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Effects of Seasonal Changes on Nutrients Composition and in *Vitro* Gas Assessment of Pigeon Pea (*Cajanus Cajan*) Foliage as a Fodder Crop

Amuda, A. J.[1]; Okunlola, D. O[2]

[1]Department of Animal Production and Health, Federal University Wukari, Taraba State, Nigeria
[2]Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomosho, Nigeria

ABSTRACT

Animal performance mainly depends on the quantity and quality of forage available as feed and animal feed represents one of the major cost in animal production. Consequently, a research was conducted to investigate the effect of seasonal changes on nutrients composition, and *in vitro* gas assessment of *Cajanus cajan* as a fodder crop. *Cajanus cajan* foliage was harvested from the pasture unit of the University farm after cut back was done and samples were collected every 28 days of every month (from July to March of the following year) and each month serve as a treatment and T_1 (July…March) T_9. The samples were oven dried to determine chemical composition, minerals, secondary metabolites and *in vitro* gas evaluation using standard techniques. The results of chemical composition showed that crude protein (CP) and crude fibre varied from 17.12 to 19.44% and 21.57 to 24.83% respectfully. Similarly, all the minerals composition (calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), manganese (Mn), copper (Cu) zinc (Zn), iron (Fe) and secondary metabolites (phenols, phytates, oxalates, alkaloids, tannins, saponins and lectins) were significantly ($P<0.05$) different across the treatments. All other parameters such as dry matter (DM), ash, ether extract (EE), nitrogen free extract (NFE), non-fibre carbohydrates (NFC) and carbohydrates (CHO) were varied significantly ($P<0.05$) except the organic matter (OM) that was similar across the treatments. Fibre fractions (Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), Hemicellulose and Cellulose) showed significant ($P<0.05$) differences except ADL that was similar throughout the season. *In vitro* fermentation study revealed that methane (CH_4), total gas volume (TGV), dry matter degradability (DMD), organic matter digestibility (OMD), metabolisable energy (ME), short chain fatty acids (SCFA), and fermentation efficiency (FE) ranged between 3.00 and 7.50ml, 7.00 and 14.00ml, 44.37 and 56.79%, 37.56 and 43.48%, 4.25 and 5.12MJ/KgDM, 0.11 and 0.28µmol, 4.00 and 6.78 respectively. Significant differences (($P<0.05$) occurred among the months regarding the *in vitro* gas parameters. evaluated. The CP levels, CF and ADF indicated that *Cajanus cajan* would be digestible. *In vitro* fermentation results showed that CH_4 ranged value was very low, an indication of low loss of energy feed if fed to ruminants. Dry matter degradability (DMD) and organic matter digestibility (OMD) of *Cajanus cajan* as affected by season change were relative low owing to relative high level of ADF, NDF and some secondary metabolites. However seasonal variation had significant ($P<0.05$) effects on the chemical composition except for Ash, OM and ADL composition that were similar across the seasons. Similarly, the results of *in vitro* gas assessment showed that seasonal change had significant influence on the parameters measured.

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KEYWORDS: *Cajanus cajan, metabolisable energy, in vitro, chemical composition*

INTRODUCTION

Cajanus cajan is a leguminous browse plant; the common names are pigeon pea, red gram, congo pea, gungo pea, no 'eye' pea (David, 2014). *Cajanus cajan* is cultivated on a large scale in 22 countries of the world and it is the sixth most important legume food crop (Varshney, 2011). It is well adapted to arid and semi-arid tropical and subtropical climates (Raju *et al.,* 2010). The numerous valuable uses of *Cajanus* *cajan* explain the cultivation of the browse in many countries (Egbe and Kalu, 2009).The forage of *Cajanus cajan* is a fodder relish by ruminants and rabbits. It is of high nutritive value and serves as vegetable for human consumption and the protein content of the seeds compares favorably with those of soybean meal and groundnut cake (Rao *et al.,* 2002, Onu and Okongwu, 2006). Production of *Cajanus cajan in* Africa was amounted to be 9.3% of world production but this is small compared to 74% contribution from India (Ojedapo *et al.,* 2009). Many legumes are found in the tropics and subtropics and very great efforts have been made on how to improve their seed yield. The chemical composition of *Cajanus cajan* as shown by Sinha (1977) contained about 57.3 – 58.7% carbohydrate, 1.2–8.2 crude fiber and 0.6 –3.2% lipids. It is a good source of dietary minerals such as calcium, phosphorus, magnesium, iron, sulphur and potassium. Furthermore, pigeon pea is a good source of soluble vitamins especially thiamine, riboflavin, niacin and choline. The Protein content of *Cajanus cajan* ranges between 20.6 and 27% and amino acids such as lysine was relatively high, methionine was relatively low with trypsin inhibitors (10.1mg/100gm) and protein energy ratio (PER) of 0.9 –1.40 (Gazzeta *et al.,* 1995). Magboul *et al.* (1976) reported that nutrient content such as ether extract and anti-trypsin activity were higher in green seeds than mature ones. Similarly, Nour and Magboul (1986) found that *Cajanus cajan* seed contain 6.1–6.2% moisture, 19.3–26.3% protein, 1.3–2.0 % fat, 6.4–8.2% fiber, 53.5–62.7% carbohydrates; in addition to 132–500 mg/g calcium and 330–354 kcal/kg of energy. However, agriculture sector is facing the direct effects of climate change and poorer developing countries are the most vulnerable. Sustainable solutions to agriculture and food security in Africa must consider more focused research efforts on locally adapted, highly nutritious and stress-tolerant crops and animals alongside with sustainable government support to agricultural research and development (Kaoneka *et al.,* 2016). Ironically, most of Africa's native stress-tolerant crops are mainly the least researched worldwide and are thus referred to as "orphan crops" (Naylor *et al.,* 2004). Availability of nutritional quality and quantity of forages in dry season feeding to meet nutritional requirements of ruminant animals is a major challenge in livestock industry. Farmers have used pigeon pea plants as animal feed for more than 100 years where after crop harvest, vegetative plant material is left in the field for grazing (Roder *et al.,* 1998). Dry pigeon pea leaves are used as fodder and the threshing from crop used as feed for dairy cattle (Matthews and Saxena, 2000). About 9% of available by-products from the seed is consumed as animal and poultry feed (Joshi *et al.,* 2001). According to Makelo (2011), the fodder of the crop has been an important factor in increasing weight of animals consuming the fodder. Considering the potential of *Cajanus cajan* seeds and foliage as feed resource, there is need to explore the potential usage of this crop as feed resource in ruminants and non-ruminants nutrition. Thus, a research was carried out to investigate effects of seasonal change on nutrients composition and *in vitro* gas evaluation of *Cajanus cajan* foliage.

