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Effect of feeding a pellet diet containing high sulfur with fresh cassava root supplementation on feed use efficiency, ruminal characteristics, and blood metabolites in Thai native beef cattle

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Abstract: The objective of this experiment was to study the effect of feeding pellet containing high sulfur (PELFUR) diet and fresh cassava root (FCR) to Thai native beef cattle on feed use efficiency, ruminal characteristics, and blood metabolites. Four male Thai native beef cattle (150 ± 15.0 kg of body weight (BW)) were allocated with a 2 × 2 factorial arrangement in a 4 × 4 Latin square design. Factor A was FCR supplementation at 15 and 20 g/kg of BW. Factor B was the sulfur level in the PELFUR ration at 15 and 30 g/kg of dry matter (DM). No interaction effect was found among FCR supplementation and PELFUR in terms of feed intake and nutrient intake ($p > 0.05$). Cyanide intake was significantly increased based on FCR supplementation ($p < 0.05$), whereas sulfur intake was increased by level addition of PELFUR levels ($p < 0.05$). There were interaction effects among FCR supplementation and PELFUR on digestibility coefficients of DM and organic matter (OM) ($p < 0.05$). FCR supplementation at

20 g/kg BW with PELFUR 30 g/kg demonstrated the highest digestibility of DM and OM. Moreover, interactions were observed between FCR and PELFUR for bacterial populations ($p < 0.01$). The populations of bacteria were highest in FCR supplementation at 20 g/kg BW with PELFUR 30 g/kg at various feeding times. An interaction effects from among feeding FCR with PELFUR was found on blood thiocyanate concentrations at various feeding times ($p < 0.01$). The highest mean values of blood thiocyanate were observed when feeding FCR at 20 g/kg BW with PELFUR at 30 g/kg. No interaction effect was found between FCR and PELFUR on total volatile fatty acids (VFA) and their profiles ($p > 0.05$). However, the proportions of the total VFA at 0 and 4 h post-feeding were increased when FCR at 20 g/kg BW was supplemented ($p < 0.01$). FCR at 20 g/kg BW could enhance propionate (C3) at 4 h post-feeding when compared with FCR at 15 g/kg BW ($p < 0.01$). Moreover, supplementation of PELFUR at 30 g/kg increased the total VFA at 0 and 4 h post-feeding, whereas the concentration of C3 at 4 h post-feeding was enhanced ($p < 0.05$). However, no significant changes were found for any parameters among treatments and between the main effect of FCR and PELFUR supplementation ($p > 0.05$). In conclusion, feeding of two combinations (FCR 20 g/kg BW with PELFUR 30 g/kg) could promote the nutrient digestibility, the bacterial populations, and the rate of disappearance of cyanide without having any adverse effect on rumen fermentation.

Keywords: Fresh cassava root, pellet containing high sulfur, ruminal characteristics, blood thiocyanate, Thai native beef cattle

1. Introduction

Cassava or tapioca (*Manihot esculenta*, Crantz) is a crop grown widely in the tropics and subtropics, especially in the northeast region of the country [1]. Cassava chip has the potential for increased use as animal feed in ruminant nutrition, and cassava chips are commonly used in animal feed formulation (40-60% DM) [2]. However, feeding cassava chips to ruminants is limited due to an increase in the demand for cassava chips leads to an increase in price. Besides its high cost, the difficulty in cassava chip production during the heavy rain season is also a constraint. Therefore, feeding fresh cassava root (FCR) to ruminants is more feasible in all seasons [3]. Supapong and Cherdthong [4] stated that feeding FCR could be another approach to increase the use of FCR without prior processing. However, cyanogenic compounds contained in FCR, linamarin, and lotaustralin, are constraints that limit cassava root usage. The compound cyanohydrin, produced via a hydrolysis process, contains linamarin as a precursor to linamarase. The more hydrolysis there is the more toxic the root is. Wanapat and Kang [5] found that FCR is composed of cyanide, ranging 15–400 mg/kg, depending on crop variety, altitude, geographic location, harvesting period, and seasonal conditions.

