


Article

Utilization of Yeast Waste Fermented Citric Waste as a Protein Source to Replace Soybean Meal and Various Roughage to Concentrate Ratios on In Vitro Rumen Fermentation, Gas Kinetic, and Feed Digestion

Chaichana Suriyapha ¹, Anusorn Cherdthong ^{1,*} , Chanon Suntara ¹ and Sineenart Polyorach ²

¹ Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; chaichana_s@kkumail.com (C.S.); chanon_su@kkumail.com (C.S.)

² Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology, Bangkok 10520, Thailand; sineenart.po@kmitl.ac.th

* Correspondence: anusornc@kku.ac.th; Tel./Fax: +66-4320-2362

Abstract: The objective of this study was to determine the application of citric waste fermented yeast waste (CWYW) obtained from an agro-industrial by-product as a protein source to replace soybean meal (SBM) in a concentrate diet. We also determined the effect of various roughage to concentrate ratios (R:C) on the gas production kinetics, ruminal characteristics, and in vitro digestibility using an in vitro gas production technique. The experiment design was a 3×5 factorial design arranged in a completely randomized design (CRD), with three replicates. There were three R:C ratios (60:40, 50:50, and 40:60) and five replacing SBM with CWYW (SBM:CWYW) ratios (100:0, 75:25, 50:50, 25:75, and 0:100). The CWYW product's crude protein (CP) content was 535 g/kg dry matter (DM). There was no interaction effect between R:C ratios and SBM:CWYW ratios for all parameters observed ($p > 0.05$). The SBM:CWYW ratio did not affect the kinetics and the cumulative amount of gas. However, the gas potential extent and cumulative production of gas were increased with the R:C ratio of 40:60, and the values were about 74.9 and 75.0 mL/0.5 g, respectively ($p < 0.01$). The replacement of SBM by CWYW at up to 75% did not alter in vitro dry matter digestibility (IVDMD), but 100% CWYW replacement significantly reduced ($p < 0.05$) IVDMD at 24 h of incubation and the mean value. In addition, IVDMD at 12 h and 24 h of incubation and the mean value were significantly increased with the R:C ratio of 40:60 ($p < 0.01$). The SBM:CWYW ratio did not change the ruminal pH and population of protozoa ($p > 0.05$). The ruminal pH was reduced at the R:C ratio of 40:60 ($p < 0.01$), whereas the protozoal population at 4 h was increased ($p < 0.05$). The SBM:CWYW ratio did not impact the in vitro volatile fatty acid (VFA) profile ($p > 0.05$). However, the total VFA, and propionate (C3) concentration were significantly increased ($p < 0.01$) by the R:C ratio of 40:60. In conclusion, the replacement of SBM by 75% CWYW did not show any negative impact on parameters observed, and the R:C ratio of 40:60 enhanced the gas kinetics, digestibility, VFA, and C3 concentration.

Keywords: citric waste; yeast waste; industrial by-product; protein source



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1. Introduction

The security of livestock feed is a fairly frequent topic of discussion in terms of quality and quantity, especially in terms of the lack of protein sources, which results in low performance. High-quality protein feed sources such as soybean meal are expensive and lead to an increase in the cost of livestock production [1]. Many researchers have attempted to look for alternative sources of protein that could help improve the production and productivity of livestock [2,3]. The utilization of agro-industrial by-products as animal feed is an interesting consideration, since it could reduce feed costs and help reduce environmental pollution [4].

Citric waste is a by-product of the citric acid industry and is generated from rice, corn, cassava, or cassava pulp that is fermented with *Aspergillus niger* [5]. The citric waste still has some nutritive value of 30–70 g/kg DM of crude protein and contain high fiber content (861.3 g/kg DM of neutral detergent fiber (NDF) and 197.4 g/kg DM of acid detergent fiber (ADF), respectively) [6]. Uriyapongson et al. [6] reported that the inclusion of 10% citric waste in the diet of buffalos did not negatively affect feed intake, average daily gain (ADG), and the feed conversion ratio (FCR), whereas the digestibility was decreased with more than 10% citric waste. This might be due to the high content of fiber limiting digestion by animals, resulting in them being able to utilize only a low amount of nutrients [7]. Therefore, for the use of citric waste as animal feed, we should improve the quality by reducing the fiber composition and enhancing another nutrients, particularly the protein content.

Yeast (*Saccharomyces cerevisiae*) is a source of probiotics that enable a positive effect on the rumen fermentation of ruminants. It has been used as a biological method to improve the protein quality of feedstuff [8]. In ethanol production processes, the initial substrates are molasses and inoculants of the yeast *S. cerevisiae*. Yeast waste is a residue generated from ethanol production. Díaz et al. [9] reported that yeast waste contains a high content of live yeast cells (about 60–70%). Furthermore, Cherdthong et al. [10] revealed that yeast waste contains around 264.0 g/kg of crude protein and is rich in vitamins and minerals. Cherdthong et al. [11] found that dried yeast waste can replace up to 100% of soybean meal in animal feed with no negative effect to feed intake, feed utilization, and ruminal ecology in beef cattle. Therefore, yeast waste obtained from industrial by-products containing many live yeast cells might be beneficial for enhancing feed quality and feed utilization in animals.

The ratio of roughage to concentrate (R:C) in feeds is important for good nutrient utilization for production. Feeds have strong ties with the ecology of the rumen, ruminal bacteria, and trends of rumen fermentation [12]. Suitable amounts of concentrate can provide fiber utilization by enhancing fermentable organic matter, nitrogen, and energy sources for ruminal microbes [3]. Generally, feeding with a concentrate can provide more fermentation end-products than feeding with only roughage. Rice straw is easily obtainable from rice cultivation areas and is usually collected by farmers for feeding cattle [13]. Hence, the feeding of ruminants with rice straw and a concentrated diet that contains a high amount of protein and energy would be beneficial for ruminant productivity [14].

