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IN SITU DEGRADATION OF HEAT-TREATED PLANT PROTEIN LEAF MEALS TO EXTRAPOLATE RUMEN BYPASS POTENTIAL IN RUMEN - FISTULATED BRAHMAN CATTLE

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ABSTRACT: Many plant protein leaf meals (LM) remain unassessed for their rumen bypass potential following heat treatment. This study aimed to evaluate whether heat treatment could reduce ruminal degradability and improve bypass protein availability in five LMs—ipil-ipil (ILM), kakawate (KaLM), moringa (MLM), kudzu (KuLM), and gumamela (GLM). A total of 60 nylon bags (3 replicates per treatment) containing untreated or heat-treated LMs were incubated for 24 h in a rumen-fistulated Brahman bull using a completely randomized design. Heat treatment reduced mean crude protein degradability (CPD) by 7.6% (81.41% untreated vs. 73.77% treated; P = 0.006), with the largest reductions recorded in ILM (−23.9%), KuLM (−14.7%), and GLM (−6.8%). In contrast, dry matter degradability (DMD: 69.76% vs. 68.65%) and organic matter degradability (OMD: 74.44% vs. 70.56%) were not significantly affected. Among the LMs, moringa consistently exhibited the highest degradability (CPD = 98.27%, DMD = 94.98%, OMD = 97.00%), whereas kudzu had the lowest (CPD = 68.69%, DMD = 48.52%, OMD = 54.21%). Degradability trends were influenced by drought-resistance, botanical classification (legume vs. non-legume), and growth form (tree, shrub, creeping). Legume LMs were 16% less digestible in protein content than non-legumes, and creeping types were 19% less digestible than non-creeping types. Heat treatment further lowered CPD in both categories, indicating its capacity to enhance bypass protein potential. These findings suggest that targeted application of heat treatment, especially for ipil-ipil, kudzu, and gumamela, offers a practical strategy to improve protein utilization efficiency in ruminant feeding systems by increasing post-ruminal protein supply.

Keywords: Brahman cattle, Heat treatment, In situ degradation, Leaf meal, Rumen.

INTRODUCTION

The rumen is a complex environment inhabited by different microbial species having different nutrient requirements and fermentative processes. In the process of fermentation, the majority of the dietary protein that the rumen microorganisms consume must be transformed into peptides that release amino acids and ammonia (NH_3) required for microbial protein synthesis (Owens and Basalan 2016). Protozoal species, which require 100% amino acids (Niepes et al., 2023) and bacteria, which require both amino acids (approximately 25%) and ammonia (approximately 75%), both use amino acids to synthesize microbial cells (Patiga et al., 2020b). As digesta flows down, these microbes will eventually give their lives to the host animal as a source of amino acids through enzymatic digestion in the abomasum and intestines. Stern et al., (2006) reported that microbial protein synthesized in the rumen accounts for 50 to 80 % of the total absorbable proteins supplied in the small intestine of the ruminants. As protozoa and bacteria are being digested in the abomasum, the amino acids produced are absorbed through the intestinal wall and carried by the blood to the different body cells, which are then synthesized into new proteins in the body (e.g., muscles, hormones, and others).

Microbial cells become the source of amino acids digested and absorbed in the abomasum and intestines, but this may be inadequate when dealing with highly productive animals such as growers, fatteners, and dairy. High-yielding animals require a higher proportion of nutrients in the diet that bypass the rumen without degradation to supply the 20 different amino acids at the intestinal level (Singh et al., 2019). Some methods of protecting the dietary protein to escape rumen fermentation have been tested to reduce the microbial degradation of protein supplements in the rumen, such as chemical treatment with tannic acid, chemical treatment with formaldehyde and heat treatment (Arisya et al., 2019; Thakur et al., 2024). In addition, the use of spent sulphite liquor and pelleting with calcium lignosulfonate has been found

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by others to be effective (Atole, 2011). However, the most common and safest way is heat treatment in the light of practical application among farmers in the humid tropics.

