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Rhodaneses Enzyme Addition Could Reduce Cyanide Concentration and Enhance Fiber Digestibility via In Vitro Fermentation Study

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Abstract: The use of cyanide-containing feed (HCN) is restricted because it causes prussic acid poisoning in animals. The objective of this study was to see how adding rhodanese enzyme to an HCN-containing diet affected gas dynamics, in vitro ruminal fermentation, HCN concentration reduction, and nutrient digestibility. A 3 × 4 factorial arrangement in a completely randomized design was used for the experiment. Factor A was the three levels of potassium cyanide (KCN) at 300, 450, and 600 ppm. Factor B was the four doses of rhodanese enzyme at 0, 0.65, 1, and 1.35 mg/10^4 ppm KCN, respectively. At 96 h of incubation, gas production from an insoluble fraction (b), potential extent (omit gas) (a + b), and cumulative gas were similar between KCN additions of 300 to 450 ppm ($p > 0.05$), whereas increasing KCN to 600 ppm significantly decreased those kinetics of gas ($p < 0.05$). Supplementation of rhodanese enzymes at 1.0 to 1.35 mg/10^4 ppm KCN enhanced cumulative gas when compared to the control group ($p < 0.05$). Increasing the dose of rhodanese up to 1.0 mg/10^4 ppm KCN significantly increased the rate of ruminal HCN degradation efficiency (DE) by 70% ($p < 0.05$). However, no further between the two factors was detected on ruminal fermentation and in vitro digestibility ($p > 0.05$). The concentration of ammonia-nitrogen (NH_3-N) increased with increasing doses of KCN ($p < 0.05$), but remained unchanged with varying levels of rhodanese enzymes ($p > 0.05$). The in vitro dry matter digestibility (IVDMD) was suppressed when increasing doses of KCH were administered at 600 ppm, whereas supplementation of rhodanese enzymes at 1.0–1.35 mg/10^4 ppm KCN enhanced IVDMD ($p < 0.05$). Increasing doses of KCN affected reduced total volatile fatty acids (TVFA) concentration, which was lowest when 600 ppm was added ($p < 0.05$). Nevertheless, the concentration of TVFAs increased when rhodanese enzymes were included by 1.0–1.35 mg/10^4 ppm KCN ($p < 0.05$). Based on this study, it could be concluded that supplementation of rhodaneses enzyme at 1.0–1.35 mg/10^4 ppm KCN could enhance cumulative gas, digestibility, and TVAF, as well as lowering ruminal HCN concentration.

Keywords: degradation efficiency; detoxify; fresh cassava root; gas production; rumen fermentation

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1. Introduction

Phytotoxins called cyanogenic glycosides are found in at least 2000 plant species, and many of them are used as animal feed in tropical regions [1,2]. Because it binds securely to cytochrome oxidase and hence inhibits respiration, cyanide (HCN) is very harmful to aerobic forms of life [3,4]. A cyanogenic plant's potential toxicity is essentially determined by its ability to create highly poisonous HCN when consumed. HCN ingestion has been linked to ruminant mortality [5]. Cassava root is typically used as a source of energy for ruminant animals. It does, however, contain a high concentration of HCN (90–114 mg/kg), which, if consumed, may cause toxicity to the animal [6]. Rumen bacteria may swiftly detoxify a low level of HCN in ruminants via rhodanese and beta mercapto

pyruvate sulfur transferase [7,8]. Providing the available sulfur sources might supply microorganisms with a synthesis rhodanese enzyme to degrade HCN. The NRC [9] noted that ruminants receiving HCN in their diet may need a high-sulfur source supply to activate sulfur to convert HCN into thiocyanate by microorganisms and eliminate it from the body. Elemental sulfur has been successfully introduced to detoxify HCN from animals fed with fresh cassava root. When 2.0% sulfur was introduced to a fermented total mixed ration (FTMR) including fresh cassava root, Supapong and Cherdthong [4] revealed that the concentration of HCN could be reduced by 99%. In addition, supplementation of the pellets containing high sulfur (PELFUR) at 3.0% decreased the concentration of HCN by 37.06–40.08% as compared to the no sulfur fed group [6]. However, feeding high amounts of elemental sulfur may cause negative effects such as decreased feed intake, diarrhea, muscular twitching, and polioencephalomalacia, reducing the performance of ruminants. Thus, detoxification of HCN using a potential alternative approach should be elucidated.