It was hypothesized that inoculated yeast waste obtained from industrial by-products could improve the quality of citric waste and could be used as a potential alternative protein source. Therefore, we investigated the utilization of citric waste fermented yeast waste (CWYW) obtained from agro-industrial by-products as a protein source to replace soybean meal in a concentrated diet, as well as the effect of various roughage to concentrate ratios (R:C) on gas production kinetics, rumen characteristics, and in vitro feed digestibility using an in vitro gas measuring technique.

2. Materials and Methods

The experimental cattle involved in this research were approved by Khon Kaen University's Animal Ethics Committee (record no. IACUC-KKU-27/64).

2.1. Preparation of Citric Waste Fermented Yeast Waste (CWYW)

Yeast waste was received as a by-product of ethanol production from KSL Green Innovation Public Company Limited (KGI), Nam Phong District, Khon Kaen Province, Thailand. Citric waste was obtained as a by-product of the citric acid industry from Sam Mor Farm Limited Partnership, Muang District, Udon Thani Province. Commercial grade urea and molasses were purchased from a local shop.

CWYW was prepared with the procedure that follows. First, 100 mL of yeast waste was added to a flask (A). Next, 20 g of brown sugar was weighed and dissolved in 100 mL of distilled water, and then 50 g of urea was mixed in (B). Yeast waste media solution was made by mixing A and B at a ratio of 1:1 and then flushing them for 16 h with air using an

air pump at room temperature. The pH was adjusted to a range of 3.9 to 4.5. After 16 h, we transferred the yeast waste media solution and mixed it with citric waste at a ratio of 1 mL to 1 g. After that, anaerobic fermentation was performed in container bottles for 14 days, followed by 48 h of sun-drying to obtain less than 10% moisture. The CWYW was packed in a plastic bag for subsequent use as an experimental ingredient for dietary treatment.

2.2. Experimental Design and Dietary Treatments

The present experiment was performed at different incubation intervals using a gas production technique. A 3×5 factorial experiment design was conducted and arranged according to a completely randomized design (CRD) with three replication runs. The experimental diets had three roughage to concentrate (R:C) ratios of 60:40, 50:50, and 40:60 with five replacing soybean meal ratios of replacement in diets (SBM:CWYW) of 100:0, 75:25, 50:50, 25:75, and 0:100. All of the experimental dietary samples were oven-dried at 72 °C and ground to pass a 1 mm sieve (Cyclotech Mill, Tecator, Sweden) for the analysis of the chemical composition and the gas production test. Experimental diets including concentrate, rice straw, and CWYW were analyzed for dry matter (DM; ID 967.03), ash (ID 492.05), and crude protein (CP; ID 984.13) content using the standard analysis of the AOAC [15]. NDF and ADF contents were determined using the procedures of Van Soest et al. [16]. Table 1 shows the diet compositions and ingredients of the concentrate, rice straw, and CWYW used in this experiment. The concentrate diets were prepared with 141.0–142.0 g/kg of CP, which is recommended for beef cattle.

Table 1. Ingredient and chemical composition of concentrates, rice straw and citric waste fermented yeast waste used in the experiment.

Item	SBM:CWYW ¹					RS ²	YW ³	CW ⁴	CWYW ⁵
	100:0	75:25	50:50	25:75	0:100				
Ingredients (kg of dry matter)									
Cassava chips	59.3	58.5	59.5	58.8	59.7	-	-	-	-
Rice bran	9.0	9.0	9.0	9.0	9.0	-	-	-	-
Soybean meal	15.0	12.3	7.5	3.8	0.0	-	-	-	-
Palm kernel meal	13.0	13.0	13.0	13.0	13.0	-	-	-	-
CWYW ¹	0.0	3.8	7.5	12.3	15.0	-	-	-	-
Urea	1.3	1.0	1.0	0.7	0.8	-	-	-	-
Mineral premix	1.0	1.0	1.0	1.0	1.0	-	-	-	-
Molasses, liquid	1.0	1.0	1.0	1.0	1.0	-	-	-	-
Pure sulfur	0.5	0.5	0.5	0.5	0.5	-	-	-	-
Salt	1.0	1.0	1.0	1.0	1.0	-	-	-	-
Chemical composition									
Dry matter (g/kg)	926	923	922	922	918	944	360	919	882
g/kg of dry matter									
Organic matter	888	882	877	861	860	827	899	889	894
Ash	112	118	123	139	140	173	101	111	106
Crude protein	141	142	141	141	142	26	315	110	535
Neutral detergent fiber	159	167	169	171	174	779	217	709	402
Acid detergent fiber	72	84	91	94	99	536	44	426	294

¹ SBM:CWYW = replacing soybean meal with citric waste fermented yeast waste ratio; ² RS = rice straw, ³ YW = yeast waste, ⁴ CW = citric waste; ⁵ CWYW = citric waste fermented yeast waste.

2.3. Animals and Ruminal Inoculums Preparation

Two male 3-year-old ruminally fistulated crossbreed (Thai × Holstein) cattle with body weights (BW) of 280 ± 15.0 kg were used as ruminal liquor donors. Ruminal liquor was obtained while the animals were fed ad libitum with roughage (rice straw) and concentrate (140 g/kg CP and 805 g/kg TDN) at 0.5% of BW daily (6:30 a.m. and 4:30 p.m.). The cattle were housed in individual pens, and clean water and mineral blocks were freely available. The cattle were fed with the diet for 21 d before collecting the rumen liquor.

