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Addition of Active Dry Yeast Could Enhance Feed Intake and Rumen Bacterial Population While Reducing Protozoa and Methanogen Population in Beef Cattle

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Abstract: Urea–lime-treated rice straw fed to Thai native beef cattle was supplemented with dry yeast (DY) (*Saccharomyces cerevisiae*) to assess total feed intake, nutrient digestibility, rumen microorganisms, and methane (CH_4) production. Sixteen Thai native beef cattle at 115 ± 10 kg live weight were divided into four groups that received DY supplementation at 0, 1, 2, and 3 g/hd/d using a randomized completely block design. All animals were fed concentrate mixture at 0.5% of body weight, with urea–lime-treated rice straw fed ad libitum. Supplementation with DY enhanced total feed intake and digestibility of neutral detergent fiber and acid detergent fiber ($p < 0.05$), but dry matter, organic matter and crude protein were similar among treatments ($p > 0.05$). Total volatile fatty acid (VFA) and propionic acid (C3) increased ($p < 0.05$) with 3 g/hd/d DY supplementation, while acetic acid (C2) and butyric acid (C4) decreased. Protozoal population and CH_4 production in the rumen decreased as DY increased ($p < 0.05$). Populations of *F. succinogenes* and *R. flavefaciens* increased ($p < 0.05$), whereas methanogen population decreased with DY addition at 3 g/hd/d, while *R. albus* was stable ($p > 0.05$) throughout the treatments. Thus, addition of DY to cattle feed increased feed intake, rumen fermentation, and cellulolytic bacterial populations.

Keywords: beef cattle; digestibility; ruminal fermentation; yeast

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1. Introduction

To develop more effective ruminant production systems, ruminants must have a high fermentation capacity. The ability of the microbial ecology to digest organic substances into milk and meat precursors is required for increased production [1]. Many feed additives, such as direct-fed microorganisms, are employed to promote livestock productivity. Yeast-derived products, such as *Saccharomyces cerevisiae*, stand out in this group because they are beneficial to animal health and ruminal enhancer [2]. In the rumen, yeast can utilize the remaining dissolved oxygen, sparing anaerobic microbes from the damaging effects of oxygen. Yeasts can increase rumen maturity and regulate ruminal pH by competing with lactic generating bacteria, minimizing the danger of acidosis [3]. Yeast products lower rumen pH by encouraging microorganisms that convert lactate into short-chain fatty acids [4]. Yeast improves cattle feed digestion and metabolism in a variety of ways, including increasing nutritional digestibility, optimizing volatile fatty acid proportions, decreasing ammonia–nitrogen, lowering pH fluctuation, and stimulating microbial communities in the rumen [5]. Furthermore, yeast provides several growth factors, pro-vitamins, and other stimulants to

rumen microbes while decreasing rumen redox potential and stimulating the growth of ruminal bacteria, primarily cellulolytic bacteria, which enhance fiber degradation [6]. Moreover, yeast enhanced total volatile fatty acids (VFAs) while decreasing acetate proportion. Supplementation of yeast can help cellulolytic bacteria and increase the digestibility of Nellore cattle [7]. This could be due to increasing cellulolytic and lactate-utilizing bacterial populations modifying lactate-to-propionate fermentative pathways [8]. Feed intake, milk yield, weight gain, digestibility of nutrients, cellulolytic bacteria numbers, and volatile fatty acid patterns have all been shown to improve with yeast supplementation in the ruminant diet [9,10]. Furthermore, there is a limitation of data because only a few researchers have studied the rumen microorganism and methane emission in cattle fed urea–lime-treated rice straw. The aim of this study was to study supplementation with *Saccharomyces cerevisiae* on feed intake, nutritional digestibility, rumen fermentation, rumen microorganism, and methane production in Thai native beef calves fed urea–lime-treated rice straw as a basal roughage.

2. Materials and Methods

The study design and plan strictly followed the norms of the Animal Ethics Committee of Nakhon Phanom University, Mueang Nakhon Phanom, Thailand (permission No. AENPU A2/2560). This study primarily involved laboratory analysis of ruminant feeds, for which permission to collect rumen fluid from animals was granted in accordance with the Thailand Ethics of Animal Experimentation of the National Research Council.

2.1. Animals, Feed, and Experimental Design

Sixteen Thai native beef cattle with 115 ± 10 kg live weight were blocked into four groups to receive active *Saccharomyces cerevisiae* (dry form) supplementation at 0, 1, 2, and 3 g/hd/day. The yeast strain *S. cerevisiae* in this study was obtained from the Renu Nakhon district, Nakhon Phanom Province, Thailand. All animals received a concentrated mixture at 0.5% body weight, while urea–lime-treated rice straw, water, and mineral blocks were available ad libitum.