The high cost of protein supplements necessitates treatment methods that increase the amount of protein (Patiga et al., 2020b and 2024) undegraded in the rumen and, therefore, increase the amount of protein of high post-ruminal degradability. However, many protein sources of plant-origin grains have not been tested for their bypass potential and whether heat treatment is effective enough to protect their protein from rumen degradation. Hence, this study will assess the effectiveness of heat treatment in reducing protein degradation in the rumen of plant protein concentrates and leaf meals as a basis for extrapolating their potential as bypass protein sources using rumen-fistulated Brahman cattle.

MATERIALS AND METHODS

Preparation of the experimental animal and area

A Brahman bull fitted with a rumen cannula (Bar Diamond Lane, Parma, ID, U.S.A.) from the Beef Cattle Project of the Department of Animal Science (DAS) was used in this experiment. The experimental animal was confined and observed for its health condition, appetite, behavior and live weight prior to the experiment. The experimental animal was injected with Ivermectin at 1 mL/50 kg BW subcutaneously to prevent internal and external parasites. The experimental area was thoroughly cleaned and disinfected a week prior to the conduct of the experiment. Likewise, the feed bin and water troughs were checked, repaired, and disinfected to secure the animal's cleanliness, comfort, health, and safety.

Preparation of the basal diet and test leaf meals

The basal diet for the experimental animal was fresh Napier grass that was chopped at about 3-4 inches (7.62 – 10.16 cm) long before feeding (Patiga et al., 2020a and Patiga et al., 2024). The test plant protein leaf meals consisted of ipil-ipil leaf meal, kakawate leaf meal, moringa leaf meal, kudzu leaf meal, and gumamela leaf meal and were collected fresh at Brgy. Canila Biliran, Biliran, sun-dried until it became crispy and was processed into leaf meal using the hammer milled at the Department of Animal Science, Visayas State University, Visca, Baybay City, Leyte. The experiment followed a completely randomized design (CRD) with three replicates and a one-way analysis of variance treatment design in one uniform rumen environment of a rumen-fistulated Brahman bull for 24 hours as shown in Table 1.

Table 1 - Treatment design for the basal diet and test leaf meals.

T_1	Without heat treatment, Ipil-ipil (*Leucaena leucocephala* Lam.) leaf meal
T_2	Without heat treatment, Kakawate (*Gliricidia sepium* Jacq.) leaf meal
T_3	Without heat treatment, Moringa (*Moringa oleifera* L.) leaf meal
T_4	Without heat treatment, Kudzu (*Pueraria phaseoloides* Roxb.) leaf meal
T_5	Without heat treatment, Gumamela (*Hibiscus* sp.) leaf meal
T_6	Heat-treated Ipil-ipil (*Leucaena leucocephala* Lam.) leaf meal
T_7	Heat-treated Kakawate (*Gliricidia sepium* Jacq.) leaf meal
T_8	Heat-treated Moringa (*Moringa oleifera* L) leaf meal
T_9	Heat-treated Kudzu (*Pueraria phaseoloides* Roxb.) leaf meal
T_{10}	Heat-treated Gumamela (*Hibiscus* sp.) leaf meal

Heat treatment of plant protein concentrates

Heat treatment of plant protein concentrates was accomplished by spreading samples in a tray at about 1-inch-thick and was placed in a forced-draft oven set at 149 °C for four hours (Glimp et al., 1967). The concentrates were then cooled down and consequently placed in the nylon bags.

Feeding of experimental cattle

The experimental animal was fed twice a day (8:00 AM and 4:00 PM) of a ration comprising 70% basal diet of Napier grass (*Pennisetum purpureum*) and 30% of the test protein concentrates in equal proportion (6% each) based on DM requirement of the cattle. Feeding was done *ad libitum*. Drinking water was also offered free choice.