In ruminants receiving cyanide, more than 300 ppm with body weight (BW) fresh matter can be toxic to the host. On a dry weight basis, feeds with more than 500 mg/kg cyanide should be considered potentially toxic [6]. In ruminants, it was believed that rumen microbes could detoxify cyanide at low levels. However, no report elucidated whether the rumen contains microbes that could degrade cyanide. Two enzymes are of particular interest in rumen microbes: rhodanase and mercaptopyruvate sulfurtransferase [4]. Rhodanase is a mitochondrial enzyme that functions to detoxify cyanide into thiocyanate, which is safe, and then excrete the compound out of the ruminant body via urine [7]. The conversion of thiocyanate from cyanide via rhodanase involves the cofactor sulfane sulfur [4]. This cofactor's availability relies on the availability of amino acids having sulfur in the structure. In diets containing low amounts of

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these amino acids, the addition of sulfur is suggested [8]. Offering FCR up to 15 g/kg of body weight with feed blocks composed of sulfur up to 40 g/kg showed no negative impact on animal health [9]. In addition, Supapong et al. [7] suggested that beef cattle fed on a fermented total mixed ration (FTMR) diet containing 400 g/kg of FCR and 20 g/kg of sulfur improved the digestibility of nutrients, bacterial protein synthesis, volatile fatty acids (VFA), and thiocyanate in blood serum. However, previous studies demonstrated that the inclusion of sulfur in feed block form [9] or supplementation in a FTMR diet [7] is limited due to the complicated preparation, required much time, high production cost, and difficult practical use for the farmers. Thus, feeding with a pellet feed containing sulfur is an interesting approach and might be more effective when compared to the previous sulfur feeding.

The flow properties and densification of pellets have been reported for waste reduction, enhancing transport or storage [10]. The physical properties of the pellets need to be determined for the proper design, handling, and transport system [11]. The benefits of pellets are not only simpler for transport and storage but also better for flow characteristics and, in particular, less dust, less waste, and lower labor costs [12]. Moreover, pellet diets are far more reliable and are easier for monitoring the desired feed rations for individual animals or groups of animals with higher nutritional needs [13]. Seankamsorn et al. [14] found that pellet diets could enhance nutrient digestibility and fermentation in the rumen. Furthermore, feeding animals with feed products such as sulfur-containing pellets require, in particular, an increase in value-added feed, the possibility of developing the product to the commercial scale, and convenient practical use for farmers. *In vitro* study by Prachumchai and Cherdthong [15] demonstrated that reductions in the cyanide content and increases in the *in vitro* feed digestion were observed when a pellet containing high sulfur (PELFUR) diet was added with FCR supplementation, whereas the *in vivo* study is necessary to confirm the results.

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It was hypothesized that FCR supplementation with PELFUR may reduce the rate of cyanide disappearance from FCR and may improve rumen ecology and feed utilization in the ruminant, with no negative affect to animal health. Therefore, the objective of this experiment was to study the effect of feeding PELFUR and FCR to Thai native beef cattle on feed use efficiency, ruminal characteristics, and blood metabolites.

2. Materials and methods

The cattle were cared for using guidelines approved by the Animal Welfare Committees of Khon Kaen University (No. ACUC-KKU 11/2563).

2.1 Pellets containing high sulfur (PELFUR)

Feed ingredients (Table 1) were prepared and milled using Cyclotech Mill (Tecator, Hoganas, Sweden). After mixing the ingredient, sulfur was added at a respective level before pelleting. The mash was moisture conditioned to 200 or 250 g/kg before pelleting. Pelleting was done with a Ryuzoukun mini (Kakiuchi co.,Ltd., Nakajima, Japan) equipped with a 50.8-mm thick die with a 4.8-mm diameter hole. Finally, the PELFUR dried under sunlight for approximately 3 days to ensure a suitable moisture content at 80–100 g/kg [14]. The 12-month-old FCR (*Manihot esculenta*, Kasetsart 50 variety) was purchased from a local producer in Khon Kaen, and FCR was chopped by machine (Root chopper C.510-1000-36, KR Strength Co., Ltd., Nakhon Ratchasima, Thailand) into thinner pieces of 0.2–0.4 mm before feeding.

