10^a$ | $26.04^b$ | $20.73^d$ | 0.15 |

***a, b, c, d, e = Value with different super scripts along the same row differ significantly (P<0.05).***

***$T_1$=Senna hirsuta seed, $T_2$ = Senna occidentalis seed, $T_3$ = Senna obtusifolia seed, $T_4$ = Pueraria phaseoloid seed, $T_5$ = Tephrosia bracteolata seed$T_6$ = Centrosema pubescence seed, $T_7$ = Mucuna pruriens seed, NDF = Neutral Detergent Fibre, ADF = Acid Detergent Fibre, ADL = Acid Detergent Lignin, HEMI = Hemicellulose, CELL= Cellulose, SEM = Standard Error of Means and PRMTS = Parameters.***

**Table 3: Some minerals composition of selected legume seeds**

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PRMTS | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | SEM |
| Ca (%) | $0.26^b$ | $0.15^c$ | $0.12^d$ | $0.15^c$ | $0.15^c$ | $0.04^e$ | $0.77^a$ | 0.00 |
| P (%) | $0.04^e$ | $0.22^a$ | $0.09^d$ | $0.15^b$ | $0.10^d$ | $0.12^c$ | $0.12^c$ | 0.00 |
| Mg (%) | $0.22^b$ | $0.50^a$ | $0.17^d$ | $0.18^c$ | $0.17^d$ | $0.17^d$ | $0.14^e$ | 0.00 |
| K (%) | $0.54^a$ | $0.36^{bc}$ | $0.48^{ab}$ | $0.41^{abc}$ | $0.29^c$ | $0.50^{ab}$ | $0.28^c$ | 0.03 |
| Na (%) | $0.54^c$ | $0.47^d$ | $0.59^b$ | $0.40^e$ | $0.36^f$ | $0.61^a$ | $0.37^f$ | 0.00 |
| Cu(mg/Kg) | $4.54^f$ | $5.24^d$ | $7.43^a$ | $4.28^g$ | $6.36^b$ | $5.42^c$ | $4.73^e$ | 0.03 |
| S (mg/Kg) | $0.27^{bc}$ | $0.39^{ab}$ | $0.47^a$ | $0.22^c$ | $0.20^c$ | $0.27^{bc}$ | $0.20^{bc}$ | 0.02 |
| Mn(mg/Kg) | $16.64^g$ | $24.30^c$ | $21.94^e$ | $26.34^b$ | $32.11^a$ | $18.98^f$ | $22.70^d$ | 0.03 |
| Zn (mg/Kg) | $18.60^f$ | $34.96^a$ | $17.31^g$ | $24.74^c$ | $21.43^e$ | $28.64^b$ | $22.44^d$ | 0.04 |
| Fe (mg/Kg) | $503.81^d$ | $549.49^b$ | $692.37^a$ | $375.64^g$ | $533.08^c$ | $422.39^e$ | $399.16^f$ | 1.34 |

***a,b,c,d,e,f,g = Value with different superscripts along the same row differ significantly (P<0.05).***

***$T_1$= Senna hirsuta seed, $T_2$ = Senna occidentalis seed, $T_3$ = Senna obtusifolia seed, $T_4$ = Pueraria phaseoloides seed, $T_5$ = Tephrosia bracteolata seed, $T_6$ = Centrosema pubescence seed, $T_7$ = Mucuna pruriens seed,***

***Ca = Calcium. P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, Cu = Copper, S = Sulphur, Mn = Manganese, Zn = Zinc and Fe = Iron, TRTS = Treatments, PRMTS = Parameters and SEM = Standard of Error Deviation.***

**Table 4: Some phytochemical properties of seven selected legume seeds**

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **PRTS(mg/100g)** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **$T_6$** | **$T_7$** | **SEM** |
| Tannins | $75.42^{b}$ | $53.69^{f}$ | $63.63^{c}$ | $56.96^{e}$ | $60.72^{d}$ | $51.91^{g}$ | $676.48^{a}$ | 0.28 |
| Phytate | $97.36^{b}$ | $90.37^{c}$ | $87.67^{d}$ | $85.27^{e}$ | $79.25^{f}$ | $74.75^{g}$ | $308.46^{a}$ | 0.17 |
| Phenol | $129.43^{a}$ | $109.78^{c}$ | $108.05^{d}$ | $121.57^{b}$ | $103.43^{e}$ | $97.53^{f}$ | $29.75^{g}$ | 0.18 |
| Saponin | $20.78^{c}$ | $18.71^{d}$ | $22.70^{b}$ | $16.27^{e}$ | $14.10^{f}$ | $12.84^{g}$ | $30.72^{a}$ | 0.12 |
| Oxalate | $30.47^{c}$ | $22.49^{f}$ | $34.84^{b}$ | $27.61^{d}$ | $20.16^{g}$ | $25.13^{e}$ | $82.25^{a}$ | 0.21 |
| Alkaloids | $16.30^{b}$ | $15.366^{b}$ | $13.29^{c}$ | $9.17^{e}$ | $11.01^{d}$ | $12.63^{c}$ | $17.45^{a}$ | 0.19 |
| Lectins | $68.10^{b}$ | $58.56^{f}$ | $62.90^{d}$ | $61.40^{e}$ | $63.95^{c}$ | $68.74^{b}$ | $74.33^{a}$ | 0.15 |