From each of the cattle, 1000 mL of ruminal liquor was collected before feeding time in the morning. Ruminal liquor was filtered through five layers of cheesecloth and then moved to the laboratory in pre-warmed thermos bottles.

The medium was prepared using the procedures reported by Makkar et al. [17] which consisted of combining 3000 mL of reduced medium with 1500 mL of ruminal liquor from cattle (2:1; reduced medium: ruminal liquor). The medium mixture was then kept under stirring at 39 °C under CO₂ with a hot plate. Each experimental bottle was filled with 40 mL of ruminal fluid mixture and incubated in a water bath at 39 °C.

2.4. In Vitro Gas Production and Ruminal Fermentation Characteristics

The gas production was measured instantly after incubation for 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, and 96 h according to the modified procedures of Cherdthong et al. [10]. Ørskov and McDonald [18] models were used for curve fitting and analysis of the kinetics of gas as follows:

$$y = a + b(1 - e^{(-ct)})$$

where a = soluble fraction from gas production, b = insoluble fraction from gas production, c = rate of gas production constant for the insoluble fraction (b), t = incubate time, $(a + b)$ = the potential extent of gas production, and y = gas produced at time ' t '.

The ruminal pH was recorded using a digital pH meter (HANNA Instrument (HI) 8424 microcomputer, Singapore) at incubation times of 0 and 4 h. The incubated ruminal liquor was divided into two parts. The first part (20 mL) was kept in 5 mL of 1 M H₂SO₄ and stored at −20 °C for ammonia nitrogen (NH₃-N) analysis according to the micro-Kjeldahl methods, and in vitro volatile fatty acid (VFA) concentration was performed according to the procedures of Samuel et al. [19] using high performance liquid chromatography (HPLC machine; Shimadzu LC-20A, Kyoto, Japan) equipped with an Inertsil ODS-3 C18 (250 mm × 4.6 mm i.d., 5 µm,) column and mobile phase: phosphoric acid 25 mM, flow rate: 1 mL/minute, detection (UV): 210 nm; injection: 20 microliters (Shimadzu LC-20A, Kyoto, Japan). The second part (1 mL) was collected in 9 mL of 10% formalin for the direct counting of protozoa [20].

In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were analyzed after incubation for 12 and 24 h using the procedures of Tilley and Terry [21].

2.5. Statistical Analysis

The data of the experiment were statistically evaluated with a 3 × 5 factorial arrangement according to CRD using the Proc. GLM procedure of SAS software version 9.4 (SAS Inst. Inc., Cary, NC, USA) [22]. All data were analyzed by the following equation:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \epsilon_{ij}$$

where: Y = observations; μ = overall mean; A_i = effect of factor A (R:C ratio at 60:40, 50:50, and 40:60; $i = 1$ to 3); B_j = effect of factor B (SBM: CWYW ratio at 100:0, 75:25, 50:50, 25:75, and 0:100; $j = 1$ to 5), AB_{ij} = the interaction effect of R:C ratio and SBM: CWYW, and ϵ_{ij} = the residual effect. Duncan's New Multiple Range Test (DMRT) performed multiple comparisons between treatment methods [23]. Differences between mean values of $p < 0.05$ were considered to represent statistically significant differences.

3. Results

3.1. Dietary Chemical Composition

Experimental dietary ingredients and chemical compositions of rice straw, concentrate, yeast waste, citric waste and CWYW are presented in Table 1. The concentrate diets were provided with almost the same protein content in each group, ranging from 141 to 142 g/kg DM, and urea was administered to adjust the CP content. The yeast waste's CP content was 315 g/kg DM. The citric waste had low CP and high fiber contents (NDF and ADF) of 110,

709, and 426 g/kg DM, respectively. After quality improvement, the CWYW product's CP was increased, and the fiber (NDF and ADF) value was reduced to 535, 402, and 294 g/kg DM, respectively.

3.2. Kinetics and Cumulative Production of Gas

The data obtained for the substrates analyzed from the kinetics and cumulative production of gas are shown in Table 2. The data demonstrated that the soluble fractions of gas production (a), insoluble fraction of gas production (b), rate of constants for the insoluble fraction (c), potential extent of gas production ($|a| + b$) and the cumulative production of gas were not affected by the interaction between the R:C ratio and SBM:CWYW ratio. In addition, the SBM:CWYW ratio did not affect the kinetics and cumulative amount of gas. However, soluble fractions of gas production (a) ranged from -3.7 to -5.3 mL/0.5 g and were decreased at the R:C ratio of 40:60 ($p < 0.01$). The value of the insoluble fraction of gas production (b) was also decreased at the R:C ratio of 40:60 ($p < 0.01$). The value of the rate of gas production (c) ranged from 0.06 to 0.08 mL/h and was increased at the R:C ratio of 40:60 ($p < 0.01$). The potential extent of gas ($|a| + b$) value and the cumulative production of gas (at 96 h of incubation) were increased ($p < 0.01$) at the R:C ratio of 40:60, and the values were about 74.5 and 77.0 mL/0.5 g, respectively.

Table 2. Effect of R:C ratio level combined with SBM:CWYW ratio level on gas kinetics and cumulative gas at 96 h after incubation.

