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Effect of Dietary Non-Fiber Carbohydrate Sources and Sulfur Supplementation on *in vitro* Ruminal Fermentation and Digestibility of the Dairy Ration

Research Article

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ABSTRACT

Synchronization of rumen degradable protein (RDP), non-fiber carbohydrate (NFC), and sulfur availability are needed for optimum microbial protein synthesis (MPS), rumen fermentation activity, and feed digestibility. Cassava and corn are rich in NFC content and have different carbohydrate characteristics. Most of the tropical feedstuff is deficient in sulfur, thus it needs to be supplemented in the ration. This study aimed to compare the effect of corn and cassava as NFC sources and sulfur supplementation on fermentability and digestibility using *in vitro* study. The experiment used a 2 × 3 factorial randomized block design with four different dairy cattle rumen liquor as replications (block). The first factor was NFC sources (corn and cassava), and the second factor was the level of sulfur supplementation (0%, 0.1%, 0.2%). Parameters observed include fermentability (rumen pH, NH_3 concentration, total volatile fatty acids (VFA), molar proportion of VFA, MPS, rumen bacteria, and protozoa population) and *in vitro* digestibility (dry matter (IVDMD) and organic matter (IVOMD)). Data were tested using ANOVA followed by the Duncan test. The result showed an interaction between rumen pH ($P<0.05$). The NH_3 concentration was low in cassava treatment, while total VFA did not have a significant effect. Corn treatment produced a higher iso-butyrate and iso-valerate than cassava treatment ($P<0.05$). The rumen microbes, MPS, IVDMD, and IVDMD, did not differ among the treatments. Cassava could replace corn as an NFC source for tropical dairy ration along with providing RDP in balance ratio and sulfur supplementation.

KEY WORDS *in vitro* digestibility, non-fiber carbohydrate, rumen degradable protein, sulfur.

INTRODUCTION

The global demand for animal foods is growing along with rising population growth and economic prosperity (Henchion *et al.* 2021). This is a challenge to providing human food and preserving human health by increasing livestock production. Milk and its product are nutrient-rich foods that largely contributes to human nutrition (Smith *et al.* 2022). Nowadays, the dairy production system has focused on their sustainability by considering animal welfare, feed efficiency, and environmental impact. Improving feed efficiency can be achieved by improving feed quality, identifying alternative feed ingredients, better explaining nutrient requirements, improving the efficiency of ruminal fermentation, and increasing dietary digestibility (Eastridge, 2006). Ruminal fermentation and digestibility is the main factor to supply nutrients for dairy cows, indeed for their maintenance and as a precursor of milk synthesis. Some factors that alter fermentation in the rumen and nutrient digestibility are the type of diet, forage-to-concentrate ratio, physical form, chewing activity, particle size (Li *et al.* 2020), rate of passage, carbohydrate source (Srakaew *et al.*

2021), dietary protein (Firkins *et al.* 2007), and rumen microbial population (Castillo-González *et al.* 2014). Determining the type of diet, mainly carbohydrates and protein, is necessary to meet microbial growth for improving ruminal fermentation and digestibility. Many previous studies have been investigated regarding arranged feed protein by supplying rumen degradable protein to undegradable protein ratio (RDPR) (Kaufman *et al.* 2018; Rosmalia *et al.* 2021; Sahroni *et al.* 2021), readily available carbohydrates reflected by non-fiber carbohydrate (NFC) (Mertens 1997; Hall *et al.* 2010; Villalba *et al.* 2021), and energy-protein synchronization (Sun *et al.* 2019; Rosmalia *et al.* 2022a).

Corn and cassava are the energy source feed ingredients that provide a fairly high NFC (Kanjanapruthipong *et al.* 2001). Corn is widely used in dairy rations and is slow degradation (Herrera-Saldana *et al.* 1990). Corn has a starch content of about 80% (Hall *et al.* 2010). Cassava meal is a feed ingredient derived from cassava (*Manihot esculenta*) and is commonly used as an energy source for livestock in tropical areas (Wanapat *et al.* 2018). As a fermentable energy source, cassava has cell walls rich in pectin (Staack *et al.* 2019).

The pectin content in the cassava cell wall is 35.7% (Nurdjanah and Elfira, 2009). The degradation rate of cassava is higher than that of corn (Wanapat *et al.* 2018). The results of previous studies stated that the type of NFC that interacts with RDP affects rumen fermentation and microbial growth (Zhao *et al.* 2020).

Besides considering the synchronization between RDP and NFC sources, sulfur supplementation in dairy rations is necessary to optimize the production of microbial protein synthesis (Rebelo *et al.* 2019). Sulfur plays a role in sulfur amino acid and vitamin synthesis (Miller, 1979). However, the sulfur content in the tropical dairy ration is deficient due to the use of agricultural by-products (Promkot and Wanapat, 2009). A sulfur deficiency was associated with reduced milk yield and milk fever (Pieper *et al.* 2016). Thus, it needs to add to the dairy ration. Investigating the association of NFC sources and sulfur supplementation in the tropical dairy ration has not been studied. Therefore, the objective of this study was to compare the effect of corn and cassava as an NFC source and sulfur supplementation on the fermentability and digestibility of dairy ration in tropical areas.

MATERIALS AND METHODS

The animal used was housed and cared for according to the Animal Ethics Committee, IPB University guidelines. This study was also proved within contract No. 047/KEH/SKE/XI/2021.

Experimental diet

The experimental diet used in this study was a 2 × 3 factorial randomized block design. The first factor was NFC sources (corn and cassava), and the second was sulfur supplementation (0%, 0.1%, 0.2%). The formulated diet is based on the NRC dairy requirement within 14% CP, 60:40 RDP to rumen undegradable protein (RUP) ratio, 35% NFC level, and set 50:50 for forage to concentrate ratio (dry matter basis). Feedstuff consisted of elephant grass, corn, rice bran, pollard, molasses, copra meal, palm kernel meal, soybean meal, corn gluten meal (CGM), and distillers dried grains with solubles (DDGS). The mineral sources were dicalcium phosphate and sodium sulfate (Na_2SO_4) as sources of sulfur. Feedstuff was finely ground and mixed through the feed formulation in Table 1. The nutrient content of the treatment diet is shown in Table 2 and it was analyzed according to the AOAC method (AOAC, 2005).

