fermentation

Article

Ruminal Fermentation, Milk Production Efficiency, and Nutrient Digestibility of Lactating Dairy Cows Receiving Fresh Cassava Root and Solid Feed-Block Containing High Sulfur

Gamonmas Dagaew [1], Anusorn Cherdthong [1,*], Metha Wanapat [1], Sarong So [1] and Sineenart Polyorach [2]

1 Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; gamonmasdagaew@gmail.com (G.D.); metha@kku.ac.th (M.W.); sarong07so@gmail.com (S.S.)

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

* Correspondence: anusornc@kku.ac.th

Abstract: This study evaluates the effects of fresh cassava root (CR) and a solid feed-block containing sulfur (S-FB) on fermentation in the rumen, feed utilization, milk yield, and milk composition in lactating dairy cows. Four Holstein-Friesian cows with 470 ± 50.0 kg body weight (BW), 10 ± 2 kg day^{-1} average milk yield, and 112 ± 15 days-in-milk were studied. A 2 × 2 factorial combination was arranged in a 4 × 4 Latin square design to evaluate the treatment-related effects. The treatments were obtained from a combination of two factors: (1) levels of CR at 10 g kg^{-1} BW (CR-1) and 15 g kg^{-1} (CR-1.5) and (2) levels of sulfur supplementation in solid feed-block at 20 g kg^{-1} (S-FB-2) and 40 g kg^{-1} (S-FB-4). The results showed that CR and S-FB had no interaction effect on feed intake, digestibility, fermentation, blood metabolites, milk yield, or its composition. Feeding CR up to 15 g kg^{-1} of the BW significantly increased ($p < 0.05$) the milk fat concentration while it decreased ($p < 0.05$) the somatic cell count. The S-FB-4 of the sulfur significantly ($p < 0.05$) increased the acid detergent fiber when compared with the S-FB-2 of the sulfur. CR could be fed up to 15 g kg^{-1} of BW with S-FB containing high sulfur (40 g kg^{-1}) in dairy cows without a negative impact.

Keywords: rumen fermentation; volatile fatty acid; somatic cell count; milk thiocyanate

Citation: Dagaew, G.; Cherdthong, A.; Wanapat, M.; So, S.; Polyorach, S. Ruminal Fermentation, Milk Production Efficiency, and Nutrient Digestibility of Lactating Dairy Cows Receiving Fresh Cassava Root and Solid Feed-Block Containing High Sulfur. *Fermentation* **2021**, *7*, 114. https://doi.org/10.3390/fermentation7030114

Academic Editors: Luca Settanni and Mohamed Koubaa

Received: 8 June 2021
Accepted: 12 July 2021
Published: 14 July 2021

1. Introduction

Fresh cassava root (CR) is high in digestible carbohydrates, mainly starch, and is considered the main energy source for ruminants. However, feeding CR to ruminants is limited in practice due to the highly toxic hydrogen cyanide (HCN) content of the roots. Sulfur has been studied and reported to reduce the cyanide toxicity [1,2]. In ruminants, HCN can be rapidly detoxified by the enzymes rhodanese and β-mercaptopyruvate sulfurtransferase, which are released by rumen microbes [3]. Rhodanese is a sulfurtransferase that catalyzes the conversion of HCN to thiocyanate, which is then subsequently excreted via the urine [4]. Sulfur (S) is an important mineral in ruminant diets. Various approaches to achieving sulfur utilization, to reduce HCN toxicity, have been conducted, such as adding elemental sulfur into a fermented total mixed ration (FTMR) containing CR for dairy cows [1,5], adding sulfur to a concentrate of cassava hay and foliage fed to dairy cows as a roughage source [4], and adding sulfur to a pellet diet with CR supplementation [6]. Adding sulfur to a solid feed-block might be an alternative way of using sulfur to reduce HCN toxicity in a diet containing CR.

The solid feed-block is normally formulated to contain many necessary nutrients, such as minerals, vitamins, nitrogen (e.g., urea), and energy sources (e.g., molasses) [2,7,8]. Solid feed-block feeding can continuously supply nitrogen, minerals, vitamins, and energy to ruminal microbes and can ensure adequate energy supply for the animal, resulting in enhanced milk production in various ruminant species [2,7]. Previous studies conducted both in vitro and in vivo in beef cattle have shown that CR supplementation with a solid feed-block containing sulfur leads to enhancement of feed efficiency, digestibility, and ruminal fermentation (e.g., increased propionate (C3) and total volatile fatty acid (VFA)) [2,9]. Enhancing feed efficiency, digestibility, and ruminal fermentation may lead to an improvement in milk production. However, the effects of feeding CR and solid feed-block containing high sulfur (S-FB) on milk yield and milk composition in lactating dairy cows have never been evaluated. Based on our companion study by Cherdthong et al. [2], finding an improvement in digestibility and rumen fermentation with CR and S-FB, a hypothesis was formulated that CR and S-FB should enhance milk production and its composition.

Therefore, this study aimed to evaluate the effects of fresh CR and S-FB on fermentation in the rumen, feed utilization, milk yield, and milk composition in lactating dairy cows.

2. Materials and Methods

2.1. Ethical Procedure

The study was conducted under approval procedure no. ACUC-KKU 45/2560 of Animal Ethics and Care issued by Khon Kaen University (Date: 15 June 2017).

