Effects of fertilizer type on protein and carbohydrate fractions of two varieties of *Arachis hypogaea* fodder

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ABSTRACT

The fractionation of protein and carbohydrate of two varieties of *A. hypogaea* affected by fertilizer type was investigated. The crude protein (CP) of the *A. hypogaea* varieties was subdivided into five fractions; A1 (crude protein soluble in the borate-phosphate buffer and tungstic acid solution), B1 (true protein soluble in buffer solution and precipitated by the tungstic solution), B2 (true protein insoluble in buffer solution but soluble in the neutral-detergent solution), B3 (true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution), and C (true protein insoluble in the acid-detergent solution). While the carbohydrate (CHO) was fractionalized into four; CA (rapidly degradable CHO), CB1 (intermediately degradable CHO), CB2 (slowly degradable CHO), and CC (completely undegradable NDF). The experiment was a 3 × 2 factorial in split-plot design, with the fertilizer type as the main plot (control, NPK, and poultry droppings) and the variety as the sub-plot (SAMNUT 22 and Local). The total treatment combination is six with three replications. The fertilizer type affected all the CP fractions except for fraction C. The interaction of fertilizer type × variety influenced all the CP fractions with a range of 65.40 ± 0.21 to 80.97 ± 0.01 g/kg DM, 5.01 ± 0.01 to 5.69 ± 0.03 g/kg DM, 42.77 ± 0.08 to 51.40 ± 0.01 g/kg DM, and 58.34 ± 0.14 to 69.93 ± 0.01 g/kg DM for CP fraction A, B1, B2, and B3, respectively with the organic fertilized local variety having the highest values. SAMNUT 22 without fertilizer recorded the highest value for CP fraction C. Varietal difference was observed for all the CP fractions. The CHO fractions were affected ($P < 0.05$) by the fertilizer type and varietal difference. The unfertilized SAMNUT 22 was observed to have the highest values for CHO fraction CA, CB1, and CC, whereas the same variety to which inorganic fertilizer was applied recorded the highest value for CB2. The organic fertilized local variety of *A. hypogaea* had a higher portion of the soluble fraction of CP, and a lower portion of cell wall bounded CP fraction making it ruminally beneficial if fed to ruminants which also reflected in the utilizable CP.

Keywords: Energy, fertilization, fiber, legumes, poultry droppings

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INTRODUCTION

The quality of forage crops generally has been adjudged to be influenced by some factors, such as the stage of maturity (Dele *et al*., 2022), species or variety, pre- and post-harvest and storage, available soil nutrient (Cherney and Hall, 2008) and fertilization (Ball *et al*., 2001). In the tropics, low soil fertility has been a major cause of concern over the decades (Sanchez, 2002; 2015; Pradhan *et al*., 2015). The utilization of forage legumes in the tropics with regard to ruminant livestock productivity and sustainability has increased in recent times (Murphy and Colucci, 1999; Dele *et al*., 2019). Low productivity is common in animals that consume lower quality forages which are typical of tropical grasses, especially at an advanced stage of growth (Paterson *et al*., 1996; Hariadi and Santoso, 2010; Pérez-Gil Romo *et al*., 2014) and Mahmoud *et al*. (2017) and Chimphango *et al.* (2020) reiterated the role of legumes to provide forages that are moderately fibrous and have high crude protein.

Legumes are known for nitrogen fixation which associative grasses benefit from but in the last two decades, there have been reports on the need for nitrogen fertilizer application for legumes on West African soil to boost their productivity (Bationo and Ntare, 2000; Abayomi *et al*., 2008; Dugje *et al*., 2009; Abdul Rahman *et al*., 2018). Nitrogen fertilization was reported by Griffith (1974) to have an improvement on yield or quality in established legume stands, especially because of its increment of protein content, invariably the non-application of fertilizer can affect both biomass yield and nutritional quality.

Protein fractionation is basically about the rate of degradation of protein which accounts for the difference between availability and utilization (Lanzas *et al*., 2007), and Fox *et al*. (2004) reported that animal products such as milk reduce when protein in the diet below energy allowable for milk production which is based on protein degradation. Protein and carbohydrate degradation if synchronized, have the potential to increase microbial protein production (Nocek and Russell, 1988), which is essential for greater efficiencies and ruminant productivity.

However, there are limited studies reported on the forage quality of legumes, especially groundnut, in terms of protein and carbohydrate fractions which is critical to ruminant livestock farmers as there is the need to improve the energy and protein usage of legume-based rations in livestock farming systems. The utilization of groundnut fodder has been reported to be a valuable feed resource with a higher premium when compared with other legumes (Samireddypalle *et al.,* 2017; Adda *et al.*, 2021), and groundnut is considered a multipurpose crop (Nigam and Blümmel, 2010; Prasad *et al.,* 2010; Blümmel *et al.,* 2012). Different varieties of groundnut have been considered for usage in previous studies (Larbi *et al.*, 1999; Konlan *et al*., 2012). Higher fodder production has been attributed to SAMNUT 22 (Oteng-Frimpong *et al.,* 2017; Ansah *et al.,* 2021) in recent times, which is a trait for a good forage resource (Dele *et al.*, 2019). The objective of this study was to investigate the effects of organic and inorganic N fertilizer types on the protein and carbohydrate fractions of two groundnut varieties in humid zones of Southwest Nigeria.

MATERIALS AND METHODS

Experiment Sites

The study was investigated at the Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta (FUNAAB). This area lies at 7°13’55° North and 3°26’11° East, at an elevation of 146 m above sea level, and the NIRS screening was in the laboratory of the International Livestock Research Institutes, Ibadan, Nigeria. This area lies at 7°30’08° North and 3°54’37° East, at an elevation of 237 m above sea level. The experimental site, rainfall lies within the savanna agro-ecological zone of Southwestern Nigeria, as reported by Dele *et al*. (2019).

Land Preparation

The experimental land was cleared, followed by plowing and harrowing after two weeks. The experimental area of 1,050 m^2 was mapped out. Before planting, soil samples were collected randomly from the plots at 0–15 cm depth with the aid of a soil auger to represent the topsoil. The soil samples were bulked and thoroughly mixed with subsamples taken for analysis to determine the pre-planting nutrient status of the soil (Dele *et al.*, 2019).