In vitro study and sample analysis

The treatment diet was carried out by *in vitro* two-stage procedures (Tilley and Terry, 1963), similar to Despal *et al.* (2022). The rumen fluid was obtained from rumen-fistulated dairy steers (510 kg of body weight) and collected before morning feeding. The McDougall solution was used as a buffer solution, which was made by diluting 9.8 g $NaHCO_3$, 4.65 g $Na_2HPO_4.2H_2O$, 0.57 KCl, 0.47 g NaCl, 0.12 g $MgSO_4.7H_2O$, and 0.04 g $CaCl_2$ into 1 L distilled water. A 0.5 g sample was weighed and placed into a fermentor tube. For ruminal fermentation (stage 1), the samples were incubated in 10 mL rumen fluid and 40 mL McDougall buffer solution for four h at 39 ˚C in a shaker water bath under anaerobic conditions. After four h of ruminal incubation, 0.5 mL, 1 mL, and 20 mL of samples were taken using a syringe for rumen bacteria and protozoa count, and microbial protein synthesis, respectively. The pH value was measured using a pH meter (Hanna Instrument, HI98191). For the molar proportion of individual volatile fatty acids (VFA), 5 mL of sample was collected, and added one drop of H_2SO_4 98% were then stored at -20 ˚C for further analysis. The fermentation process was ended up with adding two drops of $HgCl_2$ solution. The supernatant was collected by centrifugation at 3500 rpm for 15 min and then stored in a freezer to analyze of NH_3 concentration, total VFA concentration, and VFA profiles.

The *in vitro* digestibility measurements, including dry matter and organic matter (IVDMD and IVOMD), were conducted through the fermentation process (stage 1) and enzymatic process (stage 2). In the first stage, the sample was incubated with rumen and McDougall buffer solution for 48 h at 39 ˚C. Afterward, two drops of $HgCl_2$ solution were added to stop the microbial activity.

Table 1 Feed formulation of the treatment diets

Feedstuff	Corn			Cassava		
	0%S	0.1%S	0.2%S	0%S	0.1%S	0.2%S
Elephant grass	50.00	50.00	50.00	50.00	50.00	50.00
Corn	25.00	25.00	25.00	0.00	0.00	0.00
Rice bran	4.30	4.10	2.50	0.50	1.00	1.00
Pollard	2.65	2.15	2.10	3.25	2.50	1.00
Cassava	0.00	0.00	0.00	22.10	22.10	22.10
Molasses	6.30	6.50	7.65	9.00	9.00	9.00
Copra meal	2.00	2.00	2.00	2.00	2.00	2.00
Palm kernel meal	0.00	0.00	0.00	1.00	0.80	0.50
Soybean meal	9.25	9.25	9.25	6.70	6.90	7.80
CGM	0.00	0.00	0.00	3.70	3.50	2.80
DDGS	0.00	0.00	0.00	1.25	1.20	2.30
DCP	0.50	0.50	0.50	0.50	0.50	0.50
Na_2SO_4	0.00	0.50	1.00	0.00	0.50	1.00

CGM: corn gluten meal; DDGS: distillers dried grains with solubles and DCP: dicalcium phosphate.

Table 2 Chemical composition of the treatment diets

Parameters (%)	Corn			Cassava		
	0%S	0.1%S	0.2%S	0 %S	0.1%S	0.2%S
Dry matter	89.34	89.70	89.25	89.05	88.64	89.49
Ash	13.49	12.50	12.38	12.38	12.53	12.75
Ether extract	2.60	2.97	2.38	0.77	1.07	0.75
Crude protein	14.37	14.03	13.89	15.39	13.91	13.83
Crude fiber	18.16	17.86	16.41	16.33	16.02	15.95
NFE	51.39	52.64	54.95	55.13	56.47	56.72
TDN	60.38	61.74	62.85	61.98	61.95	61.53

NFE: nitrogen free extract and TDN: total digestible nutrient.
TDN= 2.79 + 1.17 CP + 1.74 EE – 0.295 CF + 0.81 NFE according to Sutardi (1980) in (Indah *et al.* 2020).

The sample was separated into supernatant and residue by centrifugation at 3500 rpm for 15 min. In the second stage, the supernatant was removed, then the sample residue was incorporated with 50 mL of pepsin-HCl solution. The pepsin solution was prepared by diluting 2 g pepsin (10000 NF U mg^{-1}, from the bovine origin, HIMedia) in 17.8 mL of 37% HCl and 1000 mL of distilled water. The tube was incubated aerobically for 48 h at 39 ˚C in a shaker waterbath. Two drops of $HgCl_2$ were added to halt enzymatic activity. Then, samples were filtered (Whatman No. 41 ashless filter paper), and the filter paper and residue were dried in an oven at 100 ˚C for 24 h and finally weighed to determine the residue's dry matter. Next, the dry residue was incinerated in an oven furnace at 650 ˚C for four h and subsequently weighed to determine the ash of the residue. The IVDMD was calculated by diminishing the dry matter of residue (corrected for the blank) from the dry matter of the sample. In comparison, the IVOMD was calculated by subtracting 100% from the ash of residue (corrected for the blank) and the initial sample.

The frozen sample of NH_3 and total VFA concentration were thawed at room temperature (25 ˚C). The NH_3 concentration was measured using the Conway microdiffusion method, while the total VFA concentration was conducted through the steam distillation method referred to (Permana *et al.* 2022).

