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Gas Kinetics, Rumen Characteristics, and *In Vitro* Degradability of Varied Levels of Dried and Fresh Cassava Leaf Top Fermented with Cassava Pulp

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ABSTRACT

The purpose of this study was to determine the impact of different levels of dried cassava leaf top (DCT) and fresh cassava leaf top (FCT) fermented with cassava pulp (CS) on the nutritional value of silage, gas kinetics, rumen characteristics, and *in vitro* degradability. Dietary treatments were administered using a completely randomized design (CRD) with eight treatments and three replicate runs. The eight treatments were as follows: 1) CS fermented no additive (nA), 2) CS fermented with additives (*Saccharomyces cerevisiae*, urea, molasses, and sugar) (CSA), 3) 95% CSA fermented with 5% DCT (5DCT), 4) 90% CSA fermented with 10% DCT (10DCT), 5) 85% CSA fermented with 15% DCT (15DCT), 6) 95% CSA fermented with 5% FCT (5FCT), 7) 90% CSA fermented with 10% FCT (10FCT), 8) 85% CSA fermented with 15% FCT (15FCT), respectively. After 21 days of fermentation, samples of the silages were taken for chemical analysis and utilized to examine the *in vitro* gas production and degradability. The results show that fermented CS with DCT at 5% to 10% DM had the highest increase in CP when compared to nA or CSA ($p<0.05$). *In vitro* dry matter disappearance (IVDMD) was significantly higher in CS fermented with 5% to 10% DCT ($p<0.01$), whereas CS fermented with FCT levels demonstrated lower IVDMD than the control group ($p<0.01$). The gas potential extent of gas production (p) and gas production from the insoluble fraction (b) did not differ significantly across treatments ($p>0.05$). However, the gas production from the immediately soluble fraction (a) was maximum when CS was fermented with 15DCT ($p<0.05$). Different treatments significantly affected the pH of the fermentation solution with the addition of 10DCT and 15DCT for 12 and 24 hours of incubation, respectively ($p<0.01$). After 12 hours of incubation, the population of protozoa was lowest when 5DCT and 10DCT were evaluated ($p<0.01$). In conclusion, CS fermented with DCT at a concentration of 5% to 10% can increase crude protein content, *in vitro* dry matter disappearance (IVDMD), and gas production from the immediately soluble fraction while decreasing the protozoa population.

Keywords: cassava top; cassava pulp; ensiled; in vitro; gas kinetic

INTRODUCTION

Cassava (*Manihot esculenta*) is a popular crop with a high yield. Fresh cassava foliage (FCF) contains 20.76%-34.8% crude protein (CP) (Leguizamón *et al.,* 2021; Mao *et al.,* 2019; Chaiareekitwat *et al.,* 2022). In cassava foliage (CF) harvested within 2 months, the DM yield was 3.2 tons/ha, and during a second harvest within 4 months, 3.3-4.6 tons/ha (Phengvilaysouk & Wanapat, 2008). Cassava pulp (CS) is a by-product that is approximately 30% of the original weight of roots (Ghimire *et al.,* 2015). CS has 1.12-3.1% CP (Tawida & Supawadee, 2019; Ornvimol *et al.,* 2018; Chauynarong *et al.,* 2015). However, additives are needed to increase the nutritional value (Dagaew *et al.,* 2021). Norrapoke *et al.* (2022) found that CP was enhanced from 2.0% DM to 14.6% DM in CS ensiled with yeast (*S. cerevisiae*) and effective microorganism (EM). The utilization of microorganisms, including yeast, has become common for ruminant feeding (Polyorach *et al.,* 2014). The potential benefits of yeast cells have been proven primarily in the growth and activity of fiber-degrading bacteria and fungi, rumen pH stabilization and lactate accumulation prevention, ruminal microbial colonization, and establishment of fermentative processes during the pre-weaning period (Suntara *et al.,* 2021).

