AGRICULTURE AND NATURAL RESOURCES

Research article

Nutrient intake, digestibility, ruminal fermentation, blood metabolites and milk production efficiency of lactating crossbred dairy cows receiving fresh cassava root and various sulfur levels

Thitiporn Kaisoda[a], Wuttikorn Srakaew[b] Tipwadee Prapaiwong[c] Pachabordee Kongphetsak[d] Sukanya Poolthajit[a], Chalong Wachirapakorn[a,*]

[a] *Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand*

[b] *Department of Animal Science and Fisheries, Faculty of Agricultural Sciences and Technology, Rajamangala University of Technology Lanna, Nan 55000, Thailand*

[c] *Department of Animal Production Technology, Faculty of Agro-industrial Technology, Rajamangala University of Technology Tawan-ok, Chanthaburi 22210, Thailand*

[d] *Dairy Farming Promotion Organization of Thailand, Saraburi 18180, Thailand*

Article Info

Abstract

Importance of work: Fresh cassava root (FCR) contains a high level of hydrogen cyanide (HCN), which is toxic when consumed by ruminants. Such adverse effects are reduced by sulfur supplementation.

Objectives: To investigate the effect of sulfur addition on intake, rumen fermentation, blood metabolites, milk production and quality in lactating dairy cows.

Materials & Methods: Four Holstein-Friesian crossbred cows with initial mean weight (± SD) of 455.1 ± 24.0 kg in mid lactation were randomly assigned according to a 4×4 Latin square design (21 d/period). The cows were assigned to receive a total mixed ration (TMR) and FCR at 0.75% bodyweight (BW; on a dry matter basis) and then sulfur was added at four levels of FCR: 0%, 0.5%, 1% and 2%.

Results: The feed intake and digestion coefficients of dry matter, protein, neutral detergent fiber and acid detergent fiber were not significantly different ($p > 0.05$). The ruminal pH, and blood metabolites were not significantly different ($p > 0.05$). Nevertheless, the total volatile fatty acids (TVFA) linearly increased ($p < 0.05$), and the ammonia-nitrogen (NH_3-N) decreased significantly ($p < 0.05$) with the increasing sulfur levels in the diets. The sulfur addition in cows fed FCR had no effect on milk yield, 4% fat-corrected milk or milk composition. Thiocyanate (SCN^-) in blood and milk linearly increased ($p < 0.01$) as the sulfur addition increased.

Main finding: FCR at 0.75% BW was promising as a supplemental feed source for lactating cows and a sulfur addition of 2% of FCR improved intake, ruminal fermentation and digestibility.

---

† Equal contribution.

* Corresponding author.

  E-mail address: chal_wch@kku.ac.th (C. Wachirapakorn)

Introduction

A good feeding program provides the correct and balanced amount of nutrients to a cow at the appropriate time to achieve optimum production, reproduction, efficiency and profitability (Maltz et al., 2013). Improper feeding due to feed quantity and quality are the primary factors influencing efficient and profitable dairy farming, particularly on smallholder farms (Wanapat et al., 2017). Many studies are being conducted to determine how to implement an effective feeding strategy to improve animal performance and reduce production costs (Bach and Cabrera, 2017).

Cassava (*Manihot esculenta*, Crantz) is a popular ruminant feed because it has a high starch degradability and is less expensive than other energy feeds (Chanjula et al., 2003). Farmers use fresh cassava root (FCR) fed to their cows during the rainy season to reduce feed costs because it is much cheaper than cassava chips (Supapong et al., 2019). However, the use of cassava is limited due to its high concentration of hydrocyanic acid or hydrogen cyanide (HCN). Cassava root, variety Kasetsart 50 (KU50), a popular variety grown throughout Thailand, contains HCN in concentrations in the range 1,427–533 mg/kg with the root age in the range 6–12 mth. After a drying process, the cassava contained HCN in the range 18–1.3 mg/kg at 6–12 mth root age (Chotineeranat et al., 2006). However, the minimum lethal dosage of HCN in cattle and sheep is about 2 mg/kg bodyweight (BW; Radostits et al., 2007). Based on the available data, regarding the effects of HCN in animal feeds, an intake lower than 500 mg HCN/kg is generally safe, 600–1,000 mg HCN/kg is potentially toxic and higher than 1,000 mg HCN/kg is dangerous to cattle and usually causes death (Kraig et al., 2012). Thus, the HCN level in dried cassava is not toxic to animals.

Hydrogen cyanide was reported to be changed to innocuous thiocyanate (SCN^-) by the action of the rhodanese enzyme that is synthesized in the liver and kidneys of an animal and participates in the detoxification of HCN to SCN^- (Cipollone et al., 2006). SCN^- is transported mainly via urine, milk and saliva for elimination from the body (Soto-Blanco and Górniak, 2003). SCN^- is a complex anion that is a potent inhibitor of iodide transport. The change of HCN to SCN^- may cause iodine deficiency because it inhibits the absorption of iodine by the thyroid gland and reduces the production of triiodothyronine (T3) and thyroxine (T4), affecting to animal performance. Furthermore, SCN^- in milk has been demonstrated to have antibacterial activity via the lactoperoxidase system, lowering the somatic cell count (SCC) of the milk (Srisaikham et al., 2018; Supapong and Cherdthong, 2020; Dagaew et al., 2021)