The new feeding innovations to reduce HCN from fresh cassava roots, particularly enzyme technology, is interesting [1]. Enzymes are excellent agents for inactivating antinutritional substances and improving the nutritional value of feeds [10,11]. Cyanase, cyanide hydratase, cyanide dihydratase, cyanide monooxygenase, and rhodanase are the five enzymes that have been identified so far as having the capacity to detoxify HCN [12]. Rhodanase (EC 2.8.1.1) is a universal enzyme found in all living species, including bacteria and humans [12,13]. Rhodanases are isolated from a variety of sources and are made up of two structurally identical domains that contain either a catalytic Cys or an inert Asp residue [14]. Cipollone et al. [15] noted that the rhodaneses enzyme has a high tolerance to a wide range of HCN compounds, which might have more potential to detoxify HCN than those other enzymes. It was hypothesized that the addition of rhodanese enzymes could reduce HCN content in the diet, whereas nutrient digestibility and ruminal fermentation might be enhanced. The goal of this study was to see how adding rhodanese enzyme to an HCN-containing diet affected gas dynamics, in vitro ruminal fermentation, HCN concentration reduction, and nutrient digestibility.

2. Materials and Methods

2.1. Experimental Design and Treatments

A 3 × 4 factorial arrangement in a completely randomized design was used for the experiment. Two factors were used in the experiments. Factor A was the three levels of potassium cyanide (KCN) (Merck, Darmstadt, Germany) at 300, 450, and 600 ppm, which was suggested by Prachumchai et al. [6]. Factor B was the four doses of rhodanese enzyme (bovine liver, Type II essentially salt-free, lyophilized powder, 100–300 units/mg solid R1756; Sigma Chemical Company, St. Louis, MO, USA) at 0, 0.65, 1, and 1.35 mg/10^4 ppm KCN, respectively. The total mixed ration (TMR) was crushed to pass through a 1-mm screen (Cyclotech Mill, Tecator, Hoganas, Sweden) and 0.5 g was added to the serum bottles, followed by the addition of KCN and rhodanese enzyme according to the experimental treatments. Table 1 lists the contents and chemical composition of the TMR diets used as substrate in the experiment.

2.2. Animals and Preparation of Rumen Inoculums

Rumen fluid was obtained from the two rumen-fistulated dairy bulls [bodyweight (BW) of 450 ± 30 kg]. Individual pens housed the animals. The concentrate diet containing 14% and 75% of CP and TDN, respectively, was fed to the cattle at 0.5% of BW in two equal portions, at approximately 08:00 h and 17:00 h. A rice straw was provided all the time. Water and mineral blocks were provided as free choices. Ruminal fluid was sampled from cattle after feeding approximately 1 h in the morning, and was filtered through layers of cheesecloth into warmed thermos bottles before being transported to the laboratory. The artificial saliva was created using Menke and Steingass' approach [16]. A mixed rumen inoculum was created by combining artificial saliva with rumen fluid in a 2:1 ratio. The serum bottles were placed in a water bath at 39 °C and replaced oxygen by flushing

CO_2 being forced down with 40 mL of the rumen solution one hour before being used as the inoculum.

Table 1. Total mixed ration diet and their composition of experiment.

Item	Concentration, % DM
Ingredients	
Rice straw	40.00
Soybean meal	8.35
Palm kernel meal	13.62
Corn	29.03
Rice bran	5.00
Urea	1.00
Mineral premix *	1.00
Molasses, liquid	2.00
Chemical composition	
Dry matter (DM), %	89.50
Organic matter (OM), %DM	90.23
Ash, %DM	9.77
Crude protein, %DM	12.00
Neutral detergent fiber, %DM	52.19
Acid detergent fiber, %DM	22.01

* Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g iron; 40 g zinc; 40 g manganese; 0.1 g cobalt; 10 g copper; 0.1 g selenium; 0.5 g iodine.