2.2. Animals, Diets, and Experimental Design

The study was conducted at the farm animal research station of the Department of Animal Science, Khon Kaen University. Due to limited research station resources and availability, only a small number of cows were used. However, the study was conducted with care and control to minimize any incidental errors originating from humans, animals, or the environment. Four Holstein-Friesian cows with 470 $\pm$ 50.0 kg body weight (BW), 10 $\pm$ 2 kg day^{-1} average milk yield, and 112 $\pm$ 15 days-in-milk were studied. A 2 $\times$ 2 factorial combination was arranged in a 4 $\times$ 4 Latin square design to evaluate the treatment-related effects. The treatments were obtained from a combination of two factors: (1) levels of CR at 10 g kg^{-1} BW (CR-1) and 15 g kg^{-1} (CR-1.5), and (2) levels of sulfur supplementation in solid feed-block at 20 g kg^{-1} (S-FB-2) and 40 g kg^{-1} (S-FB-4). The concentrate was fed to the cows at a 2:1 ratio (2 kg concentrate per 1 kg of milk yield) at 7 am and 4 pm. The cows were fed rice straw (RS) ad libitum daily with 100 g kg^{-1} refusal of the total offered amount of RS. The CR (*Manihot esculenta* Kasetsart 50) was purchased from a local farmer located in Khon Kaen province, Thailand. The CR was washed to remove soil and chopped into 3 to 5 mm sized pieces before being offered to cows at their respective levels. The concentrate and CR were fed twice daily to the cows at 7 am and 4 pm simultaneously, and RS was fed after the cows finished the concentrate and CR. The S-FBs containing 20 g and 40 g sulfur were formulated as described by Cherdthong et al. [2]. The ingredients and their proportions are shown in Table 1. All ingredients were well-mixed and put into a hydraulic compression machine (Mineral Salt Block Hydraulic Press, Zhengzhou Rephale Machinery Company, Henan, China) for 3-min to produce 1 kg of S-FB based on the fresh weight. The S-FB were sun-dried for three days to minimize the moisture content and then stored in a clean and dry place for use in this study. The cows were placed in individual pens (5 $\times$ 5 m) equipped with a cement well containing clean water; during the study, the well was cleaned daily and replenished with new and clean water. The S-FB was offered to the cows by hanging the block in individual pens and allowing the cows to access the S-FB ad libitum. The concentrate ingredients and chemical compositions of the concentrate, RS, S-FB, and CR, in addition to the HCN content of CR, are recorded in Table 1. The cows were weighed at the start of the experiment, and at the end of a period before starting a new period, the cows were weighed to adjust their dry matter intake (DMI).

Table 1. Ingredients and chemical composition of concentrate, solid feed-block, fresh cassava root (CR), and rice straw (RS).

Items	Concentrate	S-FB-2	S-FB-4	CR	RS
Ingredient Proportions, g kg^{-1} DM					
Corn	70	-	-		
Soybean pulp	40	-	-		
Cassava ship	450	-	-		
Rice bran	50	300	300		
Palm cannel meal	95	-	-		
Soybean meal	200	-	-		
Molasses	30	420	400		
Urea	25	100	100		
Di-calcium	10	-	-		
Vitamin	5	-	-		
Semen	-	110	110		
Sulfur	-	20	40		
Premixed	-	20	20		
Salt	5	10	10		
Tallow	-	20	20		
Chemical Composition					
DM, g kg^{-1}	896	791	763	385	849
OM, g kg^{-1} DM	953	900	901	986	911
CP, g kg^{-1} DM	167	305	302	23	26
NDF, g kg^{-1} DM	276	189	227	531	854
ADF, g kg^{-1} DM	118	100.1	100.3	312	476
TDN, g kg^{-1} DM	791	820	815	825	444
HCN, mg/kg	-	-	-	103.5	-
NEv, Mcal kg^{-1} DM †	1.89	2.01	1.98	1.75	0.19

S-FB-2 = solid feed-block containing high sulfur of 20 g kg^{-1}, S-FB-4 = solid feed-block containing high sulfur of 40 g kg^{-1}, DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, TDN = total digestible nutrient, HCN = hydrogen cyanide. NEv = net energy value † NEv (Mcal/kg DM) = (0.01 × (% TDN) × [2.86 − (35.5/(100−% NDF)]/(2.2 × 0.45) [10]. - ingredients were not used in the formulations.

2.3. Sample Collection and Measurements

Four 21-day periods consisting of two parts were conducted in this study; the first 14 days were used for dietary treatment adaptation and the last seven days were used for sample collection.

During the last seven days of each period, feed (RS, concentrate, and CR), refusal, and feces samples were collected daily and divided into two equal parts. The first half of the samples were analyzed daily for their DM content, while the other half of the samples were grouped by cows and periods and stored at −20 °C for analysis for their chemical composition. The feces were collected using the spot sampling technique at 50 g kg^{-1} total fresh weight. To analyze the chemical composition of the feed and refusal, the frozen feed, refusal, and fecal samples were thawed and oven-dried at 60 °C for 72 h. Then, the feed, refusal, and feces samples were ground through a 1-mm screen and analyzed for their DM, ash, and crude protein (CP) according to Association of Official Agricultural Chemists (AOAC) [11], and neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. [12]. The hydrocyanic acid (HCN) concentrations in CR were analyzed according to the method cited by Supapong and Cherdthong [1] using UV/Vis spectrophotometry. The HCN was calculated using the following: total cyanide content (mg/kg) = 396 × absorbance reading. Acid insoluble ash (AIA) was used as an indicator to estimate the apparent digestibility [13].

On day 21 of each period, rumen fluid and blood samples were collected at 0 and 4 h post-feeding. Approximately 100 mL of rumen fluid was collected via a stomach tube attached to a vacuum machine. The pH and temperature of the rumen fluid samples were measured immediately using a glass electrode pH meter (HANNA Instrument (HI) 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through a four-layer cheesecloth, kept in 1 M of sulfuric acid at a 1:9 ratio (5 mL of sulfuric acid and 45 mL of rumen fluid), and stored at −20 °C before being used for analysis. The rumen fluid samples were used to analyze the ammonia nitrogen (NH_3-N) concentration and VFA molar portions (acetate-C2, C3, and butyrate-C4). The NH_3-N concentration was analyzed according to AOAC [14]. The VFA was analyzed using high-performance liquid chromatography (HPLC; water 600 UV detector, Millipore). VFA profiles were used for methane (CH_4) prediction according to the equation of Moss et al. [15] (CH_4 = (0.45 × acetic acid) − (0.275 × propionic acid) + (0.40 × butyric acid)). The remaining rumen fluid samples were kept in 3.3 M formalin at a 1:9 ratio (1 mL formalin and 9 mL rumen fluid) and stored at 10 °C in a refrigerator to later count the bacteria, protozoa, and fungi according to Galyean [16]. Twelve milliliters of blood samples were collected from a jugular vein and divided into two equal portions. The first 6 mL of the blood samples were kept in test tubes containing Ethylenediaminetetraacetic acid (EDTA) and used to analyze the blood urea nitrogen (BUN) concentration according to Crocker [17], and the other 6 mL of the blood samples was centrifuged at 1000× *g* immediately to collect the serum samples. The serum samples were used to analyze alanine aminotransferase (ALT), aspartate aminotransferase (AST), triiodothyronine (T3), and thyroxine (T4) using automated clinical chemistry analyzers (Vitallab Flexor E, Dieren, Netherland). Blood thiocyanate (SCN^-) was analyzed according to the method described by Cherdthong et al. [2].

