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Evaluation of chemical composition, fibre fractions and carbohydrate and protein partitioning of paddy straw, Guinea grass, and hybrid Napier (CO5)#

Lasna Sahib[1*], K. Ally[2], K.S Ajith[2], Biju Chacko[1], G. Radhika[3] and K.S. Anil[4]

[1]Department of Animal Nutrition, College of Veterinary and Animal Sciences, Pookode, [2]Department of Animal Nutrition, [3]Department of Animal Genetics and Breeding, [4]Department of Livestock Production and Management, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad

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Abstract

The nutritional evaluation of fodder resources is essential for optimising ruminant feeding strategies. This study assessed the chemical composition, fibre fractions and CNCPS (Cornell Net Carbohydrate and Protein System) carbohydrate and protein partitioning of paddy straw, Guinea grass and Hybrid Napier (CO5). Four samples of each fodder were analysed for dry matter, crude protein, ether extract, ash, neutral detergent fibre, acid detergent fibre, lignin, starch, buffer soluble protein, non-protein nitrogen, neutral detergent insoluble protein, and acid detergent insoluble protein. Carbohydrates were fractioned into CA (soluble), CB1 (starch/pectins), CB2 (available fibre), and CC (unavailable fibre), while proteins were partitioned into PA (NPN), PB1 (rapidly degradable), PB2 (intermediately degradable), PB3 (slowly degradable) and PC (undegradable). Structural carbohydrates constituted the major portion, with CB2 as the predominant fraction across all fodders (P=0.021). The CB1 fraction varied significantly (P=0.015), with higher levels observed in Hybrid Napier and paddy straw. Protein fractionation revealed significant differences, with paddy straw exhibiting the highest PB1 fraction (P=0.001), Hybrid Napier the highest PB2 fraction (P<0.001) and Guinea grass the highest PB3 fraction. The PC fraction was highest in paddy straw (18.98%) (P<0.001), indicating greater undegradable protein content. These findings demonstrated that Guinea grass and Hybrid Napier provided higher fermentable fibre and protein degradability, while Paddy straw, though energy-dense, had a higher proportion of undegradable protein. This study underscores the utility of CNCPS fractionation in characterising fodder quality for precision livestock feeding.

Keywords: Guinea grass, Hybrid Napier, Paddy straw, CNCPS fractions

Fodder resources form the foundation of ruminant nutrition, supplying essential energy, protein and fibre required for optimal growth and production. Among the commonly utilised forages, paddy straw, Guinea grass (*Panicum maximum*) and Hybrid Napier (*Pennisetum purpureum* × *Pennisetum glaucum*) are economically viable and widely cultivated in tropical and subtropical regions (Jackson, 1978; Aganga and Tshwenyane, 2003; Premaratne and Premalal, 2006; Feyza, 2021). However, significant variation exists in their nutritional profiles, influencing digestibility and feed efficiency in ruminants.

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**Corresponding author: lasna@kvasu.ac.in, Ph. 9495133891*

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The Cornell Net Carbohydrate and Protein System (CNCPS) provides a refined framework for assessing the degradation kinetics of carbohydrates and proteins in the rumen, thereby enabling precision feeding (Sniffen *et al*., 1992). Conventional proximate analysis offers a general overview of nutrient composition but lacks the specificity needed to differentiate fractions influencing microbial fermentation. The CNCPS method classifies carbohydrates into rapidly fermentable (CA), intermediately degradable (CB1, CB2), and unavailable (CC) fractions, while proteins are categorised into non-protein nitrogen (PA), rapidly degradable (PB1), intermediately degradable (PB2), slowly degradable (PB3), and undegradable (PC) fractions, providing deeper insights into nutrient utilisation efficiency (Sniffen *et al*., 1992).

Despite the widespread use of these forages, limited research has characterised their CNCPS-based carbohydrate and protein fractions. Although their proximate composition and fibre content have been documented, comprehensive data on nutrient partitioning remain scarce, limiting the formulation of nutritionally balanced rations. This study aims to bridge this knowledge gap by evaluating the chemical composition and CNCPS-based nutrient fractions of these forages, enabling precise feed formulation to enhance ruminant productivity.