Experimental Design and Plot Management

The study was laid a 3 × 2 factorial arrangement in a split-plot design. The main plot dimension was 12 m × 5 m while that of the subplot was 5 m × 5 m. The fertilizer types were allotted to the main plot and the groundnut varieties to the subplot. The three fertilizer types (NPK 15:15:15 (compound) + NPK 46:0:0 urea (single), poultry (layer battery cage) manure with 30:10:10 g/kg of NPK, respectively) and unfertilized (control) and the two varieties of groundnut (SAMNUT 22 and Local) amounted to six treatment combinations with three replicates. The fodders of the groundnut varieties were harvested at 16 weeks after planting, and to determine the dry matter content, a 1 m^2 quadrat was used, which was thrown three times within a subplot at 5 cm aboveground level. Sub-samples were taken, weighed, and oven-dried at 65 °C to constant weight and stored for analysis.

Crude Protein (CP) Analysis

The oven-dried samples were milled and allowed to pass through a 1 mm screen and stored in an airtight bag for subsequent analysis at the International Livestock Research Institute, Ibadan. Five grams of each of the samples were scanned with NIRS and the crude protein content of the samples was assessed using the equation for the plant sample analysis based on the mixed feed global calibration model using the software package (Win ISI II FOSS, Denmark Model NIRS™ 5000). Spectral information was registered in the wavelength range 1,100–2,500 nm using NIR system mode 5,000 scanning monochromatic infrared spectrophotometer.

Protein Fractionation

Crude protein fractions of the samples were estimated following the procedure of Sniffen *et al*. (1992) modified by Licitra *et al*. (1996). A is the crude protein soluble in the borate-phosphate buffer and tungstic acid solution, B1 is the true protein soluble in buffer solution and precipitated by the tungstic solution, B2 is the true protein insoluble in buffer solution but soluble in the neutral-detergent solution, B3 is the true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution, and C is the true protein insoluble in the acid-detergent solution.

Carbohydrate Fractionation

Carbohydrate fractions of the samples were estimated following Cornell Net Carbohydrate and Protein (CNCP) system (Sniffen *et al*., 1992). CA is the rapidly degradable CHO composed mainly of starch, CB1 is the intermediately degradable CHO composed mainly of soluble fibers such as pectic polysaccharides, ß-glucans, and fructans, CB2 is the slowly degradable CHO composed of available NDF, and CC is the completely undegradable NDF.

Water Soluble Carbohydrate (WSC) Determination

WSC was determined according to the procedure of Hall *et al*. (1999) with some modifications. Five grams of the dried and milled samples were added to 100 mL and vortexed for 5 minutes. 5 mL of 0.1N of H_2SO_4 was added to the mixture. The acidified mixture was boiled and filtered through a Whatman No. 1 filter paper to aid the removal of proteins, and the residue was washed with distilled water. The filtrate was made to 250 mL volume. Of the filtrate, 10 mL was hydrolyzed with 5 mL of 2N H_2SO_4 and boiled in a water bath for 10 minutes. The boiled acidified filtrate was allowed to cool for 5 minutes, then neutralized with methyl red and NaOH and made up to 50 mL. Aliquots of 10 mL were analyzed using the ferricyanide method.

Utilizable Crude Protein (uCP) Estimation

The uCP was estimated according to Zhao and Cao (2004).

$$uCP = (9.95 \pm 2.73)A + (2.92 \pm 1.36)B1 + (7.24 \pm 0.86)B2 + (8.20 \pm 3.33)B3 + (17.67 \pm 3.79)C + (63.26 \pm 18.02)$$

Statistical Analysis

The data generated were subjected to Analysis of Variance using SAS (2000) statistical software and differences in means separated using turkey HSD Test at $P < 0.05$ level of probability.

RESULTS AND DISCUSSION

The crude protein content was affected ($P < 0.05$) by the fertilizer type and varietal difference (Figure 1). The local variety had a higher CP (209.21 ± 7.49 g/kg DM), and the groundnut fertilized with the inorganic fertilizer had the highest CP value. Figure 2 shows the result of the interaction of fertilizer type × variety on the CP, the highest CP recorded for the inorganic fertilized local variety (217.10 ± 13.5 g/kg DM).

The varietal difference on the CP fraction A was significant ($P < 0.05$), with the CP fraction A of the local variety 6.68% higher than that of SAMNUT 22. The effect of fertilizer type showed that the unfertilized fodder had non-protein nitrogen (NPN) component of 35.94% of the total protein, whereas the organic and inorganic fertilized fodder were made of 36.21% and 35.46% of total protein, respectively. The local variety fertilized organically had the highest NPN component of 36.95% of total protein, and unfertilized SAMNUT 22 had the least NPN component (Table 1).

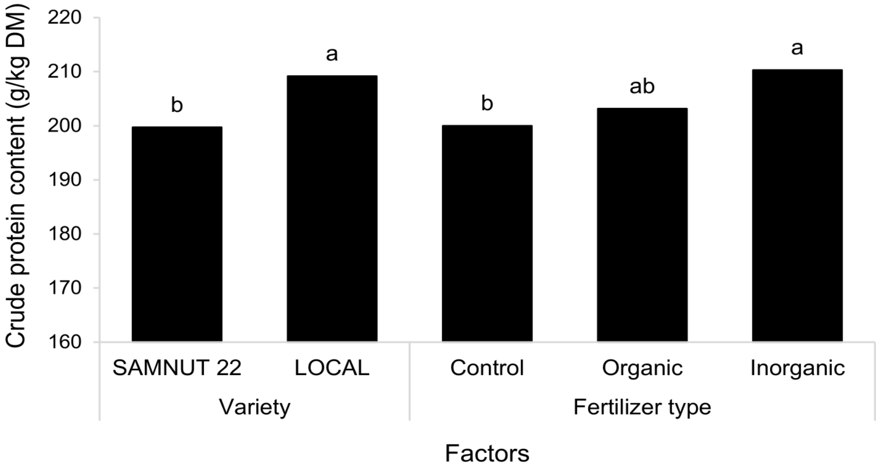

Figure 1 Main effect of fertilizer type on the crude protein content of two *Arachis hypogaea* herbage

