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Brachiaria Hybrid and *Pennisetum purpureum* Supplemented with *Pueraria phaseoloides* Increased the Concentration of Rumen-Undegradable Protein in Forages for Ruminants

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Abstract: Supplementing tropical grasses with leguminous forages is known to improve the nutritive value of forage-based diets. However, it is not clear how basal grass forages supplemented with Kudzu (*Pueraria phaseoloides*) affect the nitrogen (N) and crude protein (CP) fractions of grass/legume forages. The aim of this study was to determine the N and CP fractions and in vitro ruminal CP degradability of *Brachiaria* hybrid *(B. ruziziensis* × *B. decumbens* × *B. brizantha)* and elephant grass (*Pennisetum purpureum)* supplemented with Kudzu at a target CP content of 133 ± 0.6 g/kg of dry matter (DM). The grass/legume forages were evaluated for total N, buffer-soluble N (BSN), buffer-insoluble N (BISN), non-protein nitrogen (NPN), neutral detergent-insoluble N (NDIN), acid detergent-insoluble N (ADIN), CP fractions A, B_1, B_2, B_3, C, rumen-degradable protein (RDP), rumen-undegraded protein (RUP), and in vitro ruminal CP degradability. The CP concentration of Kudzu was 217 g/kg DM, while grass forages ranged between 79.9 and 112 g/kg DM. The BISN, CP degradability parameters *b*, and potential degradability were approximately 56, 41, and 74%, respectively, higher in grass forages supplemented with Kudzu. The concentrations of RUP in the grass forages (23.9–32.5 g/kg DM) were significantly improved when they were supplemented with Kudzu (72.0–79.9 g/kg DM). Therefore, we concluded that basal grass forages supplemented with Kudzu to a target CP content can improve the amount of RUP supplied by the forage portion of the diet. This can have a positive effect on forage utilization and animal performance while reducing the cost to feed for ruminants.

Keywords: elephant grass; leguminous forage; Mulato II; protein quality; tropical Kudzu; tropical grasses

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1. Introduction

In the Caribbean, like other tropical regions, the diet of domesticated ruminants constitutes primarily tropical grasses offered as pasture for grazing or harvested and fed in intensive systems [1,2]. However, tropical grasses are usually inadequate to satisfy the nutritional requirements of high-productivity ruminants [3]. This is because of low dry matter intake due to high fiber content or low bulk densities, especially when harvested for use in intensive systems or offered as pasture for grazing, respectively [4]. Other important limitations are seasonal fluctuations in herbage availability and nutritive value [5] and their inability to supply the required amounts of rumen-undegradable protein (RUP) needed to support high-productivity ruminants [6]. It was previously demonstrated that properly managed grasses in the Caribbean can supply adequate quantities of crude protein (CP) [7,8]. However, most of the CP supplied by these grasses is rapidly degraded in the rumen to facilitate the synthesis of microbial protein [9] and contribute to N loss in urine. As a result, the amount of RUP available to support high-productivity ruminants is inadequate [10]. Therefore, it is essential that feeding strategies are developed to improve the supply of RUP in forage-based feeding systems. Basal grass forage supplemented with feedstuffs rich in RUP such as those derived from animal by-products (e.g., blood meal, fish meal, feather meal), canola meal, and to some extent soybean meals are plausible options. However, these supplemental feedstuffs are either too expensive or not readily available to farmers in the Caribbean.

On most farms, the grass is supplemented with commercial concentrate feeds. These commercial concentrates are primarily energy feedstuffs with unknown protein quality for ruminants. A potentially suitable option may be to supplement tropical grasses with leguminous forages to improve the supply of RUP from the forage portion of the diet [6]. Indeed, it was previously confirmed that protein-rich leguminous forages such as *Macroptilium atropurpureum*, *Cassia rotundifolia,* and *Lablab purpureus* added to low-quality grasses significantly improved the supply of RUP and post-ruminal N digestion in non-lactating Holstein/Friesian cows by approximately 1.4 g/kg of dry matter (DM) [11].

More recently, estimated post-ruminal protein supplied by fifteen tropical leguminous forages range between 122 and 139 g/kg DM [6]. It is, therefore, clear that leguminous forages could become an important feedstuff for improving the supply of RUP from diets with tropical forages as the basal ingredients. *Pueraria phaseoloides* (Kudzu) is an available yet underutilized leguminous forage in Trinidad and Tobago and most other tropical countries. Recently, it was confirmed that large quantities of Kudzu (1742–2654 kg DM ha^{-1}) herbage with CP contents of 217–235 g/kg DM are available throughout the year in unutilized open grasslands in Trinidad and Tobago [2]. Compared to other popular tropical legumes, Kudzu is easy to harvest and process for feeding. Despite the high CP content, it is unknown if basal grass forages supplemented with Kudzu to a target CP content can improve the supply of RUP. Few studies indirectly supported this. For example, a reduction in ruminal CP solubility and improved fiber digestion of basal grass forages supplemented with Kudzu was previously observed [12]. Therefore, this study tested the hypothesis that Kudzu used as a supplement for basal grasses will improve the RUP content of forages for ruminants.

