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Manipulation of In Vitro Ruminal Fermentation and Feed Digestibility as Influenced by Yeast Waste-Treated Cassava Pulp Substitute Soybean Meal and Different Roughage to Concentrate Ratio

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Abstract: Cassava pulp (CS) is high in fiber and low in protein; hence, improving the nutritive value of CS is required to increase its contribution to enhancing ruminant production. The present work hypothesized that CS quality could be enhanced by fermentation with yeast waste (YW), which can be used to replace soybean meal (SBM), as well as lead to improved feed utilization in ruminants. Thus, evaluation of in vitro ruminal fermentation and feed digestibility, as influenced by YW-treated CS and different roughage (R) to concentrate (C) ratios, was elucidated. The design of the experiment was a 5 × 3 factorial arrangement in a completely randomized design. Each treatment contained three replications and three runs. The first factor was replacing SBM with CS fermented with YW (CSYW) in a concentrate ratio at 100:0, 75:25, 50:50, 25:75, and 0:100, respectively. The second factor was R:C ratios at 70:30, 50:50, and 30:70. The level of CSYW showed significantly higher ($p < 0.01$) gas production from the insoluble fraction (b), potential extent of gas production (a + b), and cumulative gas production at 96 h than the control group ($p < 0.05$). There were no interactions among the CSYW and R:C ratio on the in vitro digestibility ($p > 0.05$). Furthermore, increasing the amount of CSYW to replace SBM up to 75% had no negative effect on in vitro neutral detergent fiber degradability (IVNDFD) ($p > 0.05$) while replacing CSWY at 100% could reduce IVNDFD ($p > 0.05$). The bacterial population in the rumen was reduced by 25.05% when CSYW completely replaced SBM ($p < 0.05$); however, 75% of CSWY in the diet did not change the bacterial population ($p > 0.05$). The concentration of propionate (C3) decreased upon an increase in the CSYW level, which was lowest with the replacement of SBM by CSYW up to 75%. However, various R:C ratios did not influence total volatile fatty acids (VFAs), and the proportion of VFAs ($p > 0.05$), except the concentration of C3, increased when the proportion of a concentrate diet increased ($p < 0.05$). In conclusion, CSYW could be utilized as a partial replacement for SBM in concentrate diets up to 75% without affecting gas kinetics, ruminal parameters, or in vitro digestibility.

Keywords: yeast waste; cassava pulp; rumen fermentation; in vitro gas production technique

Citation: Dagaew, G.; Cherdthong, A.; Wongtangtintharn, S.; Wanapat, M.; Suntara, C. Manipulation of In Vitro Ruminal Fermentation and Feed Digestibility as Influenced by Yeast Waste-Treated Cassava Pulp Substitute Soybean Meal and Different Roughage to Concentrate Ratio. *Fermentation* **2021**, *7*, 196. https://doi.org/10.3390/fermentation7030196

Academic Editor: Ronnie G. Willaert

Received: 1 September 2021
Accepted: 15 September 2021
Published: 17 September 2021

1. Introduction

Cassava (*Manihot esculenta*) is a root crop planted mostly in the tropical and subtropical regions of the world. Per hectare, 25 to 60 tons is produced, and cassava is resistant to poor soils, diseases, and drought [1]. The world's cassava production is expected to be around 230 million metric tons per year, produced predominantly in Nigeria, Brazil, Thailand, Vietnam, Indonesia, and the Democratic Republic of Congo [2,3]. Cassava pulp (CS) is a byproduct of the extraction of starch from cassava roots, and its disposal can have negative consequences for the environment. As a result, the starch industry has attempted to phase it out or find alternative uses for it. The use of CS as an animal feed is an alternative to

solve this problem [4]. However, CS is high in fiber and low in protein; hence, there have been various elucidated methods to improve its nutritional value [5].

Yeast (*Saccharomyces cerevisiae*) is a source of probiotics that have a beneficial effect on rumen fermentation. Crude protein (CP) in CS increased by nearly 7% in microbial mixed culture of *S. cerevisiae* and fermentation procedures using solid media [6]. In ruminant feeding, the utilization of microorganisms, including *S. cerevisiae*, has become common [7]. Boonnop et al. [8] found that *S. cerevisiae* fermented cassava chip-enhanced CP levels from 2% to 30.4%. In addition, Polyorach et al. [9,10] reported that yeast fermented cassava chip protein (YEFECAP) might well be created to promote a CP level of up to 47%. However, *S. cerevisiae* products tend to be expensive; thus, alternate yeast sources should be considered.

Since the concentrated amounts of active yeast can be obtained from the local industry, the process of employing yeast for animal feed is exciting. In ethanol production processes, the initial substrates are molasses and inoculants of the yeast *S. cerevisiae*. Yeast waste is the byproduct of *S. cerevisiae*, fermenting sugarcane juice and molasses to produce bioethanol (YW). YW is generated throughout the year and contains 60–70% of yeast live cells and a CP content of about 30–35% [11,12]. Cherdthong et al. [13] found that using YW as a replacement for soybean meal (SBM) had no negative impact on feed intake or rumen fermentation in ruminant diets up to 100%. An earlier study demonstrated that the quality of citric waste can improve by being treated with YW, which could be a potential replacement for SBM up to 75% [14].

We hypothesized not only the significance of the costs suffered by SBM, but also the impact on the environment. The usage of cassava starch plant waste (cassava pulps, CS) and ethanol industry byproduct (yeast waste, YW) has never been reported. As a consequence, optimization of industrial use and zero waste of raw materials throughout every operation of the plant is a challenging idea, and our main goal is to enhance feed utilization in ruminants. Thus, evaluation of in vitro ruminal fermentation and feed digestibility, as influenced by CS fermented with YW (CSYW) and different roughage to concentrate ratios, was elucidated.

2. Materials and Methods

2.1. Preparation of Cassava Pulp Fermented with Yeast Waste (CSYW)

YW was supported by Khon Kaen Sugar Industry Public Co., Ltd. Cassava pulp (CS), commercial grade urea, and molasses were purchased from the local shop. The media and solution were prepared as follows: (1) CSYW was obtained by the combination of 100 mL of YW and was weighed equally into a flask containing 100 mL distilled water, then was mixed and incubated at room temperature for 2 h; (2) the medium solution was prepared by mixing 24 g of molasses and 50 g of urea into 100 mL distillation water; then the pH of the medium solution was adjusted using H_2SO_4 until the final pH was obtained at 3.5–5; (3) the solution of (1) and (2) was mixed at the ratio 1:1 and then flushed with oxygen for 18 h; (4) after 18 h, CS was mixed well with the yeast medium solution (3) at the ratio 100 g to 50 mL; (5) then the product was allowed to ferment for 14 days, followed by sun drying for 72 h to keep the moisture lower than 10%, and used for a substrate test in the in vitro gas production.