2.3. Incubation of Substrates

For an in vitro gas test, bottles were sealed with rubber and aluminum cap stoppers and incubated at 39 °C. For each sampling period, the bottles were gently shaken every 2 h. Only the rumen inoculums were provided in five of the bottles. As a control, the mean gas production values of these bottles were employed. The net gas production was calculated by subtracting the blank values from each measured value. For pH, ammonia-nitrogen (NH_3-N), and volatile fatty acids (VFA) analyses, the bottles (3 bottles/treatment × 12 treatments) were prepared individually. Another set of 36 bottles (3 bottles/treatment × 12 treatments) was used for the digestibility analysis. The concentration of HCN analysis was prepared with another set of 108 bottles (3 bottles/ treatment × 12 treatments × 3 h of incubation).

2.4. Analyses and Samples

A sample of TMR was dried at 60 °C for 48 h, then ground to pass through a 1 mm sieve (Cyclotech Mill, Tecator, Hoganas, Sweden) and used for the in vitro gas test and chemical analysis. The samples were chemically analyzed for dry matter (DM), crude protein (CP), ash, and organic matter (OM) according to the method of AOAC [17]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) in samples were estimated according to Van Soest et al. [18]. Using a calibrated syringe and a pressure transducer, data on gas generation was obtained directly after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, and 96 h. To compute cumulative gas production, the Ørskov and McDonald [19] model was employed as an equation. The following is an example:

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

where a represents gas production from the immediately soluble fraction, b represents gas production from the insoluble fraction, c represents the gas production rate constant for the insoluble fraction (b), t represents incubation time, and (a + b) represents the potential extent of gas production. Y = the amount of gas produced at time "t".

Fermentation liquor was sampled at 4 h post-inoculation for measured pH using a digital pH meter (HANNA Instrument (HI) 8424 microcomputer, Singapore). Samples were centrifuged at 16,000× *g* for 15 min, and the supernatant was stored at −15 °C before analysis of NH_3-N (Kjeldahl methods [17]) and VFA (gas chromatography, Wilmington, DE

5890A Series II gas chromatograph and a glass column (180 cm × 4 mm i.d.) packed with 100 g/L SP-1200/10 g/L H_3PO_4 on 80/100 mesh Chromosorb WAW; Supelco, Bellefonte, PA, USA). The quantities of HCN in fermentation liquor (0, 6, and 12 h after incubation) were determined using a modified version of Fisher and Brown's picric acid method [20]. With the standard KCN solutions, a linear calibration curve was created by adding 0.1 mL aliquots of a solution containing 0.5% (*w/v*) picric acid and 0.25 M Na_2CO_3 to 0.05 mL aliquots of KCN solutions (after centrifugation at 15,000× *g* for 10 min at 4 °C). The resultant solutions were boiled for 5 min, then diluted to 1 mL with 0.85 mL distilled water and chilled for 30 min in tap water. Using a spectrophotometer, the absorbance was measured at 520 nm against a blank of distilled water and picric acid reagent. The following formula was used to compute the degradation efficiency (DE) of HCN:

$$DE\ (\%) = [(Ic - Rc)/Ic] \times 100$$

where Ic = initial concentration of HCN (ppm) and Rc = residual concentration of HCN (ppm).

After a 24-h incubation period, the in vitro dry matter digestibility (IVDMD) was evaluated. The percent weight reduction was calculated and IVDMD was used to represent it. The digestibility of in vitro neutral detergent fiber (IVNDFD) and in vitro acid detergent fiber (IVADFD) were also determined [21].

2.5. Statistical Analysis

Using SAS (Cary, NC, USA) software, the data were statistically analyzed as a 3 × 4 factorial in a complete randomized design. The statistical model included terms for the level of KCN and rhodanese enzymes. Duncan's New Multiple Range Test was used to find significant changes between treatments (DMRT). To see if the differences between the means were statistically significant, a *p*-value of less than 0.05 was employed.

3. Results

3.1. Gas Kinetics and Ruminal Cyanide Concentration

Table 2 demonstrated the addition of rhodanese enzymes and KCN levels on gas kinetics, cumulative gas at 96 h after incubation, and ruminal HCN concentration. There was no interaction effect between rhodanese enzymes and KCN on gas kinetics and ruminal HCN concentration ($p > 0.05$). Gas production from the immediately soluble fraction (a) and gas production rate constant for an insoluble fraction (c) were not altered by KCN levels ($p > 0.05$). At 96 h of incubation, gas production from an insoluble fraction (b), potential extent (omit gas) (a + b), and cumulative gas were similar between KCN additions of 300 to 450 ppm ($p > 0.05$), whereas increasing KCN to 600 ppm significantly decreased those kinetics of gas ($p < 0.05$). In addition, various doses of rhodanese enzyme addition did not change all the kinetics of gas ($p > 0.05$), except for cumulative gas at 96 h of incubation ($p < 0.05$). Supplementation of rhodanese enzymes at 1.0 to 1.35 mg/10^4 ppm KCN enhanced cumulative gas when compared to the control group ($p < 0.05$).