Materials and Methods

Experimental Site

The study was carried out at Teaching and Research Farm of the Federal University Wukari, Taraba State, North–East Nigeria. Wukari is located on Latitude $7^0 51^1$ to $7^0 85^1$North and Longitude $9^0 47^1$ to $9^0 78^1$East. Wukari is situated at elevation 189 metres above the sea level and has a mean annual rainfall of 1, 300mm. The maximum annual temperature ranged between 30^0C and 39.4^0C while the minimum annual temperature is between 15^0 and 23^0C (Reuben and Mshelia, 2011).

Sampling collection

The samples were collected at the pasture unit of Federal University Wukari teaching and research farm. The established Cajanus fodder were cut back and samples were collected every 28 days of each month and the harvesting was done from July to March. The samples were air-dried and later moved to the oven regulated to 65^oc for further drying. Then, the samples were milled by 'MG 123' mixer grinder before analysed.

Chemicals and minerals composition and determination of secondary metabolites

The milled samples were analysed for percentage Dry Matter (DM), Crude Protein (CP), Crude Fibre (CF), Ether Extract (EE), Organic Matter (OM), Nitrogen Free Extract (NFE) as described by AOAC (2005) and the quantity of CP was determined (N x 6.25). Fibre Fractions (Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were analysed using the procedure of Van Soest *et al.* (1991). Hemicellulose was calculated by difference between NDF and ADF while cellulose was determined by difference between ADF and ADL (Rinne *et al.,* 1997). Non-Fibre Carbohydrates (NFC) was determined according to NRC (2001). Minerals (Ca, P, Mg, K, Na, Mn, Cu, Fe and Zn) were determined according to AOAC (2006) while secondary metabolites (Phenol, Phytates, Oxalates, Alkaloid, Tannins, Saponin and Lectin) were screened according to the methods of Sofowora (1993).

In vitro gas fermentation study

1. The *in vitro* gas assessment of triplicate samples of monthly collected *Cajanus cajan* was determined according to the method of Menke and Steingass (1988). Rumen liquor was collected from three WAD goats (fed 40% concentrates feed and 60% *Panicum maximum*) through suction tube before the morning feed. The fluid was collected into a thermos flask and taken to the laboratory. It was filtered through a four-layered cheese cloth into a warm flask, flushed with carbon dioxide (CO2) gas, and stirred using an automatic stirrer. The rumen fluid was kept at 39 ± 1^{0}C. Plastic syringes (100mL) connected with plungers were used, 200 mg DM of each sample was carefully inserted into the syringes and after that, the syringes were filled with 30ml of medium containing 10mL of rumen fluid and 20mL of buffer solution (9.8g$NaHCO_3$ + 2.77gNa_3HPO_4 + 0.57g KCl + 0.47g NaCl +0.12g l$MgSO_4.7H_2O$ + 0.16/litre $CaCl_2.H_2O$) mixed at ratio 1:4 v/v and each sample was measured three times. The syringes were tightly covered and carefully arranged in an incubator maintained at 39±1^{0}C along with three blank syringes containing 30mL of medium (rumen-buffer) only as control. The gas production was recorded at 3hours intervals (3, 6, 9, 12, 15, 18, 21 and 24hours). The content was repeatedly agitated at each time of reading. At post incubation period, 4mL of sodium hydroxide (NaOH) solution was introduced as described by Fievez *et al*. (2005) to estimate methane (CH_4). Metabolisable energy (ME, MJ/KgDM) and organic matter digestibility (OMD %) were calculated as described by Menke and Steingass, 1988) and short chain fatty acids (SCFA µmol) was determined as reported by Getachew *et al.* (1999) using post incubation. ME = 2.20 + 0.136*GV + 0.057*CP + 0.0029*CF, OMD = 14.88 + 0.889*GV- 0.0601, where GV, CP. CF and XA are net gas production (ml/200mg DM), crude protein, crude fibre and ash of the incubated samples respectively. Graphs of the volume of gas produced every 3-hour interval of the 3 replicates of each sample was 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 collected were subjected to analysis of variance using SPSS (version 23.0, 2018) and means were compared where significant using Duncan multiple range F-test at 5% probability level (Duncan, 1955).

$$\text{DMD \%} = \frac{\text{Initial dry matter input} - \text{Dry matter residues}}{\text{Initial dry matter input}} \times 100$$