Rumen incubation of unheated and heat-treated protein concentrates

The nylon bag technique estimates ruminal degradation of the plant protein concentrates. The nylon bags measuring 5 x 10 cm each were oven-dried at 65 °C for 30 minutes and their empty weights were measured immediately (Osuji et al., 1993). The nylon bags used had a pore size of ±53 microns, enough for rumen microbes to enter but small enough for

feed samples to escape from the inside (Ørskov et al., 1979; Wirayudha et al., 2022). Approximately 5g of dry weight for each dietary treatment (untreated and heat-treated concentrates) was placed inside the nylon bags and heat-sealed. These were then placed inside a lingerie bag with stainless steel pieces to prevent the nylon bags from floating on top of the rumen fluid once placed as presented in Figure 1, which may give variable degradation rates (Preston, 1986; Patiga et al., 2020.

After 24 hours of incubation, all the nylon bags were harvested and washed immediately under running water with gentle rubbing of the thumb and fingers until the water runs clear. Washed nylon bags were oven-dried at 60 °C for 48 hours and then weighed immediately using a digital weighing scale (Atole, 2011). The dry matter (DM) content of the residue was determined by subtracting the weight of the nylon bag from the gross weight of the oven-dried residue plus the nylon. The DM degradation was calculated by using this formula:

$$\text{DMD \%} = \frac{(W1 - W2)}{W1} \times 100$$

Where W1 = weight of dried sample before incubation, g; W2 = weight of dried sample after incubation, g

***In situ* degradation trial**

Day 1 – 8 adjustment period

Feeding the cattle with a 70:30 ratio diet of basal and test leaf meals was done at an amount equivalent to about 2 % body weight dry matter (DM) intake. Feeding was done twice a day with access to clean drinking water at all times.

Day 9 – 10 feed reduction period

Two days before incubation of the test diets, the amount of feed offered was reduced by half to provide space to accommodate the nylon bags with the test concentrates without adversely affecting the normal rumen function and make the recovery of bags easier (Bestil et al., 2014).

Day 11 incubation period

The bags containing feed samples of approximately 5 grams, with three samples per bag, were incubated for 24 hours. A total of 30 bags were placed inside the rumen for this study.

Day 12 recovery period

After incubation of feed samples, harvesting of the nylon bags was done. The nylon bags with feed samples were then washed under running tap water with gentle rubbing with the thumb and fingers until the water runs clear.

Data gathered

1. Percent dry matter degradation (DMD)

$$\text{DMD (\%)} = \frac{(DM1 - DM2)}{DM1} \times 100$$

Where DM1 = Dry matter weight of dried sample before incubation, g; DM2 = Dry matter weight of dried sample after incubation, g.

2. Percent organic matter degradation (OMD)

$$\text{OMD (\%)} = \frac{(OM1 - OM2)}{(OM2)} \times 100$$

Where OM1 = Organic matter weight of feed sample before incubation, g; OM2 = Organic matter weight of the residue (after incubation), g.

3. Percent crude protein degradation (CPD)

$$\text{CPD (\%)} = \frac{(CP1 - CP2)}{(CP1)} \times 100$$

Where CP1 = CP of feed sample before incubation (g); CP2 = CP of feed sample after incubation (g)

Statistical analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) using the PROC MIXED procedures of Statistical Analysis System (SAS) Version 9. Differences among treatment means were compared using group comparisons and declared significant when $P<0.05$ using adjusted Tukey's Honest Significant Difference (HSD).

RESULTS AND DISCUSSION

Nutrient analysis of plant protein leaf meals

Plant protein leaf meals untreated or heat-treated were measured to assess their potential as a bypass protein source by assessing the extent of protection from rumen degradation in fistulated cattle. Contents of dry matter (DM), organic matter, ash (minerals), and crude protein in leaf meals tested were analyzed and the results are presented in Table 2. Dry matter content among feedstuffs shows that ipil-ipil leaf meal has a high percentage of DM at 93%, and the lowest was gumamela leaf meals at 87%. For percent organic matter, ipil-ipil leaf meals at 91 % and has reverse results in ash, showing that ipil-ipil leaf meals have the lowest value of 9%. Moreover, Table 2 presents high CP in kudzu leaf

meal 27% and lowest CP in gumamela leaf meal at 12%. While the difference in crude protein content between untreated treatment and heat treatment may not be significant, there seemed to be a pattern of a slight reduction in crude protein content among the heat treatment than those untreated.

