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Potential of Combined Yeast Culture and Enzymatically Hydrolysed Yeast to Improve In Vitro Dry Matter and Nutrient Degradability of Different Feedstuffs

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Simple Summary: Enzymatically hydrolysed yeast is a novel form of yeast culture with positive effects as prebiotics used to improve gastrointestinal health in ruminants. However, their effect on ruminal nutrient digestion is unclear. This study evaluated the effects of a combined yeast culture and enzymatically hydrolysed yeast on dry matter, fibre, and crude protein ruminal digestibility in vitro. Seven different substrates inclusive of legume and grass forages and commercial supplemental feedstuffs were incubated for 24 and 48 h, with or without enzymatically hydrolysed yeast, and again for 0, 2, 4, 8, 16, 24, and 48 h to determine crude protein degradability. Enzymatically hydrolysed yeast improved the fibre digestion of *Brachiaria arrecta* by 32%, while the dry matter and crude protein digestibility in soybean meal and *Glircidia sepium* were reduced by 16.2% and 38.5%, respectively, after 24 h of incubation. Therefore, enzymatically hydrolysed yeast has potential to improve ruminal fibre digestibility and modify the crude protein degradation of different substrates, which may contribute to the improved utilization of fibrous feedstuffs and efficiency in nitrogen and crude protein metabolism in ruminants.

Abstract: Live yeast cultures have been a popular additive in ruminant feeds to improve fermentation efficiency, rumen, and intestinal health. However, very little is known about inactive yeast culture and hydrolysable yeast cells on nutrient digestibility in ruminants. Therefore, this study was conducted to determine the effects of a combined yeast culture and enzymatically hydrolysed yeast (YC+EHY) on in vitro dry matter and nutrient digestibility. Seven chemically contrasting substrates, including the leaves and petiole of forage plants (*Trichanthera gigantea*, *Gliricidia sepium*, *Leucaena leucocephala*, and *Brachiaria arrecta*), agriculture by-products (soybean meal and rice hulls), and a commercial concentrate feed, were incubated in vitro with and without YC+EHY to determine dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) digestibility after 24 and 48 h of incubation. A second experiment evaluated in vitro CP degradability by incubating substrates for 0, 2, 4, 8, 16, 24, and 48 h with and without YC+EHY. Incubation with YC+EHY reduced 24 h DM and CP digestibility in soybean meal and *G. sepium* by 16.2% and 38.5%, respectively. Conversely, the ADF digestibility of *B. arrecta* incubated with YC+EHY increased by 32%. In vitro ruminal DM and nutrient digestibility were unaffected by YC+EHY after 48 h of incubation. The rate of CP degradability in the commercial concentrate and rice hull inoculated with YC+EHY increased sharply between 16 and 24 h post-incubation and generally plateaued afterwards. Similarly, YC+EHY significantly increased CP degradability in *L. leucocephala* after 8 and 16 h of incubation. The 16 h CP degradation in *T. gigantea* without YC+EHY was significantly higher. It was therefore concluded that YC+EHY has potential to improve ruminal ADF digestibility and modify ruminal CP degradation dependent on the type of substrate.

Keywords: common feedstuffs; crude protein degradability; hydrolysable yeast; ruminal fibre digestibility; tropical forages

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1. Introduction

Traditionally, dairy cows in tropical environments are managed in rotational grazing systems [1,2] and offered tree forages, agro-industrial by-products, or commercial concentrates to supplement graze pastures [3]. Tropical graze pastures vary in their nutritional profile and are generally poorly digested by ruminants. For example, the quality and nutritive value of tropical grasses vary with the stage of regrowth, species, and season [4]. Unlike temperate regions, dry matter digestibility and the rate of ruminal degradation of tropical grasses are lower at similar stages of regrowth [4]. Low digestibility limits the supply of energy and nutrients to the animal, which negatively affects productivity. While supplementing tropical grasses with inexpensive tree forages and agricultural by-products can improve digestibility, low availability limits their use by farmers. Further, some tree forages contain anti-nutritive compounds like tannins and saponins that negatively affect ruminal digestion directly or indirectly [5]. Therefore, commercial concentrates are the most common supplemental feedstuff used by dairy farmers in the Caribbean because they are readily available, conveniently packaged, easy to use, and have no antinutritional properties. However, the amount farmers use is limited because concentrate feeds are extremely expensive. This implies that other approaches might be necessary to improve digestive efficiency and feed utilization on dairy farms.