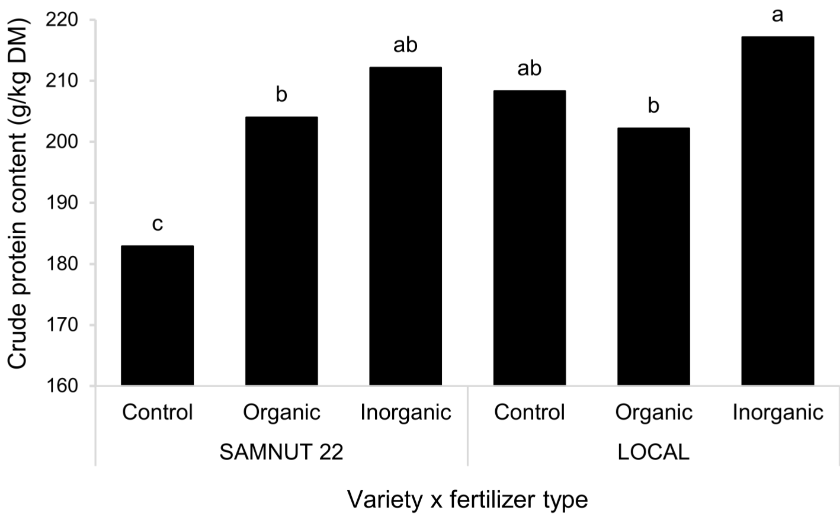

Figure 2 Interaction effect of fertilizer type and variety on the crude protein content of *A. hypogaea* herbage

The fraction A is known to be involved in the transportation and storage of N (Gierus *et al.*, 2006). The increase in the fraction A content as observed in this study with fertilizer application, indicated that an external source of additional nitrogen can help in N fixation. The higher content of fraction A for the fertilized *A. hypogeae* may negatively influence N use efficiency by ruminants (Gierus *et al.,* 2006). The fraction A is the non-protein nitrogen, soluble with a rapid ruminal degradable portion of CP, and its higher presence in fertilized legumes is also favored by the N applied, which supports the development of legumes, therefore, improving the degradation rate. The higher fraction A in this study is in line with the report of Gierus *et al.* (2016), where nitrogen application favored higher fraction A. The fraction A recorded in this study is within the range (24.4–75.4 g/kg DM) reported by Salazar-Cubillas and Dickhoefer (2021) for tropical forage legumes, except for the organically fertilized legume, which was higher than the range. The interaction of variety × fertilizer type showed that organic fertilized local variety and inorganic fertilized SAMNUT 22 were higher than the maximum fraction A reported by Salazar-Cubillas and Dickhoefer (2021) for tropical forage legumes which could be attributed to the different responses of the two varieties to the two fertilizer types different. Previous research (Johnson *et al.*, 2001; McRoberts *et al.,* 2017; Santos *et al.,* 2018) reported that the addition of N does increase nitrate accumulation which is a portion of fraction A in plants, and which might be the reason for higher fraction A with the fertilized forages. The values of fraction of CP fraction A in this study were slightly higher than that reported by Salazar-Cubillas and Dickhoefer (2021) for *A. pintoi* in the dry season, especially the fertilized *A. hypogeae*. The difference might be due to the season of the current study, which is the rainy season.

Table 1 Effects of fertilizer type on the protein fractions (g/kg DM) of two *A. hypogaea* herbage

Factors		**Protein fractions**				
Variety	**Fertilizer type**	**A**	**B1**	**B2**	**B3**	**C**
SAMNUT 22		71.60 ± 1.25^{b}	5.34 ± 0.07^{b}	47.65 ± 0.79^{b}	64.79 ± 1.28^{b}	12.25 ± 0.09^{a}
Local		76.38 ± 0.60^{a}	5.53 ± 0.03^{a}	49.55 ± 0.21^{a}	67.83 ± 0.63^{a}	11.64 ± 0.17^{b}
	Control	69.64 ± 1.82^{b}	5.20 ± 0.09^{b}	45.10 ± 1.01^{b}	61.69 ± 1.65^{b}	12.13 ± 0.20
	Organic	76.90 ± 1.15^{a}	5.56 ± 0.06^{a}	50.11 ± 0.27^{a}	68.11 ± 1.16^{a}	11.70 ± 0.19
	Inorganic	75.42 ± 0.78^{a}	5.56 ± 0.03^{a}	50.60 ± 0.33^{a}	69.13 ± 0.51^{a}	12.01 ± 0.12
SAMNUT 22	Control	65.40 ± 0.21^{d}	5.01 ± 0.01^{d}	42.77 ± 0.08^{e}	58.34 ± 0.14^{d}	12.64 ± 0.02^{a}
	Organic	72.83 ± 2.29^{c}	5.43 ± 0.35^{bc}	48.83 ± 0.53^{c}	66.29 ± 2.34^{bc}	12.28 ± 0.57^{ab}
	Inorganic	76.56 ± 0.91^{b}	5.59 ± 0.14^{ab}	51.36 ± 0.53^{a}	69.74 ± 0.95^{a}	11.84 ± 0.02^{bc}
Local	Control	73.88 ± 0.25^{bc}	5.39 ± 0.07^{c}	47.42 ± 0.08^{d}	65.05 ± 0.83^{c}	11.63 ± 0.35^{cd}
	Organic	80.97 ± 0.01^{a}	5.69 ± 0.03^{a}	51.40 ± 0.01^{a}	69.93 ± 0.01^{a}	11.12 ± 0.01^{d}
	Inorganic	74.28 ± 1.20^{bc}	5.52 ± 0.13^{abc}	49.84 ± 0.10^{b}	68.52 ± 0.33^{b}	12.18 ± 0.24^{abc}

Note: ^{a,b,c,d} Means on the same column with different superscripts are significantly different at $P < 0.05$. A = crude protein soluble in the borate-phosphate buffer and tungstic acid solution, B1 = true protein soluble in buffer solution and precipitated by the tungstic solution, B2 = true protein insoluble in buffer solution but soluble in the neutral-detergent solution, B3 = true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution, C = true protein insoluble in the acid-detergent solution.