R:C ¹	SBM:CWYW ²	Gas Kinetics ³				Cumulative Gas (mL/0.5g)
		a	b	c	$ a + b$	
60:40	100:0	−3.9	70.4	0.06	74.4	74.7
	75:25	−3.8	70.2	0.06	74.0	73.9
	50:50	−3.7	70.2	0.06	73.9	74.0
	25:75	−3.8	70.4	0.06	74.2	73.6
	0:100	−4.0	70.4	0.06	74.4	74.3
50:50	100:0	−4.9	69.8	0.07	74.8	74.8
	75:25	−5.0	69.8	0.07	74.9	75.7
	50:50	−5.0	69.7	0.07	74.8	75.6
	25:75	−5.0	69.9	0.07	74.9	76.0
	0:100	−5.1	69.7	0.07	74.9	75.7
40:60	100:0	−5.3	69.9	0.07	75.3	76.4
	75:25	−5.4	69.2	0.08	74.6	77.1
	50:50	−5.4	69.5	0.07	74.6	76.0
	25:75	−5.2	69.5	0.07	74.8	75.8
	0:100	−5.2	69.7	0.07	75.1	76.3
SEM		0.21	0.26	0.002	0.30	1.31
Comparison						
R:C ratio		<0.01	<0.01	<0.01	<0.01	<0.01
60:40		−3.8 ^a	70.3 ^a	0.063 ^c	74.2 ^b	74.5 ^c
50:50		−5.0 ^b	69.8 ^b	0.068 ^b	74.8 ^a	75.8 ^b
40:60		−5.3 ^c	69.6 ^b	0.071 ^a	74.9 ^a	77.0 ^a
SBM:CWYW ratio		0.31	0.58	0.96	0.40	0.98
100:0		−4.6	70.1	0.07	74.8	75.9
75:25		−4.6	69.9	0.07	74.5	75.8
50:50		−4.7	69.9	0.07	74.4	75.8
25:75		−4.7	69.8	0.07	74.6	75.7
0:100		−4.8	69.7	0.07	74.8	75.6
Interaction		0.94	0.96	0.39	0.95	0.99

^{a-c} Value on the same row with different superscripts differ ($p < 0.05$); ¹ R:C = roughage to concentrate ratio.

² SBM:CWYW = replacing soybean meal with citric waste fermented yeast waste ratio; ³ a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), $|a| + b$ = the gas potential extent of gas production.

3.3. In Vitro Digestibility

Table 3 shows the influence of substituting SBM for CWYW in combination with the R:C ratio on IVDMD and IVOMD. It was found that IVDMD and IVOMD did not show interaction with each other. There were no changes in IVOMD when the SBM:CWYW ratio was included. The replacement of SBM by CWYW by up to 75% did not alter IVDMD, but 100% CWYW replacement significantly reduced ($p < 0.05$) IVDMD at 24 h of incubation and the mean value ($p < 0.05$). In addition, IVDMD at 12 h and 24 h of incubation and the mean value were significantly increased ($p < 0.01$) with the R:C ratio of 40:60 (about 559, 701, and 631 g/kg, respectively). Moreover, the R:C ratio of 40:60 significantly increased ($p < 0.01$) IVOMD at 12 h, 24 h, and the mean value (706, 793, and 750 g/kg, respectively).

Table 3. Effect of R:C ratio level combined with SBM:CWYW ratio level on in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD).

R:C ¹	SBM:CWYW ²	IVDMD (g/kg)			IVOMD (g/kg)		
		12 h	24 h	Mean	12 h	24 h	Mean
60:40	100:0	541	682	622	683	770	726
	75:25	542	676	618	675	773	724
	50:50	540	675	612	675	774	725
	25:75	540	674	619	674	774	724
	0:100	539	678	611	679	768	723
50:50	100:0	553	691	630	692	781	736
	75:25	553	690	623	693	782	737
	50:50	551	688	621	692	781	736
	25:75	551	689	622	691	775	733
	0:100	551	688	622	691	775	733
40:60	100:0	560	704	634	709	792	751
	75:25	560	702	632	707	795	751
	50:50	559	702	630	706	793	749
	25:75	559	701	626	705	793	749
	0:100	559	700	621	705	793	748
SEM		0.21	0.13	0.10	0.33	0.31	0.19
Comparison							
R:C ratio		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
60:40		540 ^c	677 ^c	609 ^c	677 ^c	772 ^c	724 ^c
50:50		552 ^b	689 ^b	621 ^b	692 ^b	778 ^b	735 ^b
40:60		559 ^a	701 ^a	631 ^a	706 ^a	793 ^a	750 ^a
SBM:CWYW ratio		0.86	<0.01	0.02	0.50	0.32	0.23
100:0		551	692 ^a	622 ^a	693	781	738
75:25		551	689 ^{ab}	620 ^{ab}	692	783	737
50:50		551	688 ^{ab}	620 ^{ab}	690	782	737
25:75		551	688 ^{ab}	620 ^{ab}	691	780	735
0:100		549	686 ^c	617 ^c	692	779	735
Interaction		0.95	0.28	0.78	0.92	0.73	0.94

^{a-c} Value on the same row with different superscripts differ ($p < 0.05$); ¹ R:C = roughage to concentrate ratio.

² SBM:CWYW = replacing soybean meal with citric waste fermented yeast waste ratio.

3.4. Ruminal NH₃-N, pH and Protozoal Population

Table 4 shows the influence of substituting SBM for CWYW in combination with the R:C ratio on the ruminal NH₃-N, pH, and population of protozoa. There was no interaction effect between factors on the ruminal NH₃-N, pH, and protozoal population ($p > 0.05$). The SBM:CWYW ratio of 0:100 significantly increased the impact ($p < 0.05$) on ruminal NH₃-N at 2 h, 4 h, and the mean value with the highest values of 17.6, 19.4 and 18.2 mg/dL, respectively. However, the pH and protozoal population were not affected by the SBM:CWYW ratio ($p > 0.05$).