CS fermented with yeast waste (CSFY) did not influence gas production, *in vitro* neutral detergent fiber digestibility, or bacterial population, according to Dagaew *et al.* (2021). CF fermented with microorganism additives or lactic acid bacteria (LAB) in ruminant feed might have a beneficial effect on silage fermenta-

tion (Mao *et al.,* 2019). Providing CF at up to 2% of body weight (BW) can improve feed intake and live weight gain (LWG) in lambs (Khuc *et al.,* 2012). Ensiling with 5% molasses, cassava tops (CT), and caged layer waste improved the silage conditions and the ruminal fluid parameters in goats (Oni *et al.,* 2014). Additionally, adding 0.5% sulfur to fresh cassava foliage (FCF) increased the gas production rate, digestibility, volatile fatty acid (VFA) concentration, and microbial biomass while decreasing toxin content (Promkot *et al.,* 2007). Providing only CS did not affect feed intake, reproduction performance, methane (CH_4) production, or nutrient digestibility (Ornvimol *et al.,* 2018). Norrapoke *et al.* (2016) used 4% urea and 4% molasses in CS to improve nutritional value, gas production kinetics, and *in vitro* digestibility. However, previous reports have never elucidated the effects of dried cassava tops (DCT) and fresh cassava tops (FCT) in fermented CS on the nutritive value, gas kinetics, rumen fermentation, and *in vitro* degradability. Thus, this study aimed to determine the effects of various DCT and FCT levels fermented with CS on the nutritive quality of silage, gas kinetics, rumen characteristics, and *in vitro* degradability.

MATERIALS AND METHODS

All the procedures involving animals were performed by following the Guidelines of the Animal Care and Use for Scientific Purpose Committee, Ubon Ratchathani University, Thailand, which followed the Guidelines of the Ethical Principles for the Use of Animals for Scientific Purposes of the National Research Council of Thailand (NRCT), by approval No. MHESI0604/2167.

Preparation of Cassava Tops (CT)

FCT (KU50 variety) was purchased from a local producer in Ban Hare, Tumbon Khamkwang, Warincharab District, Ubon Ratchathani Province, Thailand. Their age of collection was 6 months at most. The FCT was chopped into 2 cm long pieces using a magnum electric motor (type mL-90S2-2, model: gs150, matched power of 3hp, rotation speed: 2800 rpm, production efficiency ≥1000kg/hr). The copped FCT was divided into two parts. The first part was used freshly to ferment CS, and the second part was used in a dry form to ferment it with CS at different levels. Dry cassava tops (DCT) were sun-dried within three days at ambient temperature in the dry season at the Experimental Field and Central Laboratory (15 °07›55.8»N 104 °55›48.2»E), Faculty of Agriculture, Ubon Ratchathani University, Thailand.

Experimental Design and Treatments

Dietary treatments were administered using a completely randomized design (CRD) with eight treatments and three replicate runs. The eight treatments were as follows: 1) CS fermented no additive (nA), 2) CS fermented with additives (*Saccharomyces cerevisiae*, urea, molasses, and sugar) (CSA), 3) 95% CSA fermented with 5% DCT (5DCT), 4) 90% CSA fermented with 10% DCT (10DCT), 5) 85% CSA fermented with 15% DCT (15DCT), 6) 95% CSA fermented with 5% FCT (5FCT), 7) 90% CSA fermented with 10% FCT (10FCT), 8) 85% CSA fermented with 15% FCT (15FCT), respectively. Each treatment was balanced with 12% CP. Table 1 shows the chemical compositions of FCT and CS.