The effect of variety on the CP fraction B1 was significant ($P < 0.05$). The local variety had higher B1 than SAMNUT 22. The fertilized fodder had a higher fraction B1 compared with the unfertilized fodder, which was 6.92% higher than the unfertilized fodder. The organically fertilized local variety had the highest CP fraction B1, and the unfertilized SAMNUT 22 recorded the least fraction B1 value. For CP fraction B2, a similar trend as observed in CP fraction B1 was observed. The local variety had 4.69% more ($P < 0.05$) CP fraction B3 than the SAMNUT 22 variety. The organic and inorganic fertilized fodder had higher fraction B3 by 10.41% and 12.06%, respectively, than the unfertilized fodder. The variety × fertilizer type interaction ($P < 0.05$) was observed and followed a similar pattern with CP fraction B1 and B2 (Table 1).

The fraction B1 recorded in this study for the unfertilized SAMNUT 22 was similar to that reported by Salazar-Cubillas and Dickhoefer (2021) for *Mucuna* spp. but the unfertilized both SAMNUT 22 and local varieties have lower values compared with the fertilized forages, which implied that N fertilizer promotes a higher fraction B1. The higher fractions B1 suggested that the additional N uptake by the fertilized groundnut was converted into protein. This was affirmed by Gierus *et al.* (2016) that moderately N fertilization of forage legumes does support the quality of CP. The fraction B1 in this study fell within the range (0.2–17.2 g/kg DM) reported by Salazar-Cubillas and Dickhoefer (2021) for tropical forage legumes.

The fraction B2 in this study fell within the range (24.2–95.7 g/kg DM) reported by Salazar-Cubillas and Dickhoefer (2021) for tropical forage legumes. The fraction B2 is known for its intermediate degradation rate in the rumen (Sniffen *et al.,* 1992). The lower fraction B2 in SAMNUT 22 corresponded with higher fiber composition (Dele *et al.*, 2019), implying that the local variety has a higher intermediate soluble portion. The higher fraction B2 of the fertilized is a reflection of N fertilizer favoring higher cell content against the cell wall translating to higher intermediate soluble protein content.

The fraction B3 portion of CP is known its a very slow degradation rate and is associated with the cell wall of the plant with a slowly available portion and another unavailable (Sniffen *et al.*, 1992). The fraction B3 with fertilization was higher compared with the unfertilized forages. The increase in fraction B3 in this study confirms the report of Cuomo and Anderson (1996) that ruminally undegraded protein increase with N fertilizer application. The N fertilizer application (organic and inorganic) increased the fraction B3 compared with the unfertilized counterpart. The result of this current study was affirmed by the report of Tran *et al.* (2009). This increase might be due to N fertilizer application which is known to be the precursor to cell multiplication (growth) thereby increasing the cell content (digestible portion), which leads to increasing the soluble protein portion of the plant. The fraction B3 in this study fell within the range (6.2–69.9 g/kg DM) reported by Salazar-Cubillas and Dickhoefer (2021) for tropical forage legumes.

SAMNUT 22 had a higher ($P < 0.05$) CP fraction C than the local variety. The CP fraction C was not significantly ($P > 0.05$) affected by the fertilizer type. Though the values were statistically similar, they were numerically different, with the fertilized fodders having lower values, the unfertilized SAMNUT 22 had the highest fraction C value while the local variety fertilized with organic fertilizer had the least CP fraction C value (Table 1).

Protein fraction C which is unavailable to the animals was significantly different between the varieties, which is why the values for SAMNUT 22 were higher, which means that the SAMNUT 22 had a higher actin-depolymerizing factor (ADF) content (Dele *et al.,* 2019; Stojanović *et al.,* 2020). The fertilizer type had no effect on the fraction C of the groundnut fodder and was similar to that reported by Rogers *et al*. (1996) that N fertilizer had no effect on acid detergent insoluble nitrogen (ADIN). The interaction of variety × fertilizer type showed that the unfertilized SAMNUT 22 had the highest ($P < 0.05$) fraction C value. This confirms the reports of Johnson *et al.* (2001) and Berça *et al.* (2021) that fraction C decrease with N fertilizer application. The slight variation might be due to higher cell wall content that is associated with unfertilized forages compared with fertilized counterparts (Dele *et al.,* 2019), as cell content is usually higher in fertilized forages, and the decrease of ADIN with N fertilization might be attributed to the difference in nutrient metabolism. The fraction C in this study fell within the range (9.6–47.0 g/kg DM) reported by Salazar-Cubillas and Dickhoefer (2021) for tropical forage legumes.

The content of different carbohydrate fractions of two varieties of *A. hypogaea* fodder as affected by fertilizer type are presented in Table 2. The CA fraction of carbohydrate was significantly ($P < 0.05$) affected by the varietal difference, with SAMNUT 22 having a higher CA fraction by 13.80% than the local variety. The fertilizer type effect showed that the unfertilized fodder had the highest ($P < 0.05$) CA fraction though statistically similar to the fodder fertilized with inorganic fertilizer. The variety × fertilizer type interaction was significant ($P < 0.05$) on the CA fraction of carbohydrate, which ranged from 75.20 to 99.24 g/kg, with unfertilized SAMNUT 22 having the highest value.

The higher fraction CA recorded for SAMNUT 22 is an indication of a higher rapidly degradable fraction of soluble sugar, a higher intermediate degradable fraction CB1, which consists of pectin and starch, and a lower fraction CB2 is an indication of a less slowly degradable fiber fraction and higher fraction CC which is the undegradable cell wall portion. This higher soluble portion of SAMNUT 22 might imply that a relatively high utilization efficiency of SAMNUT 22 is possible when fed to animals. Yu *et al.* (2003) reported that higher soluble fiber results in high efficiency of utilization in animals. The influence of fertilizer type on the fraction CA between the unfertilized and inorganic fertilized fodder was not significant but was significantly different from the organic fertilized fodder. The higher fraction CA in this study for the unfertilized fodder was similar to that reported by Hernández *et al.* (2020).