***a,b,c,d,e,f,g =Value with different upper scripts on the same differs significantly (P<0.05).***
***$T_1$= Senna hirsuta seed, $T_2$ = Senna occidentalis seed, $T_3$ = Senna obtusifolia seed, $T_4$ = Pueraria phaseoloid seed, $T_5$ = Tephrosia bracteolata seed, $T_6$ = Centrosema pubescence seed, $T_7$ = Mucuna pruriens seed TRMTS = Treatments and PRTS = Parameters.***

**Table 5: *In vitro* gas fermentation characteristics of seven selected legume seeds at 24hrs Incubation Period**

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **PRMTS** | T1 | T2 | T3 | T4 | T5 | T6 | T7 | SEM |
| a($ml^3$) | $1.33^{ab}$ | $2.00^{ab}$ | $1.33^{ab}$ | $0.00^{b}$ | $1.67^{ab}$ | $2.00^{ab}$ | $3.17^{a}$ | 0.36 |
| b($ml^3$) | $14.67^{bcd}$ | $24.00^{a}$ | $16.00^{bcd}$ | $12.00^{d}$ | $14.00^{cd}$ | $12.00^{d}$ | $19.00^{b}$ | 0.77 |
| a+b($ml^3$) | $16.00^{cd}$ | $26.00^{a}$ | $17.33^{bc}$ | $12.00^{d}$ | $15.67^{cd}$ | $19.67^{bc}$ | $22.17^{ab}$ | 0.88 |
| c(t/$ml^1$) | $0.060^{ab}$ | $0.036^{ab}$ | $0.026^{b}$ | $0.079^{a}$ | $0.080^{a}$ | $0.060^{ab}$ | $0.039^{ab}$ | 0.01 |
| t(hr) | $14.00^{a}$ | $12.00^{ab}$ | $6.00^{b}$ | $13.00^{ab}$ | $13.00^{ab}$ | $13.00^{ab}$ | $9.00^{ab}$ | 1.34 |
| Y(ml/$h^{-3}$) | $9.67^{a}$ | $11.00^{a}$ | $3.67^{c}$ | $7.33^{b}$ | $10.67^{a}$ | $10.67^{a}$ | $9.00^{ab}$ | 1.51 |

***a,b,c,d = Value with different upper scripts on the same row differs significantly (P<0.05).***
***$T_1$= Senna hirsuta seed, $T_2$ = Senna occidentalis seed, $T_3$ = Senna obtusifolia seed, $T_4$ = Pueraria phaseoloides seed, $T_5$ = Tephrosia bracteolata seed, $T_6$ = Centrosema pubescence seed, $T_7$ = Mucuna pruriens seed.***

**Table 6: *In vitro* gas fermentation parameters of seven selected forage legumes seeds at 24hrs incubation period**

| | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **PRMTS** | T1 | T2 | T3 | T4 | T5 | T6 | T7 | SEM |
| **$CH_4$ (ml)** | $9.00^{bc}$ | $12.50^{a}$ | $7.50^{bc}$ | $7.00^{c}$ | $8.00^{bc}$ | $4.50^{d}$ | $9.50^{b}$ | 0.36 |
| **TGV(ml)** | $19.67^{bc}$ | $26.00^{a}$ | $17.33^{cd}$ | $16.00^{cd}$ | $15.67^{cd}$ | $12.00^{d}$ | $23.00^{ab}$ | 0.98 |
| **ME(MJ/kg/DM)** | $5.92^{bc}$ | $7.00^{a}$ | $5.78^{bc}$ | $5.26^{cd}$ | $6.32^{b}$ | $4.93^{d}$ | $7.08^{a}$ | 0.13 |
| **SCFA ( ? )** | $0.41^{bc}$ | $0.56^{a}$ | $0.35^{cd}$ | $0.32^{cd}$ | $0.31^{cd}$ | $0.23^{d}$ | $0.49^{ab}$ | 0.02 |
| **FE** | $2.704^{bc}$ | $1.96^{c}$ | $3.09^{b}$ | $3.32^{b}$ | $3.40^{b}$ | $4.79^{a}$ | $2.28^{c}$ | 0.14 |
| **OMD (%)** | $47.70^{bc}$ | $57.45^{a}$ | $48.92^{bc}$ | $45.23^{cd}$ | $50.99^{b}$ | $41.01^{d}$ | $56.47^{a}$ | 0.88 |
| **DMD (%)** | $52.70^{b}$ | $49.90^{c}$ | $53.20^{b}$ | $56.50^{a}$ | $53.20^{b}$ | $52.95^{b}$ | $51.15^{c}$ | 0.45 |

***a,b,c,d = Value with different upper scripts on the same row differs significantly (P<0.05).***
***$T_1$= Senna hirsuta seed, $T_2$ = Senna occidentalis seed, $T_3$ = Senna obtusifolia seed, $T_4$ = Pueraria phaseoloid seed, $T_5$ = Tephrosia bracteolata seed, $T_6$ = Centrosema pubescence seed, $T_7$ = Mucuna pruriens seed, CH4 = Methane, TGV = Total Gas Volume, ME = Metabolisable Energy, SCFA = Short Chain Fatty Acid, FE = Fermentation Efficiency, OMD = Organic Matter Digestibility, DMD = Dry Matter Digestibility, SEM = Standard Error of Means and PRMTS = Parameters.***