Table 4. Effect of R:C ratio level combined with SBM:CWYW ratio level on ruminal NH₃-N, pH and protozoal population.

R:C ¹	SBM:CWYW ²	NH ₃ -N (mg/dL)			pH			Protozoal Count (×10 ⁵ cell/mL)		
		2 h	4 h	Mean	2 h	4 h	Mean	2 h	4 h	Mean
60:40	100:0	15.4	16.5	16.4	7.04	6.99	7.01	3.8	3.9	3.8
	75:25	14.8	15.8	16.0	7.10	7.04	7.07	3.8	3.9	3.8
	50:50	15.6	16.6	16.6	7.11	7.05	7.08	3.7	4.0	3.9
	25:75	15.7	16.7	17.2	7.11	7.04	7.07	3.8	3.9	3.8
	0:100	16.0	17.1	17.2	7.10	7.04	7.08	3.7	4.0	3.9
50:50	100:0	16.7	17.0	16.8	6.97	6.93	6.94	3.7	4.1	3.9
	75:25	16.7	17.8	17.6	6.97	6.93	6.95	3.7	4.1	3.9
	50:50	17.3	17.7	17.4	7.00	6.93	6.95	3.7	4.1	3.9
	25:75	17.8	18.4	17.9	6.97	6.94	6.95	3.6	4.2	3.8
	0:100	17.8	18.9	18.3	6.97	6.92	6.94	3.7	4.2	4.0
40:60	100:0	17.9	18.8	18.8	6.97	6.73	6.84	3.8	4.3	4.0
	75:25	18.6	19.6	19.3	6.97	6.73	6.84	3.8	4.3	4.0
	50:50	19.2	20.2	19.7	6.97	6.74	6.85	3.7	4.3	4.0
	25:75	19.3	20.4	19.8	6.97	6.73	6.84	3.7	4.3	4.0
SEM	0:100	19.3	20.4	19.8	6.99	6.74	6.87	3.9	4.3	4.1
		0.42	0.45	0.44	0.04	0.04	0.06	0.48	0.07	0.26
Comparison										
R:C ratio		<0.01	<0.01	<0.01	0.04	<0.01	<0.01	0.43	<0.01	0.92
60:40		15.4 ^b	16.6 ^c	16.0 ^c	7.09 ^a	7.03 ^a	7.06 ^a	3.8	3.9 ^b	3.9
50:50		16.9 ^b	17.9 ^b	17.4 ^b	6.97 ^b	6.90 ^b	6.94 ^b	3.7	4.1 ^b	3.9
40:60		18.9 ^a	19.8 ^a	19.4 ^a	6.96 ^b	6.83 ^c	6.85 ^c	3.6	4.2 ^a	3.8
SBM:CWYW ratio		0.02	0.01	0.02	0.98	0.93	0.96	0.98	0.58	0.98
100:0		16.4 ^c	17.5 ^c	16.9 ^c	7.02	6.90	6.93	3.7	4.1	3.9
75:25		16.9 ^{bc}	17.6 ^{bc}	17.2 ^{bc}	7.01	6.90	6.95	3.7	4.1	3.9
50:50		17.2 ^{abc}	18.3 ^{abc}	17.6 ^{abc}	7.01	6.90	6.95	3.7	4.1	3.9
25:75		17.4 ^{ab}	18.5 ^{ab}	18.0 ^{ab}	7.00	6.90	6.96	3.7	4.1	3.9
0:100		17.6 ^a	19.4 ^a	18.2 ^a	6.98	6.88	6.96	3.8	4.2	4.0
Interaction		0.54	0.70	0.63	0.99	0.98	0.99	0.99	0.77	0.99

^{a-c} Value on the same row with different superscripts differ ($p < 0.05$); ¹ R:C = roughage to concentrate ratio. ² SBM:CWYW = replacing soybean meal with citric waste fermented yeast waste ratio.

Additionally, the R:C ratio of 40:60 significantly increased ($p < 0.01$) the ruminal NH₃-N at 2 h, 4 h, and the mean value and the values, which were 18.9, 19.8, and 19.4 mg/dL, respectively. The ruminal pH at 2 h, 4 h, and the mean values ranged from 6.73 to 7.10, which were reduced ($p < 0.01$) by the R:C ratio of 40:60 group. The protozoal population at 2 h and the mean values remained unchanged ($p > 0.05$), while the protozoal population at 4 h was increased ($p < 0.05$) by the R:C ratio of 40:60.

3.5. In Vitro VFAs Concentration

Table 5 shows the influence of substituting SBM for CWYW in combination with the R:C ratio level on in vitro VFAs. An interaction effect was not detected between factors on the in vitro VFA concentration ($p > 0.05$). In addition, the SBM:CWYW ratio did not impact the in vitro VFA profile ($p > 0.05$). However, the total VFA at 2 h, 4 h, and the mean value were significantly increased ($p < 0.01$) by decreasing the R:C ratio to 40:60. Propionate (C3) at 2 h, 4 h, and the mean value were significantly increased ($p < 0.01$) by decreasing the R:C ratio to 40:60. Furthermore, the decrease in the R:C ratio to 40:60 decreased ($p < 0.01$) acetate (C2) at 2 h, 4 h, and the mean value. The C2 to C3 ratio (C2:C3) at 2 h, 4 h, and the mean value were decreased ($p < 0.01$) by decreasing the R:C ratio, while butyrate (C4) remained similar ($p > 0.05$).