Additives Sources and CS Fermentation

Saccharomyces cerevisiae (baker's yeast), urea, molasses, and sugar were obtained at a neighborhood grocery in the province of Ubon Ratchathani, while CS was bought at Aiemsiri Cassava Starch Powder in the Kantralak district of the province of Sisaket, Thailand. Yeast, urea, and molasses were added to the CS fermentation in different treatments. Before adding yeast, urea, and sugar, 20 g of yeast was stimulated aerobically with oxygen flushed and 40 g of sugar in 660 mL of tap water for 30 minutes (Solution A). As much as 50 mL of molasses and 4.11 g of urea were used for additive treatments, and 3.76 g, 3.41 g, and 3.07 g of urea were used in 5%, 10%, and 15% DCT. As much as 3.76 g, 3.41 g, and 3.07 g of urea were used in 5%, 10%, and 15% FCT in 830 mL of tap water and mixed well (Solution B). Urea was mixed into solutions A and B one by one. Solution C was a mix of A+B at a 1:1 ratio (v/w) and flushed with air for 1 h (Adopted from Polyrach *et al.,* 2014). After incubation, the yeast solution was applied to CS containing FCT and DCT. The FCT and DCT fermented CS, and the product was allowed to ferment for 21 days and then sampled for chemical composition analysis, followed by oven-drying at 60 °C for 72 hours to less than 10% moisture. All substrates were used for an *in vitro* test on the gas production kinetics.

Sampling and Chemical Composition Analysis

All samples were dried in a hot air oven at 60 °C for 72 hours for chemical composition analysis, ground using a Cyclotec 1093 sample mill, and passed through a 1-mm screen (Tecator, Hoganas, Sweden). The ground samples were divided into two parts: one for analyzing DM, ash, and CP, and one for ether extract (EE), according to AOAC (1997). The method of Van Soest *et al.* (1991) was used for analyzing neutral detergent fiber (NDF) and acid detergent fiber (ADF).

Table 1. Basic chemical composition in fresh cassava tops (FCT) and cassava pulp (DCS)

Chemical compositions	Assay unit (g/kg)	
	FCT	DCS
DM	192.10	888.60
Ash	71.10	64.00
OM	928.90	936.00
CP	198.80	26.60
NDF	467.00	445.00
ADF	388.30	364.90
EE	36.80	3.00

Note: FCT= fresh cassava tops, DCS= dry cassava pulp, DM= dry mater, Ash= ash, OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, EE= ether extract.

Donors of Ruminal Fluid and Substrates of Inoculum

Using a stomach tube with a vacuum pump, 2.600 mL of ruminant fluid was taken from five of 75% Holstein-Friesian crossbred dairy steers with 150±20 kg body weight (BW) and an age of 1 year before morning feeding. Homemade feed containing 12% CP was fed to the animals at 1% BW twice daily at 7:30 am and 4:00 pm. Rice straw and water were provided *ad libitum*, and the cattle were separately housed. According to the Menke & Steingass (1988) method, artificial saliva was introduced after rumen fluid had been filtered through four layers of cheesecloth and placed in pre-warmed thermos flasks.

Feedstuff samples were milled and passed through a 1.0 mm sieve, and an amount of about 200 mg was placed into 60 mL serum bottles. The bottles contained artificial saliva and rumen fluid at a 2:1 ratio. They were pre-warmed in a water bath at 39 °C and flushed with CO_2 to make them strictly anaerobic. Rumen liquor (35 mL) was added to the serum bottles using 18 a gauge, 1.5-inch needle. The bottles were then sealed with butyl rubber and metal caps and incubated at 39 °C for further measurement.

The gas production kinetics were evaluated using 3 serum bottles per group (8 groups + 3 serum bottles of blanks). All serum bottles for the experiments were shaken every 3 hours during incubation. Rumen liquor was added to the blank bottles without any substrates, and accumulated gas production was calculated by subtracting the gas yield from the average value in the experimental bottles. A 50 mL precision hypodemic glass syringe (U4520, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and an 18-gauge injection needle were used to measure gas yield production.

Fermentation Parameters and Degradability

As much as 12 mL fluid inoculum samples were collected after 12 hours and 24 hours of incubation and kept in plastic bottles containing 2 mL of 1 M sulfuric acid at -20 °C. The fluid inoculum was thawed and centrifuged at 16.000x g for 15 min to obtain the supernatant and to measure the pH using a pH meter (HI 8424 microcomputer; Hanna Instruments; Singapore) according to the AOAC (1997). To count the protozoal population, 1 mL inoculum fluid samples were taken, placed in 9 mL of 10% formalin, and stored in a refrigerator. The protozoal population was counted on a hemocytometer under a microscope, according to Galyean (1989). After all samples were taken, they were removed from a hot air oven and frozen at -20 °C to analyze DM, ash, organic matter (OM), and degradability. Before analysis, a sample from each bottle was filtered through a pre-weighed Gooch crucible and oven-dried at 105 °C for 24 hours. After drying, the Gooch crucibles were weighed and used to calculate the DM degradability by adjusting to the blank. After measuring DM degradability, the same Gooch crucibles were ashed at 550 °C for 5 hours, weighed, and used to calculate the OM degradability, according to Tilley & Terry (1963).