Table 2 - Nutrient analysis of the test leaf meal diets before incubation.

Treatment \ Nutrient analysis	%DM	%OM	% ASH	%CP
T_1	93.01	85.32	14.68	24.84
T_2	89.30	87.82	12.18	20.98
T_3	89.86	89.09	10.91	26.43
T_4	88.36	86.59	13.41	27.39
T_5	87.44	83.98	16.02	14.00
T_6	92.13	91.19	8.81	23.22
T_7	90.37	89.58	10.42	20.78
T_8	91.97	88.82	11.18	26.07
T_9	90.66	89.94	10.06	27.18
T_{10}	91.02	85.05	14.95	12.30

***In situ* degradation of plant protein leaf meals**

The degradation of dry matter, organic matter and crude protein of leaf meals are presented in Table 3. Analysis of variance across the plant protein leaf meals untreated or heat-treated showed significant differences in dry matter degradation (DMD), organic matter degradation (OMD) and crude protein degradation (CPD). Moringa leaf meal is highest in DMD and the lowest is ipil-ipil leaf meal and kudzu leaf meal significant at P<0.0001. Organic matter degradation (OMD) has the highest result in moringa leaf meal and lowest in kudzu leaf meal significant at P<0.0001. Crude protein degradation (CPD) has the highest result in moringa leaf meal, lowest in ipil-ipil leaf meal and kudzu leaf meal significant at P<0.0001. Among plant protein leaf meals tested, heat treatment appeared to be effective in protecting the protein in ipil-ipil leaf meal, kudzu leaf meal and gumamela leaf meal. Other mechanisms, such as a Browning reaction in the presence of reducing sugars in the leaf material, may also be in play. Further, heat application might increasingly protect forage leaf meals as apparently occurs when some meal is dehydrated at a temperature increasing from 65°C to 160°C (Zahari et al., 2006).

Table 3 - *In situ* degradation of dry matter, organic matter and crude protein of leaf meals.

Treatment \ Chemical analysis	DMD,%	OMD,%	CPD,%
T_1	53.25^{f}	57.40^{g}	75.30^{e}
T_2	63.76^{e}	68.38^{e}	70.51^{f}
T_3	94.98^{a}	97.00^{a}	98.27^{a}
T_4	48.52^{g}	54.21^{h}	68.69^{g}
T_5	88.55^{c}	95.23^{c}	94.28^{bc}
T_6	52.68^{f}	54.90^{h}	57.35^{i}
T_7	63.36^{ef}	65.01^{f}	70.18^{f}
T_8	93.88^{b}	96.61^{b}	94.80^{b}
T_9	46.31^{h}	51.96^{i}	58.60^{h}
T_{10}	87.04^{d}	84.34^{d}	87.91^{d}
SEM	1.7880	2.7631	2.6194
P-Value	0.0001***	0.0001***	0.0001***

Legend: DMD = Dry Matter Degradation; OMD = Organic Matter Degradation; CPD = Crude Protein Degradation

Comparison of protein leaf meals on dry matter degradation

Group comparison of protein leaf meals on DMD is presented in Table 4. Dry matter degradation was significantly different across groupings for leaf meal samples except for L_1 (untreated leaf meal vs heat treated leaf meal). For L_2 and L_3 groupings on non-drought resistant leaf meal (ipil-ipil, moringa, kudzu and gumamela) shows a higher percentage of dry matter degradation (DMD) than drought-resistant leaf meal (kakawate) in both untreated and heat-treated leaf meals samples ranging 70% to 71% resulting for drought-resistant leaf meal as a potential in reducing degradability in the rumen at 63 % -64%. On the other hand, L_4 and L_5 legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) for both

unheated and heated leaf meal samples have a lower percent DMD ranging from 64% - 65% had, reduce the outflow of dry matter from the rumen compared to non-legume leaf meal (gumamela) ranging at 87% - 89% DMD. Furthermore, L_6 and L_7 groupings for both untreated and treated leaf meals shown that shrub type leaf meal (moringa and gumamela) has a higher DMD at 90% - 92%, while tree type leaf meal (ipil-ipil and kakawate) a lower DMD ranging at 58% - 59% and therefore could bypass the dry matter at ruminal level. Lastly L_8 and L_9 for both untreated and treated leaf meals non-creeping type of leaf meal (ipil-ipil, kakawate, moringa, and gumamela) has higher DMD ranging at 74%-75% compared to creeping type of leaf meal (kudzu) at 46% - 48% DMD. Therefore, results with lower DMD could be a potential source of bypass.