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

RESULTS AND DISCUSION

Proximate composition such as Dry matter (DM), Crude Protein (CP), Ash, Ether Extract (EE), Crude Fibre (CF), Nitrogen Free Extract (NFE), Organic Matter (OM), Total Carbohydrates (TCHO) and Non-Fibre Carbohydrate (NFC)) of *Cajanus cajan* foliage as affected by seasonal change are presented in Table 1. All the parameters observed were significantly ($P<0.05$) influenced by seasonal change across the months except the ash contents and organic matter (OM) that were similar across the treatments.The DM, CP, Ash, EE, CF, NFE, OM, TCHO and NFC ranged between 90.83 and 92.94%, 17.12 and 19.44%, 11.08 and 12.68%, 1.29 and 1.98%, 21.57 and 24.83%, 43.98 and 47.57%, 87.70 and 88.92%, 66.84 and 69.14%, 0.18 and 11.96% respectively. Pigeon pea foliage is a protein rich forage fodder of about 10 – 25% CP. Young leaves contain above 20% crude protein in the DM, while old leaves may contain less than 12% CP as reported by Veloso *et al.* (2006) and Foster *et al.* (2011). The ranged values (17.12 – 19.44% CP) obtained for *Cajanus cajan* harvested from July to March is inclined to 19% and close to 20% CP reported for dried C*ajanus cajan* leaves by Adebisi *et al*. (2020) and Foster *et al.* (2011) respectively. Adebisi *et al*. (2020) reported 22.31%CP (fresh leaves), 21.20%CP (wilted leaves) and 19.00%CP (dried leaves) for pigeon leaves of different treatments. Also the crude fibre (CF) ranged values reported in this work were lower than 25.26% (fresh leaves), 26.86% (wilted leaves) and 27.12% (dried leaves) reported by Adebisi *et al*. (2020) and 30.6% reported for fresh pigeon leaves by Foster *et al*. (2009). Similarly, the ether extract (EE) contents of this work were lower than values (4.50 – 4.79) and 4.3% documented by the same authors in their finds. However, the ash contents obtained of this work were higher than 9.12% (fresh), 9.32% (wilted) and 9.45% (dried) observed by Adebisi *et al*. (2020) and 6.1% and 4.6% reported by Foster *et al.* (2009) for fresh and hay pigeon pea leaves respectively. NFE also follow similar trend, as ash content were greater than 15.98% (fresh), 24.58% (wilted) and 8.5% (dried) cajanus leaves observed by Adebisi *et al.* (2020). The variation could be attributed to season, soil fertility and ecological factor. Chemical composition of *Cajanus cajan* leaves collected from July - March suggests that seasonal variation had significant effect on the nutrient composition.

Fibre fractions of *Cajanus cajan* as affected by seasonal change

The fibre detergent fractions of *Cajanus cajan* leaves is shown in Table 2. All the fractions varied significantly (P<0.05) across the treatments except the acid detergent lignin (ADL) that was similar across the treatments. The NDF (total cell walls), ADF (lignocelluloses) and ADL ranged from 56.60 to 68.53%, 35.87% to 41.43% and 16.90 to 18.10%. The NDF, ADF and ADL values reported in this work were relatively higher than 47.5%, 30.5% and 15.1% reported (Foster *et al.,* 2009) for fresh *Cajanus cajan* leaves but lower than 78.6%, 60.2% reported for cajanus hay however ADL values were the same as 17.1%, reported for cajanus hay Foster *et al*. (2009). Furthermore, the ranged values reported for NDF, ADF and ADL of cajanus leaves harvested at different seasons were at variance with values reported for NDF, ADF and ADL (33.29%, 29.50% and10.12%) fresh, (33.52%, 29.47% and 29.40%) wilted, (33.83%, 29.40 and 12.40%) dried) of different processed cajanus leaves by Adebisi *et al.* (2020). Pigeon pea forage is protein-rich forage, but its high fibre content (particularly ADF and lignin) decreases digestibility and limits its potential use. It can be considered as a medium to low energy quality forage. The fibre fractions (NDF, ADF, and ADL) which have implication on digestibility would be expected to be high during dry season (November to March) but reverse was the case. The reason could be attributed to low soil moisture content which could have delayed the foliages regrowth before 28days of harvesting/cutting period. Thus, fibre fractions of rainy period was higher than dry season months. The neutral detergent fibre (NDF), which is a measure of the plants cell wall contents, is the chemical component of feed that determines its rate of digestion. Neutral detergnt fibre (NDF) is inversely related to the plants digestibility (McDonald *et al*., 1995 and Gillespie, 1998). The higher the NDF the lower the plants digestible energy. 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. Lignin is generally accepted as the primary component responsible for limiting the digestion of forages (Van Soest, 1994; Traxler *et al.,* 1998; Agbagla-Dohnani *et al.,* 2001). Singh and Oosting (1992) pointed out that roughage feeds containing NDF values of less than 45% could be classified as high quality those with values ranging from 45% to 65% as medium and those with values higher than 65% as low quality. High NDF values can be a limiting factor to dry matter intake, as dry matter intake and NDF content are negatively correlated (Van Soest, 1994). NDF is correlated with the level of dry matter intake by cows; the lower the NDF, the higher the level of intake. Based on this fact, the *Cajanus cajan* leaf can be categorized as medium quality forage in terms of their NDF. The hemicellulose and cellulose are cell wall constituents and polysaccharides. They are very indigestible in monogastrics but digestible in ruminants through fermentation by rumen microbes. Hemicellulose values obtained for *Cajanus cajan* leaf from rainy season to dry season ranged from 19.82 – 28.80% while cellulose ranged from 18.04 – 24.27%. These values were not too high for ruminants due to the nature of their stomach and the presence of cellulolytic bacteria and fibrolytic fungi in the rumen. According to McDonald *et al.* (1995), ruminants can be fed sole on feed that contained 40% cellulose and 20% hemicellulose. This is suggesting that ruminants can cope with feed that contain relatively high level of cellulose and hemicellulose with the aid of rumen microbes.