2.2 Experimental design and animal management

Thai native beef cattle (n = 4, 150 ± 15.0 kg) were allocated with a 2 × 2 factorial arrangement in a 4 × 4 Latin square design. Factor A was FCR supplementation at 15 and 20 g/kg of BW. Factor B was the sulfur level in the PELFUR ration at 15 and 30 g/kg of DM. PELFUR and FCR were fed twice a day at 07:30 a.m. and 15:30 p.m. PELFUR was fed at 10 g/kg of BW, whereas rice straw was fed ad libitum. Intake and the feed remaining (5–10%) from each feeding day were recorded. The animals were kept in individual pens of 3 × 4 m, and clean fresh water was provided for each animal. The feeding period lasted for 84 days divided into four periods, (21 days per period) and each period was composed of 14 days for diet adaptation and 7 days for sample collection. At the last 7 days, steers were transferred to the metabolism crates to study the digestibility. Feces and urine were collected, weighed, and recorded.

2.3 Sample collection and laboratory analysis

The feed offered and the refusal of rice straw, FCR, and PELFUR were collected during the last 7 days of each period. In addition, the total collection technique was used for feces and urine sampling at the metabolism crate. Fifty g of feces from the last 7 days were sampled and divided into two parts: one part for the DM analysis every day and two parts were kept in the refrigerator and pooled by steer at the end of each period for chemical analysis. Urine was collected using glass bottles and were preserved with 10% H_2SO_4. The urine yield was collected for each day during 7 days to study the N content using Kjeldahl methods [16] and N utilization. An oven was set at 60 °C for drying feed, refusal, and fecal samples. Then, samples were ground for nutrient content analysis. The crude protein (CP) (ID 984.13), DM (ID 967.03), and ash content (ID 942.05) were determined according to AOAC [16]. Fibrous content, namely neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined [17]. The sulfur content was determined according to Sanford and Lancaster [18]. The cyanide in FCR was determined using spectrophotometry [19], where a 510-nm

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absorbance was multiplied with the coefficient value of 396 and expressed as mg/ kg (ppm). Table 1 shows the dietary ingredients and their nutrient contents.

On day 21 of each period, 120 ml of rumen fluid from each steer was sampled via a stomach tube using a vacuum pump to withdraw rumen fluid at 0 and 4 h post feeding. The samples were analyzed immediately for pH and temperature using a glass electrode pH meter (Hanna HI-8424 Portable pH/ORP Meter, Woonsocket, USA). The samples were split into two parts. The first part was rumen fluids, which were centrifuged at 16,000 g for 15 min at 4 °C. Then, the supernatant was collected and stored frozen at -20 °C. The frozen samples were analyzed for ammonia-nitrogen (NH_3-N; ID 991.20) concentration [15]. The volatile fatty acid (VFA) was determined using high-performance liquid chromatography (ETL Testing Laboratory, Inc., Cortland, NY) [20]. Another part of the sample was stored in a 10% formalin solution (1:9 ratio) for protozoa and bacterial study using a hemocytometer (Boeco, Hamburg, Germany) [21]. In addition, on the last day of each period, the 10-ml blood sample was obtained from the jugular vein at 0 h and 4 h post morning feeding and analyzed for blood urea-nitrogen (BUN) [22] and blood thiocyanate [23]. Blood was kept in a test tube, and EDTA (10 mg) was added as an anticoagulant and centrifuged at 500× g for 15 min.

2.4 Statistical analysis and calculations

Variances were analyzed according to a 2×2 factorial arrangement in a 4×4 Latin square design using the GLM procedure of SAS [24]. Data were analyzed using the model:

$$Y_{ijk} = \mu + M_i + E_j + A_k + P_l + MEij + \varepsilon_{ijk}$$

where: Y_{ijk} is the variance, μ is the overall mean, M_i is the levels of FCR supplemented at 15 and 20 g/kg (i = 1, 2), E_j is the levels of PELFUR at 15 and 30 g/kg (j = 1, 2), A_k is the effect of the ruminant (k = 1,2,3,4); P_l is the effect of the each period (l = 1,2,3,4); ME_{ij} is the interaction effect, and ε_{ijk} is the residue. The means of the variances were reported with the

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standard error of the mean. Duncan's multiples ranging test was run to check the statistical differences of treatment means at $p < 0.05$.