Gas chromatography was run to obtain the molar proportion of individual VFA (Yulistiani *et al.* 2017). Microbial protein synthesis (MPS) was determined according to Makkar and Lowry's protein method (Makkar *et al.* 1982). The Ogimoto and Imai method calculates rumen bacteria and protozoa population (Rosmalia *et al.* 2022b).

Statistical analysis

The study used a 2 × 3 factorial randomized block design with four different dairy cattle rumen liquor as replications. Data were analyzed using the SAS OnDemand for Academic (SAS, 2004) for variance analysis. Effects of the factors were declared significant at $P \leq 0.05$ and further tested by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Ruminal fermentation

The fermentability of the treatment diet including is shown in Table 3. There was a significant interaction between NFC sources and the level of sulfur supplementation on rumen pH. A high rumen pH value resulted from cassava treatment with sulfur supplementation and corn treatment with 0.2% sulfur supplementation. NFC sources significantly influence rumen pH. Corn treatment decreased ruminal pH.

Table 3 The fermentability of the treatment diet including rumen pH, NH_3 concentration, and total VFA concentration

Parameters	Sulfur levels (%)	NFC sources		Average±SD
		Corn	Cassava	
Rumen pH	0	6.44±0.25[b]	6.57±0.23[a]	6.51±0.09
	0.1	6.44±0.24[b]	6.51±0.27[ab]	6.48±0.05
	0.2	6.55±0.28[ab]	6.52±0.26[ab]	6.54±0.02
	Average±SD	6.48±0.06[b]	6.54±0.03[a]	
NH_3 concentration (mM)	0	6.92±2.68	5.40±1.42	6.16±1.07
	0.1	6.02±1.07	5.82±1.44	5.92±0.14
	0.2	5.70±1.12	5.13±1.01	5.42±0.40
	Average±SD	6.21±0.63[a]	5.45±0.34[b]	
Total VFA concentration (mM)	0	105.05±20.95	152.14±13.50	128.59±33.30
	0.1	146.24±29.90	136.82±10.99	141.53±6.67
	0.2	127.22±28.86	143.27±20.85	135.24±11.34
	Average±SD	126.17±20.62	144.07±7.69	

VFA: volatile fatty acids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

There was no interaction between the two factors, but the main factor, NFC sources, showed a significant difference in the NH_3 concentration. The NH_3 concentration in the corn treatment was higher than in the cassava treatment. Total VFA concentration was not significantly influenced by NFC source, sulfur supplementation, or their interaction. The NH_3 and total VFA concentrations produced in this study were in the normal ranges, ranging from 5.13-6.92 mM and 105.05-152.14 mM, respectively (McDonald *et al.* 2010; Dieho *et al.* 2016).

The VFA molar proportions of the treatment diets are presented in Table 4. Based on statistical analysis, the main factors and their interaction did not affect the molar proportion of acetate, propionate, n-butyrate, n-valerate, and acetate to propionate ratio. Iso-butyrate and iso-valerate were significantly affected by NFC sources. Corn treatment produced a higher iso-butyrate and iso-valerate than cassava treatment.

Rumen microbes and microbial protein synthesis

The rumen microbes population indicates whether the rumen degradation was doing well. Ruminal microbial population (bacteria and protozoa) and MPS are shown in Table 5. There was no interaction between NFC sources and sulfur supplementation and the effects of the main factors on ruminal bacteria, protozoa population, and MPS. The number of rumen bacteria found in this study was slightly higher (10^{11}-10^{12} CFU mL^{-1}) than the normal range (10^{10}-10^{11} CFU mL^{-1}) (Bainbridge *et al.* 2018), while the protozoa population was in the normal condition (6.48-6.62 log cell mL^{-1}) (Matthews *et al.* 2019). The means of MPS ranged from 5.36-6.77 mg 10 mL^{-1}.

***In vitro* dry matter and organic matter digestibility (IVDMD and IVOMD)**

The IVDMD and IVOMD of the treatment diet are presented in Table 6.

Either the main factor and their interaction (NFC sources and sulfur supplementation) were not significantly affected by IVDMD and IVOMD. The mean of IVDMD and IVOMD in this study ranged from 68.80-69.76% and 72.27-73.15%, respectively.

The treatment diets changed rumen pH. Therefore, the ruminal pH in this study was in the normal condition (6.00-7.00) to support microbial activity and growth (McDonald *et al.* 2010). The high pH in 0.2% sulfur supplementation with NFC sources corn and cassava with all levels of sulfur (P<0.05). In contrast, a previous study found that a high sulfur diet can lower rumen pH by increasing H_2S concentration in the rumen (Felix and Loerch, 2011). The high rumen pH in this study might be due to increasing effective NDF in the ration, which leads to decreasing H_2S and H^+ (Drewnoski *et al.* 2014), and the treatment diets have a good buffering capacity (Wu *et al.* 2015). Rumen pH was influenced by diet properties, saliva secretion, osmotic pressure, volatile fatty acids, rumen water flow, and buffering power of feed (Zheng *et al.* 2020).

The NH_3 concentration produced in this study was sufficient for microbial protein synthesis. The minimum NH_3 concentration is 2.94 mM, while the optimum to support microbial growth is 5.00-17.65 mM (McDonald *et al.* 2010).

The cassava treatment had a lower NH_3 concentration than the corn treatment. A previous study also reported that the cassava diet produced low NH_3 compared to the corn diet (Putridinanti *et al.* 2019). Zheng *et al.* (2020) revealed that the inclusion of cassava in the diet reduced the NH_3 concentration related to high carbohydrate content in cassava treatment. The high availability of carbohydrates stimulated the rumen microbes to absorb the nitrogen, then decreased in the NH_3 concentration (Henning *et al.* 1991).

The total VFA produced in this study was higher than Dieho *et al.* (2016), 105.05-152.04 mM and 100.96-121.20 mM, respectively.