Ingredient compositions of concentrate mixture and nutrient composition are presented in Table 1. The rice straw-treated urea–calcium hydroxide was made by adding 2 kg of urea and 2 kg of $Ca(OH)_2$ in 100 L to 100 kg of rice straw. The quantity of urea and calcium hydroxide solution was sprayed onto rice straw bales and then covered with a sheet of plastic for at least 10 days before feeding to the beef cattle [11].

Table 1. Compositions of concentrate mixtures and urea–calcium hydroxide-treated rice straw.

Items	Concentrate	Urea–Calcium Hydroxide-Treated Rice Straw
Concentrate ingredients, % dry matter basis		
Cassava chip	63.5	
Coconut meal	10.5	
Palm kernel meal	7.5	
Rice bran	11.0	
Urea	3.0	
Molasses	2.0	
Mineral mixture	1.0	
Salt	1.0	
Sulfur	0.5	
Chemical composition		
Dry matter, %	89.7	50.7
		% of dry matter
Organic matter	94.6	90.1
Ash	5.4	9.9
Crude protein	14.0	4.3
Neutral detergent fiber	25.6	70.2
Acid detergent fiber	15.8	48.7
Total digestible nutrients (TDN) *	79.8	50.4

* Calculated value %TDN = [%digestible CP + Crude fiber (CF) + Nitrogen free extract (NFE) + (2.25 × %Digestible Ether extract (EE)].

The animals were dewormed and given a 14-day acclimation period prior to the experiment. The feeding trial lasted 90 days, with the digestibility test taking place in the final week. The individual feed was made and fed to the cattle in the morning and evening. To measure daily feed intake, the amount of feed supplied and denied was recorded every day, and to determine weight change, weighing was performed every two weeks before feeding time.

2.2. Samples Collection and Chemical Analyses of Samples

Representative feed, fecal, and urine samples were collected throughout the last 7 days, weighed, and oven-dried at 60 °C for 48 h. The composite samples were dried at 60 °C before being processed (1 mm screen using Cyclotech Mill, Tecator, 1093, Hoganas, Sweden) and tested for DM, CP, and ash [12]. The acid detergent fiber (ADF) was calculated and expressed including residual ash. Determined neutral detergent fiber (NDF) in samples with the addition of alpha-amylase but without sodium sulfite, and the findings are given inclusive of residual ash according to Van Soest et al. [13]. Nutrient digestibility was calculated using acid insoluble ash [14], and DM, OM, NDF, and ADF digestibility were determined from the ratio of AIA in feed and feces, and digestibility of nitrogen was determined from ratios of AIA and N in feed and feces.

On the final day of the trial, rumen fluid and blood samples were collected at 0 and 4 h following the morning feed. Each time, a stomach tube connected to a vacuum pump was utilized to collect approximately 200 mL of rumen fluid from the rumen. Rumen fluid pH and temperature were immediately measured, and 50 mL of rumen fluid was collected and mixed with 5 mL of 1M H_2SO_4 to stop microbial activity fermentation before centrifugation at 16,000× *g* for 15 min. A total of 20 cc of supernatant was taken and frozen at −20 °C before being analyzed in the laboratory for ammonia–nitrogen (NH_3–N) using micro-Kjeldahl methods [12].

High Performance Liquid Chromatography was used to examine rumen fluid samples for VFAs (HPLC; Model Water 600; UV detector, Millipore Corp., Milford, MA, USA). Rumen CH_4 production was approximated using equation of Moss et al. [15]. VFA proportions are as follows: production of CH_4 = 0.45 (acetate, C_2) + 0.275 (propionate, C_3) + 0.4 (butyrate, C_4).

The second portion was fixed with 10% formalin for the determined protozoal population using the direct count microscopic method as described by Galyean [16].

The community DNA was isolated from rumen fluid and digesta. QIAgen DNA Mini Stool Kit columns were used to purify the DNA (QIAGEN, Valencia, California, USA). Real-time PCR was used to determine the relative populations of total bacteria, rumen bacteria for fiber degradation (*Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens*, and *Fibrobacter succinogenes*), and methanogen. Total DNA was extracted from the samples using the method described by Stevenson et al. [17]. Extracted DNA was utilized as a template in real-time PCR experiments with specified primers to measure the microbial population of *R. albus*, and *R. flavefaciens* [18], *B. fibrisolvens* [17], *F. succinogenes* [19], and methanogen [20]. The DNA standards for real-time PCR amplification and detection were determined using a Chromo 4™ system (Bio-Rad, California, USA). The data of microbial population were transferred to log10 prior to statistical analysis.