3. Results

3.1 Nutritional contents in the PELFUR, FCR, and rice straw

The chemical compositions of the PELFUR, FCR, and rice straw are shown in Table 1. The PELFUR was formulated for supply to meet the nutritional requirements of Thai native beef cattle [25]. The PELFUR consisted of CP, NDF, ADF, and sulfur at 142–143 g/kg DM, 192–187 g/kg DM, 111–104 g/kg DM, and 15–30 g/kg DM, respectively. The FCR consisted of DM, CP, NDF, ADF, and cyanide concentration at 351 g/kg, 13 g/kg DM, 101 g/kg DM, 54 g/kg DM, and 106 mg/kg, respectively. Rice straw was used as a roughage source containing high fiber contents of NDF, and ADF at 718 and 452 g/kg DM, respectively.

3.2 Nutrient intake and apparent digestibility of nutrients

The effects of FCR supplementation with PELFUR on feed intake and digestibility in Thai native beef cattle are presented in Table 2. No interaction effect was found among FCR supplementation and PELFUR in terms of feed intake and nutrient intake ($p > 0.05$). Intakes of rice straw, PELFUR, and total intake ranged from 31.36 to 38.34 g/kg $BW^{0.75}$, 30.79 to 33.44 g/kg $BW^{0.75}$, and 78.82 to 93.40 g/kg $BW^{0.75}$, respectively. Cyanide intake was significantly increased based on FCR supplementation ($p < 0.05$), whereas sulfur intake was increased by the addition of PELFUR levels ($p < 0.05$). The intake of DM ranged from 3.54 to 4.16 kg/d. There were interaction effects among FCR supplementation and PELFUR on digestibility coefficients of DM and organic matter (OM) ($p < 0.05$). FCR supplementation at 20 g/kg BW with PELFUR 30 g/kg demonstrated the highest digestibility of DM and OM at about 0.70 and 0.74, respectively. However, coefficient digestibility of CP, NDF, and ADF were not significantly altered by FCR supplementation with PELFUR, which ranged from 0.62 to 0.66, 0.60 to 0.64, and 0.54 to 0.60, respectively ($p > 0.05$).

3.3 Ruminal fermentation parameters, microorganisms, and blood metabolites

The effect of FCR supplementation with PELFUR on ruminal fermentation parameters and microorganisms are presented in Table 3. There were no interactions between FCR and PELFUR on ruminal pH, temperature, NH_3-N, and protozoal population ($p > 0.05$), which showed a consistent range of 6.45 to 6.87, 38.58 to 39.30 °C, 15.51 to 19.78 mg/dL, and 8.25 x 10^6 to 10.75 x 10^6 cell/mL, respectively. However, interactions were observed between FCR and PELFUR for bacterial populations ($p < 0.01$). The populations of bacteria were highest in FCR supplementation at 20 g/kg BW with PELFUR 30 g/kg at 0 and 4 h post-feeding and the mean values were range from 41.63 × 10^8, 43.41 × 10^8, and 42.52 ×10^8 cell/mL, respectively.

The effect of FCR supplementation with PELFUR on blood metabolites is presented in Table 4. There was no interaction between FCR and PELFUR on BUN ($p > 0.05$). An interaction effect from feeding FCR with PELFUR was found on blood thiocyanate concentrations at various feeding times ($p < 0.01$). The highest mean values of blood thiocyanate were observed when feeding FCR at 20 g/kg BW with PELFUR at 30 g/kg, and at approximately 22.19 mg/dL.

3.4 Characteristics of ruminal VFA profiles

The effects of FCR supplementation with PELFUR on characteristics of rumen VFA profiles are presented in Table 5. No interaction effect was found between FCR and PELFUR on total VFA and their profiles ($p >0.05$). However, the proportions of the total VFA at 0 and 4 h post-feeding and the mean value were increased at approximately 5.09%, when FCR at 20 g/kg BW was supplemented ($p < 0.01$). In addition, FCR at 20 g/kg BW could enhance concentration of propionate (C3) at 4 h post-feeding by approximately 4.00% when compared with FCR at 15 g/kg BW ($p < 0.01$). Moreover, supplementation of PELFUR at 30 g/kg increased the total VFA at 0 and 4 h post-feeding and the mean value to approximately 1.98% ($p < 0.01$), whereas the concentration of C3 at 4 h post-feeding was enhanced by 3.2% ($p < 0.05$).