Additional KCN doses increased ruminal HCN concentrations significantly (Figure 1; $p < 0.05$). The highest ruminal HCN concentration was observed when supplementation of KCN was 600 ppm, while the addition of 300 ppm of KCN led to a lower ruminal HCN concentration than those other levels. In addition, ruminal HCN concentration was reduced when longer incubation was at 12 h compared to 0 and 6 h. Figure 2 illustrates the effect of rhodanese enzyme levels on the ruminal degradation efficiency (DE) of HCN at various hours of incubation ($p < 0.05$). Increasing the dose of rhodanese up to 1.0 mg/10^4 ppm KCN significantly increased the rate of ruminal HCN degradation ($p < 0.05$). However, there was no further increase in degradation rate when increasing the dose to 1.35 mg/10^4 ppm KCN ($p > 0.05$).

3.2. pH, Ammonia-Nitrogen (NH_3-N) and In Vitro Digestibility

The pH, NH_3-N and in vitro digestibility response to the addition of rhodanese enzymes and KCN levels are presented in Table 3. No interaction effect between the two

factors was detected on ruminal fermentation and in vitro digestibility ($p > 0.05$). The concentration of NH_3-N increased with increasing doses of KCN ($p < 0.05$), but remained unchanged with varying levels of rhodanese enzymes ($p > 0.05$). In addition, the dose of rhodanese enzymes and KCN dose were not altered by ruminal pH, IVNDFD, and IVADFD ($p > 0.05$). However, the IVDMD was significantly different when an individual factor was introduced ($p < 0.05$). The IVDMD was suppressed when increasing doses of KCN were administered at 600 ppm, whereas supplementation of rhodanese enzymes at 1.0–1.35 mg/10^4 ppm KCN enhanced IVDMD ($p < 0.05$).

Table 2. Addition of rhodanese enzymes and potassium cyanide (KCN) levels on gas kinetics, cumulative gas at 96 h after incubation and ruminal cyanide concentration.

Item	300 ppm KCN				450 ppm KCN				600 ppm KCN				*p*-Value			
	Concentration of Rhodanese Enzymes (mg/10^4 ppm KCN)															
	0	**0.65**	**1**	**1.35**	**0**	**0.65**	**1**	**1.35**	**0**	**0.65**	**1**	**1.35**	**A**	**B**	**A * B**	**SEM**
	Gas production kinetic															
a	−1.91	−1.41	−2.09	−2.58	−1.34	−1.87	−1.79	−1.55	−1.58	−1.87	−1.42	−1.98	0.45	0.32	0.13	0.54
b	79.85	83.99	85.93	87.08	79.93	77.53	79.23	82.10	41.96	45.29	53.70	56.16	0.02	0.45	0.22	1.49
c	0.018	0.021	0.021	0.021	0.011	0.011	0.018	0.017	0.014	0.014	0.015	0.018	0.19	0.21	0.65	0.05
a + b	77.94	82.58	83.84	84.5	78.59	75.66	77.44	80.55	40.38	43.42	52.28	54.18	0.03	0.36	0.36	1.42
Cumulative gas, mL	82.51	87.15	92.41	90.07	83.16	80.23	86.01	85.12	44.95	47.99	60.85	58.75	0.01	0.04	0.77	1.83
	Ruminal cyanide concentration, ppm															
H0	291.41	289.93	290.90	287.96	441.01	439.67	439.74	438.23	595.21	589.44	585.65	585.01	0.01	0.44	1.00	5.8
H6	254.25	228.90	145.16	143.06	395.15	355.53	225.80	224.19	555.80	525.98	438.07	440.33	0.01	0.02	0.45	4.19
H12	241.01	195.28	135.49	133.33	375.57	301.40	206.82	205.40	535.56	475.28	350.96	345.79	0.01	0.02	0.06	3.37

* A: *p*-value level of KCN in diet. B: *p*-value level of rhodanese enzymes in diet. KCN: Potassium cyanide. H0: 0 h of incubation. H6: 6 h of incubation. H12: 12 h of incubation. a: gas production from immediately soluble fraction. b: gas production from insoluble fraction. c: gas production rate constant for insoluble fraction (b). a + b: potential extent (omit gas). SEM: standard error of mean.