At the same time as the rumen fluid was collected, a 10 mL blood sample was taken from the jugular vein into a tube containing 0.1 g of ethylenediaminetetraacetic acid (EDTA). All tubes were centrifuged at 3000× *g* for 15 min to obtain plasma and then stored at −20 °C for further analyses of blood urea nitrogen (BUN) [21].

2.3. Statistical Methods

The data were analyzed using the MIXED procedure in SAS software [22]. The mathematical model assumption used was:

$$Yi = \mu + T_i + \beta_i + \varepsilon_i$$

where Y_i is the dependent variable, μ is the overall mean, T_i is the ith treatment effect (supplementation of *Saccharomyces cerevisiae* at 0, 1, 2, 3 g/hd/day), βi is the ith block effect, and $_i$ is the residual error of the ith observation. Differences among means with $p < 0.05$ were represented as statistically significant differences. Orthogonal polynomials for diet responses were determined by linear and quadratic effects.

3. Results

3.1. Feed Intake and Digestibility

DY supplementation enhanced total feed intake of urea–lime-treated rice straw by Thai native beef cattle ($p < 0.05$) but did not alter digestibility DM, OM, or CP ($p > 0.05$). Supplementation at 3 g/hd/d increased digestibility of fiber (NDF, ADF) ($p < 0.05$; Table 2).

Table 2. Effect of yeast supplementation on voluntary feed intake and nutrient digestibility in Thai native beef cattle.

Items	Yeast Supplementation (g/day)				SEM	Contrast	
	0	1	2	3		Linear	Quadratic
Dry matter intake							
Roughage intake							
kg/day	1.9 ^{a}	2.0 ^{a}	2.3 ^{b}	2.5 ^{c}	0.18	0.04	0.43
g/kg $BW^{0.75}$	65.9 ^{a}	66.0 ^{a}	68.1 ^{b}	70.6 ^{c}	0.76	0.04	0.52
Concentrate intake							
kg/day	0.7	0.7	0.7	0.7	0.31	0.17	0.41
g/kg $BW^{0.75}$	17.8	18.9	17.7	19.0	1.67	0.15	0.32
Total feed intake							
kg/day	2.6 ^{a}	2.7 ^{a}	3.0 ^{b}	3.3 ^{c}	0.09	0.04	0.05
g/kg $BW^{0.75}$	84.7 ^{a}	84.9 ^{a}	85.7 ^{b}	89.6 ^{c}	1.24	0.04	0.05
Nutrient digestibility, %							
Dry matter	57.5	58.9	60.2	60.0	0.17	0.14	0.47
Organic matter	62.8	62.7	62.4	63.4	0.05	0.25	0.32
Crude protein	58.6	59.1	59.7	60.2	0.09	0.17	0.21
Neutral detergent fiber	50.1 ^{a}	51.9 ^{a}	53.2 ^{b}	55.2 ^{c}	0.08	0.03	0.04
Acid detergent fiber	41.4 ^{a}	42.3 ^{a}	44.9 ^{b}	46.9 ^{c}	0.06	0.02	0.03

^{a,b,c} means within a row with different superscripts differ significantly ($p < 0.05$); SEM = standard error of the mean.

3.2. Rumen Fermentation, and Blood Urea Nitrogen

Table 3 shows the effect of DY on rumen fermentation and BUN. The ruminal pH (6.6–6.8) and ruminal temperature (39.0–39.5 °C) remained stable ($p > 0.05$). The concentration of NH_3–N increased in the DY supplementation groups and was highest at 3 g/hd/d but did not affect the concentration of BUN ($p > 0.05$).

Table 3. Effect of yeast supplementation on fermentation characteristics and blood urea nitrogen in Thai native beef cattle.