Several reports suggested that feed additives derived from live or inactive yeast cultures can improve digestibility in ruminants [6,7]. The current use of concentrate feeds offers the opportunity for the inclusion of additives into feeding systems for dairy cows, providing that the added benefits are clear and cost-effective. Some yeast cultures can produce useful compounds such as glucose during ruminal fermentation, which improved intake and nutrient digestibility in ruminants [8]. Similarly, other reports documented improvements in ruminal fermentation, the activity of cellulolytic microbes, and increase milk yield with the inclusion of yeast cultures [9,10]. As a result, yeast cultures, especially active yeast, have rapidly gained popularity.

However, very little is known about yeast cultures combined with enzymatically hydrolysable yeast (YC+EHY), a unique approach used to exploit the additional benefits of yeast cultures [11,12]. The production of enzymatically hydrolysed yeast involves the use of specific enzymes to produce redefined functional carbohydrates like mannooligosaccharides (MOS), D-mannose, and β-glucans from the cell wall of *Saccharomyces cerevisiae* [13,14]. These unique carbohydrates are popular components of prebiotics used in animal feed to improve gastrointestinal health [15]. Apart from a few studies that have demonstrated some benefits like methane reduction and reduced microbial colonization time in some substrates [16], increased dry matter intake, average daily gain, rumen fermentation profile, and microbial population from YC+EHY inclusion in feeds for ruminants [12,17], little is known about how YC+EHY affects nutrient digestion in the rumen. Therefore, the aim of this study was to determine the effects of a commercial YC+EHY on in vitro ruminal dry matter, NDF, ADF, and CP digestibility of contrasting feedstuffs commonly used to feed ruminants in the Caribbean. We tested the hypothesis that ruminal DM and nutrient digestibility will improve with the addition of YC+EHY.

2. Materials and Methods

2.1. Substrates

This study was performed with seven (7) contrasting feedstuffs, including three (3) high-protein tree forages (*Trichanthera gigantea*, *Gliricidia sepium*, and *Leucaena leucocephala*), one (1) grass forage (*Brachiaria arrecta*), two (2) agriculture by-products (soybean meal and rice hulls), and a commercial concentrate for lactating dairy cows. Four samples of each feedstuff were collected at the University of the West Indies Field Station or commercial dairy farms in Trinidad and Tobago over a 2-week period.

These feedstuffs are widely used to feed ruminant animals in tropical environments as either supplemental or basal feedstuffs. The grass forage was cut approximately 5 cm above ground level with a sharp knife. Rice hulls were collected from a local rice mill. Leaf

and petiole were harvested from mature *T. gigantea*, *G. sepium*, and *L. leucocephala* plants during the vegetative growth stage.

2.2. Analysis of Chemical Composition

The feedstuffs were dried to a constant weight in a force-draft (Gallenkamp, Model: OHG097.XX1.5, Manchester, M27 8WA, UK) oven set at 60 °C, followed by grinding in a hammer mill (Thomas Wiley Laboratory mill, model 4; Swedesboro, NJ, USA) to pass through a 2 mm sieve. Dried samples were completely ashed in a muffle furnace set a 550 °C for 8 h. Ash was estimated as the loss of organic matter. Complete dry matter (DM) was determined by oven-drying approximately 1.0 g of pre-dried samples at 105 °C for 24 h [18]. The concentration of crude protein (CP) was estimated from the analysis of total nitrogen (N) following the Kjeldahl method [18]. Crude protein was calculated as N × 6.25. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin were determined sequentially. Both NDF and ADF analysis were conducted with the ANKOM^{2000} fibre analyser (model: A2000I, ANKOM Technology, Macedon, NY, USA) and expressed as ash-free. Sodium sulphite and amylase (α) were included in the NDF procedure. Lignin was determined by solubilizing cellulose with 72% sulphuric acid [19].