2.2. Experimental Design and Dietary Treatments

The design of the experiment was a 5 × 3 factorial arrangement in a completely randomized design (CRD). Each treatment contained three replications and three runs. The first factor replaced SBM with CSYW in a concentrate ratio at 100:0, 75:25, 50:50, 25:75, and 0:100, respectively. The second factor was roughage (R) to concentrate (C) ratios at 70:30, 50:50, and 30:70. All samples of substrates were dried at 60 °C for 48 h. Before chemical analysis, samples were dried in an oven at lower temperatures (60 °C) for 48 h and then ground by forcing them through a 1 mm steel screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA, USA). All samples were analyzed for dry matter (DM; ID 967.03), ash (ID 492.05), ether extract (EE; ID 455.08), and CP (CP; ID 984.13) by using the procedures of

Association of Official Analytical Chemists (AOAC) [15]. Neutral detergent fiber (NDF) in substrates was measured following the work of Van Soest et al. [16], with supplementation of alpha-amylase but no sodium sulphite, and results are demonstrated with residual ash.

2.3. Ruminal Fluid Donors and Substrates of Inoculum

Two rumen fluid donors were obtained from 2-year-old dairy steers (400 ± 15.0 kg body weight; BW) and collected via fistulae rumens. The animals were fed concentrate containing CP 180 g/kg DM, OM 920 g/kg DM, NDF 220 g/kg DM, ADF 108 g/kg DM, and 806 g/kg total digestible nutrient (TDN) at 0.5% of BW (07:00 and 16:00); rice straw was provided to the animals on an ad libitum basis. The steers were housed separately and supplied with water ad libitum. Before morning feeding, 1500 mL of rumen fluid was obtained from the animals via cannula. The samples were filtered through four layers of cheesecloth and placed in a container with thermal insulation (39 °C) before being delivered to the lab in 15 min. According to Menke and Steingass [17], artificial saliva preparations contained distilled water (1095 mL), a micro mineral mixture (0.23 mL; $MnCl_2{\cdot}4H_2O$ 10.0 g/100 mL, $CaCl_2{\cdot}2H_2O$ 13.2 g/100 mL, $CoCl_2{\cdot}6H_2O$ 1.0 g/100 mL, and $FeCl_3{\cdot}6H_2O$ 8.0 g/100 mL), a macro mineral mixture (365 mL; KH_2PO_4 6.2 g/L, Na_2HPO_4 5.7 g/L, NaCl 2.22 g/L, and $MgSO_4{\cdot}7H_2O$ 0.6 g/L), a resazurin mixture 0.1% (1 mL), a reduction mixture (60 mL; $Na_2S{\cdot}9H_2O$ 80.0 mg/ 60 mL of NaOH), and a buffer mixture (730 mL; $NaHCO_3$ 35.0 g/L and NH_4HCO_3 4.0 g/L). The artificial saliva was then combined with rumen fluid (660 mL) in a non-oxygen atmosphere. Dietary treatments were weighed at 0.5 g in the 50 mL bottles; a total of 40 mL of rumen liquor medium was added to each treatment bottle using an 18 gauge × 1.5-inch needle. Finally, all experimental bottles were sealed with butyl rubber stoppers and metal caps before being incubated in a hot-air oven at 39 °C for further measurement.

Three groups of experimental bottles were established: Group 1 had gas kinetics and gas production measurement, and 3 bottles per treatment (15 treatments + 3 bottles of blank) were used. The bottles were gently shaken every 3 h throughout the incubation time, and each run included three treated bottles and three blank bottles. The blank bottles contained only rumen liquor, and net gas yield was calculated by subtracting the average value of the gas yields from experimental bottles. A 20 mL glass aloe precision hypodermic syringe (U4520, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used to measure gas production. The bottles in the heating chamber were punctured using an 18-gauge injection needle. Group 2 had the pH, ruminal NH_3-N, volatile fatty acids, and microbial count all examined in the same bottle. The samples were taken at 4 h of incubation time from three replicates of a bottle. The last nutrient degradability was measured in Group 3; samples were obtained at 12 h after incubation from three bottle replicates.

2.4. In Vitro Gas Production and Fermentation Characteristics

The amount of gas produced was measured at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, and 96 h of incubation. The bottles were divided into 3 sets. The first set was used for gas kinetics and gas production measurement. The second set was used for measurement of ruminal parameters at 4 and 8 h post-incubation, including pH (Hanna Instruments Pte Ltd., Kallang Way, Singapore), ruminal ammonia-nitrogen (NH_3-N) (Kjeldahl methods [15]), volatile fatty acids (VFA) [18], and ruminal microorganism direct counts (Boeco, Hamburg, Germany). The last set was used for the determination of in vitro degradability (IVDMD), in vitro NDF degradability (IVNDFD), and in vitro ADF degradability (IVADFD) [16,19]. The fermented residues were filtered into an Ankom filter bag (ANKOM 200, ANKOM Technology, New York, NY, USA), dried at 60 °C in an oven for 72 h, and assessed for IVDMD according to Galyean [19] IVDMD% = 100 × [(initial dry sample wt-(residue-blank))/initial dry sample wt] [19]. Dried residues were added with an NDF and ADF solution to measure IVNDFD and IVADFD [16].

2.5. Statistical Analysis

The model of Sommart et al. [20] was used for determining the kinetics of gas production.

$$Y = a + b\ (1 - e^{-ct}) \quad (1)$$

where "a" is the intercept, which ideally reflects the fermentation of the soluble fraction, "b" is the fermentation of the insoluble fraction (which is with the time fermentable), "c" is the rate of gas production, "|a| + b" is the potential extent of gas production, and "Y" is the gas produced at time "t". All data were analyzed as a 5 × 3 factorial arrangement in a completely randomized design (CRD) using the PROC GLM of SAS program [21]. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) [22]. Differences among means with $p < 0.05$ were accepted as being statistically significant.