3.5 Nitrogen (N) utilization

The effects of FCR supplementation with PELFUR on nitrogen utilization are presented in Table 6. There were no interactions between FCR and PELFUR on N intake, fecal N excretion, urinary N excretion, N absorption, N retained, and N retained/N intake in Thai native cattle ($P > 0.05$). Moreover, no significant changes were found for any parameters among treatments and between the main effect of FCR and PELFUR supplementation ($p > 0.05$). Total N intake and N retained ranged from 45.61 to 49.61 g/day and 24.15 to 30.24 g/day, respectively, for all levels of FCR supplementation with PELFUR.

4. Discussions

4.1 Nutritional contents in the PELFUR, FCR, and rice straw

PELFUR had a degree of starch gelatinization from cassava chips and other ingredients containing starch, which are produced by combining moisture, heat, and pressure on feed ingredients, resulting in rumen microbes making better use of the nutrients in PELFUR [26]. As expected, PELFUR prevents the segregation of ingredients in a mixture and could improve the palatability, density, and keeping quality of feedstuffs [27]. The dominance of PELFUR is not only easier for transportation and storage but also for improving flow properties and feeding, particularly less dust and less wastage [13]. It is known that the lack of ruminal motility and loss of appetite can be observed in cases of intoxication due to

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excessive sulfur. However, no such symptoms have been observed in the current research, which indicates the values found for sulfide in feces can be considered sufficient for consumption, despite the lack of reference values in the research. Meanwhile, the present study revealed that the total intake and digestibility coefficients ranged from 3.54 to 4.16 kg/d, and 0.66 to 0.70 was in the normal range when the ruminants received low-quality roughage, such as rice straw [28]. Therefore, it is possible that sulfur levels of 15 and 30 g/kg DM in PELFUR ensured an adequate supply for the rumen microorganisms and FCR cyanide detoxification in animals [29]. In addition, a sulfur level in PELFUR could be useful for animals because sulfur is required as a component of sulfur amino acids, such as cysteine, methionine, and cysteine, together with B vitamin thiamine and biotin for the maintenance and metabolites of many ruminal microbes, especially cellulolytic microorganisms [28,30].

The cyanide concentration in FCR was within the range noted in previous reports of 85–114 mg/kg on a fresh weight basis [5,31]. Dagaew et al. [3] noted that FCR contained HCN at 103.5 mg/kg, which was similar to the present study. However, variable levels of cassava toxicity were reported in the literature, with the total content depending on crop variety, altitude, geographic location, harvesting period, and seasonal conditions. Supapong et al. [7] reported that cassava produced in wet areas contained comparatively less cyanide than that produced in arid locations.

4.2 Nutrient intake and apparent digestibility of nutrients

FCR supplementation with PELFUR did not affect the intake of rice straw, PELFUR, and total feed intake. It was indicated that high levels of FCR supplementation and PELFUR has no negative affect nutrient intake. Sulfur intakes were in the range required for cattle fed with PELFUR 15 g/kg and PELFUR 30 g/kg, respectively. The current study demonstrated that Thai native beef cattle consumed sulfur ranging between 20–40 g/day, which was in the

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optimal range for nutrient intake and digestibility. NRC [28] demonstrated steer had a sulfur demand of about 5 g/day and a maximum tolerable level of 100 g/day. Therefore, nontoxic sulfur has been recognized as in a suitable range for steer [30]. In addition, a dose higher than 15 g/kg may be profitable for detoxifying cyanide and promoting rumen bacterial synthesis. Previous research feeding a feed block containing high sulfur (FBS; 40 g/kg) to cattle demonstrated similar total DMIs when compared to the low sulfur (20 g/kg) fed group (100-110 g/kg $BW^{0.75}$) [9]. Similarly, Supapong et al. [7] reported the sulfur supplementation at 10 and 20 g/kg in a fermented total mixed ration (FTMR) diet did not change the nutrient intake. Moreover, the daily cyanide intake was increased in steers fed with an increased FCR supplementation (217.45 to 313.01 ppm/day), but no toxicity was observed. Meanwhile, PELFUR containing sulfur may supply cyanide- reducing enzymes, such as rhodanese and β mercaptopyruvate sulfurtransferase, which are excreted by rumen microorganisms [3,4].