During the last seven days of each period, 100 mL of milk was collected daily (60 mL at the morning milking at 5 am and 40 mL at the afternoon milking at 4 pm). The milk samples were prevented from spoilage by adding potassium dichromate at 0.2 g per 100 mL of milk sample. The milk samples were analyzed for fat, protein, lactose, and solids-not-fat using Milkoscan104 (Foss Electric, Hillerod, Denmark). Somatic cell counts (SCC) were analyzed using the Fossomatic 5000 Basic (Foss, Hillerod, Denmark). The thiocyanate partition in milk (SCN^-) was analyzed according to Jacob et al. [18]. Fat-corrected milk (FCM) was calculated using the equation: 3.5% FCM = 0.35 × milk yield (kg) + 15 fat yield (kg).

2.4. Statistical Analysis

All data were subjected to ANOVA according to a 2 × 2 factorial arrangement in a 4 × 4 Latin square design using the general linear models (GLM) procedures of SAS. The analysis model is as follows Equation (1):

$$Y_{ijkl} = \mu + C_i + S_j + CS_{ij} + A_k + P_l + \varepsilon_{ijkl} \quad (1)$$

where Y_{ijk} is the observation, μ is the overall mean, C_i is the effect of CR at 10 g kg^{-1} BW and 15 g kg^{-1}, S_j is the effect of sulfur addition to a solid feed-block at 20 g kg^{-1} and 40 g kg^{-1}, CS_{ij} is the interaction effect between CR and sulfur levels in a solid feed-block, A_k is the effect from the animal, P_l is the effect from the period, and ε_{ijkl} is the residual effect. The means of treatment were statistically compared using Tukey's test [19] and significant treatment means were accepted at $p < 0.05$.

3. Results and Discussion

3.1. Chemical Composition of Feeds

The concentrate was formulated to contain 167 g kg^{-1} DM of CP to ensure the nutrient requirement for cows according to National Research Council (NRC) [20] and a cassava chip was used as an energy source. In addition to the energy source, CR feeding served as an additional energy supply. The S-FB contained approximately 300 g kg^{-1} DM of CP, which mainly consisted of urea as the nitrogen source for microbial protein synthesis [8]. The HCN concentration in CR was lower than that previously reported by Wanapat and Kang [21] (85–114 mg kg^{-1} fresh basis). The variation in HCN concentration mainly depends on breed varieties and the growth environment [2].

3.2. Effect on Intake and Digestibility

The concentrate, RS, S-FB, sulfur, CR, HCN, and total DM intake are shown in Table 2. An interactive effect between CR and S-FB for intake was not observed. The S-FB-2 and S-FB-4 were no different for the intake of RS, concentrate, S-FB, sulfur, CR, HCN, or total DM (Table 2). The sulfur intake was not over the limit recommended by NRC [20], with a minimum 1.5 g kg^{-1} and maximum 5 g kg^{-1} DM intake requirement in beef cattle. The intake of sulfur ranged from 0.01 to 0.02 kg day^{-1}, equaling 0.06% to 0.13% of total DM intake. Similarly, Supapong et al. [5] reported that sulfur supplementation at 10 and 20 g kg^{-1} DM added to the FTMR for dairy cows did not influence their feed intake. In addition, Promkot and Wanapat [4] found that supplemented elemental sulfur at 1.5 and 4 g kg^{-1} of dietary DM did not affect feed intake. In beef cattle, feed-blocks containing sulfur at 20 and 40 g kg^{-1} did not alter the feed intake [2]. The intake of concentrate, RS, S-FB, CR, sulfur, and total DM was not observed between CR-1 and CR-1.5 (Table 2). Increasing the CR supplementation caused a significant increase in HCN intake. This finding could be due to an increase in the CR feeding amount from 10 to 15 g kg^{-1} BW. The HCN intake was 600.35 mg kg^{-1} and 614.55 mg kg^{-1} for CR-1 and CR-1.5 treatment, respectively. Similarly, after Cherdthong et al. [2] fed CR at 10 and 15 g kg^{-1} BW to beef cattle, a significant increase in CR intake was noted. Promkot and Wanapat [4] reported that the HCN intake in dairy cows was significantly observed when cassava (hay and foliage) was fed at 100 g kg^{-1} DM, with HCN intakes of 1128.4 mg day^{-1} and 103.25 mg day^{-1} for cassava foliage and cassava hay feeding, respectively.

Table 2. Effect of cassava root (CR) and solid feed-block containing high sulfur (S-FB) on feed intake, nutrient intake, and nutrient digestibility in lactating dairy cows.

Items	CR-1		CR-1.5		SEM	*p*-Value		
	S-FB-2	S-FB-4	S-FB-2	S-FB-4		CR	S-FB	CR*S-FB
Rice straw, kg day^{-1}	4.0	4.0	3.8	3.7	0.33	0.51	0.88	0.96
Rice straw, g kg^{-1} BW$^{0.75}$	39.9	39.8	37.6	38.2	3.59	0.59	0.96	0.95
Concentrate, kg day^{-1}	5.8	6.8	7.3	6.3	0.56	0.39	1.00	0.09
Concentrate, g kg^{-1} BW$^{0.75}$	64.5	68.8	64.8	66.0	6.76	0.96	0.87	0.99
CR, kg day^{-1}	5.3	5.6	7.8	7.7	0.72	0.84	1.00	0.96
S-FB, kg day^{-1}	0.6	0.5	0.6	0.5	0.10	0.76	0.13	0.89
Sulfur, kg day^{-1}	0.02	0.02	0.02	0.02	0.004	0.89	0.45	0.85
HCN, mg day^{-1}	563.4	559.4	637.3	669.7	75.35	0.01	0.75	0.87
Total intake, %BW	3.2	3.3	3.6	3.6	0.19	0.92	0.79	0.67
Total intake, kg day^{-1}	15.2	15.3	16.0	16.5	0.90	0.67	0.19	0.17
Nutrient intake, kg day^{-1}								
OM	13.3	13.3	14.8	14.5	0.76	0.21	0.75	0.64
CP	3.3	3.3	3.6	3.8	0.23	0.46	0.83	0.94
NDF	5.3	5.2	6.3	5.6	0.57	0.85	0.72	0.60
ADF	3.4	3.5	4.3	3.9	0.44	0.63	0.81	0.86
Nutrient digestibility, g kg^{-1}								
DM	739	743	733	755	0.76	0.21	0.46	0.73
OM	872	873	871	874	0.34	0.25	0.45	0.93
CP	795	782	778	790	0.77	0.29	0.56	0.89
NDF	694	630	668	702	3.58	0.37	0.71	0.88
ADF	545	559	543	570	0.87	0.29	0.03	0.47