Items	Yeast Supplementation (g/day)				SEM	Contrast	
	0	1	2	3		Linear	Quadratic
Ruminal pH	6.8	6.8	6.7	6.6	0.09	0.09	0.15
Temperature, °C	39.5	39.0	39.4	39.5	0.22	0.34	0.46
NH_3–N, mg/dL	12.1 ^{a}	12.9 ^{a}	13.3 ^{b}	15.6 ^{c}	0.30	0.02	0.03
BUN, mg/dL	9.1	9.5	10.7	11.3	0.06	0.52	0.62
Total VFAs, mmol/L	90.1 ^{a}	92.8 ^{a}	96.5 ^{b}	100.3 ^{c}	0.15	0.03	0.04
VFAs, mol/100mol							
Acetic acid (C_2)	68.1^{c}	66.6 ^{b}	66.4 ^{b}	64.8 ^{a}	0.18	0.02	0.04
Propionic acid (C_3)	20.9 ^{a}	22.6 ^{b}	24.1 ^{c}	26.0 ^{d}	0.16	0.02	0.03
Butyric acid (C_4)	11.0 ^{b}	10.8 ^{b}	9.5 ^{a}	9.2 ^{a}	0.07	0.03	0.05
C_2: C_3	3.3 ^{c}	2.9 ^{b}	2.8 ^{b}	2.5 ^{a}	0.31	0.04	0.07
CH_4 (mM)	29.3 ^{c}	28.1 ^{b}	27.1^{b}	25.7 ^{a}	0.25	0.04	0.05

^{a,b,c,d} means within a row with different superscripts differ significantly ($p < 0.05$); SEM = standard error of the mean; NH_3–N = ammonia–nitrogen; BUN = blood urea nitrogen; VFAs = volatile fatty acids; CH_4 = methane production = 0.45 (C_2) − 0.275 (C_3) + 0.4 (C_4) calculated according to Moss et al. [15].

3.3. Volatile Fatty Acid (VFA) Profiles and Methane (CH_4) Production

Concentrations of total volatile fatty acid (TVFA) and propionic acid (C3) increased ($p < 0.05$) with DY supplementation, particularly for DY at 3 g/hd/d. However, acetic acid (C2) and butyric acid (C4) concentrations, C2: C3 ratio and CH_4 production reduced with the addition of DY at 3 g/hd/d.

3.4. Microbial Population

Protozoal population significantly reduced ($p < 0.05$) with LY addition at 3 g/hd/d. The bacteria, *F. succinogenes*, *B. fibrisolvens* and *R. flavefaciens* increased, whereas the methanogenic population decreased with DY addition at 3 g/hd/d. *R. albus* was stable ($p > 0.05$) throughout all treatments (Table 4).

Table 4. Effect of yeast supplementation on microbial population in Thai native beef cattle.

Items	Yeast Supplementation (g/day)				SEM	Contrast	
	0	1	2	3		Linear	Quadratic
Direct count, cell/mL							
Protozoa, × 10^6 cell/mL	8.1 ^{d}	6.9 ^{c}	5.2 ^{b}	3.5 ^{a}	0.19	0.04	0.05
Real-time PCR, copies/mL rumen content							
F. succinogenes, × 10^6	3.2 ^{a}	3.6 ^{a}	4.8 ^{b}	5.9 ^{c}	0.07	0.04	0.07
R. flavefaciens, × 10^5	2.1 ^{a}	2.4 ^{a}	3.9 ^{b}	4.8 ^{c}	0.31	0.04	0.06
R. albus, × 10^6	5.0	4.9	5.2	5.5	0.16	0.06	0.08
B. fibrisolvens, × 10^5	2.5 ^{a}	3.1 ^{a}	4.4 ^{b}	6.8 ^{c}	0.21	0.04	0.05
Methanogens, × 10^2	6.6 ^{a}	5.8 ^{a}	4.7 ^{b}	3.4 ^{c}	0.09	0.04	0.05

^{a,b,c,d} means within a row with different superscripts differ significantly ($p < 0.05$); SEM = standard error of the mean.

4. Discussion

4.1. Feed Intake and Nutrient Digestibility

Total feed intake increased with DY supplementation, with the highest found at 3 g/hd/d ($p < 0.05$), concurring with Crossland et al. [6], who found that adding yeast to cattle diet increased dry matter intake. Supplementation of DY at 3 g/hd/d also increased fiber digestibility (NDF, ADF) ($p < 0.05$) due to the ability of yeast to scavenge excess oxygen in the rumen, lower the redox potential, and enhance the degradability of NDF and ADF. Yeast provides an ecological setting that encourages the proliferation and activity of microbes, especially cellulolytic bacteria that enhance NDF and ADF breakdown. Guedes et al. [23] discovered that feeding cattle with yeast improved NDF degradation of maize silage. By contrast, Mir and Mir [24] found that supplementing cattle feed with live yeast did not impact DM and NDF degradation in the rumen. Satori et al. [10] stated that the highest total intake and average daily gain were observed in cattle supplemented with yeast at below 6 g/d.