The apparent digestibility of DM and OM increased when supplemented at 20 g/kg FCR. This could be because of the high concentration of nonstructural carbohydrates in the FCR, which can easily break down in rumen. Increases in apparent digestibility may be responsible for increasing the intake of DM because the intake of a ruminant is actively associated with the digestibility of the diet [3]. Furthermore, animals that received sulfur in PELFUR can contribute to the growth of ruminal microorganisms and result in an improved digestibility of nutrients. Supapong et al. [7] indicated that sulfur is important for the improvement of rumen microbes and for the processing of essential amino acids during bacterial protein synthesis. These findings were in agreement with Promkot and Wanapat [29], who also found that dairy cattle receiving 4 g/kg of sulfur could improve fiber digestion compared to those fed with 1.5 g/kg of sulfur. Similarly, Cherdthong et al. [9] found that the apparent digestibility of DM and OM was significantly higher in ruminants that received a high-sulfur feed block at 40 g/kg with FCR supplementation than in those that received a sulfur feed block at 20 g/kg.

4.3 Ruminal fermentation parameters, microorganisms, and blood metabolites

The pH and temperature were examined to be ideal for the activity of ruminal microbial populations in the digestion of fiber [7]. Rumen pH is one of the most important determinants of rumen action, as microbes of cellulolytic do not grow under pH 6.0, whereas a small rise in ruminal pH favors the activity of these microbes [3]. Intriguingly, the sulfur levels in PELFUR at 15 and 30 g/ kg showed no negative impacts to the rumen pH when doses of FCR increased. This may be because of sufficient synchronization of N and the energy supply for maximizing growth of rumen microbes with sulfur as an amino acid source [26]. These results were in agreement with Nocek and Russell [32], who showed that the dietary sulfur concentration enhanced microbial growth and the amount of sulfur required by rumen microorganisms for the synthesis of methionine and cysteine ranged from 0.11 to 0.20% of the total diet. Therefore, feeding different levels of FCR could maintain ruminal pH.

The concentration of ruminal NH_3-N at average values of 0 h, 4 h, and post-feeding were not affected when FCR supplementation or PELFUR levels increased. However, the values were closer to suitable concentrations of NH_3-N (15-30 mg/dL) [1] for maximizing bacterial protein synthesis, feed digestion, and feed efficiency. No increase in the NH_3-N concentration after increasing the PELFUR levels indicated that NH_3-N might meet the requirements for rumen microorganism synthesis. Similarly, Qi et al. [33] indicated that NH_3-N was not adversely affected by the addition of sulfur to a goat growth diet. Cherdthong et al. [9] also investigated that feeding FCR with a high-sulfur feed block did not change the concentration of rumen NH_3-N.

The increase in the bacterial population when levels of FCR supplementation with PELFUR increased may be because FCR provides the fermentable carbohydrates for rumen bacterial synthesis by using the amino acid sulfur from PELFUR [3]. In addition, rumen microorganisms can convert sulfate to hydrogen sulfite, which is supplied to generate cysteine and methionine for bacterial synthesis. To maintain maximum microbial growth of the rumen, sulfur is primarily required. Therefore, the rumen microbes could have improved further with a consistent supply of energy and sulfur for fermentation in the rumen. Similar to Supapong et al. [7], the present study found that populations of rumen bacteria increased when FTMR containing 400 g/kg FCR and 20 g/kg sulfur was fed to the cattle.

The average BUN concentration in animals fed FCR with PELFUR was in the normal range and was close to the numbers reported by Cherdthong et al. [9] (10–15 mg/dl), who studied beef cattle receiving FCR with FBS. These results indicated that feeding FCR and PELFUR have no adverse effects on animal health. BUN concentration is positively correlated with ruminal NH_3-N [2]. This study also found that the dietary treatments had no influence on ruminal NH_3-N, resulting in no effects to BUN. Similarly, Promkot and Wanapat [29] found that 25 g/kg BW of fresh cassava foliage did not affect BUN, liver enzymes, and triiodothyronine and thyroxine concentrations of the thyroid gland in local cattle.