Cassava root in its fresh form is frequently used as a feedstock during the rainy season or because it has a very low cost for dairy farmers (Cherdthong et al., 2018). In practice, there is limited information on the appropriate level of FCR supplementation in dairy cows. Dagaew et al. (2021) reported that adding FCR at 1% BW and 1.5% BW with solid feed block containing sulfur at 2% and 4% had no beneficial effect on milk production and feed efficiency but did reduce somatic cell counts (SCC) in milk. An *in vitro* study, the addition of FCR at more than 30% of the diet decreased gas production and digestion (Kaisoda, 2018). In addition, cassava in its fresh form may be toxic to animals due to its excessive HCN levels. Studies have shown that adding sulfur to diets with FCR could deteriorate the toxic level of HCN and have a beneficial effect in terms of intake, digestibility and animal performance (Supapong and Cherdthong, 2020; Dagaew et al., 2021). Furthermore, sulfur is required by rumen microorganisms to produce sulfur-containing amino acids and for optimal microbial growth and digestibility (Silva et al., 2014). Sulfur can be incorporated in diets containing FCR fed to animals in many ways, such as in the diet directly, in a fermented total mixed ration (FTMR) (Supapong and Cherdthong, 2020) or in a solid feed block (Dagaew et al., 2021).

Therefore, the purposes of this study were to identify by what means adding various levels of sulfur to a TMR supplemented with FCR at 0.75% BW affected nutrient intake, digestibility, rumen fermentation, blood metabolites and milk production efficiency in crossbred dairy cows.

Materials and Methods

Animal care was performed according to The Committee of Animal Experimentation, National Research Council of Thailand, and was approved by the Animal Ethics Committee of Khon Kaen University, (Record no. ACUC-KKU 32/2560).

Animals, feed and treatments

Four multiparous, Holstein-Friesian crossbred (> 75% Holstein Friesian breed) cows with mean (± SD) BW of 455.1 ± 24.0 kg in mid lactation (139 ± 42 days in milk) with parity 3–5, were assigned in a 4×4 Latin square design with four periods (21 d/period). The mean (± SD) milk yield before the study was 12.6 ± 4.7 kg/d.

The basal diet was a TMR supplemented with FCR at 0.75% BW (on DM basis) based on finding an *in vitro* study (Kaisoda, 2018). Elemental sulfur levels were added at 0.0%, 0.5%, 1.0% and 2.0% of FCR supplementation on a DM basis, equivalent to 0.0%, 0.1%, 0.3% and 0.5% of the TMR. A dietary level of 0.5% elemental sulfur ensured adequate cassava cyanide detoxification (Onwuka et al., 1992). The TMR ingredients are presented in Table 1. Concentrates and chopped rice straw were mixed using a horizontal feed mixer.

All cows were offered the TMR and FCR twice daily (after milking in the morning and the afternoon), allowing for 10% refusals. Elemental sulfur was in addition to all feeds. All cows were kept in individual pens (5 m × 5 m), with rubber mats for bedding. Clean fresh water was always freely accessible. Their feed intake was recorded for 21 d, with the last 7 d including the collection of samples of ruminal fluid, feces and blood. The FCR variety used was Kasetsart 50, bought from the local market. FCR was bought every week to ensure the freshness of cassava roots. The FCR was cleaned of dust and passed through a chopping machine and passed through an 0.8–0 cm sieve before feeding daily.

Sample collection and chemical analysis

The TMR, FCR and feces were sampled during the last 5 d of each experimental period to measure digestibility. The feces were obtained by rectal sampling, to avoid contamination from sand. The diets and feces were oven dried at 60°C, ground using a Wiley mill and passed through a 1 mm sieve before chemical analysis according to methods of Association of Official Analytical Chemists, 1995) for dry matter (DM), crude protein (CP), ether extract (EE) and ash. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using the method of Van Soest et al. (1991). The acid insoluble ash (AIA) was analyzed according to the procedure of Van Keulen and Young (1977).

The apparent digestion coefficients were calculated using AIA as an internal marker according to the equation of Schneider and Flatt (1975).

The HCN of the FCR samples of each period was evaluated followed the procedure described by Rice et al. (2012) using a spectrophotometer (UV/VIS Spectrometer; PG Instruments Ltd.; London, UK).

Table 1 Ingredients of total mixed ration and chemical composition of total mixed ration and fresh cassava root used in the present study

Item	Total mixed ration	Fresh cassava root
Ingredient (% dry matter)		
Rice straw	40.0	
Cassava chip	28.2	
Corn meal	4.2	
Soybean meal	12.0	
Fined rice bran	3.0	
Soy hulls	2.4	
Palm kernel meal	5.7	
Sugar	1.8	
Urea	1.5	
Salt	0.3	
Dicalcium phosphate	0.6	
Premix[1]	0.3	
Total	100.0	
Chemical composition		
Dry matter (%)	94.9	35.4
Organic matter (% dry matter)	92.0	97.3
Crude protein (% dry matter)	15.4	1.5
Ether extract (% dry matter)	2.7	0.9
Neutral detergent fiber (% dry matter)	43.4	12.5
Acid detergent fiber (% dry matter)	30.8	5.5
Metabolizable energy (Mcal/kg)	2.4	3.3
HCN (mg/kg)	-	108.5

[1] Premix contained (in 1 kg): vitamin A 4,000,000 IU, vitamin D 400,000 IU, vitamin E 4,000 IU, vitamin B_{12} 0.002 g, Mn 16 g, Fe 24 g, Zn 24 g, Cu 2 g, Se 0.05 g and I 0.5 g
HCN = hydrogen cyanide