Two adopted and widely used grasses in the Caribbean are *Brachiaria* hybrid (*B. ruziziensis* × *B. decumbens* × *B. brizantha*) and elephant grass (*Pennisetum purpureum*). These grasses are preferred for their high biomass yield, rapid regrowth, and suitability for different ruminant production systems. Elephant grass is commonly harvested around 8- and 12-week regrowth to optimize biomass yield and nutritive value for feeding intensively reared sheep and goats. *Brachiaria* hybrid is predominantly grazed by dairy cattle at a 4-week regrowth interval or harvested for hay or fresh feeding around 8-week regrowth. These forages can provide sufficient N to maximize microbial protein synthesis but may contribute very little RUP [6,9]. As such, this study was performed to determine the N, CP fractions, and in vitro ruminal CP degradability of *Brachiaria* hybrid and elephant grass supplemented with Kudzu to a target CP content.

2. Materials and Methods

2.1. Ethical Clearance

Ethical clearance to conduct this study was secured from the University of the West Indies (UWI) Research Ethics Committee (Ref. CREC-SA.1565/04/2022).

2.2. Forage Samples

Two adopted and widely used grass specie, *Brachiaria* hybrid Cv. Mulato II *(B. ruziziensis* × *B. decumbens* × *B. brizantha)* and elephant grass (*Pennisetum purpureum*), were harvested at 2 stages of regrowth from a commercial dairy farm in Carlsen Field (10.48702° N, 61.3910° W), Trinidad and Tobago. Both grasses were cut back in early January 2021 and allowed to regrow to the desired stages. *Brachiaria* hybrid was harvested at 4-week (*Brac.*4) and 8-week (*Brac.*8) regrowth and elephant grass was harvested at 8-week (EG.8) and 12-week (EG.12) regrowth interval. Both grasses were harvested with a sharp knife to leave a 10–15 cm stubble from 4 different locations in the respective fields to represent the replicates for this study. Kudzu was harvested at the vegetative growth stage from an open, unutilized grassland approximately 600 m from the site where grass forages were harvested. Kudzu samples included leaves with petiole and succulent vines. All forages were harvested in the dry season of 2021 between the months of February and April.

2.3. Forage Processing and Chemical Analysis

The harvested forage samples were transported to the Animal Nutrition Laboratory in the Department of Food Production, UWI, St. Augustine Campus, and dried to constant weight in a force-draft oven set at 60 °C. The oven-dried samples were ground in a stainless-steel hammer mill (Thomas Wiley Laboratory mill, model 4; Thomas Scientific, Chadds Ford Township, PA, USA) to pass through a 2 mm sieve. Grass/legume forage samples were prepared by mixing Kudzu with each of the basal grass forage to produce a combined grass/legume forage containing 133 ± 0.6 g CP/kg DM. The formulation was performed using the Pearson square technique. The amount of grass and legume forages mixed to produce the desired combined samples is described in Table 1. These grass/legume forages are assumed to contribute approximately 75% of the diet of intensively reared sheep and goats in the Caribbean.

Table 1. Proportions (g/kg) of grass and legume (Kudzu) forage.

Forages	Grass/Legume Combinations			
	***Brac.*4 + Kudzu**	***Brac.*8 + Kudzu**	**EG.8 + Kudzu**	**EG.12 + Kudzu**
Kudzu	285	363	412	467
*Brac.*4	715	-	-	-
*Brac.*8	-	637	-	-
EG.8	-	-	588	-
EG.12	-	-	-	533

Abbreviations: *Brac.*4 = 4-week regrowth *Brachiaria* hybrid; *Brac.*8 = 8-week regrowth *Brachiaria* hybrid; EG.8 = 8-week regrowth elephant grass (*Pennisetum purpureum*); EG.12 = 12-week regrowth elephant grass (*Pennisitum purpureum*).

Commercial concentrate or agro-industrial by-products usually make up the rest of the diet for the fattening sheep and goats on a typical farm in the Caribbean to achieve an average daily gain of around 150 g/day [13]. The ground samples were analyzed for dry matter (DM) at 105 °C (method 934.01; [14]). The Kjeldahl procedure for total N (method 976.05) was used to determine CP [15]. This involved the use of an automated steam distillation (Kjel Flex K-360-Meierseggstrasse 40. Postfach CH-9230, Flawil, Switzerland) unit with 1% boric acid as the receiving solution followed by manual titration with 0.1 molar hydrochloric acid. Crude protein was calculated by multiplying total N by 6.25 (CP = N × 6.25). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined from a 0.5 g ground sample sealed in an F57 filter bag for extraction in an ANKOM^{2000} Fiber Analyzer (model: A2000I–ANKOM Technology, Macedon, NY, USA). Analysis of NDF included the addition of sodium sulfite and heat-stable amylase (α) (method 13; [16]). The acid detergent fiber was determined separately from NDF (method 12; [17]). Lignin analysis was performed by solubilizing ADF residue with 72% sulfuric acid [18]. Lignin was expressed on an ash-free basis, while ADF and NDF included residual ash. Fat (ether extract, EE) was determined with the ANKOM Fat Extractor XT10 following the Soxhlet principle (method 2, 01-30-09; [19]). Non-fiber carbohydrate (NFC) was determined by computation [20] as follows:

$$\text{NFC} = 100 - [\%\text{NDF} + \%\text{CP} + \%\text{EE} + \%\text{Ash}]$$

2.4. Nitrogen and Crude Protein Fraction

Analysis of total nitrogen (N) was previously described (method 976.05; [15]). Buffer-soluble nitrogen (BSN) and buffer-insoluble nitrogen (BISN) were determined by modifying the borate–phosphate method [21]. The Daisy$^{\text{II}}$ incubator and F57 filter bag (25 micron porosity) were used instead of filtration through filter paper [22]. The estimated N in residue by the Kjeldahl procedure represented the BISN fraction. The BSN fraction was calculated as the difference between total N and BISN. The concentrations of neutral detergent-insoluble nitrogen (NDIN) and acid detergent-insoluble nitrogen (ADIN) were determined by a Kjeldahl N analysis of residues post-NDF and -ADF extraction. Non-protein nitrogen (NPN) was determined using the sodium tungstate method [21]. Fractions of N and CP were expressed as a proportion of sample DM. All analyses included at least 2 blanks.

2.5. In Vitro Ruminal Crude Protein Degradability

In vitro ruminal CP degradability was performed by incubating approximately 0.5 g of each ground forage sample in a buffered rumen fluid in the Daisy^{II} incubator (method 3; [23]). The ground samples were packaged and heat-sealed in ANKOM F57 fiber bags that were pre-rinsed in acetone. The preparation of the buffer was described by ANKOM Technology (method 3; [23]). Rumen fluid was obtained at slaughter from two seven-month-old male Anglo Nubian goats of approximately 35 ± 2 kg live weight. The goats were reared for meat at the University of the West Indies (UWI), St. Augustine campus field station. They were maintained on a diet of freshly cut *Brachiaria arrecta* forage supplemented with approximately 0.5 kg of commercial concentrate feed, which contained 14% CP, 2.5% fat, 6.75% crude fiber, and 1% calcium. Immediately after slaughter, rumen contents were emptied in a clean bucket and filtered through 4 layers of cheesecloth in pre-warmed thermos flasks under continuous purging with carbon dioxide (CO_2). About 500 g of rumen digesta was blended at high speed to dislodge and include tightly adhering microbes into the inoculum. Approximately 1600 mL of pre-warmed buffer was poured into each Daisy^{II} jar with forage samples plus 2 blanks and allowed to equilibrate at 39 °C in the rotating Daisy^{II} incubation chamber before adding 400 mL of rumen inoculum to each jar. Crude protein degradability was estimated after 0, 3, 6, 12, 24, and 48 h of incubation. For 0 h, the samples were rinsed with cold tap water, allowed to air dry and subsequently oven-dried at 65 °C for 48 h, cooled for 30 min, and reweighed. Post-incubation, forage samples were oven-dried to a constant weight at 65 °C, cooled in a desiccator for 30 min, and reweighed. Sample residues were analyzed for Kjeldahl N to determine CP degradability at the respective incubation intervals. The kinetics of ruminal CP degradation was estimated by fitting CP degradability data to the nonlinear model of [24] using Datafit 9.0 software (Oakland Engineering). The model was represented as:

$$y = a + b\left(l - e^{-c(t-lt)}\right) \quad (1)$$

In the model, y = N disappearance, a = washing loss, b = degradable part of the insoluble fraction, c = degradation rate of fraction b, $a + b$ = potential degradable N, t = incubation time (hours), and l = lag time (hours).

2.6. Calculations and Statistical Analysis

Crude protein fractions (A, B_1, B_2, B_3, and C) were defined according to Licitra et al. [21]. Fraction A described non-protein nitrogen using sodium tungstate solution. Fraction B_1 represented the fraction of CP soluble in the borate–phosphate buffer and precipitated with trichloroacetic acid. Fraction B_3 was the difference between the portions of total CP recovered in NDF (i.e., NDIN) and ADF. Fraction B_2 defined the remaining CP calculated as total CP minus the sum of fractions A, B_1, B_3, and C and fraction C, which is nitrogen-insoluble in an acid detergent solution (N residue post-ADF analysis). Rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) in wet forages were estimated with the equations proposed by the National Research Council [20] as follows:

1. RDP = A + B $[K_d/(K_d + K_p)]$
2. RUP = B $[K_p/(K_d + K_p)]$ + C

where

K_d = the rate of degradation of the B fraction, (%/h) and K_p represented the rate of passage from the rumen (%/h). The degradation rate of fraction B (K_d) was determined by fitting CP degradation data at 0, 3, 6, 12, 24, and 48 h to the exponential model of [24] using the curve fitting software DataFit 9. The rate of passage from the rumen (K_p) was calculated as $K_p = 3.054 + 0.614X_1$, where X_1 denotes dry matter intake (% BW), which was assumed to be 4 for the typical small–medium breed goat under tropical conditions [20]. Statistical analysis was performed by ANOVA following a general linear model with forages (grasses only and grass forages supplemented with Kudzu herbage) as the main effect. The model was represented as:

$$Y_{ij} = \mu + F_i \ (i = 1\text{-}9) + E_{ij}$$

where Y_{ij} = dependent variable, μ = overall mean, F_i = effect of feed type, and E_{ij}, = random error. Statistical significance was declared at $p < 0.05$. Treatment means were separated by Tukey's multiple comparison test. Statistical analysis was carried out in the Mintab 19 statistical package.

3. Results

3.1. Chemical Composition

The chemical compositions of individual forages and the basal grass forage supplemented with Kudzu differed significantly (Table 2). The lowest DM content was observed in *Brac*.8. Ash was highest in elephant grass at both stages of regrowth, while *Brac*.4 supplemented with Kudzu herbage had the highest EE content (27.5 g/kg DM). Crude protein concentration was highest in Kudzu herbage (217 g/kg DM) and lowest in 12-week regrowth elephant grass (79.9 g/kg DM). The concentrations of NDF, ADF, lignin, and NFC were generally similar in the grass forages supplemented with Kudzu herbage to a target CP content. Generally, NDF, ADF, and lignin contents were highest in grass forages harvested at the more advanced stages of maturity. Kudzu had the lowest concentration of NDF (620 g/kg DM) and ADF (562 g/kg DM), while lignin was lowest in *Brac*.4 (75.0 g/kg DM).

Table 2. Chemical composition of Kudzu (*Pueraria phaseoloides*), grass forages, and Kudzu-supplemented grass forages (values are the mean of four (4) replicates).

Forages	Chemical Compositions (g/kg DM)							
	DM (g/kg)	Ash	EE	CP	NDF	ADF	Lignin	NFC
Kudzu	926 ^{ab}	70.5 ^{c}	16.9 ^{b}	217 ^{a}	620 ^{c}	562 ^{d}	124 ^{a}	76.0 ^{a}
Brac.4	936 ^{a}	129 ^{ab}	20.7 ^{ab}	109 ^{c}	668 ^{ab}	572 ^{cd}	75.0 ^{d}	73.0 ^{a}
Brac.8	923 ^{b}	115 ^{ab}	19.0 ^{b}	96.6 ^{d}	706 ^{a}	606 ^{abc}	83.7 ^{cd}	63.9 ^{ab}
EG.8	927 ^{ab}	145 ^{a}	16.4 ^{b}	112 ^{c}	702 ^{a}	614 ^{ab}	90.7 ^{bcd}	24.8 ^{b}
EG.12	931 ^{ab}	148 ^{a}	19.9 ^{b}	79.9 ^{d}	694 ^{a}	622 ^{a}	99.2 ^{bc}	58.7 ^{ab}
Brac.4 + Kudzu	931 ^{ab}	118 ^{ab}	27.5 ^{a}	133 ^{b}	643 ^{bc}	578 ^{bcd}	87.2 ^{cd}	78.9 ^{a}
Brac.8 + Kudzu	929 ^{ab}	107 ^{b}	20.8 ^{ab}	134 ^{b}	667 ^{abc}	587 ^{abcd}	105 ^{abc}	71.1 ^{a}
EG.8 + Kudzu	930 ^{ab}	120 ^{ab}	19.0 ^{b}	131 ^{b}	661 ^{abc}	597 ^{abcd}	110 ^{ab}	68.2 ^{ab}
EG.12 + Kudzu	934 ^{a}	119 ^{ab}	21.1 ^{ab}	133 ^{b}	666 ^{abc}	602 ^{abc}	109 ^{ab}	60.0 ^{ab}
SEM	1.56	5.39	1.22	1.54	8.94	6.64	3.91	8.19
Significance	*	***	**	***	***	***	***	*

Note: $^{a–d}$ Means within columns that do not share a letter are significantly different. Abbreviations: *Brac*.4 = 4-week regrowth *Brachiaria* hybrid; *Brac*.8 = 8-week regrowth *Brachiaria* hybrid; EG.8 = 8-week regrowth elephant grass (*Pennisetum Purpureum*); EG.12 = 12-week regrowth elephant grass (*Pennisitum purpureum*); DM, dry matter; EE, ether extract; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, non-fiber carbohydrate; SEM, standard error of mean; significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.2. Nitrogen and Crude Protein Fractions