Increased levels of sulfur at 30 g/kg in PELFUR increased the blood thiocyanate concentration. It could be due to cyanide consumption from FCR and sulfur inclusion in the PELFUR. The increased levels of thiocyanate in the plasma of cows fed FCR in this study were due to a relative increase of cyanide consumption. The enzyme rhodanese is a sulfurtransferase that converts of cyanide and thiosulphate to the less toxic thiocyanate, which is eliminated through urine [29]. The NRC [28] has shown that animals receiving cyanide in their feed may need a high-sulfur supplement to stimulate sulfur detoxification by rumen bacteria and release of thiocyanate in the form of urine [4]. Similar to Promkot et al.[34], studies have shown that the addition of 5 g / kg of sulfur with fresh cassava leaves can decrease cyanide concentrations, while thiocyanate concentrations increased during *in vitro* fermentation. Supapong and Cherdthong [4] also observed that the proportion of sulfur in the diet was associated with blood thiocyanate concentrations in dairy cows that received a FTMR containing FCR 400 g/kg with 10 and 20 g/kg sulfur. In addition, the addition of 4 g/kg of elemental sulfur to fresh leaf-based cassava diets ensured sufficient cyanide detoxification and conversion to thiocyanate in cattle [34].

4.4 Characteristics of rumen VFA profiles

Feeding with exceedingly high levels of FCR (20 g/kg BW) and PELFUR (30 g/kg) had a positive effect on total VFA and C3 concentrations. This may be because FCR contains high ratio fermentable starch, which is quickly fermented into total VFA and C3 in the rumen. The C3 is absorbed into the blood and then converted into glucose in the liver. Moreover, some research has stated that the high production of VFA in rumen is related to the digestibility of feed and the number of microbes in the rumen, which may also be observed in the present work [4]. Wanapat and Khampa [2], who stated that the supplemented soluble carbohydrate from FCR in steer diets improved the concentration of C3 in the rumen, suggests this view. Similarly, Cherdthong et al. [9] revealed that feeding 15 g/kg BW of FCR with FBS increased C3 in the rumen of a steer by 16% when compared to the 10 g/kg BW group. In addition, sulfur levels in PELFUR influenced the C3 concentration, and it is capable that sulfur might meet the demand of rumen microbes for cyanide detoxification, leading to sulfur sufficiency in steer that received PELFUR containing 15 to 30 g/kg of sulfur and increasing rumen C3 concentration [29]. Moreover, when a high amount of sulfur is added, an increase in the ruminal C3 might also mean that C3 can be used as a sink for hydrogen sulfide when more ruminally available sulfide is provided in excess [3].

4.5 Nitrogen utilization

Feeding FCR with PELFUR supplementation did not change the nitrogen (N) balance parameters. Total N intakes were identical in all groups, as every steer consumed the ad libitum diet. Thus, the steers were fed the same dose of total intake and demonstrated no change in total N intake, which was similar to the range for Thai-indigenous beef cattle fed rice straw-based diets. In the present experiment, N intake, N absorption, and N retention were in the optimum range for microbial protein synthesis (30-50, 15-35, and 30-40 g/day, respectively) [7,13]. In a previous study, Supapong et al. [7] revealed that the addition of sulfur in FTMR containing FCR did not change the total N intake and N excretion, as all steers were fed the diet ad libitum. Thus, all steers consumed similar doses of DMI, which showed no difference on N utilization for all parameters.

5. Conclusions and recommendation

Based on our data, it could be concluded that feeding FCR 20 g/kg BW with PELFUR 30 g/kg was productive to Thai native beef cattle. The feeding of two combinations could promote the nutrient digestibility, the bacterial populations, and the rate of disappearance of cyanide without having any adverse effect on rumen fermentation, whereas total VFA and C3 increased when FCR was supplemented at 20 g/kg BW and PELFUR-30 g/kg. In addition, the high levels of cyanide from FCR can be converted to thiocyanate by adding PELFUR, which is a nontoxic form for ruminants. Nevertheless, further research on lactating milk production influenced by the feeding of FCR with PELFUR needs to be confirmed.

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Table 1. Ingredient and chemical composition of pellet containing high sulfur (PELFUR), fresh cassava root (FCR), and rice straw in the experiment.