CR-1 and CR-1.5 = fed CR at 10 and 15 g kg^{-1} BW, S-FB-2 and S-FB-4 = solid feed-block containing high sulfur at 20 and 40 g kg^{-1}, BW$^{0.75}$ = metabolic body weight, HCN = hydrogen cyanide, DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, SEM = standard error of mean, CR*S-FB = interaction between CR and S-FB.

The nutrient intake and digestibility are presented in Table 2. An interactive effect between CR and S-FB for nutrient intake was not observed. S-FB-2 and S-FB-4 did not influence nutrient intake. Uwituze et al. [22] found that sulfur supplementation at 4.2 and 6.5 g kg^{-1} of dietary DM in crossbred steers did not affect nutrient intake. Supapong and Cherdthong [1] added 10 and 20 g kg^{-1} into the FTMR for dairy cows and no effects on nutrient intake were revealed. CR-1 and CR-1.5 did not affect the nutrient intake (Table 3). This finding might be due to there being no negative effects of CR on feed intake, as seen in Table 2. The HCN intakes were 600.35 mg kg^{-1} and 614.55 mg kg^{-1} when fed at 10 g kg^{-1} and 15 g kg^{-1} BW of CR, respectively, which were quite high, but toxic symptoms were not observed throughout the trial. No toxic symptoms were observed even when the animals received a high amount of HCN. This finding could be explained by the high sulfur content in the solid feed-block, which would have stimulated increase rhodanese enzyme activity to break down HCN into nontoxic SCN^{-} in the liver (Table 6). An interactive effect between CR and S-FB on nutrient digestibility was not observed (Table 3). CR-1 and CR-1.5 did not influence nutrient digestibility, a finding that suggests that feeding CR at 15 g kg^{-1} of BW had no negative effects on the animal. Dagaew et al. [9] found that increasing the CR in the RS ratio resulted in a significant increase in the in vitro digestibility of DM, NDF, and ADF. The authors addressed this finding as being due to the increase in the digestible DM of CR in the substrate. S-FB-4 caused a significant increase in ADF digestibility when compared with S-FB-2. The rumen consists of a huge population of bacteria, fungi, and protozoa; some bacteria are anaerobic sulfate-reducing bacteria that use sulfur as a substrate for sulfate production and reduction into hydrogen sulfide (H_2S) in the rumen [1,2]. However, H_2S production was favored at a ruminal pH of 6.5 [22–24]. As shown in Table 4, the pH ranged from 6.6 to 6.8, a finding that suggests that this range of pH was not a favorable condition for H_2S production. Therefore, the excess sulfate production was used by other anaerobic rumen fungi and fibrolytic rumen bacteria to increase their populations by incorporating sulfur from sulfate through direct metabolism or via an indirect pathway via the sulfide pool [25]. Slyter et al. [26] revealed that sulfur could cause an increase in the activity of cellulolytic bacteria, a process that might enhance fiber degradability [27]. Dagaew et al. [9] found that a feed-block containing sulfur at 20 and 40 g kg^{-1} significantly affected only the in vitro DM digestibility. Cherdthong et al. [2] fed a sulfur-containing feed-block at 20 and 40 g kg^{-1} to beef cattle; this process caused a significant effect on the DM and OM digestibility but not the CP, NDF, or ADF digestibility. This finding might be due to the low fungal population in that study when compared with this study. In dairy cows, Promkot and Wanapat [4] found that sulfur supplementation at 1.5 and 4 g kg^{-1} DM had a significant influence on DM digestibility. The variation among these findings is not understood, although it might be due to variations in the selected sulfur form, type of animal breed, and dietary composition.

3.3. Characteristics of Rumen Ecology and Microorganism

The ruminal pH, temperature, NH_3-N, and microbial population are presented in Table 3. Interactive effects between CR and S-FB on pH, temperature, NH_3-N concentration, and bacteria and protozoal populations were not observed. S-FB-2 and S-FB-4 did not affect the pH, temperature, NH_3-N concentration, or microbial population (Table 4). The mean rumen pH ranged from 6.6 to 6.8, which was in the optimal range for microbial activity. Cherdthong et al. [2] similarly found that pH, temperature, NH_3-N, and protozoal population significantly increased when beef cattle were fed CR and feed-block containing 40 g kg^{-1} sulfur in the feed-block. Promkot and Wanapat [4] found an increase in the bacterial population with sulfur supplementation at 1.5 and 4 g kg^{-1} of DM in dairy cows fed diets containing cassava hay and foliage. The lack of effect of sulfur on the bacterial population in this study was not clear, although the bacterial population was 33.71% greater than the findings of Cherdthong et al. [2] and 60.04% greater than in the report by Promkot and Wanapat [4]. Cherdthong et al. [2] stated that sulfur is essential for microbial growth in the rumen and for microbial metabolism. Sulfur found in amino acids (e.g., methionine and cysteine) is used for microbial growth. CR feeding did not affect the pH, temperature, NH_3-N, or microbial population (Table 4). This finding could mean that the interval difference between 10 and 15 g kg^{-1} of CR was too small to produce a ruminal pH change. In addition, RS contains high indigestible fiber and was fed ad libitum; the indigestible fiber might act as a buffering agent for maintaining pH in the rumen. This result agrees with the results of Cherdthong et al. [2], who fed CR and a feed-block containing sulfur to beef cattle, and Promkot and Wanapat [4], who fed cassava hay and foliage and sulfur to dairy cows.

Table 3. Effect of fresh cassava root (CR) and a solid feed-block containing high sulfur (S-FB) on rumen ecology, microorganism, and fermentation.