Table 4 VFA molar proportion of the treatment diets

Parameters	Sulfur levels (%)	NFC sources		Average±SD
		Corn	Cassava	
Acetate (%)	0	70.19±5.76	69.23±8.07	69.71±0.68
	0.1	70.33±5.85	70.91±7.78	70.62±0.41
	0.2	68.43±9.36	70.46±7.31	69.44±1.43
	Average±SD	69.65±1.06	70.20±0.87	
Propionate (%)	0	16.79±4.96	17.13±5.95	16.96±0.23
	0.1	16.13±4.62	16.46±6.26	16.30±0.23
	0.2	18.67±8.65	16.22±4.94	17.44±1.73
	Average±SD	17.20±1.32	16.60±0.47	
Iso-butirate (%)	0	1.10±0.79	1.02±0.67	1.06±0.06
	0.1	1.22±0.80	0.90±0.68	1.06±0.23
	0.2	1.09±0.69	0.88±0.69	0.99±0.15
	Average±SD	1.14±0.07[a]	0.93±0.07[b]	
n-butirate (%)	0	8.41±1.16	9.64±3.10	9.02±0.87
	0.1	8.81±2.24	8.93±2.67	8.87±0.09
	0.2	8.61±1.73	9.58±3.36	9.09±0.69
	Average±SD	8.61±0.20	9.38±0.39	
Iso-valerate (%)	0	2.28±1.32	1.86±1.49	2.07±0.30
	0.1	2.48±1.22	1.70±1.38	2.09±0.55
	0.2	2.20±1.08	1.81±1.57	2.00±0.27
	Average±SD	2.32±0.14[a]	1.79±0.09[b]	
n-valerate (%)	0	1.22±0.41	1.13±0.49	1.18±0.07
	0.1	1.03±0.35	1.10±0.38	1.06±0.05
	0.2	1.01±0.17	1.05±0.37	1.03±0.03
	Average±SD	1.09±0.12	1.09±0.04	
C2/C3	0	4.18±1.32	4.04±1.48	4.11±0.10
	0.1	4.36±1.31	4.31±1.71	4.33±0.04
	0.2	3.67±1.76	4.34±1.42	4.00±0.48
	Average±SD	4.07±0.36	4.23±0.16	

C2/C3: acetate to propionate ratio and VFA: volatile fatty acids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 5 Ruminal microbial population and microbial protein synthesis

Parameters	Sulfur levels (%)	NFC sources		Average±SD
		Corn	Cassava	
Ruminal bacteria (log CFU mL^{-1})	0	12.34±0.16	11.72±0.58	12.03±0.44
	0.1	12.07±0.56	12.20±0.51	12.14±0.09
	0.2	11.85±0.44	12.01±0.25	11.93±0.12
	Average±SD	12.09±0.25	11.98±0.24	
Protozoa count (log cell mL^{-1})	0	6.60±0.04	6.62±0.13	6.61±0.01
	0.1	6.58±0.05	6.48±0.08	6.53±0.07
	0.2	6.59±0.06	6.57±0.09	6.58±0.02
	Average±SD	6.59±0.01	6.56±0.07	
Microbial protein synthesis (mg 10 mL^{-1})	0	6.06±1.02	7.49±4.30	6.77±1.01
	0.1	5.10±1.77	5.62±2.40	5.36±0.37
	0.2	6.71±3.77	5.40±1.99	6.06±0.93
	Average±SD	5.96±0.81	6.17±1.15	

This indicates that the treatment diet based on corn and cassava as NFC sources was highly fermentable and supplied sufficient energy for rumen microbes and the host (Wang *et al*. 2020). The high production of total VFA also indicated high organic matter that was degraded easily by rumen microbes (Joo *et al*. 2005). Although corn and cassava had different characteristics of carbohydrates, the total VFA in this study did not differ among the treatments diet.

Different types of carbohydrates could change the VFA molar proportion (Bannink *et al*. 2006). Many factors could change the total VFA concentration, such as substrate availability, rumen fermentation rate, feed consumption, VFA absorption, and liquid or solid passage (Hall *et al*. 2015).

Table 6 *In vitro* dry matter and organic matter digestibility of the treatment diets

Parameters	Sulfur levels (%)	NFC sources		Average±SD
		Corn	Cassava	
IVDMD[1] (%)	0	70.19±4.45	68.47±2.63	69.33±1.22
	0.1	69.51±2.86	69.41±0.67	69.46±0.07
	0.2	69.57±1.20	68.52±1.49	69.04±0.74
	Average±SD	69.76±0.38	68.80±0.53	
IVOMD[2] (%)	0	73.72±3.94	71.90±2.61	72.81±1.29
	0.1	72.91±2.31	72.98±0.61	72.95±0.05
	0.2	72.82±1.61	71.92±1.34	72.37±0.64
	Average±SD	73.15±0.50	72.27±0.62	

IVDMD: *in vitro* dry matter digestibility and IVOMD: *in vitro* organic matter digestibility.

Propionate is one of the precursors of lactose formation for dairy cows and a substrate for hepatic gluconeogenesis (Habel *et al*. 2022). Propionate concentration was higher in the rations with low forages (Sutton *et al.* 2003). The addition of readily degradable carbohydrates in the ration will increase propionate production (Chanjula *et al*. 2007). Based on the results of this study, sulfur treatment and NFC type did not significantly effect on the molar proportion of propionate. This aligns with a previous study that the propionate concentration between corn-based rations and cassava had no significant effect (Chanjula *et al*. 2007). This is due to the similar level of NFC in corn and cassava treatment, so the propionate production had no difference. The results of this study are also in line with Zhao *et al*. (2020), which state that Na_2SO_4 supplementation did not affect the molar proportion of propionate. *Fibrobacter* bacteria that produce succinate are not affected by Na_2SO_4 supplementation, so propionate production is not disturbed because succinate can be converted into propionate by succinate-to-propionate bacteria.