Milk yields were recorded daily for each animal. Milk samples were collected twice daily (0500 hours and 1600 hours) during the last 5 d of each experimental period and mixed together for analysis of the fat, protein, lactose, total solids and solids-not-fat contents using a Milko-Scan33 instrument (Foss Electric; Hillerod, Denmark). The SCC was analyzed using the Fossomatic 5,000 Basic instrument (Foss, Hillerod, Denmark). The milk's SCN^- was determined using the method reported by Cosby and Sumner (1945) with a spectrophotometer (UV/VIS Spectrometer; PG Instruments Ltd.; London, UK)

On day 21 of each period, 100 mL of ruminal fluid were collected using a stomach tube equipped with a vacuum pump at 0 hr and 4 hr post feeding. The ruminal fluid was strained through four layers of cloth and the pH was measured immediately using a hand-held unit (HI 8424 microcomputer; HANNA Instruments; Singapore). Next, 50 mL of ruminal fluid were added to 5 mL of 1M H_2SO_4 and centrifuged at $16,000 \times g$ for 15 min; the supernatant was collected and stored at -20 °C until being analyzed for NH_3-N concentration (Association of Official Analytical Chemists, 1995) and for volatile fatty acids (VFA) using high performance liquid chromatography (Samuel et al., 1997).

Blood samples were drawn from the jugular vein at 0 hr and 4 hr post morning feeding on day 21 of each period. Then, the blood was centrifuged at $3,000 \times g$ for 10 min and divided into two equal portions. The first portion was analyzed for the blood urea nitrogen (BUN) concentration according to Crocker (1967). The other blood portion was used to determine the alanine aminotransferase (ALT), aspartate aminotransferase (AST), triiodothyronine (T3) and thyroxine (T4) contents using automated clinical chemistry analyzers (Vitallab Flexor E; Dieren, the Netherlands). Thyroid hormones in the bovine serum samples were measured using radioimmunoassay. The blood SCN^- was analyzed using ultraviolet/VIS spectrophotometry (PG Instruments Ltd.; London, UK) according to Lambert et al. (1975).

Statistical analysis

All data were subjected to analysis of variance according to the 4×4 Latin square design using general linear model procedures. The statistical analyses of all replicated data were performed using the mixed procedure of SAS (2002). The treatment effects were tested using Duncan's new multiple range test and evaluated using the method of least significant difference at the 5% significance level ($p < 0.05$).

Results and Discussion

Nutrient intake and digestibility

Table 1 shows the chemical contents of the diets used in the experiment. The TMR contained CP and ME at 15.4% and 2.4 Mcal/kg DM, respectively. The FCR contained CP and ME at 1.5% and 3.3 Mcal/kg DM, respectively. The average HCN concentration in the FCR was 108.5 mg/kg DM, which was slightly higher than that reported by Dagaew et al. (2021) but lower than that reported by Supapong et al. (2019). In the current study, the HCN concentration in the FCR variety KU50 was lower than that reported (199.3 mg HCN/kg) by Chaengsee et al. (2020). The variation in HCN concentration depends mainly on the breed variety and the growth environment (Chaengsee et al., 2020).

The feed intake and digestibility of the cows offered TMR and FCR is presented in Table 2. The DM intake of TMR and FCR were not significantly different among the between dietary treatments and the intake of sulfur increased linearly ($p < 0.05$) as the inclusion of sulfur increased. Increased sulfur levels resulted in a linear increase in the TMR intake ($p < 0.05$), which was highest in cows that received sulfur at 2% of FCR. The average DMI of TMR was 2.2% BW when FCR was added at 0.75% BW. The total DMI expressed as % BW and g/kg $BW^{0.75}$ linearly increased ($p < 0.05$) as the sulfur content increased. This observation was similar to that reported by Supapong et al. (2019) who fed FTMR containing FCR with sulfur at 1% and 2% to dairy cows. In the current study, the animals consumed HCN in the range 350.1–383.8 mg/d or 25.8–28.8 mg/kg DM when the FCR was supplemented at 0.75% BW on a DM basis. These levels were below 500 mg/kg and so were not toxic to animals (Kraig et al., 2012).

The nutrient intakes of the cows fed the basal diet with any level of sulfur were not significantly different among treatments (Table 2); however, the CP and ADF intake linearly increased ($p < 0.05$) as the sulfur content increased. The CP and ADF intake linearly increased ($p < 0.05$) as the sulfur content increased. These increments in nutrients were due to the linear increase in the TMR intake. The CP intake of the dairy cows in this study was consistent with the recommendation of Wachirapakorn et al. (2014), who reported that CP consumption was 1.4–1.8 kg/d for lactating cows producing milk at 12 kg/d, which was comparable to the nutrient requirement for cows according to the National Research Council (2001).

This finding was consistent with that reported by Dagaew et al. (2021), who offered FCR at 1% BW and 1.5% BW with solid feed blocks containing sulfur at 2% and 4%. Supapong et al. (2019) reported that sulfur level inclusion at 1% or 2% in the FTMR for dairy cows did not influence their feed intake. In addition, Promkot and Wanapat (2009) found that supplementing elemental sulfur at 1.5% and 4% in cows fed fresh cassava leaves did not affect their feed intake. In beef cattle, feed blocks containing sulfur at 2% and 4% did not alter the feed intake (Cherdthong et al., 2018). Uwituze et al. (2011) found that sulfur supplementation at 4.2% or 6.5% of the dietary DM in crossbred steers did not affect their nutrient intake.

In the current study, the digestion coefficients of the DM and CP tended to linearly increase ($p = 0.08$) and the OM digestibility linearly increased ($p < 0.05$) by increasing the sulfur addition.

