# Comparison of protein and cell wall degradation of selected tropical and temperate roughages


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#### Abstract

Tropical roughages (cassava leaves, corn stover and rice straw) were evaluated as ruminant feeds in comparison to temperate roughages (alfalfa hay, brome grass and wheat straw). Comparisons were made using contrast procedures for comparable roughages, namely alfalfa hay vs. cassava leaves, brome grass vs, corn stover, and wheat straw vs. rice straw. Temperate roughages, as a group, were compared to tropical roughages. Nylon bag technique was used to determine protein and cell wall disappearance and their degradation characteristics. Samples were incubated in a cow rumen at 0, 6, 12, 18, 24, 48, or 72 hours.

The nutritive quality of cassava leaves were comparable to alfalfa hay, however, CP and lignin contents of cassava leaves were greater than alfalfa hay. Comparison of brome grass was similar to corn stover, with the exception of CP content of brome grass was much higher than corn stover. Comparison of wheat straw was similar to rice straw, however, both ash and protein content of wheat straw (7.0% and 3.6%) were lower than rice straw (21.8% and 7.1%), respectively. Protein disappearance and degradation characteristics were greater for alfalfa hay than cassava leaves, with exception of the potentially degradable fraction. Protein effective degradability of alfalfa hay (76.0%) was higher than cassava leaves (61.4%). Protein disappearance and degradation characteristics of brome grass were higher than corn stover. There was no significant different in protein disappearance according to incubation time between wheat and rice straw. However, soluble protein in wheat straw was higher than in rice straw, while potentially degradable protein was higher in rice straw than in wheat straw. Overall, the protein nylon bag measurements in temperate roughages were higher than in tropical roughages, with exception for potential degradation fraction. Roughages with higher arabinose : xylose ratio had neutral detergent fiber (NDF) and tended to have higher NDF degradation characteristic and effective degradability.


***Keywords:*** *degradation, roughages, rumen, temperate, tropical*

#### Introduction

In developing country, food supplies have expanded in parallel with population growth, while enhanced grain production has not resulted in grain becoming widely available for ruminant feed. In the dry season, natural pasture decreases in nutritive value and improved grasses cannot grow (Arendt et al 2016, Chu et al 2016). Therefore, it is important to find an alternative feeding system because purchased supplements are too expensive for poor farmers (Silivong and Preston 2015). Cattle and buffaloes are important on smallholder farms in most developing countries to provide meat, milk, traction power and manure in integrated crop and livestock farming systems (Preston and

Leng 2009). However, there has been increased availability of crop residues for animal feeding. The use of crop residues for feeding livestock assumes considerable importance in the overall farming system, surpassing that of grain in some circumstances (Cakir et al 2016). This situation has led to increased interest in the quality of crop residues. Notable among the potentially very useful energy crop residues in the tropic climates are rice straw (*Oriza sativa* ), corn stover (*Zea mays L*), and the protein source feedstuff, cassava leaves (*Ma nihot utilissima*). These crop residues are commonly used for the traditional ruminant feeding system in Indonesia. Because of the importance of these fibrous residues as ruminant feeds, there is considerable demand for efforts to determine their nutritive value (Chu et al 2016). Compared to common temperate roughages such as alfalfa hay, brome grass and wheat straw, very little research has been conducted on these tropical feedstuffs. The objective of this study was to provide basic comparisons (chemical compositions of neutral and acid detergent fiber, lignin, crude protein, ash and cell wall neutral sugars, as well as the rumen disappearance and degradation characteristics of crude protein and NDF) between tropical roughages and similar feeds commonly fed in temperate climates. These comparisons would provide additional information regarding the feeding value of tropical roughages.

## Materials and methods

#### Roughage samples and preparation

Twelve roughage samples consisted of tropical feeds including cassava leaves (*Manihot utilissima*), corn stover (*Zea mays L*) rice straw (*Oriza sativa*), and temperate feeds, alfalfa hay ( *Medicago sativa L*), brome grass hay (*Bromus inermis Leyss* ), and wheat straw (*Triticum vulgare*) were used in this experiment (two samples of each roughage). Tropical feeds were collected from location of crop research station areas of Sam Ratulangi University, North Sulawesi province of Indonesia, while temperate feeds were found from research station of the University of Saskatchewan, Canada. In preparation for analysis, samples were ground through a 1-mm screen. For nylon bag degradability, samples were ground through a 2-mm screen. All samples were dried at 60$^o$C prior to grinding.