VFA branched chains, such as iso-butyrate and iso-valerate, are products of oxidative deamination and decarboxylation of amino acids in the form of isoleucine, valine, and leucine (Rosmalia *et al*. 2022c). Corn treatment produced high iso-valerate and iso-butyrate compared to cassava treatment. This is in line with Zheng *et al*. (2020), who reported that the production of iso-valerate and iso-butyrate tends to decrease due to substituting of corn with cassava meal. The protein content in corn treatment was higher than in cassava treatment resulting in a high molar proportion of iso-valerate and iso-butyrate in corn treatment. Based on this research, there is no effect on the production of n-butyrate and n-valerate. Zheng *et al*. (2020) also reported that corn produced higher valerate production than cassava treatment, with no effect on butyrate. The sulfur supplementation with Na_2SO_4 did not affect the concentration of butyrate. The study's result differed from Zhao *et al*. (2020), which reported that Na_2SO_4 supplementation increases butyrate production due to increased growth of *Butyrivibrio fibrisolvens* and *Pseudobutyrivibrio* spp. Bacteria.

Acetate is the main precursor for dairy livestock to synthesize of milk fat (Urrutia and Harvatine, 2017). In this study, there was no interaction between sulfur supplementation levels and NFC types, and no significant effect of both treatments on the molar proportion of acetate and acetate to propionate ratio. This might be due to the similar NFC levels in the ration. The molar proportion of acetate will increase along with the increase in NFC levels (Rosmalia *et al*. 2022a). Chanjula *et al*. (2007) also reported that acetate concentrations did not significantly effect between corn-based rations and cassava meal. The ratio between acetate and propionate did not differ markedly as cassava meal supplementation increased into corn-based rations. In contrast, Zhao *et al*. (2020) revealed that Na_2SO_4 supplementation might increase acetate production due to increased *Ruminococcaceae* bacteria.

Rumen bacteria are the most abundant microbes in the rumen and contribute to enzymatic activities (amylase, lipase, protease, cellulase, and xylanase). Protozoa play roles in fermentation, especially in the rumen pH, and detoxification of mycotoxin. They are also closely associated with methanogenic archaea (Elghandour *et al*. 2019). The main factor of rumen microbial growth and activity include pH, temperature, buffer capacity, osmotic pressure, dry matter content, and oxidation-reduction potential. The rumen bacteria population found in this study was slightly higher than the normal range (Bainbridge *et al*. 2018). A high bacterial population in the rumen can increase the digestibility of dietary fiber and the availability of microbial protein as a protein source for dairy cows. The high energy level which is contributed by NFC sources (corn and cassava) in the treatment diet might be induced the increase of glucose fermentation by improving the levels of substrate-level phosphorylation (SLP) and electron transport phosphorylation (ETP), thereby increasing the number of rumen bacteria (Lu *et al*. 2019).

Sulfur supplementation did not influence the rumen bacteria population and protozoa. The similar results also agree with the finding of Rosmalia *et al*. (2022b), who reported that sulfur supplementation in the dairy ration with the

same RDP ratio did not affect the rumen bacteria population, as well as the protozoa population (Prachumchai *et al.* 2021; Supapong *et al.* 2019). The function of sulfur in rumen microbial cells is part of sulfuric amino acids (cysteine and methionine) and part of several enzymes (CoA, Co-enzyme A carboxylase) (Akib *et al.* 2014). Vakili *et al.* (2010) reported that NFC supplementation can reduce the population of methanogenic bacteria and protozoa in rumen fluid. Starch-type NFC has the potential to suppress rumen methanogenesis. In contrast, another study conducted by Firkins *et al.* (2007) stated that the rumen microbial population was not affected by the type of carbohydrate.

The MPS found in this study ranged from 5.36 to 6.77 mg 10 mL^{-1}. Rumen microbes can synthesize amino acids that make up their body cells from carbohydrates, non-protein nitrogen (NPN), and organic and inorganic sulfur. The microbial synthesis process will be optimal if the carbohydrate, nitrogen, and energy are available in sufficient and balanced quantities (Hackmann and Firkins, 2015). Waldi *et al.* (2017) reported that the MPS in feed containing total digestible nutrient (TDN) 59.99% – 61.75% was 123.79 – 227.56 mg 20 mL^{-1}. The composition of the feed can cause this difference, and the feed ingredients used are also different. Rodríguez *et al.* (2007) stated that MPS depends on several factors, such as carbohydrate and protein sources, feed intake levels, synchronization of rumen function, recycle of rumen microbes and antinutrients in the plants consumed. Adding starch to ruminant feed can negatively affect on microbial synthesis because starch fermentation can reduce rumen pH, fiber degradation, and amino acid synthesis. Other factors supporting optimal microbial protein synthesis are nitrogen supply, VFA concentration, protein and energy degradation rates synchronization, vitamin and mineral content, and the number of rumen microbes (Pathak, 2008).

The digestion process aims to break down the nutrient of feed (protein, lipids, carbohydrate) from large to simple molecules through mechanical and chemical digestion for absorption into the blood and lymph vessels (McDonald *et al.* 2010). Digestibility is a key factor in measuring the nutritional value of feed. The IVDMD and IVOMD in this study ranged from 68.80-69.76% and 72.27-73.15%, respectively, which indicate a moderate dairy ration in the tropical area (Lestari *et al.* 2015; Anzhany *et al.* 2022). The IVDMD and IVOMD values in this study did not differ among treatment. It might be due to the same protein content and RDP ratio in the treatment diets (Rosmalia *et al.* 2022c). Amount of the digestibility value of dry matter and organic matter is strongly influenced by the proportion of protein as a source of N for rumen microbes, carbohydrates as a carbon framework to support rumen microbial protein synthesis as well as an energy source for the ruminant, and crude fiber content (Hambakodu and Ina, 2019). This is also supported by a large number of similar bacterial populations in this study.