Supapong et al. (2019) found that the digestion coefficient of DM, but not of OM, increased following supplementation at 2% sulfur for beef cattle fed FTMR. This might have been due to increasing numbers of rumen bacteria in the cattle fed with FTMR containing a sulfur level at 2% DM compared to the 1% sulfur diet. In ruminants, sulfur is essential to improve ruminal microorganism growth and leads to an improved digestion coefficient. However, the EE digestibility linearly decreased ($p < 0.01$) as the sulfur level increased. Increased levels of sulfur decreased EE digestibility; however, the reason for this is unknown. The apparent digestibility of ADF in cows fed basal diets was not influenced ($p > 0.05$) by the various levels of sulfur supplementation. Nevertheless, NDF digestibility in the cows fed the basal diet with the addition of sulfur was higher ($p < 0.05$) than that for those fed without sulfur addition (Table 2).

Table 2 Effect of fresh cassava root and various levels of sulfur supplementation on nutrient intake and apparent digestibility of the lactating crossbred dairy cows

Item	Sulfur (% of FCR)				SEM	Contrast (p- value)		
	0.0	0.5	1.0	2.0		NS vs S	L	Q
Average LW, kg	465.4±24.10	471.3±25.87	473.6±7.31	459.4±28.45	11.68	0.84	0.75	0.36
DM intake								
TMR kg/d	9.5±1.28	10.1±1.25	10.6±0.54	10.5±1.59	0.32	0.05	0.045	0.37
FCR, kg/d	3.4±0.21	3.4±0.17	3.3±0.18	3.3±0.18	0.06	0.29	0.27	0.69
Total, kg/d	12.9±1.45	13.4±1.15	13.9±0.55	13.8±1.63	0.33	0.08	0.07	0.42
%BW	2.77±0.26	2.86±0.35	2.94±0.15	3.01±0.29	0.07	0.09	0.04	0.94
g/kgLW$^{0.75}$	128.8±12.47	132.9±14.91	137.1±6.53	139.4±13.83	3.05	0.08	0.045	0.78
Sulfur, g/d	0.0±0.00[a]	16.8±0.85[b]	33.2±1.79[c]	66.3±3.58[d]	0.79	< 0.0001	< 0.0001	< 0.0001
HCN, mg/d	367.8±22.35	362.7±18.43	358.1±19.36	358.1±19.36	6.16	0.29	0.27	0.89
Nutrient intake								
OM, kg/d	12.1±1.35	12.5±1.05	13.0±0.51	12.9±1.50	0.31	0.08	0.07	0.43
CP, kg/d	1.5±0.20	1.6±0.19	1.7±0.08	1.7±0.24	0.05	0.06	0.048	0.37
EE, kg/d	0.29±0.04	0.30±0.03	0.32±0.01	0.32±0.04	0.01	0.06	0.05	0.38
NDF, kg/d	4.7±0.60	5.0±0.55	5.2±0.24	5.2±0.72	0.15	0.06	0.05	0.38
ADF, kg/d	3.1±0.40	3.3±0.38	3.4±0.17	3.4±0.49	0.10	0.06	0.049	0.38
Energy intake[1], Mcal ME/d	32.7±3.51	33.7±2.58	34.9±1.32	34.7±3.80	0.79	0.09	0.08	0.44
Apparent digestibility								
DM, %	69.99±3.45	71.35±1.98	71.30±2.02	72.52±1.40	0.79	0.11	0.08	0.93
OM, %	72.38±3.58	73.74±2.32	74.12±1.91	75.56±1.06	0.78	0.06	0.03	0.96
CP, %	63.69±2.09	66.32±3.73	65.45±2.71	66.88±2.21	0.94	0.06	0.08	0.55
EE, %	90.27±2.41[a]	88.99±2.02[a]	88.08±1.62[a]	84.44±1.21[b]	0.64	0.003	0.006	0.12
NDF, %	47.63±1.58	54.78±6.48	50.62±5.56	52.85±1.84	1.53	0.03	0.14	0.16
ADF, %	42.11±7.21	48.46±9.23	43.24±7.82	42.19±4.09	2.83	0.50	0.71	0.24
MCP[2], kg/d	1.13±0.14	1.20±0.11	1.25±0.02	1.27±0.13	0.05	0.13	0.09	0.67

SEM = standard error of mean; LW = live weight; TMR = total mixed ration; FCR = fresh cassava root; HCN = hydrogen cyanide; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF =neutral detergent fiber; ADF = acid detergent fiber; NS vs S = non-sulfur group versus sulfur group; L = linear; Q = quadratic
[1] 1 kg DOMI = 3.8 Mcal ME/kg DM (Kearl, 1982)
[2] MCP (microbial crude protein), kg/d = 0.00825 × ME intake (MJ/d) (Agricultural Research Council, 1984)
Mean (± SD) in the same row superscripted with different lowercase letters are significantly ($p < 0.05$) different

This might have been due to sulfur enhancing rumen microbes (Supapong et al., 2019). Promkot and Wanapat (2009) found that sulfur supplementation at 1.5% or 4% had a significant influence on DM digestibility, but had no effect on OM, CP, EE or fiber digestibility. Dageaw et al. (2021) reported that the supplementation of a solid feed block containing a high level of sulfur (2% or 4%) had no impact on nutrient digestibility. However, Cherdthong et al. (2018) fed beef cattle a feed block containing sulfur at 2% or 4% and reported that this had a significant effect on the digestibility of DM and OM but not on the CP, NDF or ADF. The reasons for the inconsistencies in these findings are unknown; however they could be related to differences in the sulfur form used, the animal breed or dietary content (Cherdthong et al., 2018).