Item	CR-1		CR-1.5		SEM	*p*-Value		
	S-FB-2	S-FB-4	S-FB-2	S-FB-4		CR	S-FB	CR*S-FB
Ruminal pH								
0-h post-feeding	6.9	6.9	6.8	6.9	0.18	0.92	0.66	0.67
4-h post-feeding	6.6	6.5	6.5	6.6	0.23	0.85	0.63	0.89
Mean	6.8	6.7	6.6	6.8	0.19	0.14	0.38	0.52
Ruminal Temperature, °C								
0-h post-feeding	38.8	39.0	38.4	38.7	0.19	0.14	0.38	0.52
4-h post-feeding	39.2	39.1	38.4	38.9	0.84	0.07	0.29	0.25
Mean	39.2	39.1	38.4	38.9	0.18	0.13	0.46	0.35
Ammonia-Nitrogen, mg dL^{-1}								
0-h post-feeding	14.5	16.2	14.7	16.7	1.46	0.27	0.77	0.82
4-h post-feeding	15.3	15.7	15.9	16.2	0.79	0.48	0.92	0.72
Mean	14.9	15.9	15.3	16.4	0.61	0.66	0.19	0.82
Rumen Microbes, cells mL^{-1}								
Bacteria, $\times 10^{11}$								
0-h post-feeding	4.2	4.5	4.3	4.8	0.72	6.14	0.86	0.86
4-h post-feeding	4.7	5.0	4.8	5.3	0.73	0.86	0.62	0.86
Mean	4.5	4.6	4.7	4.8	0.68	0.85	0.72	1.00
Protozoa $\times 10^{7}$								
0-h post-feeding	1.1	1.2	1.1	1.2	0.87	0.88	0.88	0.33
4-h post-feeding	1.2	1.3	1.3	1.4	0.94	0.89	0.69	0.69
Mean	1.1	1.1	1.2	1.2	1.10	0.50	0.73	0.73
Fungi $\times 10^{6}$								
0-h post-feeding	0.2	0.3	0.2	0.3	0.62	0.84	0.55	0.55
4-h post-feeding	0.2	0.2	0.2	0.3	0.38	0.52	0.52	0.52
Mean	0.2	0.3	0.2	0.3	0.37	0.33	0.51	0.74

CR-1 and CR-1.5 = fed CR at 10 and 15 g kg^{-1} BW, S-FB-2 and S-FB-4 = solid feed-block containing high sulfur at 20 and 40 g kg^{-1}, SEM = standard error of mean, CR*S-FB = interaction effect between CR and S-FB.

3.4. Volatile Fatty Acid (VFA) and Methane Estimation

Table 4 shows the effects of CR and S-FB on VFA and CH_4 production in lactating dairy cows. Interactive effects between CR and S-FB on the total VFA, molar portions of VFA, and CH_4 estimations were not observed. CR-1 and CR-1.5 did not influence the total VFA or the C2, C3, C4, or CH_4 estimations (Table 5). This finding could be attributed to the small difference in the interval between 10 and 15 g kg^{-1} of the CR to shift the rumen fermentation. The C3 concentrations between CR-1 and CR-1.5 were 22 mole/100 mole and 23.5 mole/100 mole. In addition, it is possible that the CR and DM intakes and nutrient digestibility were not observed with 10 and 15 g kg^{-1} BW of CR. This result is similar to that of the study of Promkot and Wanapat [4], who evaluated cassava hay and foliage as roughage sources in the diets of dairy cows. Cherdthong et al. [2] found a significant increase in C3 concentration with CR 10 and 15 g kg^{-1} BW in beef cattle, which could have been due to a significant increase in the CR and DM intake when the CR increased as reported by the authors. S-FB-2 and S-FB-4 produced no effects on the total VFA or C2, C3, C4, or CH_4 estimations (Table 5). The average C3 concentrations between S-FB-2 and S-FB-4 were 22.55 mole/100 mole and 22.95 mole/100 mole. This finding might be due to a lack of influence of S-FB on the CR and DM intakes and the digestibility of DM and OM. This result was similar to the results, both in vitro and in vivo, of the previous study by Cherdthong et al. [2], who fed a feed-block containing sulfur at 20 and 40 g kg^{-1} to beef cattle, as well as Promkot et al. [3], who evaluated reducing sulfur at 2, 5, and

$10\ g\ kg^{-1}$ substrate DM via an in vitro gas technique, and Promkot and Wanapat [4], who supplemented sulfur at 1.5 and $4\ g\ kg^{-1}$ of dietary DM in dairy cows.

Table 4. Effect of fresh cassava root (CR) and solid feed-block containing high sulfur (S-FB) on ruminal volatile fatty acid (VFA) and methane estimation (CH_4).

Items	CR-1		CR-1.5		SEM	*p*-Value		
	S-FB-2	S-FB-4	S-FB-2	S-FB-4		CR	S-FB	CR*S-FB
Total VFA, mM								
0-h post-feeding	101.5	104.0	102.5	104.0	1.38	0.90	0.12	0.86
4-h post-feeding	102.5	104.3	103.7	105.1	1.37	0.41	0.49	0.52
Mean	102.0	104.2	103.1	104.6	1.35	0.60	0.63	0.42
Acetic acid (C2), mol 100 mol^{-1}								
0-h post-feeding	63.6	63.2	62.7	63.6	1.71	0.26	0.74	0.82
4-h post-feeding	64.4	65.3	63.3	62.3	1.53	0.22	0.69	0.75
Mean	64.0	64.3	63.0	62.9	0.90	0.36	0.60	0.51
Propionic acid (C3), mol 100 mol^{-1}								
0-h post-feeding	21.7	22.2	23.2	23.6	1.12	0.97	0.12	0.86
4-h post-feeding	21.6	22.4	23.5	23.6	1.20	0.89	0.73	0.52
Mean	21.7	22.3	23.4	23.6	0.77	0.65	0.43	0.99
Butyric acid (C4), mol 100 mol^{-1}								
0-h post-feeding	15.7	15.2	14.3	13.4	1.01	0.97	0.65	0.82
4-h post-feeding	15.8	12.3	13.2	14.1	1.52	0.47	0.60	0.77
Mean	15.8	13.8	13.8	13.8	0.90	0.36	0.76	0.81
C2:C3 ratio								
0-h post-feeding	2.9	2.8	2.7	2.7	0.45	0.53	0.67	0.82
4-h post-feeding	3.0	2.9	2.7	2.6	0.36	0.27	0.75	0.73
Mean	2.9	2.8	2.7	2.7	0.38	0.45	0.86	0.59
C2 + C4:C3 ratio	4.36	2.93	4.54	3.39	0.319	0.626	0.067	0.831
Methane (CH_4) †, g day^{-1}								
0-h post-feeding	27.7	27.5	28.7	27.1	0.61	0.62	0.28	0.16
4-h post-feeding	27.2	26.4	27.5	26.6	1.34	0.80	0.56	0.98
Mean	26.9	25.1	26.3	26.1	0.61	0.62	0.28	0.16