Supapong *et al.* (2019) reported that adding sulfur as much as 2% DM to fermented total mix ration (TMR) can increase DMD followed by an increase in the bacterial population. Similar results were also reported by Promkot and Wanapat (2009), which stated that the increase in fiber digestibility cattle supplemented with sulfur at the level of 0.4% DM compared to 0.15% DM in the ration. A previous study found that the NFC ratio did not affect dry matter and organic matter digestibility (Afzalzadeh *et al.* 2010). In addition, Rosendo *et al.* (2003) stated that the digestibility of NDF *in vitro* in forages added with starch, inulin, and pectin was not significantly different. Meanwhile, Gao and Oba (2016) reported that the digestibility of dry matter and organic matter was higher in starch-rich diets than in disaccharide-rich carbohydrates, which was related to the rate of passage in the digestive tract.

CONCLUSION

In conclusion, dairy cattle ration formulation based on RDP, NFC, and sulfur synchronization produced optimum microbial activity, and protein synthesis, and *in vitro* digestibility in both NFC sources. Corn as an NFC produced higher protein fermentation product (NH_3) and iso-acids (iso-valerate and iso-butyrate). However, both NFC sources failed to significantly improve in microbial protein synthesis, organic matter fermentability (VFA), and digestibility. Sulfur supplementation up to 0.2% did not improve in rumen condition, fermentation product, and digestibility. It is suggested to test sulfur supplementation at a higher level further.

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REFERENCES

Afzalzadeh A., Rafiee H., Khadem A.A. and Asadi A. (2010). Effects of ratios of non-fibre carbohydrates to rumen degradable protein in diets of Holstein cows: 1. Feed intake, digestibility and milk production. *South African J. Anim. Sci.*

40, 204-212.

Akib M.A., Setiawaty H. and Sulfiah H. (2014). Improving the quality of "leri" rice washing waste by different period of fermentation and yeast concentration as an alternative liquid organic fertilizer. *Int. J. Agric. Syst*. **2,** 153-162.

Anzhany D., Toharmat T. and Despal D. (2022). Ration to produce milk high in conjugated linoleic acid (CLA) at smallholder dairy farm: an *in vitro* reconstruction. *Am. J. Anim. Vet. Sci*. **17,** 130-138.

AOAC. (2005). Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bainbridge M.L., Saldinger L.K., Barlow J.W., Alvez J.P., Roman J. and Kraft J. (2018). Alteration of rumen bacteria ands protozoa through grazing regime as a tool to enhance the bioactive fatty acid content of bovine milk. *Front. Microbiol*. **9,** 1-13.

Bannink A., Kogut J., Dijkstra J., France J., Kebreab E., Van Vuuren A.M. and Tamminga S. (2006). Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *J. Theor. Biol*. **238,** 36-51.

Castillo-González A.R., Burrola-Barraza M.E., Domínguez-Viveros J. and Chávez-Martínez A. (2014). Rumen microorganisms and fermentation. *Arch. Med. Vet*. **46,** 349-361.

Chanjula P., Ngampongsai W. and Wanapat M. (2007). Effects of replacing ground corn with cassava chip in concentrate on feed intake, nutrient utilization, rumen fermentation characteristics and microbial populations in goats. *Asian-Australasian J. Anim. Sci*. **20,** 1557-1566.

Despal D., Manik D.T.P., Evvyernie D. and Zahera R. (2022). The accuracy of several *in vitro* methods in estimating *in vivo* digestibility of the tropical dairy ration. *IOP Conf. Ser. Earth Environ. Sci*. **951,** 120-132.

Dieho K., Dijkstra J., Schonewille J.T. and Bannink A. (2016). Changes in ruminal volatile fatty acid production and absorption rate during the dry period and early lactation as affected by rate of increase of concentrate allowance. *J. Dairy Sci*. **99,** 5370-5384.

Drewnoski M.E., Pogge D.J. and Hansen S.L. (2014). High-sulfur in beef cattle diets: A review. *J. Anim. Sci*. **92,** 3763-3780.

Eastridge M.L. (2006). Major advances in applied dairy cattle nutrition. *J. Dairy Sci*. **89,** 1311-1323.

Elghandour M.M.Y., Khusro A., Adgebeye M.J., Tan Z., Abu Hafsa S.H., Greiner R., Ugbogu E.A., Anele U.Y. and Salem A.Z.M. (2019). Dynamic role of single-celled fungi in ruminal microbial ecology and activities. *J. Appl. Microbiol*. **128,** 950-965.

Felix T.L. and Loerch S.C. (2011). Effects of haylage and monensin supplementation on performance, carcass characteristics, and ruminal metabolism of feedlot cattle fed diets containing 60% dried distillers grains. *J. Anim. Sci*. **89,** 2614-2623.

Firkins J.L., Yu Z. and Morrison M. (2007). Ruminal nitrogen metabolism: Perspectives for integration of microbiology and nutrition for dairy. *J. Dairy Sci*. **90,** 1-16.

Gao X. and Oba M. (2016). Effect of increasing dietary nonfiber carbohydrate with starch, sucrose, or lactose on rumen fermentation and productivity of lactating dairy cows. *J. Dairy Sci*. **99,** 291-300.

Habel J., Chapoutot P. and Koch C. (2022). Estimation of individual glucose reserves in high-yielding dairy cows. *Animals*. **3,** 438-464.

Hackmann T.J. and Firkins J.L. (2015). Maximizing efficiency of rumen microbial protein production. *Front. Microbiol*. **6,** 1-16.

Hall M.B., Larson C.C. and Wilcox C.J. (2010). Carbohydrate source and protein degradability alter lactation, ruminal, and blood measures. *J. Dairy Sci*. **93,** 311-322.