Ruminal characteristics and blood metabolites

The ruminal fermentation end products and blood metabolites of the cows fed dietary treatments are shown in Table 3. No significant differences were found between the dietary treatments in terms of the ruminal pH, TVFA and individual VFA profiles. The ruminal pH levels of the dietary treatments were similar in the range 6.6–6.8, which is generally thought to be in the optimal range for fermentable activity by fiber-digesting bacteria in the rumen (Musco et al., 2016). Similarly, Dagaew et al. (2021) and Cherdthong et al. (2018) reported that sulfur supplementation through a high sulfur feed block or directly added in a FTMR did not influence the ruminal pH. The NH_3-N concentration was significantly different among the treatments and linearly decreased ($p < 0.05$) as the sulfur level increased. The NH_3-N concentration in the rumen decreased, whereas the TVFA increased as sulfur supplementation increased, even though the nutrient digestibility was not influenced by sulfur supplementation. The reduction in NH_3-N was likely due to microorganisms using more NH_3-N for their growth, which accelerated the fermentation process and resulted in an increase in the TVFA. This finding was supported by Supapong et al. (2019) who reported that increased sulfur from 1% to 2% increased microbial protein synthesis.

Blood metabolites, such as BUN, AST, T3 and T4, in the blood were not significantly different among the dietary treatments. When the level of sulfur inclusion in the basal diet increased, the serum SCN^- level increased linearly ($p < 0.01$), with the highest SCN^- concentration appearing in the cows that received sulfur at the highest dose (2% FCR). These findings

Table 3 Effect of cassava root and various levels of sulfur supplementation on rumen fermentation end-products and blood metabolites of the lactating crossbred dairy cows

Item	Sulfur (% of FCR)				SEM	Contrast (p value)		
	0.0	0.5	1.0	2.0		NS vs S	L	Q
Rumen fermentation								
Ruminal pH	6.85±0.10	6.74±0.25	6.79±0.20	6.79±0.21	0.08	0.51	0.65	0.58
NH_3-N, mg/dL	15.00±2.93[a]	13.48±1.43[ab]	10.27±2.98[b]	10.92±0.90[b]	1.01	0.03	0.02	0.37
TVFA, mM	126.4±4.8[a]	146.8±22.8[ab]	150.3±14.8[b]	156.6±4.62[b]	7.27	0.01	0.011	0.39
A, mol/100 mol	64.46±2.19	64.65±1.60	66.44±8.36	64.25±2.91	1.00	0.81	0.75	0.87
P, mol/100 mol	24.01±1.21	24.00±1.27	26.47±3.49	25.58±3.44	1.40	0.39	0.28	0.76
B, mol/100 mol	11.51±1.35	11.36±0.64	9.84±1.25	10.17±2.29	0.62	0.25	0.05	0.63
A:P ratio	2.73±0.22	2.80±0.24	2.52±0.55	2.61±0.41	0.17	0.71	0.45	0.97
Blood metabolites								
BUN, mg/dL	10.50±1.55	12.08±3.12	10.00±4.69	10.08±6.37	1.58	0.93	0.71	0.70
ALT, U/L	21.17±4.49	20.08±6.26	22.58±4.93	22.67±5.82	1.43	0.85	0.62	0.85
AST, U/L	56.42±8.70	52.08±9.18	55.17±5.74	54.58±6.97	2.29	0.59	0.91	0.68
T3, ng/mL	2.00±0.93	1.06±0.21	1.69±0.32	1.97±0.42	0.23	0.32	0.67	0.42
T4, ng/mL	7.52±4.18	6.32±1.06	6.59±1.30	7.18±1.92	1.10	0.57	0.90	0.52
SCN^-, mg/L	2.51±0.00[a]	2.72±0.42[a]	5.98±4.60[a]	10.93±3.44[b]	1.31	0.01	0.003	0.13

NS vs S = non-sulfur group versus sulfur group; L = linear; Q = quadratic; SEM = standard error of mean; NH_3-N = ammonia nitrogen; TVFA = total volatile fatty acid; A = acetic acid; P = propionic acid; B = butyric acid; BUN = blood urea nitrogen; ALT = alanine aminotransferase; AST = aspartate aminotransferase; T_3 = triiodothyronine; T_4 = thyroxine; SCN^- = thiocyanate
Mean (± SD) in the same row superscripted with different lowercase letters are significantly ($p < 0.05$) different

concurred with those reported by Dagaew et al. (2021) and Cherdthong et al. (2018). However, although thiocyanate is widely accepted to cause a reduction in iodine uptake by the thyroid gland (Kittivachra, 2006), the highest SCN¯ value in the current study may not influence the transportation of iodine to the thyroid gland to produce T3 and T4, because there was no sign of iodine deficiency.

The current study found a favorable link between sulfur intake and SCN¯ in blood (correlation coefficient, $r = 0.83$, $p < 0.01$). An increase in the sulfur level resulted in an increased SCN¯ concentration, which was in line with Dagaew et al. (2021) and Supapong et al. (2019). The SCN¯ in blood is formed mostly in the rumen by sulfate-reducing bacteria via activity of the rhodanese enzyme, which is a sulfur transferase that accelerates the formation of SCN¯ from HCN (Frankenberg, 1980). Uwituze et al. (2011) found that sheep fed a cassava-based diet had a blood SCN¯ content directly proportional to the quantity of sulfur supplementation. However, Promkot and Wanapat (2009) revealed that the amount of HCN consumed and the amount of sulfur supplementation influenced the SCN¯ concentration.