3. Results

3.1. Nutritional Composition of Feed

The nutritional composition and formulation of the experimental diet are presented in Table 1. The control diet contained a high level of SBM at 180 g/kg and urea 10 g/kg as the main nitrogen source. The CP content in CS was enhanced by fermented YW, and 537 g/kg CP was obtained when the CSYW product was generated. The CSYW contained 349 g/kg DM, 243 g/kg NDF, and 113 g/kg ADF, and the CP content was high at 537 g/kg DM. SBM was replaced by CSYW as a protein source in concentrate diets from 25–100%, resulting in a reduction in the usage of urea in formulations. The CP content of the concentrate diets was similar among the formulas and ranged from 140 to 143 g/kg DM, while the ash, NDF, and ADF content increased as the quantity of CSWY was added.

Table 1. Feed ingredients and chemical composition used in the experimental ration.

Ingredients	Levels of CSYW (g/kg Dry Matter)					CSYW [1]	Rice Straw
	0	25	50	75	100		
Cassava chip	580	580	550	555	550		
Rice bran	120	150	147	122	120		
Palm kernel meal, solvent	80	80	113	135	143		
Soybean meal	180	113	75	37	0		
CSYW [1]	0	37	75	113	150		
Mineral premix	5	5	5	5	5		
Urea	10	10	10	8	7		
Molasses	10	10	10	10	10		
Pure sulfur	10	10	10	10	10		
Salt	5	5	5	5	5		
Chemical composition							
Dry matter (g/kg)	906	901	903	904	912	349	924
—g/kg of dry matter—							
Organic matter	958	930	915	901	902	845	86.5
Ash	42	70	85	99	98	103	125
Crude protein	143	141	141	140	140	537	23
Neutral detergent fiber	150	207	236	258	272	243	755
Acid detergent fiber	92	126	151	174	183	113	553

[1] CSYW = cassava pulp fermented with yeast waste.

3.2. Gas Kinetics and Cumulative Gas Production

In terms of gas production kinetics, no interactions between CSYW levels and the R:C ratio were detected ($p > 0.05$; Table 2). It was found that gas produced immediately from a soluble fraction (a) and gas rate constant for the insoluble fraction (c) did not change among treatments ($p > 0.05$). The level of CSYW showed significantly higher ($p < 0.01$) gas

production from the insoluble fraction (b), potential extent of gas production (a + b), and cumulative gas production at 96 h than the control group ($p < 0.05$). The highest b value and a + b value were 126.72 and 126.71 mL/g DM, respectively, when SBM was replaced by CSYW at 100% ($p < 0.05$). However, the R:C ratios did not alter the kinetics of gas (b or a + b) or cumulative gas ($p > 0.05$).

Table 2. Effect of cassava pulp fermented with yeast waste (CSYW) replaced soybean meal (SBM) and various roughage to concentrate ratio (R:C) on gas kinetics and cumulative gas at 96 h of incubation.

Item	SBM:CSYW	R:C	Gas Kinetics [1]				Cumulative Gas (96 h) mL/g DM Substrate
			a	b	c	$\|a\| + b$	
T1	100:0	70:30	−3.05	70.12	0.03	67.07	144.82
T2	100:0	50:50	−2.55	72.19	0.04	69.64	148.96
T3	100:0	30:70	−0.36	69.54	0.04	69.18	143.66
T4	75:25	70:30	−0.46	88.56	0.03	88.11	181.71
T5	75:25	50:50	−1.73	80.80	0.04	79.06	166.18
T6	75:25	30:70	−0.74	90.14	0.03	89.40	184.86
T7	50:50	70:30	−0.85	93.54	0.02	92.69	191.66
T8	50:50	50:50	−0.08	97.75	0.03	97.67	200.08
T9	50:50	30:70	−0.95	88.48	0.04	87.53	181.54
T10	25:75	70:30	0.10	113.81	0.02	113.91	232.20
T11	25:75	50:50	0.00	105.68	0.03	105.68	215.94
T12	25:75	30:70	−0.85	115.14	0.02	114.29	234.86
T13	0:100	70:30	−0.11	123.06	0.03	122.95	250.70
T14	0:100	50:50	0.29	137.82	0.02	138.11	280.22
T15	0:100	30:70	−0.22	119.29	0.02	119.07	243.16
SEM			1.01	25.66	0.01	21.55	18.25
***p*-value**							
SBM:CSYW			1.75	<0.01	0.17	<0.01	0.05
R:C			0.92	0.33	0.15	0.29	0.45
SBM:CSYW×R:C			0.67	0.25	0.40	0.22	0.43
Average							
SBM:CSYW	100:0		−1.99	70.62 ^{f}	0.04	68.63 ^{f}	145.81 ^{f}
	75:25		−0.98	86.50 ^{f}	0.03	85.52 ^{ef}	177.58 ^{ef}
	50:50		−0.63	93.26 ^{e}	0.03	92.63 ^{de}	191.09 ^{e}
	25:75		−0.25	115.54 ^{d}	0.03	111.29 ^{d}	227.67 ^{d}
	0:100		−0.02	126.72 ^{d}	0.02	126.71 ^{d}	258.03 ^{d}
R:C ratio	70:30		−0.87	97.82	0.03	96.94	200.22
	50:50		−0.82	98.85	0.03	98.03	202.28
	30:70		−0.63	96.52	0.03	95.98	197.61

[1] 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. $^{d–f}$ Values on the same column with different superscripts differ ($p < 0.05$); SEM = standard error of mean.

3.3. In Vitro Digestibility

The influences of the CSYW level and R:C ratio on in vitro digestibility are illustrated in Table 3. There were no interactions among CSYW and R:C ratio on the in vitro digestibility ($p > 0.05$). When a high level of concentrate diet was supplied, the IVDMD and IVADFD improved ($p < 0.05$). Furthermore, increasing the amount of CSYW to replace SBM up to 75% had no negative effect on IVNDFD ($p > 0.05$), while replacing CSWY at 100% could reduce IVNDFD ($p > 0.05$).

Table 3. Effect of cassava pulp fermented with yeast waste (CSYW) replaced soybean meal (SBM) and various roughage to concentrate ratio (R:C) on the in vitro dry matter degradability (IVDMD), in vitro neutral detergent fiber degradability (IVNDFD), and in vitro acid detergent fiber degradability (IVADFD) at 12 h of incubation.