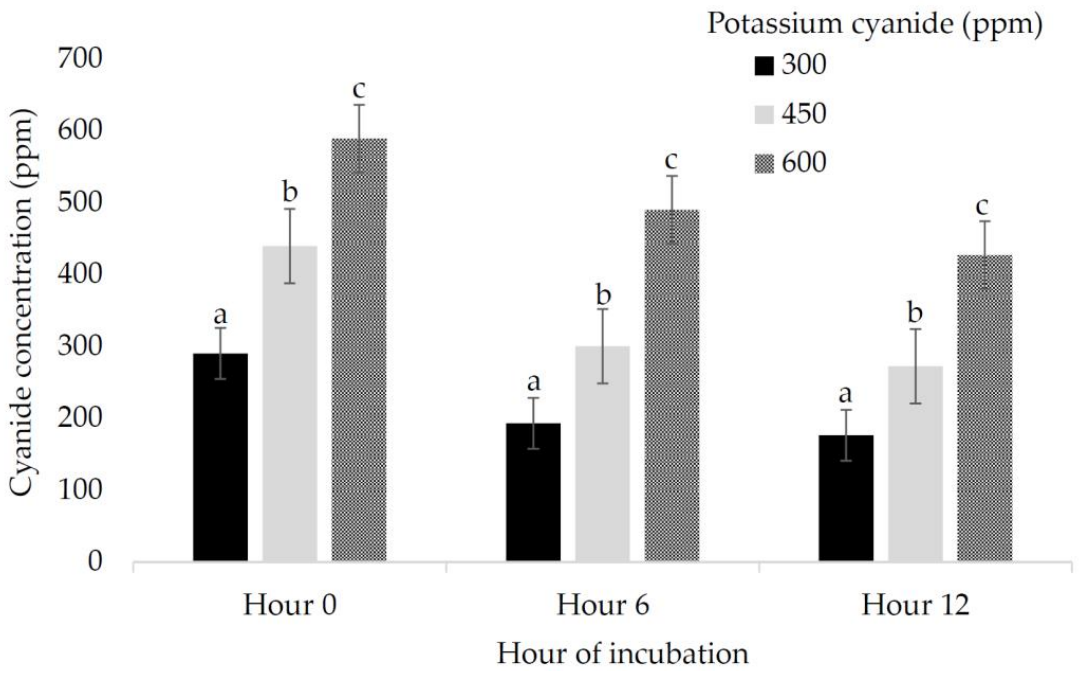

Figure 1. Influence of potassium cyanide (KCN) levels on ruminal cyanide concentration (ppm) at various hours of incubation [a–c Means in the same hour with different superscripts differ ($p < 0.05$)].