Hall M.B., Nennich T.D., Doane P.H. and Brink G.E. (2015). Total volatile fatty acid concentrations are unreliable estimators of treatment effects on ruminal fermentation *in vivo*. *J. Dairy Sci*. **98,** 3988-3999.

Hambakodu M. and Ina Y.T. (2019). *In vitro* digestibility evaluation of feed ingredients from agro-industry by-product. *J. Agripet*. **19,** 7-12.

Henchion M., Moloney A.P., Hyland J., Zimmermann J. and McCarthy S. (2021). Review: Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. *Animal*. **15,** 100287.

Henning P.H., Steyn D.G. and Meissner H.H. (1991). The effect of energy and nitrogen supply pattern on rumen bacterial growth *in vitro*. *Anim. Prod.* **53,** 165-175.

Herrera-Saldana R.E., Huber J.T. and Poore M.H. (1990). Dry matter, crude protein, and starch degradability of five cereal grains. *J. Dairy Sci.* **73,** 2386-2393.

Indah A.S., Permana I.G. and Despal D. (2020). Determination total digestible nutrient (TDN) of tropical fotage using nutrient composition. *Livest. Anim. Res.* **18,** 38-43.

Joo J.W., Bae G.S., Min W.K., Choi H.S., Maeng W.J., Chung Y.H. and Chang M.B. (2005). Effect of protein sources on rumen microbial protein synthesis using rumen simulated continuous culture system. *Asian-Australasian J. Anim. Sci*. **18,** 326-331.

Kaufman J.D., Pohler K.G., Mulliniks J.T. and Ríus A.G. (2018). Lowering rumen-degradable and rumen-undegradable protein improved amino acid metabolism and energy utilization in lactating dairy cows exposed to heat stress. *J. Dairy Sci*. **101,** 386-395.

Kanjanapruthipong J., Buatong N. and Buaphan S. (2001). Effects of roughage neutral detergent fiber on dairy performance under tropical conditions. *Asian-Aust. J. Anim. Sci.* **14,** 1400-1404.

Lestari D.A., Abdullah L. and Despal D. (2015). Comparative study of milk production and feed efficiency based on farmers best practices and national research council. *Media Peternakan*. **38,** 110-117.

Li C., Beauchemin K.A. and Yang W. (2020). Feeding diets varying in forage proportion and particle length to lactating dairy cows: I. Effects on ruminal pH and fermentation, microbial protein synthesis, digestibility, and milk production. *J. Dairy Sci*. **103,** 4340-4354.

Lu Z., Xu Z., Shen Z., Tian Y. and Shen H. (2019). Dietary energy level promotes rumen microbial protein synthesis by improving the energy productivity of the ruminal microbiome. *Front. Microbiol*. **10,** 1-14.

Makkar H.P.S., Sharma O.P., Dawra R.K. and Negi S.S. (1982).

Simple determination of microbial protein in rumen liquor. *J. Dairy Sci*. **65,** 2170-2173.

Matthews C., Crispie F., Lewis E., Reid M., O'Toole P.W. and Cotter P.D. (2019). The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes*. **10,** 115-132.

McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A. and Wilkinson R.G. (2010). Animal Nutrition. Pearson, London, United Kingdom.

Mertens D.R. (1997). Creating a system for meeting the fiber requirements of dairy cows. *J. Dairy Sci*. **80,** 1463-1481.

Miller W.J. (1979). Dairy Cattle Feeding and Nutrition. Academic Press, London, United Kingdom.

Nurdjanah S. and Elfira W. (2009). The profile of fiber composition and functional properties of dietary fiber from tuber starch residues. *J. Technol. Ind. Hesil Pertanian*. **14,** 13-23.

Pathak A.K. (2008). Various factors affecting microbial protein synthesis in the rumen. *Vet. World*. **1,** 186-189.

Permana I.G., Despal D., Rosmalia A. and Rahayu M.D. (2022). Inclusion of different level leucaena in dairy ration to balance rumen degradable and undegradable protein ratio. *IOP Conf. Ser. Earth Environ. Sci*. **1020,** 120-133.

Pieper L., Wall K., Müller A.E., Roder A.and Staufenbiel R. (2016). Untersuchungen zur schwefelversorgung von milchkühen in Deutschland. *Tierarztl. Prax. Ausg. G. Grosstiere Nutztiere*. **44,** 92-98.

Prachumchai R., Cherdthong A. and Wanapat M. (2021). Screening of cyanide-utilizing bacteria from rumen and *in vitro* evaluation of fresh cassava root utilization with pellet containing high sulfur diet. *Vet. Sci.* **8,** 1-14.

Promkot C. and Wanapat M. (2009). Effect of elemental sulfur supplementation on rumen environment parameters and utilization efficiency of fresh cassava foliage and cassava hay in dairy cattle. *Asian-Australasian J. Anim. Sci.* **22,** 1366-1376.

Putridinanti A.D., Noviandi C.T., Gunawan Agus A., Harper K. and Poppi D. (2019). A comparison of three highly fermentable carbohydrate sources (corn, cassava powder or cassava pulp) on *in vitro* digestion. *IOP Conf. Ser. Earth Environ. Sci*. **387,** 12-26.

Rebelo L.R., Luna I.C., Messana J.D., Araujo R.C., Simioni T.A., Granja-Salcedo Y.T., Vito E.S., Lee C., Teixeira I.A.M.A., Rooke J.A. and Berchielli T.T. (2019). Effect of replacing soybean meal with urea or encapsulated nitrate with or without elemental sulfur on nitrogen digestion and methane emissions in feedlot cattle. *Anim. Feed Sci.Technol.* **257,** 1-14.

Rodríguez R., Sosa A. and Rodríguez Y. (2007). Microbial protein synthesis in rumen and its importance to ruminants. *Cuban J. Agric. Sci.* **41,** 287-294.