Table 4 - Group comparison of plant protein leaf meals on *in situ* dry matter degradability.

Contrast	Group 1	Mean %	Group 2	Mean %	p-value	SEM
L_1 - UT Vs HT	T_1, T_2, T_3, T_4, T_5	69.76	$T_6, T_7, T_8, T_9, T_{10}$	68.65	0.6619^{ns}	0.68
L_2 - UT Drought Resistant Leaf Meal VS UT Non Drought Resistant Leaf Meals	T_2	63.76	T_1, T_3, T_4, T_5	71.26	0.0001***	0.74
L_3 - HT Drought Resistant Leaf Meal VS HT Non-Drought Resistant Leaf Meal	T_7	63.36	T_6, T_8, T_9, T_{10}	69.98	0.0001***	1.23
L_4 - UT Legume Leaf Meal VS UT Non Legume Leaf Meal	T_1, T_2, T_3, T_4	65.06	T_5	88.55	0.0001***	0.58
L_5 – HT Legume Leaf Meal VS HT Non Legume Leaf Meal	T_6, T_7, T_8, T_9	64.06	T_{10}	87.04	0.0001***	0.73
L_6 - UT Tree Type Leaf Meal VS UT Shrub Type Leaf Meal	T_1, T_2	58.51	T_3, T_5	91.77	0.0001***	1.23
L_7 - HT Tree Type Leaf Meal VS HT Shrub Type Leaf Meal	T_6, T_7	58.02	T8, T_{10}	90.46	0.0001***	0.59
L_8 - UT Creeping Type Leaf Meal VS UT Non-Creeping Type Leaf Meal	T_4	48.25	T_1, T_2, T_3, T_5	75.14	0.0001***	0.74
L_9 - HT Creeping Type Leaf Meal VS HT Non-Creeping Type Leaf Meal	T_9	46.31	T_6, T_7, T_8, T_{10}	74.24	0.0001***	1.23

UT = Untreated; **HT** = Heat treated

Group comparison of protein leaf meals on organic matter degradation

Group comparison of protein leaf meals on OMD is presented in Table 5. Organic matter degradation (OMD) is significantly different across groupings for leaf meal samples except for L_1 (untreated leaf meal vs heat treated leaf meal). For L_2 and L_3 groupings on non-drought resistant leaf meal (ipil-ipil, moringa, kudzu and gumamela) showing high percentage of OMD than drought-resistant leaf meal (kakawate) in both untreated and heat-treated leaf meals samples ranging 72% to 76% resulting for drought-resistant leaf meal as a potential in reducing organic matter degradability in the rumen at 65% - 68%. L_4 untreated legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) samples have a lower percent OMD at 69% had reduced organic matter's outflow from the rumen compared to untreated non-legume leaf meal (gumamela) at 95% OMD. Also, L_5 groupings for treated legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) samples still have a lower percent OMD at 67% had, reduced the outflow of samples from the rumen compared to treated non- legume leaf meal (gumamela) at 84% OMD. Furthermore, L_6 and L_7 groupings for both untreated and treated leaf meals shown that shrub type leaf meal (moringa and gumamela) has a higher OMD resulting 90% - 96% while tree type leaf meal (ipil-ipil and kakawate) a lower OMD ranging at 60% - 63% and therefore could be a good bypass of organic matter at the ruminal level. Moreover, L_8 and L_9 for both untreated and treated leaf meals non-creeping type of leaf meal (ipil-ipil, kakawate, moringa, and gumamela) has higher OMD ranging at 75%-80% compared to creeping type of leaf meal (kudzu) at 52% - 54% OMD. Therefore, OMD with a lower value could be a potential source of the bypass from the ruminal level and may increase the organic matter to the intestinal level.