CR-1 and CR-1.5 = fed CR at 10 and $15\ g\ kg^{-1}$ BW, S-FB-2 and S-FB-4 = solid feed-block containing high sulfur at 20 and $40\ g\ kg^{-1}$, SEM = standard error of mean, CR*S-FB = interaction effect between CR and S-FB. † $CH_4 = (0.45 \times \text{acetic acid}) - (0.275 \times \text{propionic acid}) + (0.40 \times \text{butyric acid})$ [14].

Table 5. Effect of fresh cassava root (CR) and solid feed-block containing high sulfur (S-FB) on thiocyanate (SCN^-), blood urea nitrogen (BUN), thyroid hormones, and liver enzymes.

Items	CR-1		CR-1.5		SEM	*p*-Value		
	S-FB-2	S-FB-4	S-FB-2	S-FB-4		CR	S-FB	CR*S-FB
Serum SCN^-, $\mu g\ mL^{-1}$	3.4	4.0	4.0	4.2	0.84	0.31	0.70	0.21
BUN, mg dL^{-1}	11.9	11.6	9.9	11.9	1.59	0.28	0.13	0.61
T3, nmol L^{-1}	0.7	0.6	0.6	0.5.1	0.76	0.51	0.64	0.78
T4, nmol mL^{-1}	69.8	46.8	52.2	40.4	1.82	0.26	0.16	0.53
ALT, units L^{-1}	15.3	14.8	15.8	14.5	1.09	0.91	0.43	0.73
AST, units L^{-1}	40.5	45.8	47.5	41.8	2.85	0.60	0.93	0.07

CR-1 and CR-1.5 = fed CR at 10 and $15\ g\ kg^{-1}$ BW, S-FB-2 and S-FB-4 = solid feed-block containing high sulfur at 20 and $40\ g\ kg^{-1}$, SEM = standard error of mean, SCN^- = thiocyanate, BUN = blood urea nitrogen, T3 = triiodothyronine, T4 = thyroxine, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CR*S-FB = interaction effect between CR and S-FB.

3.5. Blood Metabolites and Hormones

The effects of CR and S-FB on blood thiocyanate (SCN^-), blood urea nitrogen (BUN), thyroid hormones, and liver enzymes are presented in Table 5. Interactive effects between CR and S-FB on blood SCN^-, BUN, thyroid hormones (T3 and T4), and liver enzymes (ALT and AST) were not observed. CR-1 and CR-1.5 did not influence blood SCN^-, BUN, thyroid hormones, or liver enzymes (Table 5). The average SCN^- concentrations between CR-1 and CR-1.5 were 3.7 μg mL^{-1} and 4.1 μg mL^{-1}, respectively. The lack of significant differences in SCN^- after receiving CR-1 and CR-1.5 might be related to the insignificant amounts of CR and HCN intake (Table 2). Cherdthong et al. [2] fed a feed-block containing sulfur to beef cattle and found no effects on BUN; however, a concentration of blood SCN^- was observed, which could have been due to the significant intake of CR as reported by the authors. Promkot and Wanapat [4] found that cassava hay and foliage did not affect BUN concentration but significantly influenced blood SCN^- concentration, a finding that could be due to the significant intake of HCN found as a result of cassava hay and foliage feeding. The enzymes rhodanese and β-mercaptopyruvate available in animal cells and microorganisms in ruminants can be partially attributed to this finding [28]. The rhodanese enzyme is a sulfurtransferase that accelerates the formation of SCN^- from HCN. S-FB-2 and S-FB-4 did not influence blood SCN^-, BUN, thyroid hormones, or liver enzymes (Table 5). It might be that S-FB did not influence the CR or HCN intake (Table 2), thus resulting in a lack of effects on blood SCN^-. The blood SCN^- concentrations between S-FB-2 and S-FB-4 were 3.7 μg mL^{-1} and 4.1 μg mL^{-1}, respectively. Cherdthong et al. [2] found that a feed-block containing sulfur at 20 and 40 g kg^{-1} significantly affected the blood SCN^- concentration, noting that the effect of sulfur on blood SCN^- might be influenced by CR. Promkot and Wanapat [4] found that sulfur supplementation at 1.5 and 4 g kg^{-1} of dietary DM caused an increase in blood SCN^- in dairy cows; that could mainly depend on cassava hay and foliage feeding since an interactive effect between sulfur and cassava sources on blood SCN^- was observed.

3.6. Milk Production and Composition

The effects of CR and S-FB on milk SCN^-, milk yield, milk composition, and somatic cell count (SCC) are presented in Table 6. Interactive effects between CR and S-FB on milk SCN^-, milk yield, fat-corrected milk (FCM), milk composition, and SCC were not found. CR-1 and CR-1.5 did not influence milk yield, FCM, milk SCN^-, or milk composition, with the exception of fat content and SCC (Table 6). Increasing the CR from 10 to 15 g kg^{-1} BW caused a significant increase in milk fat concentration, while it caused a significant decrease in the SCC. The reason for the increase in the milk fat content after CR feeding was not clear. C2 and C4 production in the rumen are the main precursors of milk fat synthesis [1,2,8,29]; however, CR-1 and CR-1.5 did not affect the C2 and C4 concentrations in this study. This result was similar to the result of Mosavi et al. [30], who evaluated various starch sources in the diets of dairy cows that produced a significant effect on milk fat. Promkot and Wanapat [4] used cassava hay and foliage as a roughage source in dairy cows' diets and found no effect on milk yield and milk composition, which may have been due to the lower starch content in cassava hay and foliage than in CR. Increasing CR feeding led to a significant decrease in the SCC in milk, a finding that may have been due to the antimicrobial effect of SCN^-. The lactoperoxidase-thiocyanate-hydrogen peroxide (LP) system has been established as a feasible method for the temporary preservation of raw milk. The activity of the LP system against Gram-negative isolates from milk was initially applied to the reduction of bacterial flora during milk refrigeration. The LP system can inhibit many bacterial species, including a diversity of milk-borne spoilage and pathogenic microorganisms [31]. Petlum et al. [32] found that supplementation of cassava foliage led to a decrease in SCC. S-FB-2 and S-FB-4 did not affect milk yield, FCM, milk composition, milk SCN^-, or SCC (Table 6). The milk yields of S-FB-2 and S-FB-4 were 11.2 kg day^{-1} and 11.5 kg day^{-1}, respectively. The lack of difference in milk yield might be related to the lack of effect of S-FB intake and digestibility of DM and OM (Table 2). The milk SCN^- concentrations between S-FB-2 and S-FB-4 were 7.5 ppm and 7.9 ppm, respectively. Promkot and Wanapat [4] found a significant increase in milk protein in dairy cows, but other parameters did not change. These authors addressed this finding as possibly being related to greater DM, NDF, and ADF digestibility and N retention that could be affected by sulfur supplementation. Supapong and Cherdthong [1] added sulfur at 10 and 20 g kg^{-1}