Minerals composition of pigeon pea (*Cajanus cajan)* as affected by seasonal change

The minerals composition of pigeon pea *(Cajanus cajan*) as affected by seasonal change is shown in Table 3. All the minerals (calcium, phosphorus, magnesium, potassium, sodium, cupper, manganese, zinc and iron) observed in, this work varied significantly (P<0.05) across the months of harvest. Calcium (Ca), phosphorus (P) and magnesium (Mg) are required for normal skeletal development in animals and humans. The Ca, P and Mg ranged from 0.14 – 0.42%, 0.09 – 0.18% and 0.16 – 0.44% respectively. Generally, minerals composition of legumes seed is higher than leaves or foliages. The ranged values obtained for Ca, P, and Mg pigeon leaves were lower than ranged values (0.87 – 1.29%, 3.70 – 4.11% and 1.36 – 1.43%) reported by Ayuni *et al*. (2022) for seeds in this work. The values of Ca obtained in this work were lower than ranged values (0.40 – 1.5%) recommended for goats except for value (0.42%) obtained for the month of March that fall within but below 1.6% recommended for lactating goats (NRC,1981). Similarly, the P levels observed in this study were within minimum range of 1.5% recommended for small ruminants (NRC, 1981). Magnesium (Mg) levels (0.16 – 0.44%) of pigeon pea foliage cannot meet 0.6% recommended daily for sheep (John, 2006). Potassium (K) and sodium (Na) values varied from 0.11–0.64% and 0.13–0.78% across the treatments. The values of K fall below 3% daily requirement for sheep while 0.78% reported for month of March was relatively higher than 0.7% daily recommended for sheep but lower than 4% and 11% recommended for steer and dairy cow respectively. Furthermore, copper (Cu), manganese (Mn), zinc (Zn) and iron (Fe) ranged between 6.24 and 8.63mg/kg, 29.13–67.76mg/kg, 13.53–23.07mg/kg and 254.16 – 662.31mg/kg. Cu values can meet the 2-6mg/kgDM daily requirement by sheep and relatively close to minimum daily requirement (8mg/kgDM) for cattle (John, 2006). Mn values fall within minimum 20mg/kgDM and levels of 40mg/kgDM are most desirable (John, 2006). However, the values of Mn obtained in study were higher than net requirement recommendation (0.47mg/kg) of the NRC (2007) for growth in sheep. Moreover, the range values of Zn obtained here was lower than 24mg/kg recommended by NRC (2007) for growth in sheep while some authors recommended 9-14mg/kgDM. The variation could be attributed to breeds, feed, management practices, physiological and environmental conditions. The ranged values of Fe indicate that pigeon leaves as affected by season can meet the small ruminant requirements irrespective period of the season. However, for large ruminants Fe requirements varies, depending on the size and physiological state of the animal. Dairy cattle requires 30mg/kg DM feed intake while at growing, finishing, gestating, and lactating stages, cattle should be receiving about 50mg of Fe for every kg of feed/ forage they eat.