Milk production and composition

Table 4 shows the milk performance of the cows fed the basal diet with sulfur supplementation. The milk yields at 4% FCM and milk composition components, such as the fat, protein, lactose, and total solids contents were not significantly different among the treatments. The fat-to-protein ratio and feed efficiency were similar ($p > 0.05$) between the treatments. The milk yields of cows given the basal diet with varying sulfur levels were not significantly different; probably because of the similar nutrient intakes used for producing milk. This was consistent with Supapong and Cherdthong (2020) whose findings showed that cows given the FTRM added with 1% or 2% sulfur and 1.25% or 2.5% urea had identical milk yields. Similarly, Dagaew et al. (2021) investigated supplementation with FCR at 1% BW and 1.5% BW and found it did not affect milk yields. In the current study, the supplementation of sulfur did not impact milk composition. However, Dagaew et al. (2021) found that as a result of the sulfur treatment, the milk composition in dairy cows fed a diet supplemented with FCR at 1.5% BW, combined with a feed block containing a high sulfur level of 4%, increased the milk fat by 8.6%.

In the current study, sulfur levels had no significant effect on feed efficiency (measured as kilograms of milk per kilogram of feed). Nonetheless, adding FCR at 0.75% BW to lactating dairy cows resulted in a range in the feed efficiency of 0.91–0.94 kg/kg in the current study. This was consistent with the findings of Supapong et al. (2019), who fed FTMR to dairy cows. On the other hand, when FCR was supplemented to dairy cows at 1% BW or 1.5% BW, Dagaew et al. (2021) reported a feed efficiency in the range 0.71–0.74 kg/kg. High levels of FCR supplementation may result in a lower protein intake due to the lower CP content in the FCR, resulting in lower feed efficiency as well as reduced production costs.

Table 4 Effect of cassava root and various levels of sulfur supplementation on milk production and composition of the lactating crossbred dairy cows

Item	Sulfur (% of FCR)				SEM	Contrast, *p*-value		
	0.0	0.5	1.0	2.0		NS vs S	L	Q
Milk production								
Milk yield, kg/d	12.13±3.63	12.41±4.74	12.71±4.54	12.73±4.43	0.38	0.85	0.86	0.96
4%FCM, kg/d	13.73±3.55	13.01±5.26	13.82±3.45	13.71±3.60	1.36	0.93	0.94	0.90
Milk composition								
Fat, %	4.96±0.80	4.33±0.68	4.77±0.80	4.67±0.93	0.52	0.45	0.83	0.57
Protein, %	3.41±0.54	3.76±0.35	3.41±0.23	3.54±0.29	0.13	0.48	0.98	0.62
Lactose, %	4.39±0.23	4.30±0.34	4.37±0.49	4.35±0.16	0.14	0.80	0.95	0.80
SNF, %	8.88±0.62	8.91±0.61	8.74±0.45	8.94±0.37	0.12	0.97	0.99	0.78
TS, %	14.02±1.53	13.34±1.09	13.72±0.99	13.82±1.29	0.32	0.60	0.95	0.60
Fat, protein	1.45±0.08	1.16±0.25	1.40±0.19	1.31±0.18	0.11	0.15	0.62	0.28
Feed efficiency	0.94±0.24	0.91±0.27	0.91±0.31	0.91±0.24	0.19	0.86	0.91	0.93

NS vs S = non-sulfur group vs sulfur group; L = linear; Q = quadratic; SEM = standard error of mean; 4%FCM = 4% fat corrected milk; SNF = solid not fat; TS = total solids

Somatic cells and thiocyanate concentration in milk

There were no significant differences in the milk SCN^-, SCC and somatic cell score (SCS) on day 14 and day 21 of the sampling period, as presented in Table 5. The concentration of milk SCN^- linearly increased ($p < 0.05$) as the sulfur level increased. The SCC and SCS in milk were not significantly different among the treatments. There was a favorable link between serum SCN^- and milk SCN^- ($r = 0.69$, $p < 0.01$). Because of the elimination of feed HCN through its transformation to SCN^- in the liver and kidney of cows and the partial excretion in milk, the SCN^- in milk reflects the SCN^- in blood (Bafort et al., 2014). Furthermore, Srisaikham et al. (2018) discovered a favorable link between milk SCN^- and HCN in cows that had consumed fresh cassava peel.

Even though the milk SCN^- was elevated, the SCC and SCS in the milk from the cows fed the basal diet with various levels of sulfur were unaffected. This might have been due to the lower milk SCN^- concentration in the current study. However, Srisaikham et al. (2018), Supapong and Cherdthong (2020) and Dagaew et al. (2021) all reported a decrease in SCC in milk as a result of HCN in diets with sulfur supplementation. Milk SCN^- concentrations greater than 8 mg/L reduce the SCC in milk (Supapong and Cherdthong, 2020; Dagaew et al., 2021). As milk lactoperoxidase catalyzes in the presence of hydrogen peroxide (H_2O_2), SCN^- is oxidized to yield hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN;

Shin et al., 2001) and the iodide ion is oxidized to yield hypoiodite and hypoiodous acid (Bosch et al., 2000), which has potent antibacterial properties. On the other hand, the mean SCC in the milk was 2.46 log10 (288,403 cells/mL), which Thai Agriculture Commodity and Food Standard (2004) regards as acceptable.

Based on this research, it could be concluded that the inclusion of 2.0% sulfur with FCR supplementation at 0.75% BW fed to dairy cows improved intake, digestibility and ruminal fermentation. However, based on feed efficiency, FCR supplementation at 0.75% BW in cows fed TMR was a suitable level. More research is needed to elucidate the optimal level of FCR supplementation with sulfur in dairy cows that will maximize milk production efficiency and minimize cost.

Conflict of Interests

The authors declare that there are no conflicts of interest.