Fermentation Characteristics and *In Vitro* Gas Production

According to Menke & Steingass (1988), the amount of gas produced was measured at 1, 2, 3, 4, 6, 8, 12, 18, 24, 48, 60, and 84 h of incubation. Cumulative gas production data were fitted to the model of Ørskov & McDonald (1979): $y= a + b (1 – e^{-ct})$. Where *a* was the gas production from the immediately soluble fraction, *b* was the gas production from the insoluble fraction, *c* was the gas production rate constant for the insoluble fraction *(b)*, *t* was the incubation time, *p(a+b)* was the potential extent of gas production, and *y* was the gas produced at a time *"t"*. At 12 h, 24 h, and 84 h post-inoculation, samples were taken to determine the *in vitro* digestibility referring to Van Soest & Robertson (1985). True digestibility (TD)= ((DM feed taken of incubation- residues of NDF) x 100))/DM feed taken of incubation.

Statistical Analysis

All data were analyzed using a one-way ANOVA in a completely randomized design (CRD) in the Statistical Package for the Social Sciences (SPSS, version 16.0 Chicago, USA). Treatment means were compared using the Duncan Multiple Ranging Test (DMRT). For the *in vitro data,* Pearson correlation coefficients (r) were used in two sets of observations (12 h and 24 h) tested in two runs with rumen liquid to evaluate DM disappearance, ruminant fermentation, and microbial population in eight different groups with and without additives. The effects were considered significant at $p<0.05$, and trends/tendencies at $0.05<p<0.10$.

RESULTS

Chemical Composition of DCT and FCT

Table 1 shows the basic chemical makeup of FCT and CS. The FCT and DCS were analyzed prior to the experimental processes. In the FCT, the DM content was 192.10 g/kg, the ash content was 71.10 g/kg, the OM content was 928.90 g/kg, the NDF content was 467.00 g/kg, the ADF content was 388.30 g/kg, the EE content was 36.80 g/kg, and the CP content was 198.80 g/kg. In the DCS, the DM content was 888.60 g/kg, the ash content was 64.00 g/kg, the OM content was 936.00 g/kg, the NDF content was 445.00 g/kg, the NDF content was 364.90 g/kg, the EE content was 3.00 g/kg, and the CP content was 26.60 g/kg.

Chemical Composition of Silages

Table 2 displays the chemical composition of silages. The NDF and ADF compositions of the dietary treatments were not significantly different ($p>0.05$) among groups. However, DM, ash, OM, EE, and CP were significantly different ($p<0.05$). The ash composition in 5FCT had the highest amount, OM was highest in nA and CSA, and EE was highest in 15FCT. Fermented CS with DCT at 5% to 10% DM had the highest increase in CP when compared to nA or CSA ($p<0.05$).

In Vitro Disappearance

Table 3 displays the *in vitro* dry matter disappearance (IVDMD) and *in vitro* organic matter disappearance (IVOMD) after 12 h and 24 h of incubation, respectively. The DM and OM disappearance were substantially different ($p<0.05$) according to the liquor fluid incubation serum bottles within substrates established for 12 hours. IVDMD was significantly higher in CS fermented with 5% to 10% DCT ($p<0.01$), whereas CS fermented with FCT levels demonstrated lower IVDMD than the control group ($p<0.01$).

In Vitro Kinetics of Gas Production

Table 4 shows *in vitro* dry matter disappearance (IVDMD) and *in vitro* organic matter disappearance (IVOMD) after 12 and 24 hours of incubation. The gas potential extent of gas production (p) and gas production from the insoluble fraction (b) did not differ significantly across treatments ($p>0.05$). However, the gas production from the immediately soluble fraction (a) was maximal when CS was fermented with 15DCT ($p<0.05$).