Presented in Table 4 is the anti-nutritional factors of *Cajanus cajan* as affected by seasonal variation. All the anti-nutritional factors were significantly ($P<0.05$) influenced by seasonal change. Phenols, phytates, oxalates, alkaloids, tannins, saponins and lectins varied from 234.93 to 271.08mg/100g, 52.83 to 83.02mg/100g, 33.22 to 48.43mg/100g, 16.31 to 20.69mg/100g, 48.62 to 69.03mg/100g, 20.96 to 31.26mg/100g and 36.33 to 59.25mg/100g. Phenols in pigeon pea forage possess antioxidant properties, which can protect against oxidative stress and chronic diseases in humans (Yadav *et al*., 2020). Phenols have also been reported to exhibit antimicrobial and anti-inflammatory activities (Bok *et al.,* 2018). In ruminants, phenols can enhance rumen fermentation and improve nutrient utilisation (Lehloenya et al., 2019). It was reported that phenol could negatively affect feed intake, nutrients utilisation and overall animal performance. The tolerance levels of phenols in pigeon pea forage for ruminants and monogastric animals are species-specific. According to Gupta *et al.* (2018), ruminants can tolerate higher levels of phenols compared to monogastric animals due to rumen microbial degradation of phenolic compounds. However, high levels of phenols can still impair rumen fermentation and decrease nutrient digestibility in ruminants (Lehloenya *et al.,* 2019). Phenol ranged values reported in this work were higher than 12.84 and 30.72mg/100g reported by the same author for some selected legume seeds (Amuda and Okunlola, 2023). Phytates, or phytic acid, are anti-nutritional factors that bind to essential minerals, limiting their bioavailability. The tolerance levels of phytates in pigeon pea forage depend on the species and their ability to produce phytase, the enzyme that hydrolyzes phytates. Ruminants generally have higher tolerance levels owing to microbial phytase activity in the rumen (Boisen & Fernández, 1997). The values obtained in this work cannot affect the nutrients utilisation in ruminants except monogastrics. Oxalates can form insoluble calcium oxalate complexes, reducing calcium availability and leading to urinary calculi in animals. The tolerance levels of oxalates in pigeon pea forage depend on the dietary calcium-to-oxalate ratio. Ruminants can tolerate higher levels of oxalates due to ruminal microorganisms' ability to degrade oxalate (Gupta *et al*., 2017). Monogastric animals are more susceptible to oxalate toxicity, and high oxalate levels in pigeon pea forage should be avoided in their diets (Kotgire *et al.*, 2015). However, the values obtained in this work cannot have negative impacts on calcium absorption and utilisation in ruminants. Pigeon pea forage contains alkaloids such as mimosine and its metabolite, 3,4-dihydroxypyridone (DHP), which can cause a condition called "mimosine toxicity" in ruminants. The tolerance levels of alkaloids in pigeon pea forage vary among animal species. Ruminants possess rumen bacteria capable of detoxifying mimosine and DHP, allowing for higher tolerance levels (Gupta *et al.,* 2016). Ruminants have developed mechanisms to detoxify alkaloids, allowing for their utilization as a nutrient source (Gupta *et al.,* 2016). Although, the ranged values reported in this study were relatively higher than values (16.31 – 20.69mg/100g) as reported (Amuda and Okunlola 2023) for legumes seeds. Tannins are anti-nutritional factors that interfered with the digestive processes either by binding with the enzymes or by binding to feed components such as protein, minerals and form complexes with them and make it indigestible to ruminants. Tannins present in pigeon pea forage have demonstrated several health benefits for humans. They possess antioxidant and anti-inflammatory properties, which contribute to their potential role in preventing chronic diseases (Smeriglio *et al.*, 2016). Tannins also exhibit antimicrobial activity, which can help protect against pathogenic infections (Olaimat and Holley, 2012). In ruminants, tannins can improve feed efficiency and reduce methane emissions (Min *et al.*, 2005). However, the range values of tannin obtained in this study cannot have adverse effects on digestion of nutrients by ruminant animals. Saponins are glycosides found in pigeon pea forage that can disrupt cell membranes and impair nutrient absorption. Saponins also possess cholesterol-lowering properties and can aid in the prevention of cardiovascular diseases (Liu *et al.*, 2019). In ruminants, saponins can improve rumen fermentation and nutrient utilization (Kamra, 2005). The tolerance levels of saponins in pigeon pea forage vary among animal species. Ruminants, particularly those with an adapted rumen microbial population, can tolerate higher levels of saponins due to microbial degradation (Kamra, 2005). Lectins are carbohydrate-binding proteins present in pigeon pea forage that can interfere with digestive enzymes and nutrient absorption). Lectins play a role in gut health by modulating the gut microbiota (Wang *et al.*, 2019). The tolerance levels of lectins in pigeon pea forage are species-specific. Ruminants can tolerate higher levels of lectins due to rumen microbial degradation (D'Mello *et al.,* 1994). However, monogastric animals, including poultry and pigs, may be more sensitive to the negative effects of lectins, leading to reduced feed intake and nutrient utilization (Reddy *et al.,* 2015).

***In vitro* gas fermentation characteristics of *Cajanus cajan* leaves as affected by seasonal change at 24hrs incubation period**.

Presented in Table 5 is the summary of *in vitro* gas fermentation characteristics (a, b, a+b, c, t and Y) of *Cajanus cajan* as affected by seasonal change. The 'a' which indicates fermentation of soluble fraction in this study was similar across the seasons except for the month of February (T_8) which was significantly ($P<0.05$) lowest among the treatments. It enhances microbial attachment in the rumen during fermentation and induces gas production. The ranged values (1.00 – 2.33) were similar to values (0.00 – 3.17) reported for legumes seeds by Amuda and Okunlola (2023). Similarly, significant ($P<0.05$) variation occurred for the extent of gas production 'b' which depicted the fermentation of insoluble but degradable fraction of pigeon foliages collected across the seasons. The highest values (11.67 and 11.33) were obtained forT_1 and T_5 respectively. However, the values observed in this work were very low compared to grass forages which could be due to the presence of secondary metabolites such as saponins and low levels of carbohydrates being a legume fodder. The potential degradability 'a+b' of a diet shows 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. Significant ($P<0.05$) differences occurred at 24hours incubation such that T_1 (July) had the highest value (14.00) whileT_8 (February) had lowest value (7.00). The two values were very low compared to other fodder crops such as sorghum and maize. The reason for low levels of potential extent of gas production might be due to high levels of NDF, ADF coupled with low levels of fermentable carbohydrates (non-fibre carbohydrates (NFC)) in pigeon pea leaves. Getachew *et al*. (1999) reported that gas production is fundamentally a consequence of carbohydrates fermentation into volatile fatty acids (acetate, butyrate and propionate). Menke and Steingass (1988) corroborated the assertion 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 shows the reason why all the values of gas characteristics *Cajanus cajan* foliages were very low. The rate 'c' reflects gas production at time ('t') was significantly highest in T_8 (February) but similar to T_1, T_2, T_3, T_4, T_6 and T_7 respectively. The highest values reported for T_8 and T_1 suggests that the residue would stay longer in the rumen (*in vivo*) if fed to ruminants. The volume of gas 'y' at time" t"is the pinnacle 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 varied across the treatments, it means that seasonal change had effect on *Cajanus cajan* leaves concerning the "c".t and "y''characteristics of the gas. Although, 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*., 2004a) and capacity of the rumen fluid for incubation. It is possible to attain potential gas production of a feedstuff if the donor animal from which rumen liquor for incubation was obtained got the nutrient requirement met. The utilisation of roughages is mainly dependent on microbial degradation therefore, the rate and potential extent of gas production would provide a useful basis for the evaluation of the pigeon pea leaves as potential fodder feed resources for ruminants and other livestock. 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 permits evaluation of substrate and fermentability of soluble and slowly fermentable component of feeds (Getachew *et al*., 1998). Based on the above assumption, consequently, it could be adduced that among the months of collection, of cajanus leaves samples studied, T_8 (July) and T_1 (February) samples would provide minimal proportion of residue that would take up space if utilised in *in vivo* studies and also persists 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 1, 2, 4, 5 and 6 demonstrated that they possess more potentially degradable carbohydrates than T_3, T_7, T_8 and T_9 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. Generally, gas fermentation characteristics of all cajanus leaves collected at different months and seasons were very low due to high NDF levels, low NFC and presence of secondary metabolites.