2.2.1. In Vitro Ruminal DM and Nutrient Digestibility

A Holstein-graded cow of approximately 500 kg body weight that was fitted with a rumen fistula was the donor of the inoculum. The care and management of this experimental animal were guided by the recommendations of the National Research Institute [20] for the management of terrestrial animals for research purposes. The cow was housed in a well-ventilated shed with concrete floor and a galvanized roof with free access to clean drinking water and mineral block. Feeding was performed once daily at approximately 0900 h with an ad libitum supply of freshly cut *B. arrecta* grass supplemented with approximately 750 g of commercial concentrate. Inoculum and buffer preparations were performed according to ANKOM Technology, method no. 3, for in vitro true digestibility using the ANKOM DAISY^{II} Incubator. The rumen digesta was manually extracted from multiple sites within the rumen before morning feeding. The liquid from the digesta was squeezed out and immediately filtered through four layers of cheesecloth into a pre-warmed thermos (39 °C) while being purged with CO_2. The rumen fluid and approximately 400 g of rumen digesta were transported to the lab for further processing. Microbes that adhere closely to feed particles were included in the inoculum by blending the 400 g of rumen digesta at a high speed for approximately 30 s. The buffered rumen inoculum was prepared by mixing ANKOM buffer with rumen fluid in a 4:1 ratio, achieving a final pH of 6.7 under constant purging with CO_2. Each ANKOM jar contained approximately 1600 mL of buffered rumen fluid. Approximately 0.5 g of each ground substrate was heat-sealed (model: 1915/1920, ANKOM Technology, Macedon, NY, USA) into ANKOM F57 filter bags. The sealed filter bags were placed in the ANKOM jars and incubated in the rotating Daisy^{II} digestion chamber (model: D200, ANKOM Technology, Macedon, NY, USA) at 39 °C. A 10 mL aliquot of YC+EHY was added to 2 of the 4 Daisy^{II} incubation jars. The aliquot was prepared by dissolving 3 g of YC+EHY in 200 mL of pre-warmed buffer [16]. Half the samples were incubated with and without the YC+EHY. Ankom filter bags with samples were incubated for 24 and 48 h intervals in buffered inoculum with or without combined YC+EHY. Twenty-five (25) samples and three (3) blanks were distributed evenly on either side of each jar.

At the end of the incubation, the filter bags were washed thoroughly with ice water to abruptly stop the microbial fermentation, followed by oven-drying at 60 °C to constant weight. Sample residues were analysed for DM, CP, NDF, and ADF, following the previously outlined methods.

2.2.2. In Vitro Ruminal CP Degradability

A second set of samples were prepared for in vitro ruminal CP degradability and incubated for 0, 2, 4, 8, 16, 24, and 48 h intervals following the procedure previously

described with the Daisy^{II} incubator [3]. Degradability at 0 h was performed by rinsing samples in cold tap water, allowing them to air-dry, and then oven-drying them at 60 °C for 48 h. Two (2) blank samples were included in each incubation interval. The post-incubation sample residue was analysed for Kjeldahl N. [18] and converted to CP, as previously described.

2.3. Experimental Design and Statistical Analysis

The layout of this study was a 7 × 2 factorial arrangement (7 substrates × 2 treatments) in a completely randomized design with four replicates. Statistical analysis was performed with the Mintab 19 statistical software. Statistical significance was declared at $p < 0.05$ following an ANOVA by the general linear model:

$$Yij = \mu + Fi\ (i = 1 - 2) + Eij$$

where Yij = dependent variable (DM and nutrient degradability), μ = overall mean, Fi = effect of YC+EHY additive, and Eij = random error. Substrates incubated with or without YC+EHY were the main effects. Tukey's multiple comparison test separated the treatment means.

3. Results

3.1. Chemical Composition of Substrates

The substrates selected for this study (Table 1) had contrasting chemical compositions ($p < 0.001$). The ash, NDF, and ADF were highest in *B. arrecta*. The concentrations of CP were the highest in soybean meal and lowest in rice hull. Soybean meal also had the lowest fibre and lignin contents. *T. gigantea* had the lowest NDF and ADF contents among the forages.

Table 1. Dry matter and chemical composition of substrates.