#### Forage analysis

Dry matter was determined according to AOAC (1990). Protein content was determined by Kjeldhal method (AOAC 1990). Cell wall constituents were determined by methods of Goering and Van Soest (1970) modified by Van Soest and Robertson (1985). Cell wall neutral sugars were obtained by acid hydrolysis of the NDF fraction using gas chromatographic determination (Harris et al 1988).

#### Nylon bag degradation characteristic determination

Nylon bags (9 cm x 14 cm, Â± 43 Âµm pore size) were doubly stitched with nylon thread. The bags contained 20 mg of sample cm$^{-2}$ of bag surface area. The sample containing nylon bags presoaked with water (approximately 5 minutes) and then incubated in the cow rumen for 6, 12, 18, 24, 48, or 72 hours. There were two bags per sample per incubation time. Incubations were replicated two times (runs) on different days. The bags were inserted at different intervals and removed simultaneously. Once removed, the bags were washed thoroughly with gentle squeezing in tap water until the water was clear. Following washing, the bags were dried in forced oven at 60$^o$C until the weight was constant. To assess the washing loss, two additional samples from each of roughages were soaked and washed thoroughly with gentle squeezing in tap water until water was clear and dried at 60$^o$C. The equation of Orskov and McDonald (1979) was used to determine degradation characteristics (equation 1) by a direct non linear least square estimating procedure based on Marquardt’s method (SAS 1990). Effective degradability (ED) (equation 2) was calculated according to the equation of Orskov and McDonald (1979).

$p = a + b (1 – e^{-ct})$ (1)

$ED = a + bc / (c + k)$ (2)

a, b, and c are constant;

p = the proportion of degraded fraction at time t;

a = the extrapolated zero value taken as soluble fraction;

b = the insoluble but degradable fraction;

e = degradation rate constant;

k = rate of passage or rumen flow (assumed as 5% $h^{-1}$).

#### Statistical analysis

A completely randomized design was used to analyze the disappearance and degradation characteristics of CP and NDF of roughages (Steel and Torrie 1980, Byrkit 1987). The data for 12 roughage samples with two replicates were subjected to one-way analysis of variance using the General Linear Model (GLM) procedure of the Statistical Analysis Systems Institute, Inc. (SAS 1990). Comparisons were made using contrast procedures for comparable roughages, namely alfalfa hay vs cassava leaves (contrast A), brome grass vs corn stover (contrast B), and wheat straw vs rice straw (contrast C). Temperate crops, as a group, were compared to tropical crops (contrast D).

### Results

#### Roughage compositions

Compositions of the roughages were shown in Table 1. The average of NDF, ADF, and hemi cellulose contents of alfalfa hays were comparable to cassava leaves, however, cellulose, lignin and crude protein (CP) of cassava leaves were higher than alfalfa hays. Composition of brome grass was similar to corn stover, with the exception of CP content, brome grass (9.3%) was much higher than corn stover (4.7%). Comparison of wheat straw was similar to rice straw, however, both ash and protein content of wheat straw (7.0% and 3.6%) were lower than rice straw (21.8% and 7.1%), respectively.

**Table 1.** Chemical composition of roughages (Dry Matter basis g 100g $^{-1}$)

| Item[1] | Roughages[2] | | | | | |
|---|---|---|---|---|---|---|
| | Alfalfa hay | Cassava leaves | Brome grass | Corn stover | Wheat straw | Rice straw |

| | | | | | | |
|---|---|---|---|---|---|---|
| OM | 92.2 Â± 1.1 | 89.5 Â± 1.2 | 91.9 Â± 0.4 | 91.7 Â± 0.4 | 93.0 Â± 1.3 | 78.2 Â± 2.1 |
| NDF | 44.3 Â± 7.7 | 45.3 Â± 1.4 | 64.7 Â± 4.1 | 64.4 Â± 2.0 | 79.6 Â± 4.5 | 81.6 Â± 4.6 |
| ADF | 33.0 Â± 4.8 | 34.4 Â± 4.3 | 37.6 Â± 4.8 | 38.1 Â± 1.1 | 51.5 Â± 5.2 | 54.2 Â± 4.1 |
| Lignin | 7.2 Â± 1.5 | 14.1 Â± 3.8 | 3.8 Â± 0.3 | 2.9 Â± 0.4 | 5.9 Â± 1.4 | 5.5 Â± 0.2 |
| CP | 14.7 Â± 1.5 | 24.9 Â± 1.4 | 9.3 Â± 1.4 | 4.7 Â± 0.2 | 3.6 Â± 0.5 | 7.0 Â± 1.2 |