***In vitro* gas fermentation parameters of *Cajanus cajan* leaves as affected by seasonal change at 24hrs incubation period**.

The *in vitro* gas fermentation parameters of *Cajanus cajan* foliage as affected by seasonal variation at 24hrs are presented in Table 6 and significant ($P<0.05$) differences occurred in all the parameters observed at 24hrs incubation period. The methane (CH_4), total gas volume (TGV), dry matter degradability (DMD %), organic matter digestibility (OMD %), metabolisable energy (ME MJ/KgDM)), short chain fatty acids (SCFA µmol) and fermentation efficiency (FE) varied from 3.00 to 7.50 ml, 7.00 to 14.00, 44.21 to 56.79%, 37.56 to 43.48%, 4.25 to 5.12MJ/KgDM, 0.11 to 0.28µmol and 4.00 to 6.78. Proximate composition is usually the basic and the most common form of feed evaluation by animal nutritionists. A more reliable technique of estimating livestock feed is *in vitro* gas fermentation (Menke and Steingass, 1988). Although the two methods are independent of each other however, they are interrelated. Gas production is an indication of microbial degradability of samples (Babayemi *et al*., 2004b, Fievez *et al*., 2005). This trend of gas production was inversely related to the fibre fractions of forages as forage with higher NDF and ADF content as lower gas production. Monacao *et al*. (2014) had pointed out the structural arrangement of the cell wall components influence the extent of degradation which implies that increase or decrease in gas production depend on chemical composition of the forage. The ranged values (3.00 –7.50ml) of methane (CH_4) gas production indicate that the forage is legume. Legume forage usually contain secondary metabolite such as phenols, tannins and saponins which usually reduce the activities of methanogenic bacterial in the rumen by inhibit them from converting carbon from CO_2 with gaseous H_2 in rumen during fermentation to methane. In most cases, feedstuffs that showed high capacity for gas production were also observed to be synonymous for high methane production. However, 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. The low levels of CH_4 production indicates that energy loss would decline and more energy would be available to ruminants. It is also an indication that the feed is environmental friendly regarding the effect of CH_4 on ozone (O_3) layer depletion and consequential effect on global warming and climate change. Methane (CH_4) and total gas volume (TGV) obtained in this study were very low compared to values reported for some selected legumes seed by Amuda and Okunlola (2023) and some legumes forage by Babayemi (2007). The Dry Matter Degradability (DMD %) of *Cajanus cajan* forages were significantly ($P<0.05$) different across the treatments. Dry matter degradability (DMD %), value of 56.79% obtained for T_5 (November*)* was the highest while the least value of 44.37% was recorded for T_9 (March*)*. The DMD value is a good measure of the amount of dry matter (DM) in the feed that can be degraded by microbes in the rumen of ruminants. The result obtained indicates that T_5 will do better compared to others. Organic Matter Digestibility (OMD %) of legume forage ranged from 37.56 (March) T_9 to 43.48% (July) T_1. The organic matter digestibility (OMD) could be described as a measure of degradability potentials of the microbes on the substrates especially in the presence of sufficient ammonia nitrogen (NH_3-N) which has impact on bacterial fermentation, was highest in T_1. The total gas volume (TGV)) in this study is directly proportional to organic matter digestibility (OMD), Short chain fatty acid (SCFA) and metabolisable energy (ME) as reported by Menke *et al*., (1979). The Metabolisable Energy (ME) ranged between 4.25 and 5. 72 (MJ/kg DM). 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. 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 acids level indicates that the energy is available to the animal and it contributes up to 80% of animal daily energy requirement (Fellner, 2004). The short chain fatty acid (SCFA) mean values were significantly different across the treatments. Furthermore the SCFA mean values of

SCFA ranged from 0.11 to 0.28µml. The short chain fatty acids (SCFA) reported in this work is directly proportional to metabolisable energy (ME) which further buttressed the observation made by Menke *et al*. (1979) and Amuda and Okunlola (2023).

Conclusion

The seasonal change, as observed in this study, had significant (P<0.05) effects on chemical, fibre fractions, minerals and secondary metabolites except ash, organic matter (OM) and ADL of the *Cajanus cajan* foliage samples that were similar across the seasons and months examined. Also percentage dry matter (DM %) composition increased towards dry season period. Similarly, the results of *in vitro* gas assessment characteristics (a, b, a+b, c, t and y) and parameters (CH_4ml, TGVml, DMD%, OMD%, ME MJ/KgDM and SCFA µmol) observed decreased significantly ((P<0.05) as season tends towards dry season except the FE that increased. The increase in fermentation efficiency (FE) implies that the soluble carbohydrates decreased progressively toward dry season which resulted to low gas production. However, all the samples regardless of the season are moderately digestible.