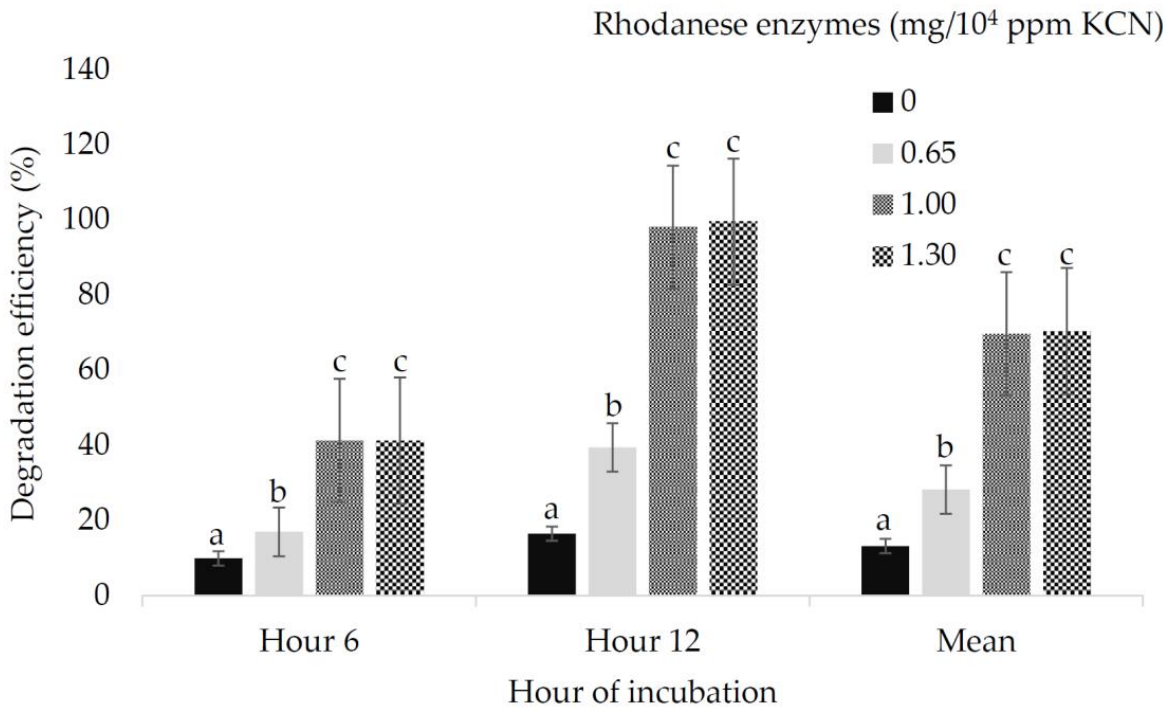

Figure 2. Effect of rhodanese enzyme levels on ruminal degradation efficiency of cyanide (%) at various hours of incubation [a–c Means in the same hour with different superscripts differ ($p < 0.05$)].

Table 3. Addition of rhodanese enzymes and potassium cyanide (KCN) levels on pH, ammonia-nitrogen (NH_3-N) and in vitro digestibility.

Item	300 ppm KCN				450 ppm KCN				600 ppm KCN				*p*-Value			
	Concentration of Rhodanese Enzymes (mg/10⁴ ppm KCN)															
	0	**0.65**	**1**	**1.35**	**0**	**0.65**	**1**	**1.35**	**0**	**0.65**	**1**	**1.35**	**A**	**B**	**A * B**	**SEM**
pH	6.99	6.98	6.99	6.98	7.02	7.03	7.02	7.04	7.07	7.06	7.05	7.07	0.11	0.97	0.99	0.53
NH_3-N, mg%	11.88	11.99	12.21	12.33	13.10	13.58	13.55	13.44	14.01	14.09	15.64	17.81	0.02	0.97	0.19	1.07
IVDMD, %	67.43	67.87	73.08	75.59	64.34	65.21	70.84	72.83	47.52	53.04	60.54	62.69	0.02	0.03	0.94	1.51
IVNDFD, %	38.30	40.31	45.69	50.74	41.81	41.76	42.36	43.55	39.53	40.67	43.83	44.05	0.29	0.16	0.49	0.99
IVADFD, %	24.96	26.59	27.51	30.83	22.45	23.56	25.86	28.15	20.12	22.82	23.63	24.89	0.66	0.61	0.19	0.45

* A: *p*-value level of KCN in diet. B: *p*-value level of rhodanese enzymes in diet. KCN: potassium cyanide. IVDMD: in vitro dry matter digestibility. IVNDFD: in vitro neutral detergent fiber digestibility. IVADFD: in vitro acid detergent fiber digestibility. SEM: standard error of mean.

3.3. Total Volatile Fatty Acids (TVFAs) and Their Profiles

The effects of rhodanese enzymes and KCN levels on TVFAs and VFA profiles are presented in Table 4 and no interaction effect was found between the two factors on those observations. The VFA profiles such as acetate (C2), propionate (C3), and butyrate (C4) did not differ when rhodanese enzymes and KCN were added ($p > 0.05$). Increasing doses of KCN affected reduced TVFAs concentration, which was lowest when 600 ppm was added ($p < 0.05$). Nevertheless, the concentration of TVFAs increased when rhodanese enzymes were included by 1.0–1.35 mg/10^4 ppm KCN ($p < 0.05$).

Table 4. Addition of rhodanese enzymes and potassium cyanide (KCN) levels on in vitro total volatile fatty acids (VFAs) and VFA profiles.