Table 2 Effects of fertilizer type on the carbohydrate fractions (g/kg DM) of two *A. hypogaea* herbage

Factors		**Carbohydrate fractions**			
Variety	**Fertilizer type**	**CA**	**CB1**	**CB2**	**CC**
SAMNUT 22		94.11 ± 3.50^{a}	41.17 ± 0.48^{a}	436.80 ± 1.48^{b}	75.16 ± 1.02^{a}
Local		81.12 ± 2.42^{b}	40.16 ± 0.45^{b}	440.09 ± 1.08^{a}	68.31 ± 1.55^{b}
	Control	90.26 ± 3.76^{a}	39.87 ± 0.39^{b}	445.64 ± 1.03^{a}	74.60 ± 2.21^{a}
	Organic	82.74 ± 5.14^{b}	39.83 ± 0.71^{b}	435.15 ± 1.54^{b}	68.85 ± 1.74^{b}
	Inorganic	89.84 ± 2.96^{a}	42.30 ± 0.38^{a}	434.55 ± 1.52^{b}	71.76 ± 1.13^{ab}
SAMNUT 22	Control	99.24 ± 0.75^{a}	38.60 ± 0.20^{c}	444.98 ± 1.91^{a}	80.52 ± 0.22^{a}
	Organic	90.27 ± 1.06^{abc}	41.99 ± 0.91^{ab}	432.60 ± 1.74^{c}	74.99 ± 1.47^{b}
	Inorganic	92.82 ± 1.60^{ab}	42.91 ± 0.02^{a}	432.82 ± 0.85^{bc}	69.96 ± 0.03^{bc}
Local	Control	81.28 ± 2.09^{bc}	41.13 ± 0.42^{b}	446.29 ± 0.12^{a}	68.68 ± 3.29^{c}
	Organic	75.20 ± 0.04^{c}	37.67 ± 0.01^{c}	437.70 ± 0.05^{b}	62.70 ± 0.02^{d}
	Inorganic	86.87 ± 5.69^{abc}	41.68 ± 0.71^{ab}	436.28 ± 2.89^{bc}	73.56 ± 2.13^{bc}

Note: [a,b,c] Means on the same column with different superscripts are significantly different at $P < 0.05$. CA = rapidly degradable carbohydrate composed mainly of starch, CB1 = intermediately degradable carbohydrate composed mainly of soluble fibers such as pectic polysaccharides, ß-glucans, and fructans, CB2 = slowly degradable carbohydrate composed of available neutral detergent fiber (NDF), CC = completely undegradable NDF.

The CB1 fraction of carbohydrate was affected (P < 0.05) by the variety, with SAMNUT 22 having a higher value than the local variety. The CB1 fraction was influenced by the fertilizer type, the inorganic fertilized fodder had the highest value, approximately 6.20% higher than that of the organic fertilized fodder. The interaction effect of variety × fertilizer type on the CB1 fraction of carbohydrate was significant (P < 0.05). The inorganic fertilized SAMNUT 22 recorded the highest CB1 value 42.91 g/kg, which was 13.91% higher than that of the CB1 of the organic fertilized local variety that recorded the least CB1 value. The CB2 fraction value of the local variety was higher (P < 0.05) than that of SAMNUT 22 fodder. The fertilizer type influenced the CB2 significantly, with the values of unfertilized fodder greater than the fertilized fodders. The CB2 fraction, as influenced by the interaction of variety and fertilizer type, was observed to be significant, with the unfertilized local variety having the highest value though not statistically different from the unfertilized SAMNUT 22 and organic fertilized SAMNUT 22 having the least CB2 value. The varietal effect on the CC fraction of carbohydrate was significant (P < 0.05), with the SAMNUT 22 value higher than that of the local variety by 9.11%. The effect of fertilizer type was also significant on the CC fraction, but the fertilized fodders were reduced by 7.71% and 3.81% for organic and inorganic fertilized fodders, respectively. The two-way interaction (variety × fertilizer type) indicated that unfertilized SAMNUT 22 had the highest CC fraction of carbohydrate while the organic fertilized local variety recorded the least CC fraction value (Table 2). The higher CB1 with the inorganic fertilized forages in this study is in line with the report of Neumann *et al.* (2017). The fraction CC is adjudged to be the non-degradable portion of the neutral detergent fiber (Sniffen *et al*., 1992). The higher fraction CC of the unfertilized forage is an indication of lesser energy value because of the higher structural indigestible portion (Jung and Allen, 1995). The higher fraction CC of the unfertilized in this study is in line with the report of Hernández *et al*. (2020).

The varietal difference reflected (P < 0.05) on the WSC, WSSC:CP ratio, and uCP. Higher values of WSC and WSC:CP ratio were observed for the SAMNUT 22 whereas the local variety had higher uCP values. The WSC, as influenced by fertilizer type, was significant (P < 0.05), with the inorganic fertilized forage having the highest value. The unfertilized forage recorded the highest WSC:CP ratio, and the organically fertilized forage recorded the least value for both parameters. The uCP values of the fertilized forages were similar (P > 0.05) and significantly (P < 0.05) higher than the unfertilized counterpart. The interaction of variety × fertilizer type on the parameters in Table 3 was significant. The WSC ranged from 96.28 to 107.79 g/kg DM, with the organic fertilized SAMNUT 22 having the highest WSC value and the organic fertilized local variety recording the highest uCP (258.98 ± 1.04 g/kg DM) value.

Water-soluble carbohydrates are components that digest completely and have an essential role they play in animal nutrition, as they are a major source of the readily available energy necessary for efficient microbial fermentation in the rumen (Jafari, 2012). The variation in the WSC based on variety in this study is supported by the report of Buxton and Fales (1994) who stated that the carbohydrate content of forages could be a production of the plant and its environment such as temperature, sunlight, and nutrient/fertilization. In this study, N fertilization was interacted with differently by the two varieties. It has been reported that higher WSC results in higher energy value and utilization efficiency of nutrients for animal productivity (Miller *et al*., 2001). This higher WSC for the organic fertilized SAMUNT 22 will support the consumption of lesser dietary N and conversion of dietary N to animal products.