Table 2. Chemical composition of silages

Variables (g/kg dry matter)	Treatments								SEM	p value
	nA	CSA	5DCT	10DCT	15DCT	5FCT	10FCT	15FCT		
DM	292.80^{bc}	307.40^{abc}	279.90^{bc}	279.10^{bc}	302.60^{abc}	264.00^{c}	322.10^{ab}	351.50^{a}	7.08	0.02
Ash	71.70^{d}	72.90^{d}	91.10^{b}	89.80^{bc}	87.80^{bc}	98.90^{a}	85.70^{c}	91.60^{b}	1.90	0.01
OM	928.30^{a}	927.10^{a}	908.90^{c}	910.20^{bc}	912.10^{bc}	901.10^{d}	914.30^{b}	908.40^{c}	1.90	0.01
NDF	484.3	461.9	438.8	484	463.5	482.2	495	495.6	6.40	0.32
ADF	384.8	381.4	378.5	395.2	414.6	392.5	401.6	403.8	3.90	0.27
EE	2.50^{e}	2.60^{e}	5.70^{d}	8.40^{bc}	8.90^{bc}	7.90^{c}	9.50^{b}	10.90^{a}	0.60	0.01
CP	29.90^{b}	86.50^{ab}	110.50^{a}	103.50^{a}	101.70^{a}	97.50^{ab}	94.50^{ab}	99.30^{a}	5.00	0.01

Note: nA= CS fermented no additive, CSA= CS fermented with additives (*Saccharomyces cerevisiae*, urea, molasses, and sugar), 5DCT= 95% CSA fermented with 5% DCT, 10DCT= 90% CSA fermented with 10% DCT, 15DCT= 85% CSA fermented with 15% DCT, 5FCT= 95% CSA fermented with 5% FCT, 10FCT= 90% CSA fermented with 10% FCT, 15FCT= 85% CSA fermented with 15% FCT (15FCT), DM= dry mater, OM= organic matter, NDF= neutral detergent fiber, ADF= acid detergent fiber, EE= ether extract, CP= crude protein, $^{a-d}$ Means in the same row with different superscripts differ significantly ($p<0.05$), SEM= standard error of mean.

Table 3. *In vitro* dry matter disappearance (IVDMD) and *in vitro* organic matter disappearance (IVOMD) at 12 h and 24 h of incubation

Variables (g/kg dry matter)	Treatments								SEM	p value
	nA	CSA	5DCT	10DCT	15DCT	5FCT	10FCT	15FCT		
Liquor fluid incubation in 12 h, disappearance (g/kg)										
DM	321.80^{ab}	325.40^{ab}	338.70^{a}	341.30^{a}	330.70^{a}	289.00^{b}	283.60^{b}	269.60^{b}	6.1	<0.01
OM	410.50^{a}	411.20^{a}	405.90^{a}	423.70^{a}	402.60^{a}	343.10^{b}	338.00^{b}	318.70^{b}	8.4	<0.01
Liquor fluid incubation in 24 h, disappearance (g/kg)										
DM	434.40^{ab}	476.30^{a}	452.40^{ab}	414.10^{b}	413.30^{b}	457.90^{ab}	447.40^{ab}	425.60^{b}	6.2	0.04
OM	561.40^{a}	543.80^{ab}	508.60^{bc}	486.20^{c}	490.90^{c}	530.10^{abc}	532.90^{c}	492.20^{abc}	6.9	0.03

Note: DCT= dried cassava tops, FCT= fresh cassava tops, nA= CS fermented no additive, CSA= CS fermented with additives (*Saccharomyces cerevisiae*, urea, molasses, and sugar), 5DCT= 95% CSA fermented with 5% DCT, 10DCT= 90% CSA fermented with 10% DCT, 15DCT= 85% CSA fermented with 15% DCT, 5FCT= 95% CSA fermented with 5% FCT, 10FCT= 90% CSA fermented with 10% FCT, 15FCT= 85% CSA fermented with 15% FCT (15FCT), $^{a-c}$ Means in the same row with different superscripts differ significantly ($p<0.05$), SEM= standard error of mean.