Total nitrogen and N fractions (Table 3) and CP fractions (Table 4) differed significantly between forages. The NDIN fractions were similar for the basal grass forages that were supplemented with Kudzu. The highest concentrations of ADIN (16.9 g/kg DM), BISN (25.4 g/kg DM), BSN (10.5 g/kg DM), and NPN (8.8 g/kg DM) were observed in elephant grass, while these fractions were the lowest in EG.12. The fractions of BISN and BSN for grass forages supplemented with Kudzu were relatively similar. Elephant grass at 12-week regrowth had the lowest CP fractions A, B_1, B_2, C, and RDP. The B_3 fraction of *Brac.*8 (5.4 g/kg DM) was the lowest, while *Brac.*4 (23.9 g/kg DM) had the lowest RUP fraction. The B_1, B_2, B_3, and RUP fractions were generally similar for grass forages supplemented with Kudzu. Both RDP (92.7 g/kg DM) and RUP (124.1 g/kg DM) were highest in Kudzu herbage. The addition of Kudzu herbage to the basal grass forages increased the concentrations of RUP to 72.0–79.9 g/kg DM compared to 23.9–32.5 g/kg DM in the grass forages alone.

Table 3. Total nitrogen and N fractions of Kudzu (*Pueraria phaseoloides)*-supplemented grass forages (values are the mean of four (4) replicates).

Forages	Total Nitrogen and N Fractions (g/kg DM)					
	Total N	**NDIN**	**ADIN**	**BISN**	**BSN**	**NPN**
Kudzu	34.7 ^{a}	18.3 ^{a}	16.9 ^{a}	25.4 ^{a}	10.5 ^{a}	8.8 ^{a}
*Brac.*4	17.5 ^{c}	7.3 ^{c}	4.9 ^{e}	13.6 ^{bc}	5.1 ^{b}	7.1 ^{ab}
*Brac.*8	15.5 ^{d}	7.4 ^{c}	6.5 ^{d}	11.8 ^{cd}	4.9 ^{b}	4.9 ^{cd}
EG.8	17.9 ^{c}	8.3 ^{c}	6.5 ^{d}	13.7 ^{bc}	5.3 ^{b}	6.8 ^{abc}
EG.12	12.8 ^{e}	5.8 ^{d}	4.0 ^{e}	9.3 ^{d}	4.7 ^{b}	3.9 ^{d}
*Brac.*4 + Kudzu	21.2 ^{b}	11.2 ^{b}	9.4 ^{c}	16.7 ^{b}	5.7 ^{b}	4.8 ^{cd}
*Brac.*8 + Kudzu	21.5 ^{b}	11.8 ^{b}	9.4 ^{c}	16.6 ^{b}	6.1 ^{b}	5.0 ^{bcd}
EG.8 + Kudzu	21.0 ^{b}	11.8 ^{b}	9.9 ^{bc}	16.2 ^{b}	6.0 ^{b}	4.6 ^{d}
EG.12 + Kudzu	21.3 ^{b}	11.7 ^{b}	10.8 ^{b}	16.6 ^{b}	5.9 ^{b}	4.9 ^{cd}
SEM	0.25	0.18	0.13	0.50	0.48	0.33
Significance	***	***	***	***	***	***

Note: $^{a–e}$ Means within columns that do not share a letter are significantly different. Abbreviations: *Brac.*4 = 4-week regrowth *Brachiaria* hybrid; *Brac.*8 = 8-week regrowth *Brachiaria* hybrid; EG.8 = 8-week regrowth elephant grass (*Pennisetum purpureum*); EG.12 = 12-week regrowth elephant grass (*Pennisitum purpureum*). Nitrogen fraction: total N, total nitrogen; NDIN, neutral detergent-insoluble nitrogen; ADIN, acid detergent-insoluble nitrogen; BISN, buffer-insoluble nitrogen; BSN, buffer-soluble nitrogen; NPN, non-protein nitrogen; SEM = standard error of mean; significance: *** $p < 0.001$.

Table 4. CP fractions of Kudzu (*Pueraria phaseoloides)*-supplemented grass forages (values are the mean of four (4) replicates).