Substrates	DM (g/kg)	Chemical Composition (g/kg DM)				
		Ash	CP	NDF	ADF	Lignin
Com. Concentrate	906 ^{a}	93.0 ^{de}	209 ^{b}	378 ^{d}	222 ^{d}	40.0 ^{de}
G. sepium	221 ^{c}	101 ^{cd}	204 ^{b}	694 ^{bc}	599 ^{b}	139 ^{c}
L. leucocephala	280 ^{b}	64.0 ^{f}	208 ^{b}	771 ^{ab}	644 ^{b}	293 ^{a}
Rice hull	898 ^{a}	126 ^{b}	67.9 ^{e}	687 ^{bc}	626 ^{b}	179 ^{b}
Soybean meal	890 ^{a}	75.0 ^{ef}	483 ^{a}	210 ^{e}	125 ^{e}	10.0 ^{e}
B. arrecta	158 ^{d}	115 ^{bc}	123 ^{d}	837 ^{a}	715 ^{a}	71.0 ^{d}
T. gigantea	160 ^{d}	242 ^{a}	153 ^{c}	628 ^{c}	542 ^{c}	174 ^{b}
SEM	3.70	3.50	1.30	9.90	11.5	5.40
Significance (*p*-value)	0.000	0.000	0.000	0.000	0.000	0.000

^{a,b,c,d,e,f} Means with a column that do not share a letter are significantly different. DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; SEM = standard error of the mean.

3.2. In Vitro Ruminal Dry Matter and Nutrient Digestibility

Incubation with combined YC+EHY had no effect ($p > 0.05$) on the 24 h in vitro ruminal dry matter and nutrient digestibility, except for DM in soybean meal, CP in *G. sepium*, and ADF in *B. arrecta* (Table 2). The addition of YC+EHY caused a reduction in 24 h DM digestibility in soybean meal by 16.2% and CP digestibility in *G. sepium* by 38.5%. On the other hand, the ADF digestibility in *B. arrecta* increased by approximately 32% when incubated with YC+EHY. The addition of YC+EHY did not affect the 48 h in vitro ruminal dry matter and nutrient digestibility (Table 3).

Table 2. Effect of YC+EHY on 24 h in vitro ruminal dry matter and nutrient digestibility.

Substrate	YC+EHY (+/−)	DM (g/kg)	24 h In Vitro Digestibility (g/kg DM)		
			CP	NDF	ADF
Com. Concentrate	(+)	571 ^{b}	109 ^{b}	129 ^{fg}	27.0 ^{fg}
	(−)	607 ^{b}	97.0 ^{b}	130 ^{fg}	25.0 ^{fg}
G. sepium	(+)	557 ^{b}	59.0 ^{c}	398 ^{ab}	315 ^{a}
	(−)	560 ^{b}	96.0 ^{b}	402 ^{a}	312 ^{a}
L. lecocephala	(+)	239 ^{ef}	59.0 ^{c}	302 ^{cd}	162 ^{cd}
	(−)	233 ^{ef}	54.0 ^{c}	291 ^{cd}	164 ^{cd}
Rice hull	(+)	215 ^{ef}	17.0 ^{ef}	120 ^{fg}	79.0 ^{ef}
	(−)	271 ^{de}	4.00 ^{f}	162 ^{ef}	103 ^{de}
Soybean meal	(+)	625 ^{b}	199 ^{a}	72 ^{g}	50.0 ^{g}
	(−)	746 ^{a}	217 ^{a}	101 ^{fg}	50.0 ^{efg}
B. arrecta	(+)	361 ^{c}	47.0 ^{cd}	315 ^{bc}	297 ^{a}
	(−)	344 ^{cd}	35.0 ^{cde}	275 ^{cd}	225 ^{b}
T. gigantea	(+)	239 ^{ef}	34.0 ^{cde}	271 ^{cd}	191 ^{bc}
	(−)	172 ^{f}	27.0 ^{def}	229 ^{de}	177 ^{bc}
SEM		14.6	4.40	14.9	9.80
Significance (*p*-value)					
Substrate		0.000	0.000	0.000	0.000
YC+EHY		0.043	0.728	0.797	0.666
Substrate × YC+EHY		0.000	0.000	0.135	0.001

^{a,b,c,d,e,f,g} Means within a column that do not share a letter are significantly different (effect of YC+EHY within substrate). DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM = standard error of the mean; YC+EHY (+/−), substrates incubated with (+) or without (−) yeast culture and enzymatically hydrolysed yeast.

Table 3. Effect of YC+EHY on 48 h in vitro ruminal dry matter and nutrient digestibility.