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Table 1: Proximate composition of *Cajanus cajan* (pigeon pea) as affected by seasonal change

TRMT	PRMT (%)								
	DM	CP	Ash	EE	CF	NFE	OM	TCHO	NFC
T_1	91.21^{b}	18.09^{bc}	12.30	1.43^{ab}	24.20^{ab}	43.98^{b}	87.70	68.18^{bc}	0.18^{d}
T_2	91.42^{b}	19.04^{a}	11.08	1.89^{a}	22.47^{bcd}	45.53^{ab}	88.92	67.99^{bc}	2.73^{d}
T_3	$91,12^{b}$	17.60^{c}	11.60	1.67^{ab}	21.57^{d}	47.57^{a}	88.40	69.14^{ab}	0.60^{d}
T_4	90.83^{b}	17.80^{c}	12.47	1.81^{ab}	22.20^{cd}	45.73^{ab}	87.53	67.93^{bc}	1.36^{d}
T_5	92.90^{a}	18.90^{ab}	11.17	1.82^{ab}	22.31^{cd}	45.81^{ab}	88.83	68.11^{bc}	8.04^{c}
T_6	92.75^{a}	17.12^{c}	11.40	1.29^{b}	24.83^{a}	43.36^{ab}	88.60	70.19^{a}	10.79^{ab}
T_7	92.80^{a}	17.26^{b}	12.68	1.50^{ab}	22.31^{cd}	49.25^{b}	87.32	68.56^{abc}	11.96^{a}
T_8	92.94^{a}	19.44^{a}	11.83	1.89^{a}	22.53^{bcd}	46.31^{ab}	88.17	66.84^{c}	8.84^{bc}
T_9	92.77^{a}	17.84^{c}	12.03	1.98^{a}	23.61^{abc}	44.53^{b}	87.97	68.14^{bc}	11.06^{a}
SEM	0.21	0.17	0.40	0.10	0.31	0.40	0.40	0.36	0.49

a,b,c,d = Value with different superscripts in a column differ significantly (P<0.05).

T1 = July, T2 = August, T3 = September, T4 = October, T5 = November, T6 = December, T7 = January, T8 = February and T9 = March.

CP = Crude protein, EE = Ether Extract, CF = Crude Fibre, DM = Dry Matter, NFE = Nitrogen Free Extract, OM = Organic Matter, TCHO =Total Carbohydrates and NFC = Non-Fibre Carbohydrates, TRMTS = Treatments and PRMT = Parameters.

Table 4.2: Fibre fractions of *Cajanus cajan* (pigeon pea) as affected by seasonal change

Treatments	Parameters				
	NDF	ADF	ADL	HEMICELL	CELL
T1	68.00^{a}	41.43^{a}	17.17	26.57^{ab}	24.27^{a}
T2	65.27^{ab}	41.34^{a}	16.90	23.93^{abc}	24.44^{a}
T3	68.53^{a}	39.73^{ab}	17.37	28.80^{a}	22.37^{abc}
T4	66.57^{ab}	40.83^{a}	17.10	25.73^{abc}	23.73^{ab}
T5	60.07^{c}	38.20^{abc}	16.30	21.87^{bcd}	21.90^{abc}
T6	59.40^{cd}	36.47^{bc}	17.23	22.93^{bcd}	19.23^{bc}
T7	56.60^{e}	35.87^{c}	17.62	20.73^{cd}	18.25^{c}
T8	58.00^{cde}	36.14^{c}	18.10	21.86^{bcd}	18.04^{c}
T9	57.08^{de}	37.27^{bc}	17.93	19.82^{d}	19.33^{bc}
SEM	0.49	0.63	0.38	0.93	0.82

a,b,c,d = Value with different superscripts in a column differ significantly (P<0.05).

T_1 = July, T_2 = August, T_3 = September, T_4 = October, T_5 = November, T_6 = December, T_7 = January, T_8 = February and T_9 = March

NDF = Neutral Detergent Fibre, ADF =Acid Detergent Fibre, ADL=Acid Detergent Lignin and HEMICELL= Hemicellulose.

Table 3: Effect of seasonal change on mineral composition of pigeon pea (*Cajanus cajan*)

TRTS	PRTS								
	Ca (%)	P (%)	Mg (mg/Kg)	K (%)	Na (%)	Cu (mg/Kg)	Mn (mg/Kg)	Zn (mg/Kg)	Fe (mg/Kg)
T_1	0.19e	0.18a	0.30b	0.64a	0.68b	7.87b	67.76a	15.53c	601.45b
T_2	0.17f	0.15c	0.19d	0.49b	0.50c	7.04d	43.90f	14.70c	311.24e
T_3	0.14g	0.14d	0.16g	0.22c	0.31d	6.24h	29.13h	13.53d	254.16f
T_4	0.28c	0.12e	0.21c	0.19d	0.21g	6.41g	36.50g	15.17c	396.99d
T_5	0.18e	0.15c	0.18ef	0.21c	0.27e	7.19c	47.67e	17.13b	367.27d
T_6	0.31b	0.11f	0.19d	0.21c	0.24f	6.44f	48.28e	18.03b	368.35d
T_7	0.26d	0.16b	0.17f	0.11e	0.21g	6.76e	58.85c	23.07a	320.25e
T_8	0.28c	0.11f	0.18ef	0.11e	0.13h	7.02d	52.22d	15.34c	450.30c
T_9	0.42a	0.09g	0.44a	0.64a	0.78a	8.63a	62.66b	17.45b	662.31a
SEM	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.22	2.20

a, b, c,d,e,f,g,h = Means on the same row with different superscripts differ significantly (P < 0.05

T_1 = July, T_2 = August, T_3 = September, T_4 = October, T_5 = November, T_6 = December, T_7 = January, T_8 = February and T_9 = March

Table 4: Effect of seasonal change on Anti-nutritional Factors (mg/100g) of Pigeon pea (*Cajanus cajan*)