Item	300 ppm KCN				450 ppm KCN				600 ppm KCN				*p*-Value			
	Concentration of Rhodanese Enzymes (mg/10^4 ppm KCN)															
	0	**0.65**	**1**	**1.35**	**0**	**0.65**	**1**	**1.35**	**0**	**0.65**	**1**	**1.35**	**A**	**B**	**A * B**	**SEM**
Total VFA, mmol/L	94.28	98.55	104.86	106.35	90.68	95.12	102.15	104.15	71.12	73.58	91.26	93.50	0.25	0.02	0.4	2.33
	VFA profiles, mol/100 mol															
Acetic acid	64.18	59.49	66.95	65.22	68.52	64.74	63.56	64.48	66.71	66.54	67.92	60.19	0.79	0.52	0.53	2.24
Propionic acid	27.11	29.84	25.02	25.71	23.58	27.43	27.46	26.75	25.00	25.20	24.13	29.70	0.87	0.56	0.57	1.86
Butyric acid	8.73	10.68	8.03	9.08	7.90	7.83	9.00	8.78	8.30	8.26	7.95	10.13	0.55	0.52	0.46	1.16

* A: *p*-value level of KCN in diet. B: *p*-value level of rhodanese enzymes in diet. KCN: potassium cyanide. VFA: volatile fatty acids. SEM: standard error of mean.

4. Discussion

The negative impact of a high dose of KCN on the kinetics of gas and cumulative gas production was detected. In particular, the cumulative gas was suppressed by 60.4% when 600 ppm of KCN was supplemented, while it could be maintained when KCN was supplemented at 450 ppm compared to 300 ppm KCN. The suitable level of HCN in the rumen can be removed by rumen bacteria that use it. HCN may be supplied as a nitrogen source for bacterial growth through enzymes which catalyze the conversion of the sulfur species to rhodanese and mercaptopyruvate sulfurtransferase [6]. However, adding a high level of HCN may exceed the capacity to detoxify rumen microorganisms, thus resulting in inhibited feed digestion and gas production. HCN might be interfering with bacterial growth by inhibiting the mechanism of the cytochrome respiratory chain and the electron transport chain, leading to the low potential removal of HCN from the rumen fluid [22]. Furthermore, Prachumchai et al. [6] demonstrated that the number of cyanide-using rumen bacteria was depressed by up to 90% when increasing the dose of HCN supplementation. In agreement with previous work by Sumadong et al. [8], who reported that the gas production from the insoluble fraction (b) and gas production at 96 h after incubation significantly declined by 12.3% and 15.0%, respectively, when increasing fresh cassava root as a HCN source from 300 mg to 400 mg. Therefore, the present result indicated that the maximum dose of KCN which did not adversely affect the kinetics of gas was not more than 450 ppm.

The alternative feeding approach to mitigate HCN from feedstuff, including enzyme additives, is focused and has not yet been clearly investigated in ruminant animals. Rhodanese is a mitochondrial enzyme that functions to detoxify HCN into thiocyanate, which is safe, then excrete it out of the ruminant via urine [4]. According to Aminlari et al. [23], the rumen of ruminant animals has high activity of the rhodanese enzyme, which ranges from 10.3 to 16.3 U/mg protein for sheep and cattle, respectively. In this study, adding an additional rhodanase enzyme could affect cumulative gas production and mitigate ruminal HCN concentration. The cumulative gas production increased by 7.8–9.6% when rhodanase enzyme was supplemented at 1.0–1.35 mg/10^4 ppm KCN compared to the no enzyme-fed group. It is possible that the rhodanase enzyme will aid in the nutrient digestion process of feed, resulting in increased gas production. Furthermore, it has been clearly established that the addition of a rhodanase enzyme could increase the efficiency of HCN degradation. No enzyme-fed group obtained 13% DE of HCN, which may be the activity of individual rumen bacteria removing HCN. However, additional rhodanase enzyme at 1.0–1.35 mg/10^4 ppm KCN led to an increase in DE rate by 70%. Similarly, Cipollone et al. [15] noted that the rhodaneses enzyme has high tolerance to a wide range of HCN compounds, which might have more potential to detoxify HCN than those other enzymes. In addition, Latif et al. [1] indicated that using Multifect® enzyme containing cellulase, with side activities of hemicellulase, xylanase, and beta-glucanase, which were produced from *Trichoderma reesei* (6200 international units (IU)/mL), showed a HCN content reduction of 82% from cassava leaves after the incubation process. Moreover,

Sornyotha et al. [24] noted that a linamarin content in cassava root decreased by 90.3% after incubation using xylanase and cellulase.