Materials and methods

Four samples of paddy straw, Guinea grass and Hybrid Napier **(**CO5) were collected from farms of Kerala Veterinary and Animal Sciences University and analysed in duplicate. Samples were oven-dried at 60°C and ground for subsequent analyses.

Chemical composition and fibre fractions

The samples were analysed following AOAC (2016) methods for various components: dry matter (DM) (method 930.15), ash (method 942.05), crude protein (CP) (method 2001.11) and ether extract (EE) (method 2003.06). Organic matter (OM) content was determined by subtracting the ash content (on DM basis) from 100. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured according to (Goering and Van Soest, 1970; Van Soest *et al*., 1991), with modifications excluding the use of sodium sulphite and heat-stable amylase in the NDF procedure. All fibre fractions were reported, excluding residual ash.

Carbohydrate fractionation

Lignin (ADL) content was quantified following the AOAC (2016) (method 973.18). The analysis involved solubilising cellulose in the ADF residue using 72 per cent (w/w) sulphuric acid. The cellulose content was subsequently calculated as the difference between the ADF and lignin contents, obtained through sequential analysis. Hemicellulose (HC) content was indirectly estimated by calculating the difference between NDF and ADF.

Starch analysis followed the procedure as per AOAC (2016) (method 920.44). Ground feed sample of 200 mg was mixed with 50 mL of cold water and stirred for one hour in a rotary shaker. The mixture was filtered with Whatman No. 1 filter paper using 250 mL of cold water. The insoluble residue was refluxed with 200 mL of water and 20 mL of 2N HCl for 2.5 h in a reflux condenser. After cooling, the solution was neutralised with NaOH using litmus paper as a guide. The solution was diluted to 250 mL in a volumetric flask and then filtered. A 0.1 mL aliquot was diluted to one mL, mixed with six mL of GOD-POD reagent (std. kits), and incubated at 50°C for 20 min. Absorbance was measured at 505 nm within 30 min. Glucose concentration was estimated by plotting a standard curve of absorbance against glucose concentration, and starch content was determined by multiplying the glucose concentration by a factor of 0.9.

Protein fractionation

The feed ingredients were analysed in duplicate for buffer soluble protein (BSP) and non-protein nitrogen (NPN) following the procedure recommended by Licitra *et al.* (1996). Neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were determined from the residues obtained after NDF and ADF procedures. These residues were quantitatively transferred to digestion tubes and nitrogen content was determined using the standard Kjeldahl procedure.

Soluble protein (Buffer soluble protein)

Ground dry sample (0.5 g) was weighed into a 125 mL Erlenmeyer flask. Fifty mL of borate-phosphate buffer and one mL of freshly prepared 10 per cent sodium azide solution were added to control microbial growth. The mixture was left to stand at room temperature for three hours. It was then filtered through Whatman 54 filter paper, and the residue was washed with 250 mL of cold distilled water. Nitrogen in the residue was estimated by Kjeldahl method, yielding the insoluble protein fraction. Soluble protein (BSP) was calculated by subtracting the insoluble protein (BIP) from the total CP.

Non-protein nitrogen (NPN) using trichloroacetic acid

Ground dry sample (0.5 g) was weighed into a 125 mL Erlenmeyer flask and 50 mL of distilled water was added. The mixture was allowed to stand for 30 min. Then, 10 mL of 10 per cent trichloroacetic acid was added and left to stand for 20-30 min. The mixture was filtered on Whatman 54 paper by gravity, with the funnel covered to prevent evaporation. The residue was washed twice with trichloroacetic acid solution. The filter paper was transferred to digestion tubes to determine residual nitrogen. Non-protein nitrogen (NPN) was calculated by subtracting the residual nitrogen from the total nitrogen.