Acknowledgments

Table 5 Effect of cassava root and various levels of sulfur supplementation on thiocyanate, somatic cell count and somatic cell score in milk of the lactating crossbred dairy cows

Item	Sulfur (% of FCR)				SEM	Contrast, *p*- value		
	0.0	0.5	1.0	2.0		NS vs S	L	Q
Milk thiocyanate, mg/L								
14 d	3.88±0.49[a]	4.37±0.26[a]	5.13±0.45[ab]	6.49±1.69[b]	0.50	0.046	0.009	0.42
21 d	4.12±0.50[a]	4.30±0.59[ab]	4.72±0.33[b]	5.38±0.79[c]	0.13	0.004	0.0003	0.11
Mean	4.00±0.49[a]	4.34±0.41[ab]	4.92±0.36[b]	5.94±0.91[c]	0.24	0.008	0.001	0.20
Somatic cell count, Log10								
14 d	2.69±0.55	2.64±0.65	2.32±0.35	2.23±0.48	0.15	0.14	0.047	0.92
21 d	2.24±0.68	2.54±0.26	2.62±0.54	2.37±0.47	0.14	0.15	0.47	0.10
Mean	2.47±0.33	2.59±0.45	2.47±0.44	2.30±0.48	0.14	0.31	0.11	0.56
Somatic cell score (SCS)								
14 d	5.30±1.81	5.12±2.17	4.05±1.17	3.77±1.58	0.51	0.14	0.047	0.92
21 d	3.79±2.27	4.78±0.87	5.05±1.78	4.24±1.58	0.47	0.15	0.46	0.10
Mean	4.55±1.09	4.95±1.51	4.55±1.47	4.00±1.58	0.45	0.31	0.11	0.57

NS vs S = non-sulfur group versus sulfur group; L = linear; Q = quadratic; SEM = standard error of mean
Mean ± SD in the same row superscripted with different lowercase letters are significantly ($p < 0.05$) different

References

Association of Official Analytical Chemists. 1995. Official Methods of Analysis, 16th ed. AOAC International. Arlington, VA, USA.

Agricultural Research Council. 1984. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux. Farnham Royal, UK.

Bach, A., Cabrera, V. 2017. Robotic milking: Feeding strategies and economic returns. J. Dairy Sci. 100: 7720–7728. doi.org/10.3168/jds.2016-11694

Bafort, F., Parisi, O., Perraudin, J.P., Jijakli, M.H. 2014. Mode of action of lactoperoxidase as related to its antimicrobial activity: A review. Enzyme Res. 2014: 517164. doi.org/10.1155/2014/517164

Bosch, E.H., van Doorne, H., de Vries, S. 2000. The lactoperoxidase system: The influence of iodide and the chemical and antimicrobial stability over the period of about 18 months. J. Appl. Microbiol. 89: 215–224. doi.org/10.1046/j.1365-2672.2000.01098.x

Chaengseea, P., Kongsila, P., Siriwong, N., Kittipadakula, P., Piyachomkwanc, K., Petchpoung, K. 2020. Potential yield and cyanogenic glucoside content of cassava root and pasting properties of starch and flour from cassava Hanatee var. and breeding lines grown under rain-fed condition. Agr. Nat. Resour. 54: 237–244. doi.org/10.34044/j.anres.2020.54.3.02

Chanjula, P., Wanapat, M., Wachirapakorn, C., Uriyapongson, S., Rowlinson, P. 2003. Ruminal degradability of tropical feeds and the potential use in ruminant diets. Asian-Aust. J. Anim. Sci. 16: 211–216. doi.org/10.5713/ajas.2003.211

Cherdthong, A., Khonkhaeng, B., Seankamsorn, A., et al. 2018. Effects of feeding fresh cassava root with high-sulfur feed block on feed utilization, rumen fermentation and blood metabolites in Thai native cattle. Trop. Anim. Health Prod. 50: 1365–1371. doi.org/10.1007/s11250-018-1569-8

Chotineeranat, S. 2006. Process development of low cyanide cassava flour from Kasetsart 50 variety and its utilization in food products. Ph.D. Thesis, Faculty of Agro-Industry, Kasetsart University. Bangkok, Thailand.

Cipollone, R., Ascenzi, P., Frangipani, E., Visca, P. 2006. Cyanide detoxification by recombinant bacterial rhodanese. Chemosphere 63: 942–949. doi.org/10.1016/j.chemosphere.2005.09.048

Cosby, E.L., Sumner, B. 1945. Rhodanese. Archives. Biochem. 7: 457–460.

Crocker, C.L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol. 33: 361–365.

Dagaew, G., Cherdthong, A., Wanapat, M., So, S., Polyorach, S. 2021. Ruminal fermentation, milk production efficiency, and nutrient digestibility of lactating dairy cows receiving fresh cassava root and solid feed-block containing high sulfur. Fermentation 7: 114. doi.org/10.3390/fermentation7030114

Frankenberg, L. 1980. Enzyme therapy in cyanide poisoning: Effect of rhodanese and sulfur compounds. Arch. Toxicol. 45: 315–323. doi.org/10.1007/BF00293812

Kaisoda, T. 2018. Inclusion of fresh cassava root in total mixed ration with high level of sulfur on feed utilization and milk production in lactating dairy cows. M.Sc. Thesis, Graduate School, Khon Kaen University. Khon Kaen, Thailand.

Kearl, L.C. 1982. Nutrient Requirements of Ruminants in Developing Countries. International Feedstuff Institute. Utah State University, Logan, UT, USA.

Kittivachra, R. 2006. Effects of cassava on thyroid gland in rats. Thai J. Pharm. Sci. 30: 57–62.