Substrate	YC+EHY (+/−)	DM (g/kg)	48 h In Vitro Digestibility (g/kg DM)		
			CP	NDF	ADF
Com. Concentrate	(+)	719 ^{b}	157 ^{b}	201 ^{b}	71.0 ^{d}
	(−)	714 ^{b}	147 ^{b}	192 ^{b}	77.0 ^{d}
G. sepium	(+)	746 ^{b}	166 ^{b}	515 ^{a}	419 ^{a}
	(−)	739 ^{b}	162 ^{b}	516 ^{a}	408 ^{ab}
L. lecocephala	(+)	378 ^{d}	71.0 ^{c}	383 ^{a}	237 ^{c}
	(−)	378 ^{d}	63.0 ^{cd}	391 ^{a}	242 ^{c}
Rice hull	(+)	306 ^{d}	38.0 ^{d}	150 ^{b}	108 ^{d}
	(−)	326 ^{d}	34.0 ^{d}	167 ^{b}	126 ^{d}
Soybean meal	(+)	972 ^{a}	450 ^{a}	204 ^{b}	123 ^{d}
	(−)	970 ^{a}	446 ^{a}	201 ^{b}	112 ^{d}
B. arrecta	(+)	551 ^{c}	83.0 ^{c}	457 ^{a}	386 ^{ab}
	(−)	531 ^{c}	74.0 ^{c}	444 ^{a}	370 ^{ab}
T. gigantea	(+)	513 ^{c}	57.0 ^{cd}	412 ^{a}	327 ^{bc}
	(−)	539 ^{c}	62.0 ^{cd}	401 ^{a}	358 ^{ab}
SEM		19.6	5.9	24.9	15
Significance (*p*-value)					
Substrates		0.000	0.000	0.000	0.000
YC+EHY		0.872	0.171	0.936	0.740
Substrate × YC+EHY		0.954	0.914	0.999	0.827

^{a,b,c,d} Means within a column that do not share a letter are significantly different. DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre. SEM = standard error of the mean; YC+EHY (+/−), substrates incubated with (+) or without (−) yeast culture and enzymatically hydrolysed yeast.

3.3. In Vitro Ruminal Crude Protein Degradability

The in vitro ruminal CP degradability was unaffected by YC+EHY up to 16 h post-incubation in most substrates except *T. gigantea* and *L. leucocephala* (Figure 1). The rate of CP degradability in the commercial concentrate and rice hull increased sharply between 16 h and 24 h post-incubation and generally plateaued afterwards when inoculated with YC+EHY. Similarly, YC+EHY significantly increased CP degradability after 8 h in *L. leucocephala* and 24 h in *T. gigantea* and the commercial concentrate.