Table 3 Effects of fertilizer type on the WSC, WSC:CP ratio, and utilizable crude protein of two *A. hypogaea* herbage

Factors		WSC (g/kg DM)	WSC:CP	uCP (g/kg DM)
Variety	**Fertilizer type**			
SAMNUT 22		105.11 ± 0.73[a]	0.53 ± 0.01[a]	244.52 ± 2.81[b]
Local		96.52 ± 1.51[b]	0.46 ± 0.01[b]	252.16 ± 1.30[a]
	Control	100.84 ± 1.55[ab]	0.53 ± 0.01[a]	237.94 ± 2.03[b]
	Organic	98.58 ± 2.51[b]	0.47 ± 0.02[c]	253.43 ± 2.83[a]
	Inorganic	103.01 ± 0.88[a]	0.49 ± 0.01[b]	253.66 ± 1.43[a]
SAMNUT 22	Control	105.40 ± 0.11[ab]	0.58 ± 0.00[a]	230.09 ± 0.19[c]
	Organic	107.79 ± 1.69[a]	0.53 ± 0.01[b]	247.90 ± 4.96[b]
	Inorganic	102.12 ± 0.00[b]	0.48 ± 0.01[d]	255.56 ± 2.11[ab]
Local	Control	96.28 ± 2.09[c]	0.48 ± 0.01[d]	245.78 ± 0.07[b]
	Organic	89.37 ± 0.01[d]	0.41 ± 0.00[e]	258.95 ± 1.04[a]
	Inorganic	103.89 ± 1.76[ab]	0.50 ± 0.01[c]	251.75 ± 1.81[ab]

Note: [a,b,c] Means on the same column with different superscripts are significantly different at $P < 0.05$.
WSC = water-soluble carbohydrate, CP = crude protein, uCP = utilizable crude protein.

The WSC:CP ratio of the forage in this study is lower than the proposed WSC:CP ratio of 0.70 to improve dietary N and for the reduction of N loss through urine (Edwards *et al.*, 2007). The unfertilized SAMNUT 22 was observed to have a slightly higher WSC:CP ratio value than alfalfa (da Silva *et al.*, 2014). This implied that the unfertilized SAMNUT 22 higher WSC:CP with lower CP value will improve dietary N utilization but lower forage yield (Dele *et al.*, 2019).

The value of uCP reflects the undegraded forage protein and microbial protein synthesis. The local variety had higher uCP than the SAMNUT 22 which is a reflection of the CP as well as the soluble portion of the protein fraction and non-protein nitrogen. The higher uCP estimation as related to the fertilizer type for the fertilized forage is in line with the report of Falahatizow *et al.* (2019). This is an indication that fertilizer application favors true protein (Peyraud and Astigarraga, 1998; Abbasi *et al*., 2012).

CONCLUSION

It can be concluded that the organic fertilized local variety (local × organic fertilizer) of *A. hypogaea* had the better potential of providing forage resources with a higher soluble fraction of CP and lower portion of cell wall bounded CP fraction as well as the highest uCP making it ruminally beneficial if fed to ruminants. It is therefore recommended as a better feedstuff for protein supplementation. The unfertilized SAMNUT 22 was concluded to be a forage resource with a better balance of energy to protein feedstuff and recommended as feedstuff for energy-protein balancing candidates.

REFERENCES

Abayomi, Y.A., T.V. Ajibade, O.F. Sammuel and B.F. Sa'adudeen. 2008. Growth and yield response of cowpea (*Vignia unquiculata* (L.) Walp.) genotypes to nitrogen fertilizer (NPK) application in southern guinea savanna zone of Nigeria. Asian J. Plant Sci. 7(2): 170–176.

Abbasi, D., Y. Rouzbehan and J. Rezaei. 2012. Effect of harvest date and nitrogen fertilization rate on the nutritive value of amaranth forage (*Amaranthus hypochondriacus*). Anim. Feed Sci. Technol. 171(1): 6–13.

Abdul Rahman, N., A. Larbi, B. Kotu, F.M. Tetteh and I. Hoeschle-Zeledon. 2018. Does nitrogen matter for legumes? Starter nitrogen effects on biological and economic benefits of cowpea (*Vigna unguiculata* L.) in Guinea and Sudan savanna of West Africa. Agronomy. 8: 120.

Adda, K.J., W. Addah, N. Abdul Rahman and T.A. McAllister. 2021. Inter-row plant spacing effects on grain and fodder yields, growth performance, digestibility and manure quality of sheep. Peanut Sci. 48(2): 144–152.

Ansah, T., A. Sahoo, N. Abdul Rahman, P.K. Kumawat and R.S. Bhatt. 2021. *In vitro* digestibility and methane gas production of fodder from improved cowpea (*Vigna unguiculata* L.) and groundnut (*Arachis hypogaea* L.) varieties. Sci. Afr. 13: e00897.

Ball, D.M., M. Collins, G.D. Lacefield, N.P. Martin, D.A. Mertens, K.E. Olson, D.H. Putnam, D.J. Undersander and M.W. Wolf. 2001. Understanding Forage Quality. American Farm Bureau Federation Publication 1-01, Park Ridge, Illinois, USA.

Bationo, A. and B.R. Ntare. 2000. Rotation and nitrogen fertilizer effects on pearl millet, cowpea, and groundnut yield and soil chemical properties in a sandy soil in the semi-arid tropics, West Africa. J. Agric. Sci. 134: 277–284.

Berça, A.S., A.D.S. Cardoso, V.Z. Longhini, L.O. Tedeschi, R.M. Boddey, R.A. Reis and A.C. Ruggieri. 2021. Protein and carbohydrate fractions in warm-season pastures: effects of nitrogen management strategies. Agronomy. 11: 847.

Blümmel, M., P. Ratnakumar and V. Vadez. 2012. Opportunities for exploring variations in haulm fodder traits of intermittent drought tolerant lines in a reference collection of groundnut (*Arachis hypogea* L). Field Crops Res. 126: 200–206.

Buxton, D.R. and S.L. Fales. 1994. Plant environment and quality, pp. 155–199. *In*: G.C. Fahey Jr., (Ed), Forage Quality, Evaluation, and Utilization. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Cherney, J.H. and M.H. Hall. 2008. Forage quality in perspective. Penn State Extension. Agronomy Facts 30. Penn State College of Agricultural Sciences, The Pennsylvania State University, Pennsylvania, USA.