Table 5. Effect of R:C ratio level combined with SBM:CWYW ratio level on in vitro volatile fatty acids (VFAs).

R:C ¹	SBM:CWYW ²	Total VFA (mmol/L)			C2 (mol/100 mol)			C3 (mol/100 mol)			C4 (mol/100 mol)			C2:C3 Ratio		
		2 h	4 h	Mean	2 h	4 h	Mean	2 h	4 h	Mean	2 h	4 h	Mean	2 h	4 h	Mean
60:40	100:0	85.7	86.9	81.3	65.0	63.0	63.9	25.1	27.0	26.1	10.0	10.1	10.0	2.6	2.3	2.5
	75:25	84.7	86.9	80.8	65.3	63.3	64.3	26.1	28.0	27.0	8.7	8.7	8.6	2.5	2.3	2.4
	50:50	84.6	86.8	80.7	65.8	63.8	63.7	25.9	27.8	26.8	8.4	8.5	8.4	2.5	2.3	2.4
	25:75	84.3	86.5	80.4	65.6	64.0	64.7	25.7	27.8	26.8	8.7	8.3	8.4	2.6	2.3	2.5
	0:100	84.1	86.6	80.3	65.8	63.8	64.8	25.6	27.5	26.6	8.6	8.7	8.6	2.6	2.3	2.5
50:50	100:0	93.2	88.7	85.9	62.9	61.3	62.2	26.7	28.6	27.6	10.4	10.1	10.2	2.4	2.1	2.5
	75:25	93.5	88.5	85.9	63.2	61.5	62.3	26.9	28.9	27.9	10.0	9.7	9.8	2.4	2.1	2.3
	50:50	93.1	88.6	85.8	63.5	61.0	62.3	26.6	28.6	27.6	10.0	10.4	10.1	2.4	2.1	2.3
	25:75	94.6	88.6	86.6	63.5	61.5	62.5	26.0	28.0	27.0	10.5	10.5	10.5	2.4	2.2	2.2
	0:100	93.2	87.7	85.4	63.6	61.6	62.6	26.1	27.9	26.9	10.3	10.6	10.4	2.4	2.2	2.3
40:60	100:0	98.3	91.2	89.8	61.4	59.4	60.4	29.2	30.3	29.7	9.4	10.3	9.8	2.1	2.0	2.2
	75:25	97.0	91.1	89.1	61.9	60.4	61.1	29.0	30.5	29.7	9.1	9.1	9.1	2.1	2.0	2.0
	50:50	97.7	90.6	88.8	61.5	59.5	60.5	28.8	30.1	29.4	9.7	10.5	10.0	2.1	2.0	2.1
	25:75	97.1	90.1	88.9	61.8	59.8	60.8	28.6	30.5	29.5	9.6	9.8	9.7	2.2	2.0	2.0
	0:100	97.2	90.5	88.8	62.0	60.2	61.2	28.6	29.6	29.3	9.4	9.9	9.6	2.2	2.0	2.1
SEM		0.52	0.93	0.60	0.73	0.61	0.65	1.14	1.13	1.14	1.39	1.26	1.31	0.11	0.10	0.10
Comparison																
R:C ratio		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.32	0.22	0.27	<0.01	<0.01	<0.01
60:40		74.7 ^c	86.7 ^c	80.7 ^c	65.4 ^a	63.5 ^a	64.5 ^a	25.7 ^b	27.7 ^b	26.7 ^b	8.9	8.8	8.9	2.6 ^a	2.3 ^a	2.4 ^a
50:50		83.5 ^b	88.4 ^b	86.0 ^b	63.4 ^b	61.3 ^b	62.4 ^b	26.4 ^b	28.2 ^b	27.4 ^b	9.4	10.2	10.2	2.3 ^b	2.1 ^b	2.2 ^b
40:60		87.3 ^a	90.7 ^a	89.1 ^a	61.8 ^c	59.8 ^c	60.8 ^c	28.8 ^a	30.2 ^a	29.5 ^a	10.2	9.9	9.4	2.2 ^b	1.9 ^c	2.0 ^c
SBM:CWYW ratio		0.18	0.88	0.60	0.76	0.72	0.75	0.97	0.95	0.97	0.97	0.90	0.95	0.92	0.97	0.93
100:0		82.3	88.9	85.7	63.8	61.9	62.2	27.0	28.6	27.8	8.9	8.8	8.9	2.4	2.1	2.3
75:25		82.2	88.8	85.3	63.6	61.8	62.6	27.3	29.1	28.2	9.4	10.2	10.2	2.3	2.1	2.3
50:50		81.7	88.6	85.1	61.6	61.7	62.5	27.1	28.8	27.9	10.2	9.9	9.4	2.4	2.1	2.2
25:75		81.6	88.3	85.3	65.4	61.4	62.7	26.8	28.7	27.8	8.9	8.8	8.9	2.4	2.1	2.3
0:100		81.5	88.2	84.9	63.1	61.3	62.8	26.8	28.5	27.6	9.4	10.2	10.2	2.4	2.1	2.3
Interaction		0.42	0.98	0.95	0.98	0.96	0.998	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

^{a-c} Value on the same row with different superscripts differ ($p < 0.05$); ¹ R:C = roughage to concentrate ratio. ² SBM:CWYW = replacing soybean meal with citric waste fermented yeast waste ratio.