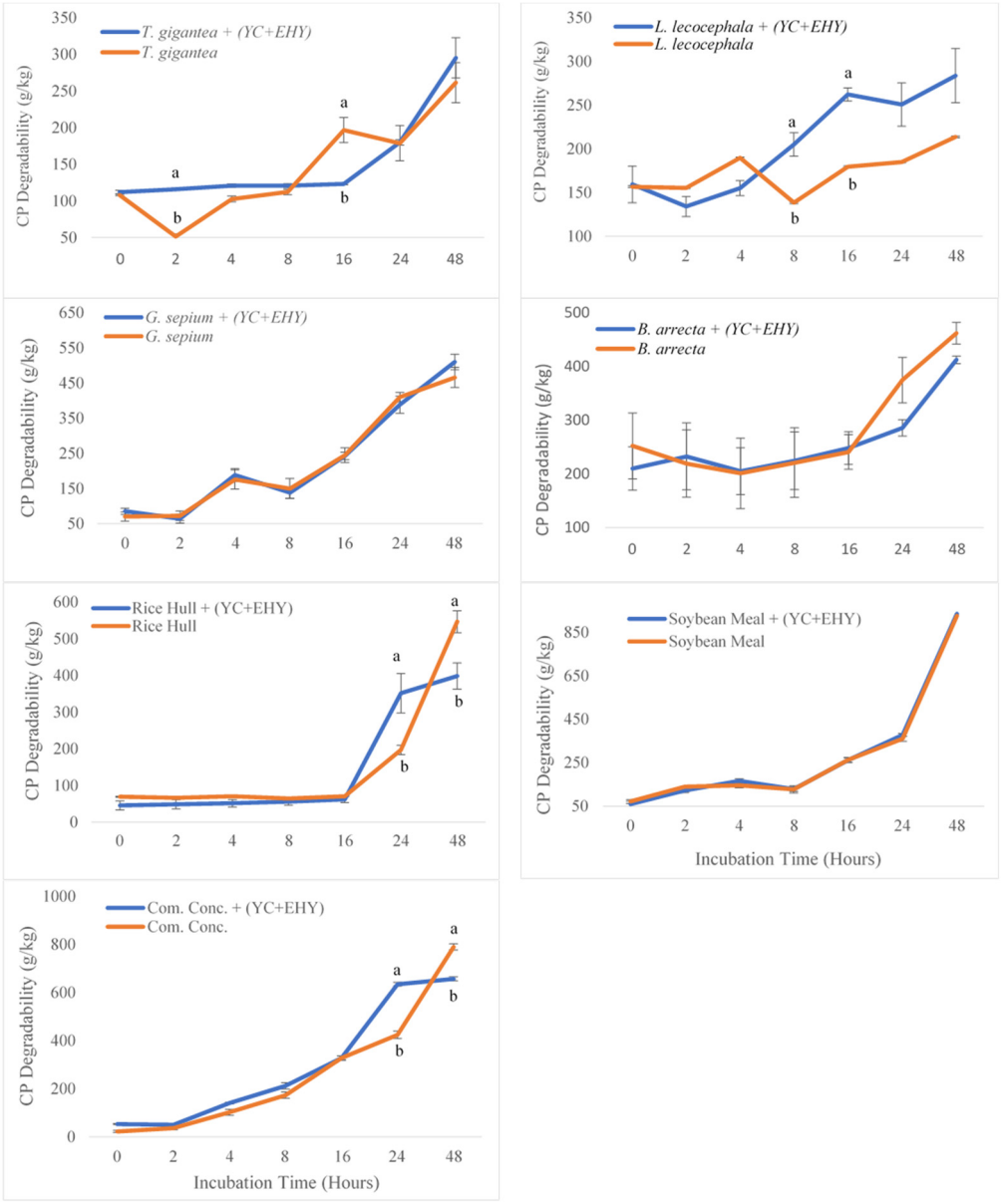

Figure 1. In vitro ruminal CP degradability substrates incubated with and without YC+EHY. [a,b] CP degradability at specific incubation times that do not share a letter is significantly different ($p < 0.05$).

4. Discussion

4.1. Chemical Composition of Substrates

The CP content of soybean meal in the present study is like other reports [21]. Soybean meal is the most popular protein feedstuff used in animal feeds [21,22]. It is also one of the most expensive feedstuffs [23]. Leguminous tree forages such as *G. sepium* and *L. leucocephala* had CP contents above 200 g/kg DM, making them potentially cheap

alternatives (partial or complete) to soybean meal [24,25]. However, high concentrations of fibre and lignin in *G. sepium* and *L. leucocephala* may reduce intake and digestibility [3]. Also, CP utilization can be limited by tannins and other phenolic compounds that are usually present in high concentrations in these browse forages [24,26] unless mitigating measures are adopted. The high fibre and lignin contents of the grass and tree forages in the present study are likely attributed to an advance stage of maturity [1]. Low-quality feedstuff like rice hull is mainly used as a source of roughage when the supply of grass forages is limited, e.g., in the dry season. With CP content below 80 g/kg DM, rice hull will supply less than the minimum amount of nitrogen needed to satisfy the rumen microbial requirements and maintain optimum rumen function [27]; therefore, careful supplementation with a good-quality N/protein source is required.