Chimphango, S.B.M., L.H. Gallant, Z.C. Poulsen, M.I. Samuels, D. Hattas, O.E. Curtis, A.M. Muasya, C. Cupido, J.S. Boatwright and J. Howieson. 2020. Native legume species as potential fodder crops in the Mediterranean renosterveld shrubland, South Africa. J. Arid Environ. 173: 104015.

Cuomo, G.J. and B.E. Anderson. 1996. Nitrogen fertilization and burning effects on rumen protein degradation and nutritive value of native grasses. Agron. J. 88(3): 439–442.

da Silva, M.S., G.F. Tremblay, G. Bélanger, J. Lajeunesse, Y.A. Papadopoulos, S.A.E. Fillmore and C.C. Jobim. 2014. Forage energy to protein ratio of several legume–grass complex mixtures. Anim. Feed Sci. Technol. 188: 17–27.

Dele, P., B. Akinyemi, O. Omikunle, F. Enwete, O. Aiyesa, O. Awoola, C. Anotaenwere, T. Amole, A. Jolaosho and O.M. Arigbede. 2022. Protein fraction of soybean herbage as influenced by the stage of growth. Animal - Science Proceedings. 13(1): 14.

Dele, P.A., O.B. Kasim, B.T. Akinyemi, O. Kenneth-Obosi, F.E. Salawu, C.C. Anotaenwere, A.O. Jolaosho and O.M. Arigbede. 2019. Forage yield and nutritive quality of two groundnut (*Arachis Hypogaea* L) varieties as influenced by fertilizer types. J. Agric. Sci. & Env. 19(1–2): 1–16.

Dugje, I.Y., L.O. Omoigui, F. Ekeleme, A.Y. Kamara and H. Ajeigbe. 2009. Farmers' Guide to Cowpea Production in West Africa. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Edwards, G.R., A.J. Parsons, S. Rasmussen and R.H. Bryant. 2007. High sugar ryegrasses for livestock system in New Zealand. Proceedings of the New Zealand Grassland Association. 69: 161–171.

Falahatizow, J., M. Danesh Mesgaran, A.R. Vakili, M. Kafi and M.D. Stern. 2019. *In vitro* utilizable crude protein at the duodenum of dairy cows of various ecotypes of *Kochia scoparia* fertilized with nitrogen. Iran. J. Appl. Anim. Sci. 9(3): 409–418.

Fox, D.G., L.O. Tedeschi, T.P. Tylutki, J.B. Russell, M.E. Van Amburgh, L.E. Chase, A.N. Pell and T.R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112: 29–78.

Gierus, M., A. Herrmann, S. Kruse, J. Kleen and F. Taube. 2006. Variation in the non-protein nitrogen content (fraction A) of several forages during the growing period, pp. 595–597. *In*: Proceedings of the 21st General Meeting of the European Grassland Federation. 3–6 April 2006, Badajoz, Spain.

Gierus, M., E.M. Pötsch and F. Weichselbaum 2016. Influence of nitrogen fertilization on the crude protein fractions of grassland forage, pp. 245–247. *In*: M. Hoglind, A.K. Bakken, K.A. Hovstad, E. Kallioniemi, H. Riley, H. Steinshamn and L. Øsstrem, (Eds), The Multiple Roles of Grassland in the European Bioeconomy. Grassland Science in Europe. Volume 21. Norwegian Institute of Bioeconomy Research, Norway.

Griffith, W.K. 1974. Satisfying the nutritional requirements of established legumes, pp. 147–169. *In*: D.A. Mays, (Ed), Forage Fertilization. Soil Science Society of America, Madison, USA.

Hall, M.B., W.H. Hoover, J.P. Jennings and T.K. Miller-Webster. 1999. A method for partitioning neutral detergent-soluble carbohydrates. J. Sci. Food Agric. 79(15): 2079–2086.

Hariadi, B.T. and B. Santoso. 2010. Evaluation of tropical plants containing tannin on *in vitro* methanogenesis and fermentation parameters using rumen fluid. J. Sci. Food Agric. 90: 456–461.

Hernández, E.A., F.I.J. Lagunes, A.N. Pell, M.M. Lagunes, J.M.P. Rodríguez and R.W. Blake. 2020. *In vitro* ruminal degradation of carbohydrate fractions in tropical grasses fertilized with nitrogen. Rev. Mex. Cienc. Pecu. 11(1): 266–282.

Jafari, A.A. 2012. Environmental and genetic variation for water soluble carbohydrate content in cool season forage grasses, pp. 373–388. *In*: C.F. Chang, (Ed), Carbohydrates – Comprehensive Studies on Glycobiology and Glycotechnology. InTech, Rijeka, Croatia.

Johnson, C.R., B.A. Reiling, P. Mislevy and M.B. Hall. 2001. Effects of nitrogen fertilization and harvest date on yield, digestibility, fiber, and protein fractions of tropical grasses. J. Anim. Sci. 79(9): 2439–2448.

Jung, H.G. and M.S. Allen. 1995. Characteristics of plant cell wall affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73(9): 2774–2790.

Konlan, S., J. Sarkodies-Addo, E. Asare and M.J. Kombiok. 2012. Groundnut (*Arachis hypogaea* L.) varietal response to spacing in the Guinea Savanna agro-ecological zone of Ghana: nodulation and nitrogen fixation. Agric. Biol. J. N. Am. 4(3): 324–335.

Lanzas, C., L.O. Tedeschi, S. Seo and D.G. Fox. 2007. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. J. Dairy Sci. 90: 507–521.

Larbi, A., D.D. Dung, P.E. Olorunju, J.W. Smith, R.J. Tanko, I.R. Muhammad and I.O. Adekunle. 1999. Groundnut (*Arachis hypogaea*) for food and fodder in crop livestock systems: forage and seed yields, chemical composition and rumen degradation of leaf and stem fractions of 38 cultivars. Anim. Feed Sci. Technol. 77: 33–47.

Licitra, G., T.M. Hernandez and P.J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57(4): 347–358.