[1] *OM = organic matter, NDF = neutral detergent fibre, ADF = acid detergent fiber, CP = crude protein.*
[2] *Figures were average of two samples Â± standard deviation*

**The cell wall neutral sugars of roughages**

Quantitative differences in structural polysaccharides were indicated by the cell wall neutral sugar components expressed as proportion of the total neutral sugar (TNS) (Table 2). Glucose, the most predominant neutral sugar of the cell wall, and indicative of cellulose content, comprised 62.1 to 74.8% of TNS, followed by xylose (13.7 to 31.7%). Xylose concentration was considered indicative of hemicelluloses. The proportion of xylose in alfalfa hay was higher (19.6%) than in cassava leaves (13.6%). Brome grass contained similar xylose proportion (30.2%) to corn stover (31.4%), while xylose content in wheat straw (31.7%) was higher than in rice straw (27.7%).

**Table 2.** Total cell wall neutral sugar composition of roughages (g 100g $^{-1}$)

| **Item** | **Roughages** [1] | | | | | |
|---|---|---|---|---|---|---|
| | **Alfalfa hay** | **Cassava leaves** | **Brome grass** | **Corn stover** | **Wheat straw** | **Rice stra** |
| Glucose | 71.0 Â± 5.80 | 74.8 Â± 8.34 | 62.9 Â± 2.90 | 62.1 Â± 4.74 | 63.9 Â± 6.15 | 66.7 Â± |
| Xylose | 19.6 Â± 0.28 | 13.7 Â± 1.20 | 30.2 Â± 0.00 | 31.4 Â± 2.19 | 31.7 Â± 0.49 | 27.7 Â± |
| Arabinose | 3.9 Â± 0.57 | 3.2 Â± 0.28 | 5.3 Â± 0.49 | 5.0 Â± 0.21 | 3.9 Â± 0.07 | 4.1 Â± 0. |

| | | | | | | |
|---|---|---|---|---|---|---|
| Galactose | 2.8 Â± 0.42 | 5.4 Â± 0.14 | 1.5 Â± 0.07 | 1.5 Â± 0.07 | 0.5 Â± 0.07 | 1.6 Â± 0. |
| Mannose | 2.8 Â± 0.14 | 3.0 Â± 0.28 | - | - | - | - |
| Ara:xyl[2] | 0.2 Â± 0.50 | 0.2 Â± 0.00 | 0.18 Â± 0.02 | 0.16 Â± 0.01 | 0.15 Â± 0.00 | 0.12 Â± ( |

[1] *Figures were average of two samples Â± standard deviation*
[2] *Ara:xyl = arabinose:xylose ratio*

**Nylon bag disappearance and degradation characteristic parameters**

The nylon bag procedure provided measurement of sample disappearance over time, which may be interpreted in term of digestion kinetics.

**Protein**

Protein degradation characteristics of roughages were presented in Table 3. Alfalfa hay protein contained more soluble fraction (a) ($P<0.001$), less potentially degradable fraction (b) ($P<0.01$), and had a higher degradation rate (c) (P<001) than cassava leaves (Contrast A).

**Neutral detergent fiber (NDF)**

Neutral detergent fiber (cell wall) degradation characteristics were shown in Table 3. There was no significant difference in NDF soluble fraction (a), potentially degradable (b), potential degradability (a + b), degradation (c) in alfalfa hay and cassava leaves ($P>0.05$), however, NDF effective degradability (ED) in alfalfa hay was lower ($P<0.05$) than cassava leaves (Contrast A). There was no significant difference in NDF degradation characteristics of brome grass and corn stover ($P>0.05$; Contrast B). Effective degradability (ED) ($P<0.01$) and soluble fraction (a) ($P<0.01$) in wheat straw were significant lower than rice straw (Contrast C). There was no significant difference between temperate and tropical degradation characteristics, except for soluble fraction (a) and effective degradability (ED) (Contrast D).