Table 4. Gas kinetics production (GKP) and disappearance at 84 h of incubation

Variables		Treatments								SEM	p value
		nA	CSA	5DCT	10DCT	15DCT	5FCT	10FCT	15FCT		
Gas kinetics (mL/g DM substrate)	a	-1.43^{c}	-2.21^{c}	2.76^{ab}	-1.33^{c}	4.07^{a}	1.39^{b}	3.00^{ab}	-1.95^{c}	2.65	<0.01
	b	49.4	48.4	46.17	44.6	45.17	46.53	49.03	45.53	2.64	0.18
	c	0.05^{ab}	0.06^{ab}	0.03^{ab}	0.07^{b}	0.02^{ab}	0.03^{ab}	0.02^{ab}	0.05^{ab}	0.02	<0.01
	p	50.8	50.8	48.97	45.93	49.23	48.2	52	47.5	2.02	0.24
Disappearance (g/kg)	DM	744.20^{bc}	733.80^{c}	766.00^{a}	751.90^{b}	720.40^{d}	716.70^{d}	776.90^{a}	776.40^{a}	0.48	<0.01
	OM	842.10^{c}	846.30^{c}	862.00^{b}	843.00^{c}	835.40^{c}	816.10^{c}	876.10^{a}	841.40^{c}	0.37	<0.01

Note: 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), p(a+b)= the gas potential extent of gas production, DCT= dried cassava tops, FCT= fresh cassava tops, nA= CS fermented no additive, CSA= CS fermented with additives (*Saccharomyces cerevisiae*, urea, molasses, and sugar), 5DCT= 95% CSA fermented with 5% DCT, 10DCT= 90% CSA fermented with 10% DCT, 15DCT= 85% CSA fermented with 15% DCT, 5FCT= 95% CSA fermented with 5% FCT, 10FCT= 90% CSA fermented with 10% FCT, 15FCT= 85% CSA fermented with 15% FCT (15FCT), a-d Means in the same column with different superscripts differ significantly ($p<0.05$), SEM= standard error of mean.

Protozoal Population

Table 5 shows the ruminal pH and protozoal population at 12 h and 24 h. Different treatments significantly affected the pH of the fermentation solution, with the addition of 10DCT and 15DCT for 12 and 24 hours of incubation, respectively ($p<0.01$). After 12 hours of incubation, the protozoa population was lowest when 5DCT and 10DCT were evaluated ($p<0.01$).

Pearson Correlation Coefficients

Table 6 shows Pearson correlation coefficients (r) of dry matter degradability, pH, and gas generation after 12 h and 24 h of incubation. The results revealed that the dietary treatments for IVDMD were significant within 12 h ($p<0.01$) but not in 24 h or 12 h vs 24 h. On the other hand, it has a very low correlation in 24 h, with $R^2=0.25$. The pH exhibited a substantial difference between 12 h and 24 h, as well as a strong correlation between 12 h and 24 h, $R^2=0.75$. In contrast, there is no significant ($p<0.01$) correlation in gas production, and it was weakly correlated in 12 h against 24 h by $R^2=0.34$. Separately, the microbial population was significant in three times (12 h, 24 h, and 12 h vs 24 h) ($p<0.01$), with the strongest correlation in 12 h, $R^2=0.75$.

DISCUSSION

The FCT composition analysis indicated that the OM, NDF, and ADF contents were low, and the CP content was 198.80 g/kg, which was consistent with earlier findings (Leguizamón *et al.*, 2021; Oni *et al.*, 2014; Mao *et al.*, 2019). DCS had a CP content of 26.6 g/kg, which is similar to the values of 11.2-31 g/kg reported by previous studies (Tawida & Supawadee, 2019; Ornvimol *et al.*, 2018; Chauynarong *et al.*, 2015; Polyorach *et al.*, 2014) and 20.2 g/kg reported by Chirinang & Oonsivilai (2018). The difference in these results could be due to cultivar, harvest time, or various climatic zones of crops **(**Ornvimol *et al.*, 2018; Burns *et al.*, 2012).