Item	SBM:CSYW	R:C	IVDMD (g/kg)	IVNDFD (g/kg)	IVADFD (g/kg)
T1	100:0	70:30	440	608	234
T2	100:0	50:50	549	613	210
T3	100:0	30:70	634	637	283
T4	75:25	70:30	509	557	155
T5	75:25	50:50	529	566	239
T6	75:25	30:70	545	633	293
T7	50:50	70:30	502	575	174
T8	50:50	50:50	484	580	227
T9	50:50	30:70	601	624	276
T10	25:75	70:30	427	570	202
T11	25:75	50:50	502	573	201
T12	25:75	30:70	647	615	185
T13	0:100	70:30	456	430	200
T14	0:100	50:50	512	435	187
T15	0:100	30:70	576	512	231
SEM			5.39	3.33	5.31
***p*-value**					
SBM:CSYW			0.26	<0.01	0.92
R:C			<0.01	0.50	<0.01
SBM:CSYW×R:C			0.06	0.14	0.06
Average					
SBM:CSYW	100:0		541	619 ^{a}	222
	75:25		528	585 ^{a}	197
	50:50		529	593 ^{a}	201
	25:75		522	586 ^{a}	202
	0:100		515	459 ^{b}	194
R:C ratio	70:30		467 ^{b}	548 ^{b}	193 ^{b}
	50:50		515 ^{b}	553 ^{b}	213 ^{b}
	30:70		600 ^{a}	604 ^{a}	253 ^{a}

^{a,b} Values on the same column with different superscripts differ ($p < 0.05$); SEM = standard error of mean.

3.4. Ruminal pH, Ammonia-Nitrogen (NH_3-N) Concentration, and Microorganisms

There were no interactions on the ruminal pH, NH_3-N, and microbial population between the CSYW level and R:C ratio ($p > 0.05$; Table 4). The pH and NH_3-N levels in the rumen were measured and ranged from 6.81 to 7.04 and 15.79 to 17.89 mg/dL, respectively. Except for fungal zoospore, the quantity of bacteria and protozoa changed significantly when the concentrate diet was high (bacteria, 36.58×10^7 and protozoa, 3.93×10^5; $p < 0.05$). The bacterial population in the rumen was reduced by 25.05% when CSYW completely replaced SBM ($p < 0.05$); however, 75% of CSWY in the diet did not change the bacterial population ($p > 0.05$).

Table 4. Effect of cassava pulp fermented with yeast waste (CSYW) replaced soybean meal (SBM) and various roughage to concentrate ratio (R:C) on pH, ammonia-nitrogen (NH_3-N), and ruminal microorganisms at 4 h of incubation.

Item	SBM:CSYW	R:C	pH	NH_3-N (mg/dL)	Bacteria ($\times 10^7$ cells/mL)	Protozoa ($\times 10^5$ cells/mL)	Fungal Zoospore ($\times 10^4$ cells/mL)
T1	100:0	70:30	7.04	15.79	25.50	2.05	7.00
T2	100:0	50:50	6.90	15.38	29.75	2.70	10.00
T3	100:0	30:70	6.87	16.24	44.50	4.30	11.25
T4	75:25	70:30	6.96	15.63	27.75	2.25	10.25
T5	75:25	50:50	6.92	17.04	27.25	2.20	11.50
T6	75:25	30:70	6.86	16.43	38.50	4.00	13.50
T7	50:50	70:30	6.95	17.43	31.20	1.55	7.50
T8	50:50	50:50	6.94	16.12	29.00	2.85	10.50
T9	50:50	30:70	6.88	15.90	33.00	4.10	11.25
T10	25:75	70:30	7.10	16.27	26.00	1.90	10.50
T11	25:75	50:50	6.94	17.11	29.75	2.25	12.00
T12	25:75	30:70	6.80	16.09	34.75	4.50	15.00
T13	0:100	70:30	7.03	16.29	18.75	2.15	8.75
T14	0:100	50:50	6.91	15.95	19.75	2.50	9.00
T15	0:100	30:70	6.81	17.89	32.15	2.75	9.50
SEM			0.07	0.66	3.06	0.70	2.07
***p*-value**							
SBM:CSYW			0.41	0.49	<0.05	0.70	0.22
R:C			0.06	0.86	<001	<001	0.07
SBM:CSYW×R:C			0.09	0.45	0.34	0.42	0.99
Average							
SBM:CSYW	100:0		6.94	15.80	33.25 [a]	3.02	9.99
	75:25		6.91	16.37	31.17 [a]	2.82	9.42
	50:50		6.92	16.48	31.07 [a]	2.83	11.75
	25:75		6.94	16.49	30.17 [a]	2.88	9.75
	0:100		6.92	16.71	23.55 [b]	2.47	12.50
R:C ratio	70:30		7.02	16.28	25.84 [b]	1.98 [b]	9.08
	50:50		6.92	16.32	27.10 [b]	2.50 [b]	8.80
	30:70		6.84	16.51	36.58 [a]	3.93 [a]	10.60

[a,b] Values on the same column with different superscripts differ ($p < 0.05$); SEM = standard error of mean.

3.5. Volatile Fatty Acid

The in vitro total VFAs and proportion of VFAs are shown in Table 5. No interaction occurred between the CSYW level or R:C ratio ($p > 0.05$). The total VFAs and VFA profiles in the rumen did not change when CSYW was replaced by SBM ($p > 0.05$), except the concentration of C3 was changed ($p < 0.05$). The concentration of C3 decreased upon an increase in the CSYW level, which was lowest with the replacement of SBM by CSYW up to 75%. However, various R:C ratios did not influence total VFAs or the proportion of VFAs ($p > 0.05$), except the concentration of C3 increased when the proportion of a concentrate diet increased ($p < 0.05$).

Table 5. Effect of cassava pulp fermented with yeast waste (CSYW) replaced soybean meal (SBM) and various roughage to concentrate ratio (R:C) on concentrations of volatile fatty acid (VFA), acetate (C2), propionate (C3), and butyrate (C4) at 4 h of incubation.