Group comparison of protein leaf meals on crude protein degradation

Group comparison of protein leaf meals on CPD is presented in Table 6. The table presented that CPD was significantly different across all groupings for leaf meal samples. L_1 group for untreated leaf meals had high CPD at 81% compared to heat-treated leaf meals at 74%. This confirms the report of Aguilera et al. (1992) that heating decreases protein solubility and renders it more resistant to ruminal degradation. For L_2 and L_3 groupings on non-drought resistant leaf meals (ipil-ipil, moringa, kudzu and gumamela) showing high percentage of CPD in both untreated and heat-treated leaf meals samples ranging 75% to 84% than drought-resistant leaf meal (kakawate) ranging at 70% - 71% CPD this result shows potential in reducing protein degradability in the rumen. Some range plants, which are drought-tolerant environmental, can be used as protein supplements to straws for ruminants (Al-masri, 2007). L_4 untreated legume leaf meal samples (ipil-ipil, kakawate, moringa and kudzu) have a lower percent CPD at 78% reduced organic matter's outflow

from the rumen compared to untreated non-legume leaf meal (gumamela) at 94% CPD. Also, L_5 groupings for treated legume leaf meal (ipil-ipil, kakawate, moringa and kudzu) samples still have a lower percent CPD at 70% had reduce the outflow of samples in the ruminal level compared to treated non- legume leaf meal (gumamela) at 88% CPD. Furthermore, L_6 and L_7 groupings for both untreated and treated leaf meals showed that shrub type leaf meal (moringa and gumamela) has a higher CPD resulting in 91% - 96%, while tree type leaf meal (ipil-ipil and kakawate) a lower CPD ranging at 64% - 73% and therefore could be a good bypass of crude protein at the ruminal level. Moreover, L_8 and L_9 for both untreated and treated leaf meals non-creeping type of leaf meal (ipil-ipil, kakawate, moringa, and gumamela) have higher CPD ranging at 78%-85% compared to creeping type of leaf meal (kudzu) at 59% - 69% CPD. Therefore, CPD with lower value could be a potential source of the bypass from the ruminal level and may increase the crude protein to the intestinal level.

Table 5 - Group comparison of plant protein leaf meals on *in situ* organic matter degradability.

Contrast	Group 1	Mean %	Group 2	Mean %	P-value	SEM
L_1 - UT Vs HT	T_1, T_2, T_3, T_4, T_5	74.44	$T_6, T_7, T_8, T_9, T_{10}$	70.56	0.7881^{ns}	0.07
L_2 - UT Drought Resistant Leaf Meal Vs UT Non Drought Resistant Leaf Meals	T_2	68.38	T_1, T_3, T_4, T_5	75.96	0.0001***	0.68
L_3 - HT Drought Resistant Leaf Meal Vs HT Non-Drought Resistant Leaf Meal	T_7	65.01	T_6, T_8, T_9, T_{10}	71.95	0.0001***	0.74
L_4 - UT Legume Leaf Meal Vs UT Non Legume Leaf Meal	T_1, T_2, T_3, T_4	69.25	T_5	95.23	0.0001***	1.23
L_5 – HT Legume Leaf Meal Vs HT Non Legume Leaf Meal	T_6, T_7, T_8, T_9	67.12	T_{10}	84.34	0.0001***	0.58
L_6 - UT Tree Type Leaf Meal Vs UT Shrub Type Leaf Meal	T_1, T_2	62.89	T_3, T_5	96.12	0.0024**	0.73
L_7 - HT Tree Type Leaf Meal Vs HT Shrub Type Leaf Meal	T_6, T_7	59.96	T8, T_{10}	90.48	0.0001***	1.23
L_8 - UT Creeping Type Leaf Meal Vs UT Non-Creeping Type Leaf Meal	T_4	54.21	T_1, T_2, T_3, T_5	79.50	0.0001***	0.59
L_9 - HT Creeping Type Leaf Meal Vs HT Non-Creeping Type Leaf Meal	T_9	51.96	T_6, T_7, T_8, T_{10}	75.22	0.0001***	0.74

UT = Untreated; **HT** = Heat treated

Table 6 - Group comparison of plant protein leaf meals on *in situ* crude protein degradability.