4. Discussion

4.1. Dietary Chemical Composition

In this study, the CP content of non-fermented citric waste was 110 g/kg DM, which was comparable to the result reported by Silva et al. [5] (79.3–110.8 g/kg DM of CP). However, a lower content of CP of 61.1 g/kg DM in citric waste was found in a study of Tanpong et al. [7]. The NDF and ADF contents in non-fermented citric waste were lower than in the report by Uriyapongson et al. [6] (861.3 and 197.4 g/kg DM, respectively). This was probably due to the differences between raw materials used for citric production, such as the variety, age of harvest, and soil fertilizer [24,25]. In addition, the processing of citric production might influence the different nutrient compositions [5].

The CP content in the yeast waste used in this study was 315 g/kg DM, which is close to the result from Bátori et al. [26], who reported 320 g/kg DM. However, the CP content in yeast waste could vary from about 182.5 to 296 g/kg DM [10,27–29]. This might be caused by substrate used in ethanol production, heat from the fermentation process, yeast strain, and freshness of the yeast stillage, which is a complex medium [26,30,31].

After improving the citric acid waste quality by yeast waste fermentation, CWYW was found to have increased CP to 535 g/kg DM when compared with non-fermented citric waste. This could be due yeast waste containing high protein content and being rich in essential amino acids [9,10]. In addition, the inclusion of media solution containing urea in the CWYW fermented product during the fermentation process might enhance the CP content [3]. Fiber contents were reduced when citric acid waste was fermented with yeast waste. Similarly, Suntara et al. [32] indicated that some strains of yeast could produce cellulolytic enzymes to break down fiber in plant materials. Furthermore, the fermentation process with a media containing urea as an alkaline agent might degrade the structure fiber of CWYW and lead to the fiber structure decreasing [33].

4.2. Kinetics and Cumulative Production of Gas

Yeast waste rich in *S. cerevisiae* could promote the microorganism in the rumen and improve the incubated substrate's digestibility, thus improving the kinetics of gas production and increasing the total production of gas [34]. However, in this study, the kinetics of gas was not changed by the SBM:CWYW ratio. This is probably due to the fermentation of protein not leading to the production of gas [35]. This agreed with the results of Cherdthong et al. [10], who stated that the gas and fermentation kinetics was not changed by the substitution of soybean meal with yeast waste.

Moreover, soluble fraction (a), rate of gas production (c), potential extent of gas production ($|a| + b$), and the cumulative production of gas were significantly affected by the R:C ratio. The addition of a high concentration ratio contributes to an improvement in the rate of fermentation and soluble fraction of the rumen [34]. Starch degradation is an important factor in regulating energy utilization for the growth of rumen microorganisms, increasing the rumen population, and increasing digestion [36]. The potential extent of gas production ($|a| + b$) is known to be essentially the result of the carbohydrates fermented into acetate, propionate, and butyrate [37].

The present results demonstrated that the intercept value of (a) was negative in this study. This was a result of the delay in ruminal microbial growth of the substrates during the early stage of incubation. The data show that there is a lag period after the soluble part of the substrate is ingested but before the cell walls are fermented [38,39]. Several researchers [35,40] have also stated that, when using mathematical models to match the kinetics of gas output, there were negative values for different substrates. It is understood that it is possible to use the absolute value of a, ($|a|$), to define the ideal fermentation of the soluble fraction. In this experiment, the absolute gas production was the highest for the R:C ratio of 40:60. The soluble fraction makes it easy for rumen microbes to bind and contribute to the greater production of gas [41]. The results revealed that the insoluble fraction (b) at the R:C ratio of 60:40 was significantly the highest value. The high fiber in feed had an effect of the increase in (b), which increases the polysaccharides and activities of

glycoside hydrolase against lignified plant tissues [3]. Particularly, the NDF degradability was substantially associated with the NDF fraction. Similarly, Phesatcha et al. [34] revealed that (b) of gas production increased as the ratio of concentrates in the diet decreased.

4.3. In Vitro Digestibility

The yeast *S. cerevisiae* can scavenge the accessible oxygen to support metabolic activity, thus reducing the ruminal redox potential and stimulating the ruminal microbes to have a higher rate of feed digestion. This improves the digestibility of nutrients [34]. In addition, the findings of Cherdthong et al. [11] showed that 100% of yeast waste could be used to replace SBM as a source of protein in concentrated diets without detrimental effects on digestibility. The present results indicated that CWYW can replace SBM at up to 75% without a negative impact on IVDMD and IVOMD.

During its metabolic activities, *S. cerevisiae* may be responsible for secreting extracellular enzymes into the citric waste mash, such as lignocellulose peroxidase, lignin peroxidase, cellulase, and hemicellulose [32,42]. This results in yeast proliferation. Additionally, this could happen because the alkaline agents (ammonium hydroxide; NH_4OH) produced from urea during the fermentation process of CWYW cause the hemicellulose–lignin complex in citric waste to swell [33]. The concentrated alkaline agents can physically swell structural fibers by chemically degrading their ester bonds [43]. This could help enable the extracellular enzymes from *S. cerevisiae* to attack the structural carbohydrates more easily and increase the degradability of CWYW.

However, replacement with SBM:CWYW at up to 100% decreased IVDMD at 24 h and the mean value. This could be due to the structural carbohydrates content in the CWYW negatively affecting digestibility in vitro. Uriyapongson et al. [6] reported that the use of citric waste at more than 10% in the diet results in the digestibility decreasing because of the high fiber content. It was concluded that changes in cell-wall composition involving structural carbohydrate contents in CWYW restricted the possible degree of digestion, while chemical factors other than the crystalline or physical nature of the fiber limited the rate of digestion [44].