Item	SBM:CSYW	R:C	Total VFA (mmol/L)	Molar Proportions of VFA (mmol/L)			C2:C3 Ratio
				C2	C3	C4	
T1	100:0	70:30	64.36	71.03	15.63	10.56	4.55
T2	100:0	50:50	73.81	67.20	25.54	12.26	2.63
T3	100:0	30:70	79.11	61.46	27.56	14.86	2.23
T4	75:25	70:30	68.10	72.67	15.91	12.57	4.57
T5	75:25	50:50	64.95	74.16	26.95	11.08	2.75
T6	75:25	30:70	77.50	74.23	24.28	9.95	3.06
T7	50:50	70:30	68.56	71.00	20.41	10.50	3.48
T8	50:50	50:50	69.97	69.40	20.64	11.53	3.36
T9	50:50	30:70	69.54	64.94	25.68	10.94	2.53
T10	25:75	70:30	67.17	65.89	20.75	10.55	3.17
T11	25:75	50:50	74.02	64.52	18.76	8.85	3.44
T12	25:75	30:70	66.02	70.78	26.82	12.27	2.64
T13	0:100	70:30	66.27	71.68	18.07	12.55	3.97
T14	0:100	50:50	66.97	66.35	21.00	9.74	3.16
T15	0:100	30:70	64.69	62.52	20.12	8.84	3.11
SEM			3.19	3.82	1.41	1.99	0.67
***p*-value**							
SBM:CSYW			0.23	0.18	0.05	0.69	0.07
R:C			0.11	0.34	0.01	0.83	0.70
SBM:CSYW × R:C			0.08	0.58	0.09	0.61	1.00
Average							
SBM:CSYW	100:0		72.43	66.56	22.91 ^{a}	12.56	3.14
	75:25		70.19	73.69	22.38 ^{a}	11.20	3.46
	50:50		69.36	68.45	22.24 ^{a}	10.99	3.12
	25:75		69.07	67.06	22.11 ^{a}	10.56	3.08
	0:100		65.98	66.85	19.73 ^{b}	10.37	3.41
R:C ratio	70:30		66.89	70.45	18.74 ^{c}	11.35	3.79
	50:50		69.94	68.33	22.58 ^{b}	10.69	3.07
	30:70		71.37	66.78	25.05 ^{a}	11.37	2.63

$^{a–c}$ Values on the same column with different superscripts differ ($p < 0.05$); SEM = standard error of mean.

4. Discussion

4.1. Chemical Composition

The chemical composition of CSYW in this experiment had lower OM, NDF, and ADF content than the compositions within the study conducted by Sommai et al. [5]. These variations may be a result of different materials, growing locations, and plant factory processing [23]. However, the OM, NDF, and ADF contents in CSYW were similar to those of the report of Chuelong et al. [24], with 845, 243, and 113 g/kg DM, respectively. Furthermore, the use of CSYW instead of SBM resulted in increased ash and fiber content in the concentrate diet, while the CP in the formula was regulated at the same level to investigate the probable use of CSYW replacement.

Combining CS and YW could deliver a product with a high CP content of 537 g/kg DM. The apparent increase in CP could be explained by an increase in microorganisms contained in YW and proliferation in the form of single-cell proteins occurring throughout the fermentation process [6]. Before YW was fermented with CS, the quantity of carbon and nitrogen sources in the medium solution was the key factor that differentiated the amount of yeast and CP contained in the product. Polyorach et al. [9] discovered that protein and lysine levels in cassava chips increased from 3.4% to 32.5% and 3.8% to 8.5%, respectively, when the *S. cerevisiae* grew in media solution containing 9.6% molasses and 19.2% urea. Similarly, Khampa et al. [25] found that *S. cerevisiae* grown in a media solution containing

10% molasses and 24% urea could increase the amount of protein in cassava chips by up to 36.1%. In addition, 23.3% of CP was obtained from CS fermented *S. cerevisiae* with a media solution containing 12% molasses and 25% urea [5].

The greater CP in this study could be related to the product of yeast that was used as a starter. YW obtained from bioethanol production contains a high content of 60% to 70% live cells of *S. cerevisiae* [12]. In the preliminary investigation, the amount of *S. cerevisiae* in YW was found to be around 3.1×10^{13} cells/mL. This indicates that higher protein levels can be obtained than in previous studies utilizing baker's yeast, which had a low yeast cell count (around 10^6 to 10^8 cells/mL, [26]). As a consequence, this experiment implies that utilizing YW to ferment CS has a higher protein productivity potential than previous CS improvement approaches. Its properties could be used as a protein source in animal diets, provided it is economically viable.

4.2. Effects on Gas Kinetics and In Vitro Digestibility

The level of CSYW substitution for SBM in the concentrate diet altered the in vitro rumen gas kinetics. The volume of gas produced from the insoluble fraction (b) increased as CSYW was raised, which is the main reason why CSYW has a significant impact on the prospective scope of the potential extent of gas production (a + b). The use of CSYW could increase the cumulative gas production because the product containing yeast may promote the growth of some cellulolytic bacteria in the rumen. According to Sommai et al. [5], yeast-fermented CS can activate the cellulolytic bacterial population from 2.0×10^9 to 5.6×10^9 cfu/mL, meaning that the more products supplied, the more cellulolytic bacteria there are. These findings were in accordance with Chuelong et al. [24], who confirmed that *S. cerevisiae* fermented with CS increased bacterial populations by 32.2%.

As the proportion of concentrate diet increased from 30% to 70%, the in vitro digestibility of DM and ADF improved by 449 and 308 g/kg DM, respectively. This was in agreement with the statement of Polyorach et al. [7], who found that increasing the concentrate diet from 20% to 80% raised IVDMD by about 13%. In this study, a 30:70 R:C ratio diet provided more readily available energy, which resulted in improved bacterial growth and digestibility [27]. Furthermore, this study confirmed that a concentrate diet increased the ruminal microbiota, particularly bacteria, by approximately 55%. Hungate [28] suggested a more significant effect in the rumen when carbohydrate, rather than forages, is used. These findings corroborated previous research by Sommai et al. [5].

The IVNDFD was maintained when CSYW replaced SBM up to 75%. This relates to the volume of gas produced from the previously mentioned insoluble fraction (b-value). In addition, cellulolytic bacterial colonization of plant cell walls is supported by probiotic yeasts. This effect has many mechanisms of action, one of which is the distribution of thiamin, a vitamin that rumen microorganisms need [14]. Chuelong et al. [24] stated that, when yeast was added to the diet, the activity of most polysaccharidase and glycosidehydrolase enzymes increased and rumen digestion fiber was improved. In addition, the ability of yeast cells to scavenge oxygen is one of the key factors that may justify the beneficial effect of live yeasts on fiber-degrading bacteria [29]. Furthermore, the media solution containing urea might act as an alkaline substance (ammonium hydroxide) and result in a breakdown of the fiber structure in CS [14]. The alkaline substance may then support enzyme activity from yeast to degrade the NDF content contained in CS.