TRTS	PRTS(mg/100g)						
	Phenols	Phytates	Oxalates	Alkaloids	Tannins	Saponins	Lectins
T_1	266.02^{b}	79.93^{b}	45.16^{b}	16.80^{e}	69.03^{b}	29.12^{b}	54.18^{b}
T_2	271.08^{a}	83.02^{a}	46.91^{a}	17.75^{cde}	74.10^{a}	31.26^{a}	59.25^{a}
T_3	251.96^{c}	72.63^{d}	41.03^{c}	16.31^{e}	67.07^{b}	28.29^{b}	52.22^{b}
T_4	246.08^{f}	68.74^{f}	40.51^{c}	17.35^{de}	58.39^{de}	24.84^{de}	44.40^{d}
T_5	251.37^{d}	65.98^{g}	41.50^{c}	20.15^{ab}	52.57^{f}	22.65^{f}	40.28^{e}
T_6	234.93^{h}	52.83^{h}	33.22^{d}	18.38^{cd}	48.62^{g}	20.96^{g}	36.33^{f}
T_7	249.36^{e}	66.98^{g}	42.12^{c}	18.51^{cd}	59.43^{d}	25.62^{d}	47.14^{c}
T_8	266.71^{b}	77.00^{c}	48.43^{a}	20.69^{a}	61.44^{c}	26.48^{c}	49.15^{c}
T_9	236.09^{g}	70.77^{c}	44.51^{b}	19.09^{bc}	56.68^{e}	24.43^{d}	44.39^{d}
SEM	0.19	0.24	0.31	0.27	0.39	0.40	0.40

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

T_1 = July, T_2 = August, T_3 = September, T_4 = October, T_5 = November, T_6 = December, T_7 = January, T_8 = February and T_9 = March.

Table 5: *In vitro* gas characteristics of *Cajanus cajan* (Pigeon pea) as affected by seasonal change

TRTS	PRTS					
	a (ml^3)	b (ml^3)	a+b (ml^3)	c (mlh^{-1})	t (hr)	Y(l/h^{-3})
T_1	2.33^{a}	11.67^{a}	14.00^{a}	0.081^{ab}	13.00^{ab}	9.33^{a}
T_2	2.33^{a}	10.00^{ab}	12.33^{ab}	0.058^{abc}	11.00^{ab}	7.00^{abc}
T_3	2.00^{ab}	8.00^{bc}	10.00^{bc}	0.052^{abc}	11.00^{ab}	5.33^{bcde}
T_4	2.33^{a}	9.67^{ab}	12.00^{ab}	0.056^{abc}	9.00^{ab}	6.00^{bcd}
T_5	1.33^{ab}	11.33^{a}	12.67^{ab}	0.046^{bc}	10.00^{ab}	5.67^{bcde}
T_6	1.33^{ab}	10.00^{ab}	11.33^{ab}	0.074^{abc}	14.00^{a}	7.67^{ab}
T_7	1.67^{ab}	6.00^{c}	7.67^{cd}	0.064^{abc}	13.00^{ab}	5.00^{cde}
T_8	1.00^{b}	6.00^{bc}	7.00^{d}	0.089^{a}	9.00^{ab}	4.00^{de}
T_9	1.67^{ab}	6.00^{c}	7.67^{cd}	0.041^{c}	8.00^{b}	3.33^{e}
SEM	0.20	0.50	0.52	0.00	0.90	0.45

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

T_1 = July, T_2 = August, T_3 = September, T_4 = October, T_5 = November, T_6 = December, T_7 = January, T_8 = February and T_9 = March. a = zero time which idealy reflects the fermentation of 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,Y = volume of gas produce at time (t).

Table 6: *In vitro* fermentation parameters of *Cajanus cajan* as affected by season at 24hrs incubation period

Treatments	Parameters (%)						
	CH4 (ml)	TGV (ml)	DMD (%)	OMD (%)	ME (MJ/KgDM)	SCFA (µmol)	FE
T_1	7.50^a	14.00^a	55.10^{ab}	43.48^a	5.12^a	0.28^a	4.00^{cd}
T_2	5.00^{bc}	12.33^{ab}	51.99^{bc}	41.63^{ab}	4.95^{ab}	0.24^{ab}	4.22^d
T_3	4.50^c	10.00^{bc}	49.80^{cd}	39.24^{bc}	4.56^{cd}	0.18^{bc}	5.01^{bcd}
T_4	5.50^b	12.00^{ab}	52.67^{bc}	41.68^{ab}	4.84^{abc}	0.23^{ab}	4.48^{cd}
T_5	4.50^c	12.67^{ab}	56.79^a	41.95^{ab}	4.99^{ab}	0.24^{ab}	4.60^{cd}
T_6	5.50^b	11.33^{ab}	52.56^{bc}	40.08^{bc}	4.72^{bc}	0.21^{ab}	4.71^{cd}
T_7	3.00^d	7.67^{cd}	48.40^d	37.72^c	4.25^d	0.12^{cd}	6.78^a
T8	3.00^d	7.00^d	46.92^{de}	37.56^c	4.28^d	0.11^d	6.33^{ab}
T_9	3.50^d	7.67^{cd}	44.37^e	37.56^c	4.28^d	0.12^{cd}	5.99^{abc}
SEM	0.14	0.52	0.62	0.49	0.07	0.01	0.28

a,b,c,d = Value with different superscripts in a column differ significantly ($P<0.05$).

T_1 = July, T_2 = August, T_3 = September, T_4 = October, T_5 = November, T_6 = December, T_7 = January, T_8 = February and T_9 = March.

CH_4 = Methane, TVG = Total Gas Volume, DMD = Dry Matter Degradability OMD = Organic Matter Digestibility,, ME = Metabolisable Energy, SCFA = Short Fatty Acid,.

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