CNCPS carbohydrate and protein fractions

Equations as mentioned below were used to fractionate carbohydrates according to degradation rates as: CA - fast degrading (sugars and organic acid), CB1 - intermediate degrading (starch and pectins), CB2 - slow degrading (available cell wall), and CC – non-degrading (unavailable cell wall) (Sniffen *et al.*, 1992).

CHO (% DM) = 100- CP (% DM) - EE (% DM) – TA (% DM)

CC (% CHO) = 100 x [NDF (% DM) x 0.01 x Lignin (% NDF) x 2.4] / CHO (% DM)

CB2(% CHO) = 100 x [(NDF (% DM) – NDIP (% CP) x 0.01 x CP (% DM) - NDF (% DM) x 0.01 x Lignin (% NDF) x 2.4) / CHO (% DM)]

NSC (% CHO) = 100 - CB2(% CHO) - CC (% CHO)

CB1(% CHO) = [Starch (% NSC) x NSC (% CHO)] / 100

CA(% CHO) = [(100 - Starch (% NSC)) x NSC (% CHO)] / 100

Where, CHO – carbohydrate, NSC - non-structural carbohydrate, CP - crude protein, EE – ether extract, TA – total ash, NDF - neutral detergent fibre, NDIP - neutral detergent insoluble protein, DM – dry matter.

Feed protein was fractioned into three fractions: PA (NPN), PB (true protein), and PC (unavailable or bound true protein, non-degradable) using equations mentioned below (Sniffen *et al.*, add space 1992). The true protein fraction (PB) was further divided into three sub-fractions based on their inherent rates of ruminal degradation: PB1 – rapidly degrading, PB2 – intermediate degrading, and PB3 – slow degrading.

PA (% CP) = NPN (% SOLP) x 0.01 x SOLP (% CP)

PB1(% CP) = SOLP (% CP) - PA (% CP)

PC(% CP) = ADIP (% CP)

PB3(% CP) = NDIP (% CP) - ADIP (% CP)

PB2(% CP) = 100 - PA (% CP) - PB1(% CP) - PB3 (% CP) - PC (% CP)

Where, NPN - non-protein nitrogen, SOLP - soluble protein, ADIP - acid detergent insoluble protein, NDIP - neutral detergent insoluble protein

Data were analysed using one-way ANOVA for normally distributed variables and Kruskal-Wallis test for non-parametric data.

Result and discussion

The chemical composition of the fodders analysed in this study is presented in Table 1. From this initial compositional data, primary carbohydrate (CHO) and protein fractions were derived, as shown in Table 2 and Table 3. Based on these primary fractions, the CNCPS (Cornell Net Carbohydrate and Protein System) fractions of carbohydrates and proteins were calculated to offer a more refined understanding of the nutritional profiles of the fodders. These are presented in Table 4 detailing the CNCPS carbohydrate fractions, such as CA (soluble carbohydrates), CB1 (starch and pectins), CB2 (available fibre) and CC (unavailable fibre) and CNCPS protein fractions, including PA (NPN), PB1 (rapidly degraded true protein), PB2 (intermediately degraded true protein), PB3 (slowly degraded true protein) and PC (undegradable protein).The carbohydrate and protein fractions of Guinea grass, Hybrid Napier and paddy straw are discussed below to provide comprehensive insights into their nutritional composition.

Table 1. Chemical constituents of fodder, % DM

Nutrients	Guinea grass	Hybrid Napier	Paddy straw
DM (% as fed)	95.84 ± 1.28	95.19 ± 0.93	92.98 ± 0.44
OM	89.30 ± 1.54	92.31 ± 0.74	85.66 ± 0.84
CP	15.52 ± 2.01	17.33 ± 2.51	5.45 ± 0.44
EE	3.84 ± 0.41	4.78 ± 0.59	0.67 ± 0.13
Ash	10.70 ± 1.54	7.69 ± 0.74	14.34 ± 0.84
NDF	71.95 ± 0.84	69.07 ± 2.15	72.42 ± 0.74
ADF	39.97 ± 1.00	35.39 ± 1.53	40.79 ± 0.43
ADL	4.60 ± 0.60	3.92 ± 0.20	5.42 ± 0.21
HC	31.98 ± 1.61	33.68 ± 1.03	31.63 ± 0.96
Cellulose	35.37 ± 0.45	31.48 ± 1.33	35.37 ± 0.43