Forages	CP fractions (g/kg DM)						
	A	**B_1**	**B_2**	**B_3**	**C**	**RDP**	**RUP**
Kudzu	54.8 ^{a}	65.7 ^{a}	44.4 ^{a}	8.42	105.9 ^{a}	92.7 ^{a}	124.1 ^{a}
*Brac.*4	44.4 ^{ab}	31.8 ^{b}	39.9 ^{ab}	14.6	30.7 ^{e}	85.6 ^{ab}	23.9 ^{c}
*Brac.*8	30.6 ^{cd}	30.4 ^{b}	27.9 ^{ab}	5.4	40.5 ^{d}	64.1 ^{bcd}	32.5 ^{c}
EG.8	42.6 ^{abc}	33.4 ^{b}	33.8 ^{ab}	11.5	40.6 ^{d}	80.2 ^{abc}	31.6 ^{c}
EG.12	24.2 ^{d}	29.5 ^{b}	21.8 ^{b}	11.3	24.9 ^{e}	50.9 ^{d}	29.0 ^{c}
*Brac.*4 + Kudzu	29.8 ^{cd}	35.8 ^{b}	34.0 ^{ab}	11.5	58.8 ^{c}	58.7 ^{cd}	73.9 ^{b}
*Brac.*8 + Kudzu	31.5 ^{bcd}	38.0 ^{b}	30.3 ^{ab}	15.1	58.7 ^{c}	58.8 ^{cd}	75.6 ^{b}
EG.8 + Kudzu	28.5 ^{d}	37.6 ^{b}	27.5 ^{ab}	11.7	61.9 ^{bc}	59.0 ^{cd}	72.0 ^{b}
EG.12 + Kudzu	30.4 ^{cd}	36.7 ^{b}	30.7 ^{ab}	5.8	67.5 ^{b}	53.2 ^{d}	79.9 ^{b}
SEM	2.1	3.0	3.0	1.8	0.8	4.7	4.1
Significance	***	***	*	*NS*	***	***	***

Note: $^{a–e}$ Means within columns that do not share a letter are significantly different. Abbreviations: *Brac.*4 = 4-week regrowth *Brachiaria* hybrid; *Brac.*8 = 8-week regrowth *Brachiaria* hybrid; EG.8 = 8-week regrowth elephant grass (*Pennisetum purpureum*); EG.12 = 12-week regrowth elephant grass (*Pennisitum purpureum*). CP fractions: A, fraction of CP that is instantaneously soluble; B_1, fraction of true protein that rapidly degrades in the rumen; B_2, difference between insoluble proteins and proteins insoluble in neutral detergent; B_3, fraction of true protein that is insoluble in neutral detergent but soluble in acid detergent; C, ruminal unavailable (cell wall-bound protein, nitrogen associated with lignin); RDP, protein fraction that is degraded in the rumen; RUP, protein fraction that escapes rumen degradation (bypass protein), significance: * $p < 0.05$; *** $p < 0.001$; *NS*, not significant.

3.3. In Vitro Ruminal CP Degradability

In vitro ruminal CP degradability ($p < 0.01$) after 6 h and 24 h of incubation and the CP degradation rate ($p < 0.001$) differed significantly between forage types (Table 5). After 6 h of incubation, the CP degradability of Kudzu herbage (245 g/kg) was similar to the grass forages supplemented with Kudzu. The EG.8 recorded the highest 6 h CP degradability. However, after 24 h of incubation, CP degradability was highest in *Brac*.4 (590 g/kg). Ruminal CP degradability post-6 h and -24 h and CP degradation rates were similar for basal grass forages that were supplemented with Kudzu. The lowest CP degradation rate was observed in elephant grass at both stages of regrowth. The Kinetics of ruminal CP degradability are presented in Table 6. Ruminal degradation of the immediately soluble CP fraction (*a*) was highest in *Brac*.4 and EG.8 (276 g/kg) and lowest in EG.8 + Kudzu (146 g/kg). There were significant differences ($p < 0.05$) in the in vitro ruminal degradability of the insoluble CP fraction (*b*) (706 g/kg) and potential CP degradation (*PD*) (919 g/kg), which were highest in EG.12 + Kudzu. The CP degradability rate constant for the insoluble fraction (*c*) was highest in *Brac*.8 (6.3 g/kg) and lowest in both Kudzu and EG.12 + Kudzu (2.6 g/kg). The lag phase (*l*) was similar in all forages.

Table 5. In vitro ruminal CP degradability of Kudzu (*Pueraria Phaseoloides)*-supplemented grass forages (values are the mean of four (4) replicates).

Forages	Ruminal CP Degradability (g/kg) and CP Degradation Rate (g/h)		
	6 h	24 h	CP Degradation Rate
Kudzu	245^{b}	508^{abc}	10.0^{a}
Brac.4	350^{ab}	590^{a}	7.1^{bcd}
Brac.8	313^{ab}	560^{ab}	8.1^{abc}
EG.8	393^{a}	541^{ab}	6.6^{cd}
EG.12	290^{ab}	450^{c}	5.2^{d}
Brac.4 + Kudzu	249^{b}	496^{bc}	8.8^{abc}
Brac.8 + Kudzu	260^{b}	507^{abc}	9.2^{ab}
EG.8 + Kudzu	246^{b}	541^{ab}	9.7^{a}
EG.12 + Kudzu	255^{b}	549^{ab}	10.4^{a}
SEM	17.4	14.4	0.41
Significance	**	**	***

Note: $^{a–d}$ Means within columns that do not share a letter are significantly different. Abbreviations: *Brac*.4 = 4-week regrowth *Brachiaria* hybrid; *Brac*.8 = 8-week regrowth *Brachiaria* hybrid; EG.8 = 8-week regrowth elephant grass (*Pennisetum Purpureum*); EG.12 = 12-week regrowth elephant grass (*Pennisitum purpureum*); 6 h, 6 h ruminal CP degradability; 24 h, 24 h ruminal CP degradability; SEM, standard error of mean; significance: *** $p < 0.001$; ** $p < 0.01$.