Cherdthong and Supapong [27] found that supplementing yeast would increase the bacterial population by 3.6 times, resulting in enhanced NDF digestibility, as shown in Tables 3 and 4. This agrees with Boonnop et al. [8], who indicated that feeding yeast-fermented cassava chip (YEFFECAP) to dairy steers could increase feed consumption and nutritional digestibility. In addition, yeast efficacy was frequently established when used in combination with low-quality roughage. Tang et al. [30] observed that feeding *S. cerevisiae* to low-quality roughage enhanced in vitro digestibility. These results indicated that CSYW at 75% could be incorporated with no influence on rumen digestibility when added to

concentrate diets. However, replacement of SBM by CSYW at 100% could reduce IVNDFD, which might be due to limited high fiber content, resulting in reduced fiber digestibility.

4.3. Ruminal Fermentation and Quantity of Rumen Microorganisms

The ruminal fermentation parameters did not change when SBM was replaced by CSYW, and the value remained stable between 6.82 and 7.05. Wanapat and Cherdthong [31] suggested that the optimum level of pH in the rumen for microbial digestion of fiber and protein is 6.5 to 7.0. Furthermore, raising the CSYW and R:C ratio did not affect the concentrations of NH_3-N and ranged from 15.88 to 17.99 mg/dL in the rumen fluid, which is considered acceptable. The NH_3-N was under the optimum concentrations for bacterial growth and microbial activity in the range of 5–25 mg/dL [31]. This range would be improved by voluntary feed consumption and microbial protein synthesis [32].

Several factors influence the organization of the ruminal microbial community, and diet is a key to rumen community composition [33]. Our investigation showed that CSYW could replace SBM up to 75% without any negative effects on microbial activity. In particular, the bacterial population was comparable to the use of 100% SBM in the concentrate diet. Sommai et al. [5] revealed that yeast fermentation of CS has no negative impact on the bacteria population in Thai native beef cattle. This is in agreement with Cherdthong and Supapong [27], who found that using *S. cerevisiae*-fermented cassava bioethanol waste (YECAW) seems to have no adverse influence on bacterial, protozoa, or fungal populations or values in dairy calf rations. Our study observed that increasing the level of concentrate diet enhanced bacterial and protozoa populations (with a 70% concentration diet, the bacteria and protozoa increase was 41.6% and 98.5%, respectively) in the rumen fluid. The increased fermentable substrate (sugar and starch) in the concentrate diet may have favored the growth of bacteria and protozoa, resulting in a change in the structure and diversity of microbial populations [34]. Accordingly, Phesatcha et al. [35] also noticed an enhancement in the total amount of ruminal bacteria, with a comparable shift in rumen microbial population numbers in animals that were fed a high-concentrate diet versus animals that were fed a low-concentrate diet vs. those fed a low level of concentrate diet. This demonstrated that, when a rapid fermentation carbohydrate is supplied, microbial bacteria in the rumen tend to be enhanced. Cherdthong and Wanapat [36] found that ruminal microbial bacteria's synthesis depends on an appropriate carbohydrate supply of NH_3-N, which is used to synthesize peptide bonds and as an energy source. In accordance with Anantasook and Wanapat [37], when a high-concentrate diet is included in the formula, the bacterial population in the rumen increases dramatically. However, the replacement of CSWY did not affect bacterial populations until 100% SBM was replaced. SBM replacement with CSYW at 100% resulted in a decrease in the bacteria population, possibly because of the high fiber content of CS, which inhibited bacterium digestion and utilization. Therefore, the advice for those using CSYW is that the user should carefully assess the replacement quantity to SBM and that further experiments should be carried out on animals.

4.4. Ruminal Volatile Fatty Acid (VFA)

The TVFA did not change when CSWY was utilized instead of SBM at any level. In this experiment, the concentration of rumen VFA ranged from 64.36 to 79.11 mmol/L, which was close to the previous study (68.8 to 89.7 mmol/L [38]; 50.1 to 68.5 mmol/L [7]).

The use of CSYW instead of SBM in the concentrate diet can be employed up to 75% without altering the VFA profile, which demonstrates that the product has a potential use as animal feed as compared to SBM. According to Cherdthong et al. [13], the highest amounts of SBM were replaced by YECAW, with no alterations in TVFA concentration or VFA profiles. In addition, Polyorach et al. [7] found no difference in TVFA and VFA profiles when YEFECAP was used at the 80% SBM substitution level. However, the concentration of C3 was slightly lowered after CSWY was completely substituted with SBM in the concentrate diet. It could be that the high fiber content and low fermentation fraction in CSWY lead to a low substrate supply to generate C3 in the rumen [39,40]. In addition, C3 is

a product of the rumen's bacterial fermentation activity; a change in the number of bacteria in the rumen can impact C3. Our studies have revealed that using 100% CSYW reduces the amount of bacterial population. The decrease in bacteria could be related to the increase in fiber; when the quantity of fiber in the composition is higher, the amount of digestible nutrients is significantly lower and results in a reduction of the fermentation yield [41,42]. This incidence was similar to that reported by Polyorach et al. [7], who found that replacing the SBM with 100% yeast-fermented cassava chip protein lowered the amount of C3.

The concentrated diet ratio, C3, increased by 22.6%. Normally, C3 is generated from the rumen fermentable starch, which is caused by bacteria in the rumen. Thus, high-fermentable starch in the concentrate diet resulted in a high concentration of C3 production [43,44]. This agrees with Cherdthong et al. [40], who revealed that the addition of a high-concentrated diet supplied an enhanced proportion of C3. Furthermore, the C3 content could significantly increase when a substrate containing 80% of the concentrate was tested [35].

5. Conclusions

The quality of CS could be improved by using YW and the optimum media solution. CSYW could be utilized as a partial replacement for SBM in concentrate diets up to 75% without affecting gas kinetics, ruminal parameters, or in vitro digestibility. Furthermore, a 30:70 R:C ratio may be useful for gas kinetics, ruminal ecology, digestibility, volatile fatty acids, and propionic acid. However, more in vivo investigations are needed to determine the success of animal production.