Mean ± SE of four samples in duplicate

Table 2. Primary carbohydrate fractions of fodder

Nutrient	Guinea grass	Hybrid Napier	Paddy straw
CHO (%DM)	69.94 ± 3.37	67.86 ± 4.17	79.55 ± 0.47
NSC (% DM)	7.61 ± 1.09	9.88 ± 1.34	10.05 ± 0.49
SC (% DM)	62.33 ± 2.34	58.96 ± 7.07	69.51 ± 0.57
NSC (% CHO)	10.78 ± 1.11	9.51 ± 2.02	12.63 ± 0.61
SC (% CHO)	89.22 ± 1.11	85.07 ± 3.46	87.37 ± 0.61
Starch (% DM)	1.43 ± 0.17	1.84 ± 0.14	2.34 ± 0.13
Starch (%CHO)	2.05 ± 0.21	2.68 ± 0.03	2.94 ± 0.16
Starch (% NSC)	19.58 ± 3.43	19.63 ± 3.70	23.77 ± 2.29
ADL (% NDF)	6.41 ± 0.90	5.67 ± 0.19	7.49 ± 0.28

Mean ± SE of four samples in duplicate

Table 3. Primary protein fractions of fodder

Nutrient	Guinea grass	Hybrid Napier	Paddy straw
TP (% DM)	11.56 ± 1.48	12.60 ± 1.16	4.24 ± 0.37
NPN (% DM)	3.96 ± 0.71	5.72 ± 1.36	1.37 ± 0.11
NPN (%CP)	25.35 ± 2.97	29.20 ± 3.43	25.07 ± 1.20
BIP (% DM)	11.44 ± 1.50	12.96 ± 1.40	3.72 ± 0.36
BSP (%DM)	4.08 ± 0.72	5.98 ± 1.34	1.73 ± 0.15
BSP (%CP)	26.15 ± 3.12	30.69 ± 3.13	32.00 ± 2.05
NPN (% SP)	97.01 ± 0.60	94.83 ± 1.82	75.84 ± 2.66
NDIP (% DM)	8.69 ± 9.62	8.69 ± 6.82	2.91 ± 0.28
NDIP (% CP)	59.36 ± 60.82	38.65 ± 40.28	53.26 ± 1.82
ADIP (% DM)	0.98 ± 0.95	0.84 ± 0.98	1.02 ± 0.05
ADIP (%CP)	6.26 ± 0.44	5.88 ± 0.72	18.98 ± 1.19

Mean ± SE of four samples in duplicate

Table 4. CNCPS carbohydrate and protein fractions of fodders

Fractions		Guinea grass	Hybrid Napier	Paddy straw	p value
Carbohydrate fractions	CA (%CHO)	8.74 ± 1.19	7.51 ± 1.39	9.69 ± 0.70	0.335
	CB1 (%CHO)	2.05^{b} ± 0.21	2.68^{a} ± 0.03	2.94^{a} ± 0.16	0.015
	CB2 (%CHO)	73.42^{ab} ± 2.59	78.16^{a} ± 2.07	71.01^{b} ± 0.71	0.021
	CC (%CHO)	15.80 ± 1.97	13.89 ± 0.35	16.37 ± 0.64	$0.069^{\#}$
Protein fractions	PA (%CP)	25.35 ± 2.97	29.20 ± 3.43	25.07 ± 1.20	0.433
	PB1 (%CP)	0.79^{b} ± 0.21	1.48^{b} ± 0.40	8.18^{a} ± 1.17	0.001
	PB2 (%CP)	7.02^{c} ± 1.75	30.21^{a} ± 1.39	14.76^{b} ± 1.48	<0.001
	PB3 (%CP)	54.56^{a} ± 4.94	34.41^{b} ± 2.33	33.37^{b} ± 2.65	0.003
	PC (%CP)	6.26^{a} ± 0.44	5.88^{a} ± 0.72	18.98^{b} ± 1.19	<0.001