4.2. Rumen Ecology and Blood Urea-Nitrogen

Ruminal pH and temperature values for all DY supplementations were reported in the optimal range by Phesatcha et al. [11]. In general, rumen pH stability benefits acid-sensitive cellulolytic bacteria and is extremely beneficial to beef cattle, especially fattening cattle. Monnerat et al. [25] and Ghasemi et al. [26] reported that adding yeast to high concentrate cattle feed did not affect rumen pH.

The NH_3–N concentration increased with DY supplementation at 3 g/hd/d. The concentration of BUN was similar among treatments and ranged between 9.1and 11.3 mg/dl, and in the normal range as reported by Wanapat and Pimpa [27]. By contrast, Li et al. [28] found that the addition of yeast to cattle feed decreased BUN.

4.3. Ruminal Volatile Fatty Acid (VFA) Profiles and Methane (CH_4) Production

In this study, beef calves fed DY had higher total VFA levels and higher C3 levels, while the C2, C4, and C2 to C3 ratio were lower than those no supplemented group. This was due to an increase in the lactate-utilizing bacteria *Selenomonas ruminantium* and *Megasphaera elsdenii* that convert lactate to C3, with their growth stimulated by yeast supplementation [10,29]. When compared to the control, yeast supplementation increased total VFA, C3, and valeric acid but decreased C2 and the C2 to C3 ratio [30,31]. Dawson et al. [32] found that for yeast supplements containing in vitro total VFA, the molar proportion of C3 increased while C2 decreased. Variable effects of yeast on rumen fermentation efficiency can be attributed to dose, diet type, different yeast strains, animal physiological stage, and feeding systems [7,33].

Major alterations of CH_4 in ruminants are produced through propionate fermentation, and CH_4 production decreased with yeast supplementation. This result concurred with Phesatcha et al. [11] and Wang et al. [34], who found that CH_4 production decreased with yeast supplementation, while Munoz et al. [35] observed that DY supplementation increased CH_4 production in lactating dairy cows and Bayat et al. [36] determined that yeast did not influence CH_4 emissions. Diverse effects of yeast supplementation on CH_4 synthesis were attributed to varying yeast strains, dosages, and diets utilized in the trials [28]. Yeast can be used to minimize CH_4 emissions and was shown to lower methane production in the rumen by encouraging acetogens to use more hydrogen in the process of acetate formation by Darabighane et al. [37]. In this study, the methanogen population was reduced with yeast supplementation and was lowest at 3 g/hd/d.

The protozoal population was reduced with DY supplementation. Microbial populations studied using real-time PCR revealed that *R. flavefaciens*, *B. fibrisolvens*, and *F. succinogenes* increased, while methanogen population decreased with yeast supplementation at 3 g/hd/d. This result concurred with Sousa et al. [7], who reported that the addition of yeast significantly increased the relative population of *R. flavefaciens*. The addition of DY stimulated the growth of cellulolytic bacterial populations (*R. flavefaciens* and *F. succinogenes*), while suppressing growth of the lactate-producing bacterium (*Streptococcus bovis*), thereby improving the consistency of rumen fermentation [38]. Enhanced fiber degradation increased total cellulolytic bacteria in the rumen. Ding et al. [39] found that the addition of yeast increased bacteria, fungi, protozoa, lactate-utilizing bacteria, and rate of fiber decomposition. Growth factors induced by organic acids and vitamins provided by yeast may enhance cellulolytic bacterial and fungal colonization in the rumen. Yeast promoted microbial proliferation, specifically lactic acid-utilizing bacteria, and reduced acidosis [3,9]. Furthermore, as a facultative anaerobe organism, yeast gathers available oxygen on the surface of freshly swallowed meals to sustain metabolic activity, thereby lowering rumen redox potential. Removal of oxygen improves growth conditions for strict anaerobic cellulolytic bacteria, increasing their adherence to fodder particles and shortening the cellulolytic process [33]. Jiang et al. [29] examined the ruminal microbiota of cows fed with different amounts of yeast. They found that the number of *Butyrivibrio fibrisolvens*, an important hemicellulolytic species, was reduced in cows supplemented with a high dose of yeast. In our study, methane production and methanogen population reduced with yeast supplementation, and the lowest values were found at 3 g/hd/d. By contrast, Lu et al. [40] reported that adding yeast at 6 and 12 g/d decreased methane production without affecting the number or diversity of methanogens.

5. Conclusions

The addition of DY at 3 g/hd/d enhanced total feed intake, rumen fermentation, and total bacteria populations while reducing protozoal population and CH_4 production in beef cattle fed with urea–lime-treated rice straw. However, there are certain drawbacks related to the fattening beef cattle influenced by DY addition, which requires further study.

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