Supplementation of rhodanase enzyme in the diet containing HCN could maintain ruminal pH in the suitable range for rumen microorganisms to act on feed digestion. Ruminal pH ranged from 6.99 to 7.06 and was close to that reported by Wanapat and Cherdthong et al. [25], who noted ruminal pH of about 6.5–7.0. Furthermore, the concentration of NH_3-N ranged from 12.1 to 15.4 mg%, which might be suitable for supply as an N source for microbial bacteria synthesis. The addition of KCN at 600 ppm increased the NH_3-N concentration by 21.3% compared to 300 ppm KCN. This might be explained by the degradation of KCH, which could provide an available N source in the fluid liquor, leading to an enhanced NH_3-N concentration with 600 ppm of KCN. However, at the highest dose of KCN at 600 ppm, 26.9% of IVDMD was reduced, possibly due to KCN toxicity on rumen microbes and resulting depressed feed digestion.

Furthermore, digestion of in vitro DM increased by 12.3–15.1% when supplemented with rhodanase enzyme at 1.0–1.35 mg/10^4 ppm KCN compared to the no enzyme-fed group. It could possibly be that the rhodanase enzyme could directly affect feed digestion and lead to enhanced IVDMD. Similarly, Kondratovich et al. [26] found that supplementing fibrolytic enzymes with fiber sources commonly fed to cattle improved digestion and may have additional benefits when used on unprocessed fiber diets, as well as stimulated intake and had positive effects on ruminal fermentation in beef cattle growing diets. By dephosphorylation and hydrolysis, the phytase enzyme releases phosphorus (P) from phytate [myo-inositol hexakisphosphate (InsP6)] and lowers inositol phosphates [27]. Exogenous phytase supplementation boosted the ruminal degradation of InsP6 by around four levels, according to Brask-Pedersen et al. [28]. Another reason is that KCN could be detoxified by the rhodanase enzyme, leading to the formation of a less toxic thiocyanate for rumen microbes and providing bacteria with greater feed digestibility than those with low levels of rhodanase enzyme.

Ruminal fermentation produces huge amounts of TVFA, which are critical since they provide more than 70% of the ruminant's energy [29,30]. The greater TVFA in enzyme-added treatments could be attributed to an increase in IVDMD, which would then provide enough glucose for VFA synthesis [31,32]. Present results demonstrate that rhodanase enzyme addition at 1.0–1.35 mg/10^4 ppm KCN could enhance VFAs concentration by 14.1–15.8% compared to no enzyme supplementation. This result might be related to the rhodanase enzyme improving feed digestion. This outcome was consistent with earlier research on enzyme supplementation. When compared to no enzyme addition, So et al. [33] found that adding cellulase enzyme at 10^4 U/kg fresh matter of feed increased IVDMD and improved TVFA from 23 to 29 mM. Furthermore, in this investigation, cumulative gas production was higher in the rhodanase enzyme treatment group, which could explain the increased TVFA compared to the control group. However, increasing KCN up to 600 ppm results in the lowest TVFA when compared to 300 and 450 ppm KCN, respectively. The negative effect of high-dose KCN on feed digestion and toxicity to microbes plays a major role in lowering TVFA.

5. Conclusions

Based on this study, it could be concluded that an increasing dose of KCN up to 600 ppm has adversely influenced the kinetics of gas, digestibility, and ruminal fermentation. In addition, supplementation of rhodaneses enzyme at 1.0–1.35 mg/10^4 ppm KCN could enhance cumulative gas, digestibility, and TVAF, whereas ruminal HCN concentration was removed by 70%. As a result, HCN-containing feedstuffs could be combined with supplements containing the rhodanese enzyme, which can detoxify HCN content without harming animal health. This is the first report in the field of ruminant nutrition on the use of rhodanese enzyme to mitigate HCN. Thus, further work should be applied to in vivo experiments to observe the possible use of feedstuffs containing HCN and supplemented with rhodanase enzyme.

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