The NDF degradation at 6-h and after 24-h fermentation in alfalfa hay were significantly lower than in cassava leaves (Contrast A). Brome grass NDF disappearances were significantly higher than corn stover both 6 and 72-h fermentation (Contrast B). NDF disappearance at 6 and after 24-h fermentation in wheat straw was significantly lower than in rice straw (Contrast C). NDF disappearance of temperate roughages was lower compared to tropical roughages at 6-h and after 24-h incubation period (Contrast D).

**Table 3.** Protein and neutral detergent degradation characteristics according to the equation p = a + b (1 –

| Degradation characteristics[1] | Roughages[2] | | | | | |
|---|---|---|---|---|---|---|
| | Alfalfa hay | Cassava | Brome | Corn | Wheat | Rice straw |

| | | leaves | grass | stover | straw | |
|---|---|---|---|---|---|---|
| **Protein:** | | | | | | |
| a (%) | 41.2 Â± 0.85 | 25.1 Â± 2.12 | 29.2 Â± 2.90 | 26.0 Â± 1.56 | 30.7 Â± 2.97 | 20.0 Â± 8.20 |
| B (%) | 49.7 Â± 2.97 | 59.9 Â± 5.09 | 55.5 Â± 6.65 | 49.8 Â± 5.66 | 26.0 Â± 9.26 | 41.8 Â± 19.9 |
| (a + b) (%) | 90.9 Â± 2.12 | 85.0 Â± 7.21 | 84.7 Â± 9.55 | 75.8 Â± 4.10 | 56.7 Â± 6.29 | 61.8 Â± 11.7 |
| C (% $h^{-1}$) | 11.9 Â± 3.39 | 8.1 Â± 1.77 | 7.0 Â± 0.78 | 3.0 Â± 0.28 | 5.6 Â± 2.26 | 4.8 Â± 1.84 |
| ED (%) | 76.0 Â± 4.10 | 61.4 Â± 8.34 | 61.1 Â± 5.09 | 44.1 Â± 0.00 | 42.6 Â± 2.97 | 39.2 Â± 2.90 |
| **Neutral detergent fiber (NDF):** | | | | | | |
| a (%) | -4.5 Â± 3.04 | -3.3 Â± 3.11 | -0.9 Â± 1.48 | -1.8 Â± 1.06 | -0.8 Â± 0.49 | 13.1 Â± 0.28 |
| B (%) | 52.0 Â± 9.40 | 62.1 Â± 3.68 | 83.2 Â± 10.5 | 80.8 Â± 0.64 | 66.1 Â± 2.26 | 55.6 Â± 0.14 |
| (a + b) (%) | 47.5 Â± 6.36 | 58.8 Â± 6.79 | 82.3 Â± 9.05 | 79.0 Â± 1.70 | 65.4 Â± 1.77 | 68.7 Â± 0.42 |
| C (% $h^{-1}$) | 6.4 Â± 0.85 | 7.4 Â± 1.20 | 3.4 Â± 0.28 | 3.3 Â± 0.14 | 3.0 Â± 0.21 | 3.0 Â± 0.85 |
| ED (%) | 24.2 Â± 3.96 | 32.5 Â± 7.85 | 31.3 Â± 4.10 | 29.1 Â± 1.98 | 22.3 Â± 0.14 | 33.1 Â± 4.60 |

[1] a = soluble fraction, b = potentially digestible fraction, (a + b) = potential degradation, c = degradation ra
(assumed as 5% h [-1]).

[2] Figures were average of two samples Â± standard deviation.

[3] SEM = standard error of the mean.

[4] Contrast A = alfalfa vs cassava leaves, Contrast B = brome grass hay vs corn stover, Contrast C = whea
roughages, ns = non significant (P>0.05), * = P<0.05, ** = P<0.01, *** = P<0.001.