into the FTMR for dairy cows and found that sulfur addition did not affect milk yield; however, milk fat, SCN^-, and SCC were influenced by sulfur supplementation. Supapong and Cherdthong [1] stated that an increase in milk fat with sulfur supplementation was found because sulfur affected the C2 and C4 concentrations since they acted as substrates for milk fat synthesis, while the increase in milk SCN^- might be related to the increase in blood SCN^-, which may partition into milk.

Table 6. Effect of fresh cassava root (CR) and a solid feed-block containing high sulfur (S-FB) on milk yield and its composition in lactating dairy cows.

Items	CR-1		CR-1.5		SEM	*p*-Value		
	S-FB-2	S-FB-4	S-FB-2	S-FB-4		CR	S-FB	CR*S-FB
Milk yield, kg day^{-1}	11.0	11.3	11.4	11.8	0.48	0.90	0.92	0.81
3.5% FCM †, kg day^{-1}	11.3	11.5	11.8	12.4	0.61	0.81	0.49	0.23
Milk fat, g kg^{-1}	31	32	35	38	0.30	0.04	0.59	0.21
Protein, g kg^{-1}	34	36	35	34	0.14	0.49	0.93	0.23
Lactose, g kg^{-1}	44	43	45	43	0.08	0.49	0.30	0.64
Solid-not-fat, g kg^{-1}	73	85	71	82	0.87	0.78	0.21	0.92
Total solids, g kg^{-1}	115	128	122	125	0.50	0.27	0.88	0.20
SCN^-, ppm	7.1	7.3	8.0	8.6	1.14	0.28	0.79	0.61
SCC, $\times 10^3$ cell mL^{-1}	285.5	249.5	110.5	102.3	0.20	0.02	0.21	0.26

CR-1 and CR-1.5 = fed CR at 10 and 15 g kg^{-1} BW, S-FB-2 and S-FB-4 = solid feed-block containing high sulfur at 20 and 40 g kg^{-1}, SCN^- = thiocyanate, SEM = standard error of mean, CR*S-FB = interaction effect between CR and S-FB. † FCM = Fat-corrected milk.

4. Conclusions

CR and S-FB had no interactive effect on feed intake, digestibility, blood metabolites milk yield, or milk composition. A 15 g kg^{-1} BW, CR produced a significantly greater milk fat concentration, while significantly lower SCC was found when compared with 10 g kg^{-1} BW of CR. The solid feed-block containing sulfur up to 40 g kg^{-1} presented significantly greater ADF digestibility when compared with solid feed-block containing sulfur up to 20 g kg^{-1}.

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

Funding: The authors express their most sincere gratitude to the Research Program on the Research and Development of Winged Bean Root Utilization as Ruminant Feed; the Increase Production Efficiency and Meat Quality of Native Beef and Buffalo Research Group; Research and Graduate Studies, Khon Kaen University (KKU). Thailand Research and Researcher for Industry (RRi- MSD60I0023) and Agricultural Research Development Agency (Public Organization- PRP6005020500) is gratefully acknowledged. Gamonmas Dagaew was granted by Animal Feed Inter Trade Co., Ltd and Thailand Science Research and Innovation (TSRI) through the RRi programme (contract grant PHD62I0021).

Institutional Review Board Statement: The study was conducted under approval procedure no. ACUC-KKU 45/2560 of Animal Ethics and Care issued by KKU (Date: 15 June 2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Thanks to the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, KKU for the use of its research facilities.