Contrast	Group 1	Mean %	Group 2	Mean %	P-value	SEM
L_1 - UT Vs HT	T_1, T_2, T_3, T_4, T_5	81.41	$T_6, T_7, T_8, T_9, T_{10}$	73.77	0.0060^{ns}	0.45
L_2 – UT Drought Resistant Leaf Meal Vs UT Non Drought Resistant Leaf Meals	T_2	70.51	T_1, T_3, T_4, T_5	84.14	0.0034**	1.02
L_3 - HT Drought Resistant Leaf Meal Vs HT Non-Drought Resistant Leaf Meal	T_7	70.18	T_6, T_8, T_9, T_{10}	74.67	0.0001***	2.05
L_4 - UT Legume Leaf Meal Vs UT Non Legume Leaf Meal	T_1, T_2, T_3, T_4	78.19	T_5	94.28	0.0001***	3.86
L_5 - HT Legume Leaf Meal Vs HT Non Legume Leaf Meal	T_6, T_7, T_8, T_9	70.23	T_{10}	87.91	0.0001***	2.24
L_6 - UT Tree Type Leaf Meal Vs UT Shrub Type Leaf Meal	T_1, T_2	72.91	T_3, T_5	96.28	0.0002***	1.20
L_7 - HT Tree Type Leaf Meal Vs HT Shrub Type Leaf Meal	T_6, T_7	63.77	T8, T_{10}	91.36	0.0003***	1.01
L_8 - UT Creeping Type Leaf Meal Vs UT Non-Creeping Type Leaf Meal	T_4	68.69	T_1, T_2, T_3, T_5	84.59	0.0001***	0.69
L_9 - HT Creeping Type Leaf Meal Vs HT Non-Creeping Type Leaf Meal	T_9	58.60	T_6, T_7, T_8, T_{10}	77.56	0.0001***	1.13

UT = Untreated; **HT** = Heat treated

CONCLUSION AND RECOMMENDATION

While heat treatment had no effect on dry matter and organic matter degradability, it did considerably lower crude protein degradability—by up to 24% in ipil-ipil and 15% in kudzu—implying increased potential for post-ruminal protein supply. The inherent poorer digestibility of legume and creeping-type leaf meals suggests a structural or chemical resistance to rumen decomposition, which heat treatment accentuates. These studies demonstrate the combined effect of botanical kind and processing method on optimizing bypass protein sources for bovine diets.

Among protein-rich leaf meals, heat treatment has been observed to reduce protein digestibility, particularly in ipil-ipil (*Leucaena leucocephala*), kudzu, and gumamela leaf meals. Therefore, further studies on the potential of these plant-based protein sources as bypass protein are recommended. Specifically, different categories of leaf meals such as drought-resistant species, legumes, and those classified as tree, shrub, or creeping types should be thoroughly investigated. Evaluating these factors in relation to rumen bypass characteristics and their effects on cattle performance will contribute valuable knowledge to the scientific community and support the development of more efficient feeding strategies.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical considerations

This study adhered to the Philippines' Animal Welfare Act, which ensured the humane treatment of rumen-fistulated Brahman cattle through suitable housing, feeding, veterinary care, and gentle handling. Approval was obtained on March 19, 2019, under the IACUC code MHAM-0219-119-01.

Authors' contribution

Cindy Cortez managed fund acquisition, experimentation, data collection, conceptualization, analysis, and drafting; Lolito C. Bestil supervised methodology and revisions; Manuel D. Gacutan Jr. oversaw data collection, analysis, and writing assistance; Marisel A. Leorna handled literature review, interpretation, and proofreading; Elmar Patiga conducted experimentation, data collection, figure preparation, writing, editing, and proofreading of the manuscript.

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Competing interests

The authors declare no competing interests in this research and publication.

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