Table 6. In vitro ruminal CP degradability kinetics of Kudzu (*Pueraria Phaseoloides)*-supplemented grass forages (values are the mean of four (4) replicates).

Forage/Diets	In Vitro Ruminal CP Degradability (g/kg) Parameters				
	a	*b*	*PD*	*c* (g/h)	*l* (hours)
P. phaseoloides	209^{ab}	661^{a}	870^{ab}	2.6^{b}	1.9
Brac.4	276^{a}	365^{bcd}	642^{bcd}	5.2^{ab}	1.1
Brac.8	176^{b}	411^{bcd}	587^{cd}	6.3^{a}	1.0
EG.8	276^{a}	338^{cd}	613^{bcd}	5.2^{ab}	0.67
EG.12	221^{ab}	316^{d}	539^{d}	4.4^{ab}	1.2
Brac.4 + Kudzu	202^{ab}	557^{abcd}	759^{abcd}	3.2^{ab}	1.6
Brac.8 + Kudzu	215^{ab}	599^{ab}	814^{abc}	2.8^{b}	1.7
EG.8 + Kudzu	146^{b}	580^{abc}	726^{abcd}	3.8^{ab}	0.27
EG.12 + Kudzu	214^{ab}	706^{a}	919^{a}	2.6^{b}	2.1
SEM	14.5	46.2	48.8	0.66	0.49
Significance	**	***	**	*	*NS*

Note: $^{a–d}$ Means within columns that do not share a letter are significantly different. Abbreviations: *Brac*.4 = 4-week regrowth *Brachiaria* hybrid; *Brac*.8 = 8-week regrowth *Brachiaria* hybrid; EG.8 = 8-week regrowth elephant grass (*Pennisetum Purpureum*); EG.12 = 12-week regrowth elephant grass (*Pennisitum purpureum*). CP degradability parameters: *a*, CP degradability from the immediately soluble fraction; *b*, CP degradability from the insoluble fraction; *PD*, potential CP degradability; *c*, CP degradability rate constant for the insoluble fraction; *l*, colonization period/lag phase; SEM, standard error of mean; significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *NS*, not significant.

4. Discussion

4.1. Chemical Composition

Generally, the chemical composition of Kudzu herbage in this study was comparable to common tropical leguminous tree forages, such as *Gliricidia sepium* and *Leucaena leucocephala* [7,25], and creeping legumes, such as *Centrosema* spp. [26]. The CP content of Kudzu in the present study was lower than those harvested at the reproductive stage in an earlier report [27]. Seasonal and environmental differences at the time of harvesting may be the underlining factors responsible for the differences in CP contents.

The CP contents of *Brachiaria* hybrid in the present study were significantly lower than those of similar maturity and appended with 25–75 kg N/fertilizer/ha^{-1} [8], suggesting *Brachiaria* hybrid is highly responsive to N fertilization. The CP contents of EG.8 and EG.12 were low but are typical of the species for the stage of maturity [28]. Therefore, it is necessary to include a supplemental protein source in feeds where elephant grass forms the basal diet. The basal grass forages that were supplemented with Kudzu herbage showed a marginal decline in NDF and ADF and an increase in NFC contents because leguminous forages contain lower concentrations of cell walls. The reduced cell wall and higher NFC are expected to improve intake and digestibility of the combined forage [11] because NFC is an important source of energy and fermentable carbohydrate for ruminal microbial protein synthesis [29,30].

4.2. Nitrogen and Crude Protein Fractions

With the exception of CP fraction A, the observed CP fractions for both grass and legume forages were different from other reports [10]. Non-protein nitrogen is a major constituent of BSN. With almost twice the amount of BSN and much higher NPN and B_1 CP fraction in Kudzu herbage, it may be a suitable supplemental feedstuff for poor-quality grasses that usually have inadequate soluble N [22]. If fed as the basal forage or the sole feed, the concentration of soluble N in Kudzu herbage would likely exceed the threshold needed for efficient ammonia utilization, leading to significant N loss into the environment [31,32]. Also, Kudzu is reported to contain secondary metabolites, such as tannins, saponins, alkaloids, and cyanogenic glycosides [1,33], which could further limit its use as the basal or sole forage. Adequate soluble dietary N is essential for rumen fermentation and synthesis of microbial protein [22]. It is estimated that approximately 280–460 g BSN/kg dietary N is required to optimize rumen ammonia concentration to maximize the synthesis of microbial protein in dairy cattle [34,35]. This suggests that the concentrations of BSN of the basal grass forages that were supplemented with Kudzu (280 g BSN/kg dietary N equivalent) are around the lower limit of the requirements to optimize rumen ammonia concentration for efficient microbial protein synthesis.