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

References

1. Supapong, C.; Cherdthong, A. Effect of sulfur concentrations in fermented total mixed rations containing fresh cassava root on rumen fermentation. *Vet. Sci.* **2020**, *7*, 98. [CrossRef]
2. Cherdthong, A.; Khonkhaeng, B.; Seankamsorn, A.; Supapong, C.; Wanapat, M.; Gunun, N.; Gunun, P.; Chanjula, P.; Polyorach, S. 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.* **2018**, *50*, 1365–1371. [CrossRef]
3. Promkot, C.; Wanapat, M.; Wachirapakorn, C.; Navanukraw, C. Influence of sulfur on fresh cassava foliage and cassava hay incubated in rumen fluid of beef cattle. *Asian-Australas. J. Anim. Sci.* **2007**, *20*, 1424–1432. [CrossRef]
4. Promkot, C.; Wanapat, M. Effect of elemental sulfur supplementation on rumen environment parameters and utilization efficiency of fresh cassava foliage and cassava hay in dairy cattle. *Asian-Australas. J. Anim. Sci.* **2009**, *22*, 1366–1376. [CrossRef]
5. Supapong, C.; Cherdthong, A.; Wanapat, M.; Chanjula, P.; Uriyapongson, S. Effects of sulfur levels in fermented total containing fresh cassava root on feed utilization, rumen characteristics, microbial protein synthesis, and blood metabolites in Thai native beef cattle. *Animals.* **2019**, *9*, 261. [CrossRef] [PubMed]
6. Prachumchai, R.; Cherdthong, A.; Wanapat, M. Screening of cyanide-utilizing bacteria from rumen and *in vitro* evaluation of fresh cassava root utilization with pellet containing high sulfur diet. *Vet. Sci.* **2021**, *8*, 10. [CrossRef]
7. FAO. Feed supplementation block technology—Past, present and future. In *Feed Supplementation Blocks—Urea-Molasses Multinutrient Blocks: Simple and Effective Feed Supplement Technology for Ruminant Agriculture*; Makkar, H.P.S., Sánchez, M., Speedy, A.W., Eds.; Food and Agriculture Organization of the United Nations: Rome, Italy, 2007; pp. 1–12.
8. Cherdthong, A.; Wanapat, M.; Rakwongrit, D.; Khota, W.; Khantharin, S.; Tangmutthapattharakun, G.; Kang, S.; Foiklang, S.; Phesatcha, K. Supplementation effect with slow-release urea in feed blocks for Thai beef cattle-nitrogen utilization, blood biochemistry and hematology. *Trop. Anim. Health Prod.* **2014**, *46*, 293–298. [CrossRef]
9. Dagaew, G.; Cherdthong, A.; Wanapat, M.; Chanjula, P. In vitro rumen gas production kinetics, hydrocyanic acid concentration and fermentation characteristics of fresh cassava root and feed block sulfur concentration. *Anim. Prod. Sci.* **2020**, *60*, 659–664. [CrossRef]
10. Belyea, R.L.; Ricketts, R.E. Forages for Cattle: New Methods of Determining Energy Content and Evaluating Heat Damage. University of Missouri Extension. 1993. Available online: https://extension.missouri.edu/publications/g3150 (accessed on 30 June 2021).
11. AOAC. *Official Methods of Analysis*, 19th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA, 2012.
12. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
13. Van Keulen, J.; Young, B.A. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* **1997**, *44*, 282–287. [CrossRef]
14. AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA, 1990.
15. Moss, A.R.; Jouany, J.P.; Newbold, J. Methane production by ruminants: Its contribution to global warming. *Anim. Res.* **2000**, *49*, 231–253. [CrossRef]
16. Galyean, M. *Laboratory Procedures in Animal Nutrition Research*; New Mexico State University: Las Cruces, NM, USA, 1989.
17. Crocker, C.L. Rapid determination of urea nitrogen in serum or plasma without deproteinization. *Amer. J. Med. Technol.* **1967**, *33*, 361–365.
18. Jacob, B.M.; Antony, K.E.; Sreekumar, B.; Haridas, M. Thiocyanate mediated antifungal and antibacterial property of goat milk lacto-peroxidase. *Life Sci.* **2000**, *66*, 2433–2439. [CrossRef]
19. Tukey, J.W. Comparing individual means in the analysis of variance. *Biometrics.* **1949**, *5*, 99–114. [CrossRef] [PubMed]
20. NRC. *Nutrient Requirements of Dairy Cattle, 7th ed*; The National Academies Press: Washington, DC, USA, 2001.
21. Wanapat, M.; Kang, K. Cassava chip (*Manihot esculenta* Crantz) as an energy source for ruminant feeding. *Anim. Nutr.* **2015**, *1*, 266–270. [CrossRef] [PubMed]
22. Uwituze, S.; Parsons, G.L.; Karges, K.K.; Gibson, M.L.; Hollis, L.C.; Higgins, J.J.; Drouillard, J.S. Effects of distillers grains with high sulfur concentration on ruminal fermentation and digestibility of finishing diets. *J. Anim. Sci.* **2011**, *89*, 2817–2828. [CrossRef]
23. Fang, H.; Liu, H. Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresour. Technol.* **2002**, *82*, 87–93. [CrossRef]
24. Shah, A.M.; Ma, J.; Wang, Z.; Hu, R.; Wang, X.; Peng, Q.; Amevor, F.K.; Goswami, N. Production of hydrogen sulfide by fermentation in rumen and its impact on health and production of animals. *Processes.* **2020**, *8*, 1169. [CrossRef]
25. McSweeney, C.S.; Denman, S.E. Effect of sulfur supplements on cellulolytic rumen micro-organisms and microbial protein synthesis in cattle fed a high fibre diet. *J. Appl. Microbiol.* **2007**, *103*, 1757–1765. [CrossRef]
26. Slyter, L.L.; Chalupa, W.; Oltjen, R.R.; Weaver, J.M. Sulfur influences on rumen microorganisms in vitro and in sheep and calves. *J. Anim. Sci.* **1986**, *63*, 1949–1959. [CrossRef]
27. Boucher, S.E.; Ordway, R.S.; Whitehouse, N.L.; Lundy, F.P.; Kononoff, P.J.; Schwab, C.G. Effect of incremental urea supplementation of a conventional corn silage-based diet on ruminal ammonia concentration and synthesis of microbial protein. *J. Dairy Sci.* **2007**, *90*, 5619–5633. [CrossRef] [PubMed]
28. Frankenberg, L. Enzyme therapy in cyanide poisoning: Effect of rhodanese and sulfur compounds. *Arch. Toxicol.* **1980**, *45*, 315–323. [CrossRef] [PubMed]

29. So, S.; Wanapat, M.; Cherdthong, A. Effect of sugarcane bagasse as industrial by-products treated with *Lactobacillus casei* TH14, cellulase and molasses on feed utilization, ruminal ecology and milk production of mid-lactating Holstein Friesian cows. *J. Sci. Food Agri.* **2021**, *101*, 4481–4489. [CrossRef]
30. Mosavi, G.H.R.; Fatahnia, F.; Mirzaei, A.H.R.; Mehrabi, A.A.; Darmani, K.H. Effect of dietary starch source on milk production and composition of lactating Holstein cows. *S. Afr. J. Anim. Sci.* **2012**, *42*, 201–209. [CrossRef]
31. Zapico, P.; Gaya, P.; Nuñez, M.; Medina, M. Activity of goat's milk lactoperoxidase system on *Pseudomonas fluorescens* and *Escherichia coli* at refrigeration temperatures. *J. Food Prot.* **1995**, *58*, 1136–1138. [CrossRef]
32. Petlum, A.; Surachai, B.; Werachai, T.; Kanawit, P.; Phanompon, W.; Anurak, P.; Ladda, P.; Manoch, K. Effect of ensiled cassava foliage supplementation on milk yield and milk quality of lactating dairy cows in smallholder farms. *Khon Kaen Agric. J.* **2012**, *40*, 114–117.