The R:C ratio of 40:60 improved the in vitro digestibility. This may have been due to increased levels of concentrate, which would supply energy that is more readily available, thereby improving the subsequent degradability by ruminal microbes. The concentrate diet has a pronounced stimulatory effect on the ruminal microflora that is achieved more readily from carbohydrates than from forages in the rumen [45]. These studies agree with Cherdthong et al. [12], who demonstrated that when the fiber value was reduced, particularly with a higher concentrate level, ruminal microbe activity could be encouraged [46]. However, in buffalo, the in vitro organic matter digestibility (IVOMD) increased with the increase in concentrate in the diet, while the cumulative gas production showed an irregular trend and was not closely correlated to digested OM [47].

4.4. In Vitro Ruminal $\text{NH}_3\text{-N}$ Concentration and Ruminal pH

In the present study, the ruminal $\text{NH}_3\text{-N}$ concentration was increased with higher levels of concentrate diet and levels of CWYW used to replace soybean meal. This is probably due to CWYW containing yeast waste, which has a high protein content of 315 g/kg DM. Thus, substantial increases in $\text{NH}_3\text{-N}$ concentrations occur in response to the microbial degradation of yeast cells [48,49]. Additionally, it could be due to the ability to provide stimulatory factors and even protein [50,51] to ruminal bacteria, or by changing in the abundance of microbes with proteolytic activity [52].

Another reason is likely the NPN-urea level in CWYW, which was higher than in previous studies by Polyorach et al. [3], where increased levels of urea-N in feed resulted in an increase in ruminal $\text{NH}_3\text{-N}$ concentration from the dissolution of urea. The rapid hydrolysis of NPN-urea to rumen $\text{NH}_3\text{-N}$ by microbial enzymes is another possible cause in the present study [10]. The amount of N actually digested in the rumen increased as the proportion of concentrate in the diet increased, which is likely to be a key explanation

for enhancing in the concentration of $\text{NH}_3\text{-N}$ in the rumen [14]. Additionally, decreasing the R:C ratio from 60:40 to 40:60 in the diet increased the ruminal $\text{NH}_3\text{-N}$ concentration. Similarly, Suriyapha et al. [46] and Matra et al. [53] revealed that the concentrations of rumen $\text{NH}_3\text{-N}$ increased significantly with a decreasing R:C ratio.

The ruminal pH is an important parameter that reflects the internal homeostasis of the rumen environment. Normally, ruminants have a highly balanced ecology for preserving a ruminal pH range of 6.0–7.0 [14]. The yeast motivates lactate users and enhances their population, but it also serves as a contender with the producers of lactate [54]. However, the data of this study revealed that the ruminal pH was not changed by the influence of the SBM: CWYW ratio. Similarly, Cherdthong et al. [10] found that 100% of yeast waste used to replace soybean meal did not change the ruminal pH in vitro, and saw no negative impact on the ruminal pH in Thai native bulls [11]. Additionally, ruminal pH at 4 h and the mean value were decreased by the higher R:C ratio. This agrees with Cherdthong et al. [12], who reported that a high ratio of concentrate diet usually results in a significant drop in ruminal pH, which decreases the activity of cellulolytic bacteria and slows digestion.

4.5. *In Vitro* Protozoal Population

This study revealed that the number of protozoa did not change when changing the SBM: CWYW ratio. However, the protozoal counts at 4 h was increased by the highest concentrate ratio, which agrees with Cherdthong et al. [12]. This might have happened because of the role of protozoal in starch utilization, which progressively increase when a carbohydrate with fast fermentation is added [34]. In contrast, Van Soest [55] demonstrated that feeding over a certain level of concentrate diet could reduce the population of protozoa. Suriyapha et al. [46] and Matra et al. [53] revealed that an experimental diet with an R:C ratio higher than 30:70 decreased the protozoal population. This is probably due to the increased concentrate diet leading to a high fermentation rate, which results in a lower pH that is unsuitable for the rumen ecology and decreases protozoal populations [3].

4.6. *In Vitro* VFAs

When replacing SBM with CWYW at up to 100%, the concentration of VFA and VFA profiles could be maintained. Similar results on VFA production between CWYW and SBM indicate that CWYW has similar nutritional quality and that it could be comparable to SBM when used to enhance ruminal end-products. Increased concentrate levels enhanced in vitro VFA, which could be supported by the fact that a concentrate diet contains a fraction of highly degradable carbohydrates, particularly starch. The high level of starch in concentrate diet appeared to increase the total VFA and C3, while C2 and the C2:C3 ratio were decreased with an expanding concentrate level [45,56].

In particular, C3 is obtained by the fermentation of soluble carbohydrates with more concentrate diet by ruminal bacteria activity [57]. This agrees with Cherdthong et al. [11], who also reported that the fermentation of a high concentrate level resulted in a greater molar concentration of ruminal C3. In addition, Phesatcha et al. [34] reported that increasing the ratio of a concentrate diet to 80% could increase VFA and C3, whereas C2 and the C2:C3 ratios decreased.

5. Conclusions

Citric waste can improve the nutritional values by being fermented with yeast waste and appropriate media solutions. No interaction effect was found between the R:C ratio and SBM: CWYW for all parameters. CWYW could be substituted for SBM in concentrate diets at up to 75% by without negative impact on gas kinetics, ruminal parameters, and in vitro digestibility. In addition, the R:C ratio of 40:60 could be beneficial for gas kinetics, ruminal ecology, digestibility, volatile fatty acids, and propionic acid concentration. However, more in vivo trials should be conducted in order to determine the success of animal production.

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