Author Contributions: Planning and design of the study, G.D. and A.C.; conducting and sampling, G.D., A.C. and C.S.; sample analysis, G.D., A.C. and C.S.; statistical analysis, G.D. and A.C.; manuscript drafting, G.D., A.C. and C.S.; manuscript editing and finalizing, G.D., A.C., C.S., S.W. and M.W. All authors have read and agreed to the published version of the manuscript.

Funding: The authors express their most sincere gratitude to the Research Program on the Research and Development of Winged Bean Root Utilization as Ruminant Feed, Increase Production Efficiency and Meat Quality of Native Beef and Buffalo Research Group, and Research and Graduate Studies, Khon Kaen University (KKU). Gamonmas Dagaew was granted by Animal Feed Inter Trade Co., Ltd. and Thailand Science Research and Innovation (TSRI) through the RRi program (contract grant PHD62I0020).

Institutional Review Board Statement: The study was conducted under approval procedure no. IACUC-KKU-108/63 of Animal Ethics and Care issued by Khon Kaen University.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to express our sincere thanks to the KSL Green Innovation Public Co. Limited, Thailand, and the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University (KKU), for the use of the research facilities.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chauynarong, N.; Elangovan, A.V.; Iji, P.A. The potential of cassava products in diets for poultry. *World's Poult. Sci. J.* **2009**, *65*, 23–36. [CrossRef]
2. FAOSTAT. Food and Agricultural Commodities Production. Food and Agriculture Organization of the United Nations Statistics Database. Available online: http://faostat.fao.org/ (accessed on 1 April 2021).
3. Sugiharto, S. A review on fungal fermented cassava pulp as a cheap alternative feedstuff in poultry ration. *J. World's Poult. Res.* **2019**, *9*, 1–6. [CrossRef]
4. Rukboon, P.; Prasanpanich, S.; Kongmun, P. Effects of cassava pulp mixed with monosodium glutamate by-product (CPMSG) as a protein source in goat concentrate diet. *Indian J. Anim. Res.* **2019**, *53*, 774–779. [CrossRef]

5. Sommai, S.; Ampapon, T.; Mapato, C.; Totakul, P.; Viennasay, B.; Matra, M.; Wanapat, M. Replacing soybean meal with yeast-fermented cassava pulp (YFCP) on feed intake, nutrient digestibilities, rumen microorganism, fermentation, and N-balance in Thai native beef cattle. *Trop. Anim. Health Prod.* **2020**, *52*, 2035–2041. [CrossRef]
6. Pilajun, R.; Wanapat, M. Chemical composition and in vitro gas production of fermented cassava pulp with different types of supplements. *J. Appl. Anim. Res.* **2018**, *46*, 81–86. [CrossRef]
7. Polyorach, S.; Wanapat, M.; Cherdthong, A. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and microorganisms using in vitro gas production technique. *Asian-Australas. J. Anim. Sci.* **2014**, *27*, 36–45. [CrossRef]
8. Boonnop, K.; Wanapat, M.; Nontaso, N.; Wanapat, S. Enriching nutritive value of cassava root by yeast fermentation. *Sci. Agric.* **2009**, *66*, 629–633. [CrossRef]
9. Polyorach, P.; Wanapat, M.; Wanapat, S. Increasing protein content of cassava (Manihot esculenta, Crantz) using yeast in fermentation. *Khon Kaen Agr. J.* **2012**, *40* (Suppl. S2), 178–182.
10. Polyorach, S.; Wanapat, M.; Wanapat, S. Enrichment of protein content in cassava (Manihot esculenta Crantz) by supplementing with yeast for use as animal feed. *Emirat. J. Food Agric.* **2013**, *25*, 142–149. [CrossRef]
11. Laluce, C.; Leite, G.R.; Zavitoski, B.Z.; Zamai, T.T.; Ventura, R. *Fermentation of Sugarcane Juice and Molasses for Ethanol Production. Sugarcane-Based Biofuels and Bioproducts*; John Willey & Sons: Hoboken, NY, USA, 2016. [CrossRef]
12. Díaz, A.; Ranilla, M.J.; Saro, C.; Tejido, M.L.; Pérez-Quintana, M.; Carro, M. Influence of increasing doses of a yeast hydrolyzate obtained from sugarcane processing on in vitro rumen fermentation of two different diets and bacterial diversity in batch cultures and Rusitec fermenters. *Anim. Feed Sci. Technol.* **2017**, *232*, 129–138. [CrossRef]
13. Cherdthong, A.; Prachumchai, R.; Supapong, C.; Khonkhaeng, B.; Wanapat, M.; Foiklang, S.; Polyorach, S. Inclusion of yeast waste as a protein source to replace soybean meal in concentrate mixture on ruminal fermentation and gas kinetics using in vitro gas production technique. *Anim. Prod. Sci.* **2019**, *59*, 1682–1688. [CrossRef]
14. Suriyapha, C.; Cherdthong, A.; Suntara, C.; Polyorach, S. 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. *Fermentation* **2021**, *7*, 120. [CrossRef]
15. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 1995.
16. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
17. Menke, K.H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim. Res. Develop.* **1988**, *28*, 7–55.
18. Osaki, T.Y.; Kamiya, S.; Sawamura, S.; Kai, M.; Ozawa, A. Growth inhibition of *Clostridium difficile* by intestinal flora of infant faeces in continuous flow culture. *J. Med. Microbiol.* **1994**, *40*, 179–187. [CrossRef]
19. Galyean, M. *Laboratory Procedure in Animal Nutrition Research*; Department of Animal and Life Science, New Mexico State University: Las Cruces, NM, USA, 1989; p. 188.
20. Sommart, K.; Parker, D.S.; Rowlinson, P.; Wanapat, M. Fermentation characteristics and microbial protein synthesis in an in vitro system using cassava, rice straw and dried ruzi grass as substrates. *Asian-Australas. J. Anim. Sci.* **2000**, *13*, 1084–1093. [CrossRef]
21. SAS (Statistical Analysis System). *SAS/STAT User's Guide*, 4th ed.; Statistical Analysis Systems Institute, Version 9; SAS Institute Inc.: Cary, NC, USA, 2013.
22. Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistics: A Biometerial Approach*, 2nd ed.; McGraw-Hill: New York, NY, USA, 1980.
23. Kamphaya, S.; Kumagai, H.; Angthong, W.; Narmseelee, R.; Bureenok, S. Effects of different ratios and storage periods of liquid brewer's yeast mixed with cassava pulp on chemical composition, fermentation quality and in vitro ruminal fermentation. *Asian-Australas. J. Anim. Sci.* **2017**, *30*, 470–478. [CrossRef]
24. Chuelong, S.; Siriuthane, T.; Polsit, K.; Ittharat, S.; Koatdoke, U.; Cherdthong, A.; Khampa, S. Supplementation levels of palm oil in yeast (*Saccharomyces cerevisiae*) culture fermented cassava pulp on rumen fermentation and average daily gain in crossbred native cattle. *Pak. J. Nutr.* **2011**, *10*, 1115–1120. [CrossRef]
25. Khampa, S.; Chaowarat, P.; Singhalert, R.; Wanapat, M. Supplementation of yeast fermented cassava chip as a replacement concentrate on rumen fermentation efficiency and digestibility on nutrients in cattle. *Asian-Australas. J. Anim. Sci.* **2009**, *3*, 18–24. [CrossRef]
26. Promkot, C.; Wanapat, M.; Mansathit, J. Effects of yeast fermented-cassava chip protein (YEFECAP) on dietary intake and milk production of Holstein crossbred heifers and cows during pre-and post-partum period. *Livest. Sci.* **2013**, *154*, 112–116. [CrossRef]
27. Cherdthong, A.; Supapong, C. Improving the nutritive value of cassava bioethanol waste using fermented yeast as a partial replacement of protein source in dairy calf ration. *Trop. Anim. Health Prod.* **2019**, *51*, 2139–2144. [CrossRef]
28. Hungate, R.E. *The Rumen and Its Microbes*; Academic Press: New York, NY, USA, 1966; p. 53.
29. Habeeb, A.A.M. Importance of yeast in ruminants feeding on production and reproduction. *Ecol. Evol.* **2017**, *2*, 49.
30. Tang, L.; Wang, W.; Zhou, W.; Cheng, K.; Yang, Y.; Liu, M.; Cheng, K.; Wang, W. Three-pathway combination for glutathione biosynthesis in *Saccharomyces cerevisiae*. *Microb. Cell Factories.* **2015**, *14*, 1–12. [CrossRef]