## Discussion

### The cell wall neutral sugars of roughages

Arabinose was the third most abundant (3.2 to 5.3%) (Bailey 1973). Moreover, galactose ranged from 0.5 to 5.4% (Bailey 1973). Mannose was similarly low in alfalfa and cassava samples, and absent in graminaceous roughages. Finding of no mannose in grass agreed with Bailey (1973), but disagreed with Wedig et al (1983) and Buxton et al (1987) who concluded that mannose proportion in TNS was small, but greater in legume than grass stem. Arabinose:xylose ratio has been positively related to hemicelluloses polymer branching (Rui and Anderson 2016, Dumont et al 2015). A high arabinose:xylose ratio indicated a low degree of polymerization with much branching and inter-chain bonding. Arabinose:xylose ratio ranged from 0.12 to 0.24, this indicated differences between legumes and graminaceous roughages (Li et al 2015).

### Protein

Protein disappearance of roughages according to incubation times was shown in Figure 1. The differences resulted in the proportion of protein degraded at 6 to 48-h fermentation of alfalfa hay being higher in alfalfa hay than cassava leaves, however, as fermentation progressed to 72-h, the disappearance was not significantly different (Figure 1). These results agreed with Wright (1992) who reported that proportion of material degraded at 6-h incubation was more dependent on soluble fraction, while disappearance after 6 to 18-h depended on the balance between the soluble fraction and potentially degradable fractions. The disappearance after 24-h was mostly dependent on potential degradation and degradation rate (c). Due to protein degradation rate (c) in alfalfa hay was higher (11.9%) than cassava leaves (8.1% $h^{-1}$),this difference resulted in protein effective degradability (ED) for alfalfa hay was higher (76%) than cassava leaves (61.4%).

When brome grass and corn stover was compared (Contrast B), protein soluble fraction (a) (P<0.05), potential degradation (a + b) (P<0.05), and degradation rate (c) (P<0.01) in brome grass were higher than in corn stover. This resulted in the protein disappearance over all incubation periods and effective degradability (ED) (P<0.001) were greater in brome grass than in corn stover.

There was no significant difference in protein disappearance and degradation characteristics between wheat straw and rice straw, except for soluble fraction in wheat straw was higher than in rice straw (P<0.001), while rice straw contained more potentially degradable fraction (P<0.001). Comparing temperate and tropical roughages (Contrast D), soluble fraction (a) (P<0.001), potentially degradable fraction (b) (P<0.01), degradation rate (c) (P<0.01), and effective degradability (ED) of protein (P<0.001) in temperate roughages were higher than in tropical roughages. These results agreed with study by Thivierge et al (2016). The differences in structural as well as chemical characteristics of the protein of these roughages may have affected both solubility and the rate of protein degradation in the rumen.

#### Neutral detergent fiber (NDF)

The NDF disappearance according to incubation time was presented on Figure 2. Cell wall neutral sugar compositions of roughages may explain differences in NDF disappearance and degradation characteristics (Dumont et al 2015, Ding et al 2016). When comparing NDF (cell wall) disappearance of roughages (alfalfa hay vs. cassava leaves, brome grass vs. corn stover, wheat straw vs rice straw) according to incubation time and their degradation characteristics, roughages with a higher arabinose:xylose ratio had a higher NDF (cell wall) disappearance and tended to have a higher degradation characteristics. These results did not agree with some researchers (Buxton et al 1987) who found that branched xylans (as measured by arabinose:xylose ratio) had a negative on digestibility.

This discrepancy may be explained because the degree of branching or substitution of polysaccharide chains affected solubility by regulating the extent to which they can associate with other polysaccharides through hydrogen bonds (Hatfield 1989, Rachmilevitch et al 2015). Carpita and Gibeaut (1993) reported that side chain did not only prevent hydrogen bonding between linear xylans to cellulose, bur also rendered the side chains water soluble. McNeil et al (1975) reported that lower arabinose branching probably increased the propensity for formation of non-covalent bonds between xylose and cellulose. Such an association would increase the rigidity of cell walls, and might lower digestibility (Rui and Anderson 2016).

**Figure 1.** Protein disappearance of roughages according to incubation time

**Figure 2.** Neutral detergent fiber (NDF) disappearance of roughages according to incubation time

## Conclusion

- Nutritive qualities of tropical roughages were comparable to the temperate counterparts.
- Temperate roughages had higher protein degradability than tropical roughages.
- Arabinose:xylose ratio affecting cell wall solubility and degradability and separation of the cell wall constituents into their monosaccharides could explain the differences in digestibility between plant species.

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