Mean ± SE of four samples in duplicate

$^{\#}$Independent-Samples Kruskal-Wallis Test

Carbohydrates are essential for rumen fermentation, microbial protein synthesis, and energy supply. The total carbohydrate content ranged from 67.86 per cent to 79.55 per cent, with structural carbohydrates (SC) comprising 58.96 per cent to 69.51 per cent of dry matter (DM) and non-structural carbohydrates (NSC) ranging from 7.61 per cent to 10.05 per cent. These components play a crucial role in providing fermentable fibre for microbial growth. Among the carbohydrate fractions, soluble carbohydrates (CA) ranged from 7.51 per cent to 9.69 per cent (P=0.335), indicating comparable availability across fodders. The CB1 fraction, which includes starch and pectins, varied significantly (P=0.015), with Hybrid Napier (2.68%) and paddy straw (2.94%) exhibiting higher levels than Guinea grass (2.05%), suggesting their superior potential as energy sources. The CB2 fraction, indicative of available fibre, was dominant across all fodders, with Hybrid Napier (78.16%) showing the highest value and paddy straw (71.01%) the lowest (P=0.021). These findings are consistent with the reports of Gupta *et al*. (2011) and Das *et al*. (2015), who also highlighted the dominance of CB2 in Guinea grass, Hybrid Napier and paddy straw. The CC fraction, representing unavailable fibre, was highest in paddy straw (16.37%), though it did not differ significantly among the fodders (P=0.069). Patidar *et al.* (2022) reported a higher CC fraction of 21.03 per cent in paddy straw compared to that observed in this study.

Protein fractionation provides insight into nitrogen utilisation for microbial synthesis. The crude protein (CP) content ranged from 5.45 per cent to 17.33 per cent, with true protein (TP) forming the major portion and non-protein nitrogen (NPN) varying between 1.37 per cent and 5.72 per cent. The PA fraction, representing non-protein nitrogen (NPN), ranged from 25.07 to 29.20 per cent and showed no significant differences (P=0.433), suggesting comparable availability of rapidly degradable nitrogen across the fodders. However, significant variations were observed in the PB fractions. The PB1 fraction, representing rapidly degradable true protein, was highest in paddy straw (8.18%) compared to Hybrid Napier (1.48%) and Guinea grass (0.79%) (P=0.001), indicating its potential for quick nitrogen release. The PB2 fraction, which includes intermediately degradable protein, was highest in Hybrid Napier (30.21%) (P<0.001), supporting its role in prolonged microbial activity. The PB3 fraction, representing slowly degradable protein, was highest in Guinea grass (54.56%), significantly exceeding the values reported by Das *et al*. (2015) (28.1%) and Gupta *et al.* (2011) (25.64%), underscoring the potential of Guinea grass for sustained nitrogen availability. Conversely, the PC fraction, indicating undegradable protein, was highest in paddy straw (18.98%) (P<0.001), reinforcing its limited protein availability.

Conclusion

The study demonstrated that Guinea grass and Hybrid Napier provided higher levels of fermentable fibre and degradable protein, supporting efficient microbial fermentation and protein synthesis in the rumen. In contrast, paddy straw, though rich in carbohydrates, had a higher proportion of undegradable protein, limiting microbial nitrogen synthesis and affecting animal performance. These variations in nutrient composition underscore the importance of precise feed evaluation methods. The CNCPS fractionation approach provides a structured framework for assessing forage quality by categorising feed components based on their degradation characteristics, enabling accurate nutrient assessment and guiding the formulation of balanced rations to enhance rumen efficiency and livestock productivity.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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