4.2. In Vitro Ruminal Dry Matter and Nutrient Degradability

The ruminal DM and nutrient digestibility post 48 h of incubation were unaffected by YC+EHY, while the 24 h ruminal digestibility of DM, CP, and ADF was influenced by YC+EHY in some substrates. This is an indication that the activity of YC+EHY may be short-term and is limited to the early periods post-feeding where rumen fermentation is highest. In the present study, a significant 16.2% reduction in the dry matter digestibility of soybean meal and a 38.5% reduction in CP digestibility in *G. sepium* were observed with the addition of combined YC+EHY post 24 h of incubation. On the other hand, ruminal ADF digestion in *B. arrecta* increased by approximately 32%. The significant reduction in DM digestibility in soybean meal without a significant concomitant reduction in the more resistant fractions like NDF and ADF was surprising, notwithstanding a nominal decline in NDF digestion. This was particularly unexpected since hydrolysable yeast is known to promote the development of cellulolytic bacterial population such as *Ruminococcus* spp., which increases fibre digestion [28]. This was most likely the reason for the significant increase in ADF digestion in *B. arrecta* forage incubated with YC+EHY in this study. However, some in situ studies reported that hydrolysable yeast did not affect fibre digestion [12,17]. Variations in fibre digestion are likely due to the availability of soluble nutrients and the concentration of fibre, especially the cellulose and spatial distribution of lignin in the cell wall of the substrate. Most studies where hydrolysable yeast did not affect fibre digestion used substrates with total fibre below 400 g/kg DM [12,17], like soybean meal and the commercial concentrate in the present study. It is important to note that *B. arrecta* forage had the highest fibre contents in the present study, suggesting the potential of YC+EHY to stimulate ruminal fibre digestion, particularly in substrates with extremely high fibre. The reduction in the 24 h CP digestibility in *G. sepium* incubated with YC+EHY is contrary to a previous report [12] where a significant increase in CP digestibility in beef cattle supplemented with hydrolysable yeast was observed. The presence of fermentable carbohydrates and highly soluble protein such as amino acids and nucleotides in YC+EHY could enhance ruminal CP digestibility [12]. Therefore, it is possible that the additional protein provided by YC+EHY in the present study could have caused more protein to be bound to tannin in *G. sepium*, resulting in a reduction in ruminal CP degradability. However, YC+EHY did not have a similar effect on *L. lecocephala*, a tannin-rich forage like *G. sepium*. *Leucaena lecocephala* is believed to have a higher tannin content and protein binding potential than *G. sepium* [29]. Therefore, the reason for the reduction in CP digestibility in *G. sepium* is not clear.

Few studies reported contrasting results regarding the influence of hydrolysable yeast on ruminal or total tract CP digestion in different livestock species, [12,30] but no previous work was found reporting the effects of combined YC+EHY on ruminal CP degradability at different incubation intervals. Yeast-based additives have gained popularity for modulating ruminal fermentation, but their mechanisms of action are not clearly understood [31]. This is especially the case with ruminal CP degradability. Many factors affect CP degradability in the rumen, like the relative concentrations of non-protein nitrogen and the physical and chemical characteristics of the protein that comprise the true protein fraction of the

feedstuff [32]. In experiment 2 of the present study, the ruminal CP degradability was unaffected by YC+EHY up to 16 h post-incubation in most substrates except *T. gigantea* and *L. leucocephala*. The decline observed in the 2 h and 24 h CP degradability in *T. gigantea* may be accidental. The limited effect of YC+EHY on the in vitro ruminal CP degradability within the initial 16 h of fermentation could be an indication that the supply of readily soluble N was adequate to satisfy the microbial requirements during the early stages of digestion and/or YC+EHY had little to no effect on the microbial colonization in some substrates. Interactions with phenolic compounds like tannins, saponins, and alkaloids known to be present in these browse forages can also be a possible cause [24,33]. The suppressive mechanism of YC+EHY on CP degradability in *L. leucocephala* post the 4 h incubation interval is not clear, but it can be important in increasing the supply of rumen undegradable protein to the small intestine. Low CP degradability in *T. gigantea* is suspected to be resulting from high-fibre-bound N, rendering most of the protein insoluble and inaccessible to rumen microbes [33]. However, significant increases in CP degradability after 8 h in *L. leucocephala* and 24 h in rice hull and the commercial concentrate could be pointing to the potential of YC+EHY to increase the ruminal degradation of the more slowly degrading protein fraction. Further, improved CP degradability in beef cattle was attributed to the additional supply of highly soluble protein by the hydrolysable yeast [12].

5. Conclusions

This study demonstrated that combined YC+EHY has the potential to improve ruminal ADF digestibility and modify ruminal CP degradation dependent on the type of substrate.

This can be an indication that supplementation with YC+EHY can improve the utilization of fibrous feedstuffs in particular and enhance the efficiency of nitrogen and crude protein metabolism in ruminants. Further studies are recommended to determine the nutritional mechanisms and mode of actions of YC+EHY. For example, it is worth knowing if and how YC+EHY modifies rumen microbial populations and if there are interactions with phenolic compounds in some browse forages.

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