The BISN, like the B_3 and to some extent B_2 CP fractions, describes dietary true protein that is slowly degraded in the rumen or will escape rumen fermentation and contribute to the pool of protein available for post-ruminal absorption [6]. Fraction C, like ADIN, also contributes to the pool of N and true protein that bypasses rumen fermentation but is considered unavailable because it is fiber-bound [32]. The high fiber-bound protein of the grass forages supplemented with Kudzu herbage suggests that some of this protein may be unavailable for absorption in the small intestine [36], despite an increase in RUP. Approximately 5–15% of the total CP is usually cell wall-bound in most feedstuffs [37]. However, a much higher proportion was observed in the present study, possibly because of the spatial distribution of N [38] and high cell wall contents in Kudzu herbage tissues. Additionally, fiber-bound N is associated with the rapid growth of tropical forages causing a reduction in the leaf:stem ratio and an incremental increase in fiber and lignin content [8]. Therefore, management approaches like nitrogen fertilizer application and harvesting forages at an earlier stage of maturity can reduce the accumulation of N in the cell wall. The unavailable cell wall-bound protein in the present study was almost double the highest value reported from twenty-three tropical grass forages, while the B_3 fraction was approximately three times higher in the same report [6]. Since the B_3 fraction represents feed protein likely to bypass rumen degradation, high concentrations of fraction C and the low B_3 fraction may restrict post-ruminal protein absorption. Nonetheless, an increase in the RUP shows that basal grass forges supplemented with Kudzu have the potential to increase the concentration of RUP. Increasing the content of RUP will provide more amino acids for absorption in the small intestine to support high levels of production. For example, a daily intake of around 51–69 g of RUP by male Farahani lambs is needed to achieve an average daily gain of 200 g/day or higher [39]. Therefore, if daily dry matter intake of approximately 1 kg can be achieved by post-weaned fattening sheep and goats, the basal grass forages that were supplemented with Kudzu may be able to supply around 80 g of RUP/day, enough to achieve growth rates similar to those suggested earlier [39], assuming other nutrients are adequate.

4.3. In Vitro Ruminal CP Degradability Kinetics

Though not statistically significant, the basal grass forages supplemented with Kudzu to a target CP content slightly reduced 6 h and 24 h CP degradability. This could be a result of more rumen-resistant protein, particularly from CP fractions C, $B_{2,}$ and B_3 from Kudzu herbage. The ruminal CP degradability of grass forages supplemented with Kudzu post-24 h of incubation was only 496–549 g/kg CP. Low ruminal CP degradation suggested there is a lower chance of excess ammonia buildup, nitrogen loss, and an increase in dietary bypass protein [22]. In fact, lower ruminal protein digestibility is usually compensated by higher intestinal protein digestion. For example, when ruminal protein digestibility was around 70%, intestinal CP digestibility of grass silage increased to 81%. Similarly, ruminal CP digestibility of fresh forages was approximately 57% and intestinal CP digestibility ranged between 83 and 91% [40]. Therefore, the total track CP digestibility of the basal grass forages plus Kudzu herbage could be high. Ruminal CP degradability from the immediately soluble "*a*" and insoluble "*b*" fractions are generally high in leguminous forages [41]. However, in this study, the younger grasses, *Brac.*4 and EG.8, were highest in these parameters despite having lower NPN and CP fraction A. This could be an indication that the rumen-degradable portion of the B_1 and B_2 fractions is proportionally larger in the younger grasses. Additionally, the grass forages with significantly lower fiber-bound N would have a negative influence on 24 h CP degradability. However, adding Kudzu to the basal grass forages improved the CP degradation rate of the grass/legume forages, which is a positive indication that rapidly soluble N was not limited despite the slight decline in 6 h and 24 h of CP degradability. It has been suggested that the immediately soluble CP fraction (*a*) must constitute a minimum of 10% ruminal CP degradability as an indicator of adequate soluble N [41]. Crude protein degradability of this fraction ranged between 14.6 and 21.5% in grass forages supplemented with Kudzu, which further confirmed the adequacy of rumen-soluble N.

5. Conclusions

Basal grass forages supplemented with Kudzu herbage can improve the amount of RUP supplied by the forage portion of the diet for domesticated ruminants. This will have a positive impact on forage utilization and animal performance while reducing feeding costs, particularly in resource-poor countries that are dependent on imported grains. However, further investigation into post-ruminal digestion of fiber-bound protein in Kudzu herbage is recommended.

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