CS without additives had 484.30 g/kg of NDF and 29.90 g/kg of CP in DM. Previous studies found 360 g/kg of NDF and 23.0 g/kg of CP in DM (Ornvimol *et al.*, 2018). Our findings were quite similar to those of Norrapoke *et al.* (2016), who discovered 452 g/kg of NDF and 18.0 g/kg of CP, and Napasirth *et al.* (2015), who noticed that 254.0 g/kg of NDF and 28.0 g/kg of CP when CS fermented without additives. Fermented CS with additives contained 461.90 g/kg of NDF and a CP content of 86.50 g/kg in DM, and there was decreased fiber and increased CP in Table 2. These results are comparable to those of an earlier study using CS fermented yeast waste (CSYW) containing *S. cerevisiae,* which found 243 g/kg of NDF and a CP content of 537 g/kg DM (Dagaew *et al.*, 2021). However, Norrapoke *et al.* (2017) reported that the CP concentration was high at 940 g/kg DM in silage when CS fermented urea at 4% and 2% molasses.

This result is unsupported by results from Pilajun & Wanapat (2018). CS fermented with yeast and molasses revealed minimized NDF and higher CP at 13.3 g/kg DM. This was similar to a study by Napasirth *et al.* (2015), who used FCT fermented Chikuso-1 (CH, *L. plantarum* and Snow Lact L (SN, *L. rhamnosus*). They found 550.3 g/kg of NDF and 148.8 g/kg of CP in DM. Some nutrient parameters were different, which may be from various levels of additive source supplementation or the effects of some chemicals in CT and CS on the fermentation process. Nevertheless, those nutrient contents can be used for animal feedstuffs to improve animal productivity.

Table 5. Ruminal pH and protozoal population at 12 h and 24 h

Variables	Treatments								SEM	p value
	nA	CSA	5DCT	10DCT	15DCT	5FCT	10FCT	15FCT		
					Ruminal pH					
12 h	6.61^{d}	6.72^{d}	6.54^{e}	6.83^{a}	6.78^{b}	6.76^{bc}	6.65^{d}	6.71^{c}	0.02	<0.01
24 h	6.66^{c}	6.70^{bc}	6.66^{c}	6.72^{ab}	6.74^{a}	6.68^{c}	6.60^{d}	6.68^{c}	0.01	<0.01
					Ruminal protozoa (log cells/mL)					
12 h	5.39^{c}	5.39^{c}	5.15^{e}	5.35^{d}	5.46^{b}	5.60^{a}	5.62^{a}	5.62^{a}	0.03	<0.01
24 h	5.64^{a}	5.65^{a}	5.68^{a}	5.66^{a}	5.68^{a}	5.44^{b}	5.46^{b}	5.22^{c}	0.03	<0.01

Note: nA= CS fermented no additive, CSA= CS fermented with additives (*Saccharomyces cerevisiae*, urea, molasses, and sugar), 5DCT= 95% CSA fermented with 5% DCT, 10DCT= 90% CSA fermented with 10% DCT, 15DCT= 85% CSA fermented with 15% DCT, 5FCT= 95% CSA fermented with 5% FCT, 10FCT= 90% CSA fermented with 10% FCT, 15FCT= 85% CSA fermented with 15% FCT (15FCT), $^{a-d}$ Means in the same row with different superscripts differ significantly ($p<0.05$), SEM= standard error of mean.