Rosendo O., Hall M.B., Staples C. and Bates D. (2003). The effect of different neutral-detergent-soluble polysaccharides in digestive cynetics *in vitro* of neutral detergent forrage fiber and the synthesis of microbial protein. *Rev. Cient. Fac. Cienc. Vet. Univ. Zulia*. **13,** 18-27.

Rosmalia A., Astriani A., Sahroni W.P., Permana I.G. and Despal D. (2022a). Effect of rumen degradable protein and sulfur supplementation on *in vitro* digestibility and ruminal fermentation. *IOP Conf. Ser. Earth Environ. Sci*. **951,** 12013.

Rosmalia A., Dewi N.A., Permana I.G. and Despal D. (2022b). Reformulation of dairy cattle concentrate based on rumen degradable protein to undegradable protein ratio at different energy levels: *in vitro* study. *IOP Conf. Ser. Earth Environ. Sci*. **1020,** 12008.

Rosmalia A., Permana I.G. and Despal D. (2022c). Synchronization of rumen degradable protein with non-fiber carbohydrate on microbial protein synthesis and dairy ration digestibility. *Vet. World*. **15,** 252-261.

Rosmalia A., Permana I.G. and Despaland Zahera R. (2021). Estimation rumen degradable protein of local feeds in dairy cattle using *in sacco* method. *IOP Conf. Ser. Earth Environ. Sci*. **883,** 12010.

Sahroni W.P., Permana, I.G. and Despal D. (2021). Reformulation of dairy cow diets based on rumen degradable protein and total digestible nutrient with varying levels on *in vitro* fermentability and digestibility. *IOP Conf. Ser. Earth Environ. Sci*. **888,** 12075.

SAS Institute. (2004). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Smith N.W., Fletcher A.J., Hill J.P. and McNabb W.C. (2022). Modeling the contribution of milk to global nutrition. *Front. Nutr*. **8,** 1-7.

Srakaew W., Wachirapakorn C., Cherdthong A.and Wongnen C. (2021). Ruminal degradability and bypass nutrients of alkaline or steam-treated cassava chip and corn grain. *Trop. Anim. Sci. J*. **44,** 451-461.

Staack L., Pia E.A.D., Jorgensen B., Pettersson D. and Pedersen N.R. (2019). Cassava cell wall characterization and degradation by a multicomponent NSP-targeting enzyme (NSPase). *Sci. Rep.* **9,** 10150-10161.

Sun F., Aguerre M.J. and Wattiaux M.A. (2019). Starch and dextrose at 2 levels of rumen-degradable protein in iso-nitrogenous diets: Effects on lactation performance, ruminal measurements, methane emission, digestibility, and nitrogen balance of dairy cows. *J. Dairy Sci*. **102,** 1281-1293.

Supapong C., Anusorn C., Wanapat M., Chanjula P. and Uriyapongson S. (2019). Effects of sulfur levels in fermented total mixed ration containing fresh cassava root on feed. *Animals*. **9,** 1-11.

Sutton J.D., Dhanoa M.S., Morant S.V., France J., Napper D.J. and Schuller E. (2003). Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *J. Dairy Sci*. **86,** 3620-3633.

Tilley J.M.A. and Terry R.A. (1963). A two‐stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.* **18,** 104-111.

Urrutia N.L. and Harvatine K.J. (2017). Acetate dose-dependently stimulates milk fat synthesis in lactating dairy cows. *J. Nutr*. **147,** 763-769.

Vakili A., Mesgaran M.D., Jahani-Azizabadi H., Ghovvati S., Milani E. and Rezaee F. (2010). The effect of non-fibre carbohydrates supplementation on methanogenesis bacteria and protozoa populations in rumen fluid as determined by real-time polymerase chain reaction. *Adv. Anim. Biosci.* **1,** 253-253.

Villalba J.J., Ates S. and MacAdam J.W. (2021). Non-fiber carbohydrates in forages and their influence on beef production systems. *Front. Sustain. Food Syst.* **5,** 1-12.

Waldi L., Suryapratama W. and Suhartati F.M. (2017). Pengaruh penggunaan bungkil kedelai dan bungkil kelapa dalam ransum berbasis indeks sinkronisasi energi dan protein terhadap sintesis protein mikroba rumen sapi perah. *J. Livest. Sci. Prod.* **1,** 1-12.

Wanapat M., Foiklang S., Sukjai S., Tamkhonburi P., Gunun N., Gunun P., Phesatcha K., Norrapoke T. and Kang S. (2018). Feeding tropical dairy cattle with local protein and energy sources for sustainable production. *J. Appl. Anim. Res.* **46,** 232-236.

Wang L., Zhang G., Li Y. and Zhang Y. (2020). Effects of high forage/concentrate diet on volatile fatty acid production and the microorganisms involved in VFA production in cow rumen. *Animals*. **10,** 1-12.

Wu H., Meng Q. and Yu Z. (2015). Effect of pH buffering capacity and sources of dietary sulfur on rumen fermentation, sulfide production, methane production, sulfate reducing bacteria, and total Archaea in *in vitro* rumen cultures. *Bioresour. Technol.* **186,** 25-33.

Yulistiani D., Puastuti W., Haryanto B., Purnomoadi A., Kurihara M. and Thalib A. (2017). Complete rumen modifier supplementation in corn cob silage basal diet of lamb reduces methane emission. *Indonesian J. Agric. Sci.* **18,** 33-42.

Zhao Y., Xie B., Gao J. and Zhao G. (2020). Dietary supplementation with sodium sulfate improves rumen fermentation, fiber digestibility, and the plasma metabolome through modulation of rumen bacterial communities in steers. *Appl. Environ. Microbiol.* **86,** 1-18.

Zheng Y., Xue S., Zhao Y. and Li S. (2020). Effect of cassava residue substituting for crushed maize on *in vitro* ruminal fermentation characteristics of dairy cows at mid-lactation. *Animals*. **10,** 1-13.