Mahmoud, A.E.M., M.S. Abbas, A. Cieslak and M. Szumacher-Strabel. 2017. Evaluation of chemical composition and *in vitro* dry and organic matter digestibility of some forage plant species derived from Egyptian rangelands. J. Anim. Plant Sci. 27(5): 1573–1581.

McRoberts, K.C., D. Parsons, Q.M. Ketterings, T.T. Hai, N.H. Quan, N.X. Ba, C.F. Nicholson and D.J.R. Cherney. 2017. Urea and composted cattle manure affect forage yield and nutritive value in sandy soils of south-central Vietnam. Grass Forage Sci. 73(1): 132–145.

Miller, L.A., J.M. Moorby, D.R. Davies, M.O. Humphreys, N.D. Scollan, J.C. MacRae and M.K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late lactation dairy cows. Grass Forage Sci. 56(4): 383–394.

Murphy, A.M. and P.E. Colucci. 1999. A tropical forage solution to poor quality ruminant diets: a review of *Lablab purpureus*. Livest. Res. Rural. Dev. 11(2): 21.

Neumann, M., J.L. Nörnberg, G.F.M. Leão, E.H. Horst and D.N. Figueira. 2017. Chemical fractionation of carbohydrate and protein composition of corn silages fertilized with increasing doses of nitrogen. Cienc. Rural. 47(5): e20160270.

Nigam, S.N. and M. Blümmel. 2010. Cultivar-dependent variation in food feed-traits in groundnut (*Arachis hypogea* L.). Anim. Nutr. Feed Technol. 10S: 39–48.

Nocek, J.E. and J.B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71(8): 2070–2107.

Oteng-Frimpong, R., S.P. Konlan and N.N. Denwar. 2017. Evaluation of selected groundnut (*Arachis hypogaea* L.) lines for yield and haulm nutritive quality traits. Int. J. Agron. 2017: 7479309.

Paterson, J., R. Cochran and T. Klopfenstein. 1996. Degradable and undegradable protein response of cattle consuming forage-based diets. *In*: M.B. Judkins and F.T. McCollum III, (Eds), Proceedings of the 3rd Grazing Livestock Nutrition Conference. Proc. West. Sec. Amer. Soc. Anim. Sci. 47(Suppl. 1): 94–103.

Pérez-Gil Romo, F., M.E. Carranco Jáuregui, M. Calvo Carrillo, L. Solano and T.D.J. Martínez Iturbe. 2014. Chemical characterization of native panicles and seed pods of the State of Guerrero, Mexico, for use in animal nutrition. Rev. Mex. Cienc. Pecu. 5(3): 307–319.

Peyraud, J.L. and L. Astigarraga. 1998. Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance. Anim. Feed Sci. Technol. 72: 235–259.

Pradhan, P., G. Fischer, H. van Velthuizen, D.E. Reusser and J.P. Kropp. 2015. Closing yield gaps: how sustainable can we be?. PLoS ONE 10(6): e0129487.

Prasad, K.V.S.V., A.A. Khan, S. Vellaikumar, R. Devulapalli, Ch. Ramakrishna Reddy, S.N. Nigam and M. Blümmel. 2010. Observations on livestock productivity in sheep fed exclusively on haulms from ten different genotypes of groundnut. Anim. Nutr. Feed Technol. 10S: 121–126.

Rogers, J.R., R.W. Harvey, M.H. Poore, J.P. Mueller and J.C. Barker. 1996. Application of nitrogen from swine lagoon effluent to bermudagrass pastures: seasonal changes in forage nitrogenous constituents and effects of energy and escape protein supplementation on beef cattle performance. J. Anim. Sci. 74(5): 1126–1133.

Salazar-Cubillas, K.C. and U. Dickhoefer. 2021. Evaluating the protein value of fresh tropical forage grasses and forage legumes using *in vitro* and chemical fractionation methods. Animals. 11: 2853.

Samireddypalle, A., O. Boukar, E. Grings, C.A. Fatokun, P. Kodukula, R. Devulapalli, I. Okike and M. Blümmel. 2017. Cowpea and groundnut haulms fodder trading and its lessons for multidimensional cowpea improvement for mixed crop livestock systems in West Africa. Front. Plant Sci. 8: 30.

Sanchez, P.A. 2002. Soil fertility and hunger in Africa. Science. 295(5562): 2019–2020.

Sanchez, P.A. 2015. En route to plentiful food production in Africa. Nat. Plants. 1: 14014.

Santos, C.B., K.A. de Pinho Costa, W.F. de Souza, V.C. e Silva, P.S. Epifanio and H.S. Santos. 2018. Protein and carbohydrates fractionation in Paiaguas palisadegrass intercropped with grain sorghum in pasture recovery. Acta Sci. 41: e42693.

SAS. 2000. SAS/E-miner 3.0 Program. SAS Institute Inc., Cary, North Carolina, USA.

Sniffen, C.J., J.D. O’Connor, P.J. Van Soest, D.G. Fox and J.B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. carbohydrate and protein availability. J. Anim. Sci. 70(11): 3562–3577.

Stojanović, B., N. Đorđević, A. Simić, A. Božičković, V. Davidović and A. Ivetić. 2020. The *in vitro* protein degradability of legume and sudan grass forage types and ensiled mixtures. Ankara Univ. Vet. Fak. Derg. 67: 419–425.

Tran, H., P. Salgado and P. Lecomte. 2009. Species, climate and fertilizer effects on grass fibre and protein in tropical environments. J. Agric. Sci. 147(5): 555–568.

Yu, P., D.A. Christensen, J.J. McKinnon and J.D. Markert. 2003. Effect of variety and maturity stage on chemical composition, carbohydrate and protein subfractions, *in vitro* rumen degradability and energy values of timothy and alfalfa. Can. J. Anim. Sci. 83(2): 279–290.

Zhao, G.Y. and J.E. Cao. 2004. Relationship between the *in vitro*-estimated utilizable crude protein and the Cornell Net Carbohydrate and Protein System crude protein fractions in feeds for ruminants. J. Anim. Physiol. Anim. Nutr. (Berl). 88(7–8): 301–310.