Kraig, R., Dave, M., Dale, B., John, H. 2012. Prussic Acid Poisoning. Kansas State University. Manhattan, KS, USA.

Lambert, J.L., Ramasamy, J., Paukstelis, J.F. 1975. Stable reagents for the colorimetric determination of cyanide by modified Konig reactions. Anal. Chem. 47: 916–918. doi.org/10.1021/ac60356a036

Maltz, E., Barbosa, L.F., Bueno, P., Scagion, L., Kaniyamattam, K., Greco, L.F., De Vries, A., Santos, J.E.P. 2013. Effect of feeding according to energy balance on performance, nutrient excretion, and feeding behavior of early lactation dairy cows. J. Dairy Sci. 96: 5249–5266. doi.org/10.3168/jds.2013-6549

Musco, N., Koura, B.I., Tudisco, R., et al. 2016. Nutritional characteristics of forage grown in south of Benin. Asian-Australian J. Anim. Sci. 29: 51–61. doi.org/10.5713/ajas.15.0200

National Research Council (NRC). 2001. Nutrient Requirements of Dairy Cattle, 7th ed. National Academic Press. Washington, DC, USA.

Onwuka, C.F.I., Akinsoyinua, A.O., Tewe, O.O. 1992. Role of sulphur in cyanide detoxification in ruminants. Small Ruminant Res. 8: 277–284. doi.org/10.1016/0921-4488(92)90209-M

Promkot, C., 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-Aust. J. Anim. Sci. 22: 1366–1376. doi.org/10.5713/ajas.2009.90141

Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D. 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Goats, Pigs, and Horses, 10th ed. Saunders Elsevier. London, UK.

Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S. 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF). Washington, DC, USA.

Samuel, M., Sagathewan, S., Thomus, J., Mathen, G. 1997. An HPLC method for estimation of volatile fatty acids of rumen fluid. Indian J. Anim. Sci. 67: 805–807.

Schneider, B.H., Flatt, W.P. 1975. The Evaluation of Feed through Digestibility Experiments. The University of Georgia Press. Athens, GA, USA.

Shin, K., Hayasawa, H., Lönnerdal, B. 2001. Inhibition of *Escherichia coli* respiratory enzymes by the lacto-peroxidase hydrogen peroxide-thiocyanate antimicrobial system. J. Appl. Microbiol. 90: 489–493. doi.org/10.1046/j.1365-2672.2001.01268.x

Silva, C.J.D., Leonel, F.D.P., Pereira, J.C.M., Costa, G., Moreira, L.M., Oliveira, T.S.D., Abreu, C.L.D. 2014. Sulfur sources in protein supplements for ruminants. Rev. Bras. Zootec. 43: 537–543. doi.org/10.1590/S1516-35982014001000005

Soto-Blanco, B., Górniak, S.L. 2003. Milk transfer of cyanide and thiocyanate: Cyanide exposure by lactation in goats. Vet. Res. 34: 213–220. doi.org/10.1051/vetres:2002068

Srisaikham, S., Suksombat, W., Lounglawan, P. 2018. Fresh cassava peel in dairy cattle diet: Effects on milk production, hygienic quality of raw milk and somatic cell counts. J. Sci. Technol. 40: 977–984. doi:10.14456/sjst-psu.2018.107

Statistical Analysis System. 2002. User's Guide: Statistics, Version 9.1. SAS. Inst. Inc. Cary, NC, USA.

Supapong, C., Cherdthong, A. 2020. Effect of sulfur concentrations in fermented total mixed rations containing fresh cassava root on rumen fermentation. Anim. Prod. Sci. 16: 1429–1434. doi.org/10.1071/AN18779

Supapong, C., Cherdthong, A., Wanapat, M., Chanjula, P., Uriyapongson, S. 2019. Effects of sulfur levels in fermented total mixed ration containing fresh cassava root on feed utilization, rumen characteristics, microbial protein synthesis, and blood metabolites in Thai native beef cattle. Animals 9: 261. doi.org/10.3390/ani9050261

Thai Agricultural Commodity and Food Standard. 2004. Raw Milk. TACFS 6003–2004. Office of Thai Agricultural Commodity and Food Standard. Bangkok, Thailand.

Uwituze, S., Parsons, G.L., Schneider, C.J., Karges, K.K., Gibson, M.L., Hollis, L.C., Higgins, J.J., Drouillard, J.S. 2011. Evaluation of sulfur content of dried distillers grains with solubles in finishing diets based on steam-flaked corn or dry–rolled corn. J. Anim. Sci. 89: 2582–2591. doi.org/10.2527/jas.2010-3103

Van Keulen, J., Young, B.A. 1977. Evaluation of acid insoluble ash as a neutral marker in ruminant digestibility studies. J. Anim. Sci. 44: 282–287. doi.org/10.2527/jas1977.442282x

Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fiber neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583–3597. doi.org/10.3168/jds.S0022-0302(91)78551-2

Wachirapakorn, C., Parmaluk, P., Wanapat, M., Pakdee, P., Cherdthong, A. 2014. Effects of levels of crude protein and ground corn cobs in total mixed ration on intake, rumen fermentation and milk production in crossbred Holstein Friesian lactating dairy cows, J. App. Anim. Res. 42: 263–268. doi.org/10.1080/09712119.2013.842483

Wanapat, M., Foiklang, S., Sukjai, S., et al. 2017. Feeding tropical dairy cattle with local protein and energy sources for sustainable production. J. App. Anim. Res. 46: 232–236. doi.org/10.1080/09712119.2017.1288627