31. Wanapat, M.; Cherdthong, A. Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in swamp buffaloes. *Cur. Microbiol.* **2009**, *58*, 294–299. [CrossRef] [PubMed]
32. Wanapat, M.; Pimpa, O. Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. *Asian-Australas. J. Anim. Sci.* **1999**, *12*, 904–907. [CrossRef]
33. Bi, Y.; Zeng, S.; Zhang, R.; Diao, Q.; Tu, Y. Effects of dietary energy levels on rumen bacterial community composition in Holstein heifers under the same forage to concentrate ratio condition. *BMC Microbiol.* **2018**, *18*, 69. [CrossRef]
34. Fernando, S.C.; Purvis, H.; Najar, F.; Sukharnikov, L.; Krehbiel, C.; Nagaraja, T.; Roe, B.; Desilva, U. Rumen microbial population dynamics during adaptation to a high-grain diet. *Appl. Environ. Microbiol.* **2010**, *76*, 7482–7490. [CrossRef]
35. Phesatcha, K.; Phesatcha, B.; Wanapat, M.; Cherdthong, A. Roughage to concentrate ratio and *Saccharomyces cerevisiae* inclusion could modulate feed digestion and in vitro ruminal fermentation. *Vet. Sci.* **2020**, *7*, 151. [CrossRef]
36. Cherdthong, A.; Wanapat, M. Rumen microbes and microbial protein synthesis in Thai native beef cattle fed with feed blocks supplemented with a urea–calcium sulphate mixture. *Arch. Anim. Nutr.* **2013**, *67*, 448–460. [CrossRef]
37. Anantasook, N.; Wanapat, M. Influence of rain tree pod meal supplementation on rice straw based diets using in vitro gas fermentation technique. *Asian-Australas. J. Anim. Sci.* **2012**, *5*, 325–334. [CrossRef] [PubMed]
38. Suntara, C.; Cherdthong, A.; Uriyapongson, S.; Wanapat, M.; Chanjula, P. Comparison effects of ruminal Crabtree-negative yeasts and Crabtree-positive yeasts for improving ensiled rice straw quality and ruminal digestion using in vitro gas production. *J. Fungi* **2020**, *6*, 109. [CrossRef]
39. Terry, S.A.; Badhan, A.; Wang, Y.; Chaves, A.V.; McAllister, T.A. Fibre digestion by rumen microbiota—A review of recent metagenomic and metatranscriptomic studies. *Can. J. Anim. Sci.* **2019**, *99*, 678–692. [CrossRef]
40. Cherdthong, A.; Wanapat, M.; Kongmun, P.; Pilajun, R.; Khejornsart, P. Rumen fermentation, microbial protein synthesis and cellulolytic bacterial population of swamp buffaloes as affected by roughage to concentrate ratio. *J. Anim. Vet. Adv.* **2010**, *9*, 1667–1675. [CrossRef]
41. Gunun, P.; Gunun, N.; Wanapat, M.; Cherdthong, A.; Polyorach, S.; Kang, S. In vitro rumen fermentation and methane production as affected by rambutan peel powder. *J. Appl. Anim. Res.* **2018**, *46*, 626–631. [CrossRef]
42. Kang, S.; Wanapat, M.; Cherdthong, A. Effect of banana flower powder supplementation as a rumen buffer on rumen fermentation efficiency and nutrient digestibility in dairy steers fed on high concentrate diet. *Anim. Feed Sci. Technol.* **2014**, *196*, 32–41. [CrossRef]
43. Anantasook, N.; Wanapat, M.; Cherdthong, A.; Gunun, P. Changes of microbial population in the rumen of dairy steers as influenced by plant containing tannins and saponins and roughage to concentrate ratio. *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 1583–1591. [CrossRef]
44. Anantasook, N.; Wanapat, M.; Cherdthong, A.; Gunun, P. Effect of plants containing secondary compounds with palm oil on feed Intake, digestibility, microbial protein synthesis and microbial population in dairy cows. *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 820–826. [CrossRef]