Table 6. Pearson correlation coefficients (r) of dry matter degradability, pH, and gas production at 12 h and 24 h of incubation

Group							SEM	p value			r		
nA	CSA	5DCT	10DCT	15DCT	5FCT	10FCT	15FCT		12h	24h	12hx24h	12h	24h	12hx24h
DM_d							0.48	<0.01	0.74	0.14	-0.68	0.25	-0.31
pH							0.02	0.33	0.35	<0.01	0.21	-0.2	0.75
GP							2.02	0.24	0.25	0.1	0.24	-0.24	0.34
Protozoa (log.cell/mL)							0.03	<0.01	<0.01	0.04	0.75	-0.80	-0.42

Note: Statistical significance of (r): ***. $p<0.001$; DMd= dry matter degradability, CP= crude protein, GP= gas production, pH= hydrogen potential, nA= CS fermented no additive, CSA= CS fermented with additives (*Saccharomyces cerevisiae*, urea, molasses, and sugar), 5DCT= 95% CSA fermented with 5% DCT, 10DCT= 90% CSA fermented with 10% DCT, 15DCT= 85% CSA fermented with 15% DCT, 5FCT= 95% CSA fermented with 5% FCT, 10FCT= 90% CSA fermented with 10% FCT, 15FCT= 85% CSA fermented with 15% FCT (15FCT), SEM= standard error of mean.

DCT and FCT fermented CS additives were tested for gas production from an immediately soluble fraction (a) and for the gas rate constant for the insoluble fraction (c), which were different among treatments ($p<0.05$). Gas production from the insoluble fraction (b) and the potential extent of gas production p (a+b) was not changed within 84 h of incubation ($p>0.05$). 5DCT, 10FCT, and 15FCT had DM digestibility of 76.6 g/kg, 776.90 g/kg, and 776.40 g/kg, which were higher than in other groups ($p<0.05$). Dagaew *et al.* (2021) had a different result when they used CSYW and found significant gas production from the insoluble fraction (b), potential extent of gas production p (a+b), and net gas production at 96 h compared to a control group ($p<0.05$), and DM degradability was 576 g/kg.

According to Pilajun & Wanapat (2018), the increases in the gas produced from the soluble fraction (a), rate of gas production (c), and potential of gas production (p) could result from molasses addition. Paengkoum & Bunnakit (2012) showed that average gas production was significantly higher in a mixture of CS and urea than in control.

The protozoa population was lowest after 12 h of incubation when 5DCT and 10DCT were examined. The protozoal concentration decline may have resulted from toxicity when we used DCT during incubation for 24 h. DCT contains cyanogenic glycoside, a toxic compound in cassava leaves, and anti-nutrients (e.g., tannin, polyphenols, and phylic acid) (Latif & Müller, 2015). Tannin has a high molecular weight and is composed of phenolic hydroxyl groups that form strong interactions with proteins (Supapong *et al.*, 2017). It inhibits digestion and nutrient absorption (Wobeto *et al.*, 2007). Small Entodinium species respond to bacterial CP return and can contribute more than 50% of the biomass in the rumen (Newbold *et al.*, 2015). In agreement with Wanapat *et al*. (2018), feeding cows 1.5 kg/day of cassava top silage reduced protozoal concentration by 62% compared to the control group. However, Sommai *et al.* (2020) reported that yeast-fermented CS did not affect the bacterial concentration in Thai native beef cattle. This was agreed by Cherdthong & Supapong **(**2019**)**, who used yeast-fermented cassava bioethanol waste (YECAW) and found no significant effect on bacteria and protozoa concentrations in dairy calves.

After 12-24 h of rumen incubation, the pH was 6.54-6.74. These findings contradicted to those of Napasirth *et al.* (2015), who discovered that CS fermented with additives had a pH of less than 4.0. However, our results were comparable to those of Dagaew *et al.* (2021), who employed CSYW and discovered a pH of 6.91. The pH range of 6.5-7.0 has been proposed as the ideal level for rumen microbial degradability of fiber and protein (Souza *et al.*, 2022). Thus, the research findings differ from earlier results, which are most likely attributable to differences in feedstuff chemical composition and incubation period.

CONCLUSION

Based on this study, CS fermented with DCT at 5% to 10% DM can enhance crude protein content in silage, *in vitro* dry matter disappearance (IVDMD), and gas production from the immediately soluble fraction while lowering the protozoa population. Further studies should be conducted *in vivo* to validate the impact of CS ensilage with DCT.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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