Item	PELFUR 15	PELFUR 30	FCR	Rice Straw
Ingredients, g/kg DM				
Cassava chips	523	518		
Soybean meal	151	146		
Rice bran	150	145		
Coconut meal	45	45		
Palm kernel meal	55	55		
Minerals and vitamins	10	10		
Sulfur powder	15	30		
Urea	14	14		
Salt	10	10		
Molasses	27	27		
Chemical composition				
Dry matter, g/kg	912	921	351	929
Organic matter, g/kg DM	936	934	963	896
Crude protein, g/kg DM	142	143	13	25
Neutral detergent fiber, g/kg DM	192	187	101	718
Acid detergent fiber, g/kg DM	111	104	54	452
Sulfur, g/kg DM	15	30	-	-
Cyanide, ppm	-	-	106	-

PELFUR 15, pellet containing high sulfur at 15 g/kg; PELFUR 30, pellet containing high sulfur at 30 g/kg; FCR, fresh cassava root

Table 2. Feed intake and digestibility of Thai native beef cattle fed fresh cassava root (FCR) with pellet containing high sulfur (PELFUR).

Items	FCR 15 g/kg BW		FCR 20 g/kg BW		SEM	P-value		
	PELFUR 15	PELFUR 30	PELFUR 15	PELFUR 30		F	P	F x P
DM intake								
Rice straw								
kg/day	1.63	1.44	1.65	1.41	0.14	0.98	0.15	0.87
g/kg $BW^{0.75}$	38.34	31.98	37.12	31.36	3.71	0.81	0.13	0.94
PELFUR								
kg/day	1.42	1.38	1.49	1.45	0.07	0.31	0.54	0.95
g/kg $BW^{0.75}$	33.02	30.79	33.44	32.03	0.94	0.39	0.08	0.67
Sulfur intake								
kg/day	0.02	0.04	0.02	0.04	0.01	0.31	<0.01	0.78
g/kg $BW^{0.75}$	0.51	0.94	0.52	0.97	0.26	0.40	<0.01	0.55
FCR								
kg/day	0.79	0.72	1.02	1.04	0.06	<0.01	0.68	0.46
g/kg $BW^{0.75}$	18.35	16.06	22.83	22.99	1.16	<0.01	0.37	0.31
Cyanide intake								
ppm/day	237.89	217.45	307.07	313.01	17.23	<0.01	0.68	0.46
g/kg $BW^{0.75}$	0.006	0.005	0.007	0.007	0.001	<0.01	0.37	0.31
Total intake								
kg/day	3.84	3.54	4.16	3.89	0.17	0.07	0.13	0.93
g/kg $BW^{0.75}$	89.71	78.82	93.40	86.39	4.22	0.21	0.06	0.65
Nutrient intake, kg/d								
Dry matter	3.84	3.54	4.16	3.89	0.17	0.07	0.13	0.93
Organic matter	3.55	3.27	3.86	3.61	0.16	0.06	0.12	0.92
Crude protein	0.25	0.25	0.27	0.26	0.01	0.21	0.75	0.96

aNeutral detergent fiber	1.52	1.36	1.58	1.39	0.10	0.72	0.12	0.89
Acid detergent fiber	0.94	0.83	0.97	0.84	0.07	0.75	0.10	0.89
Digestibility coefficients								
Dry matter	0.65[a]	0.66[a]	0.67[b]	0.70[c]	0.01	<0.01	<0.01	<0.05
Organic matter	0.70[a]	0.72[b]	0.73[c]	0.74[d]	0.01	<0.01	<0.01	<0.05
Crude protein	0.62	0.65	0.66	0.66	0.03	0.38	0.59	0.54
aNeutral detergent fiber	0.60	0.60	0.64	0.60	0.04	0.59	0.53	0.52
Acid detergent fiber	0.55	0.54	0.60	0.54	0.04	0.47	0.38	0.59

[a,b]Means in the same row with different superscripts differ ($p < 0.05$). F: p-value level of fresh cassava root. P: p-value level of sulfur in pellet. SEM: standard error of mean. FCR 15, fresh cassava root at 15 g/kg BW; FCR 20, fresh cassava root at 20 g/kg BW; PELFUR 15, pellet containing high sulfur at 15 g/kg; PELFUR 30, pellet containing high sulfur at 30 g/kg

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Table 3. Effects of fresh cassava root (FCR) with pellet containing high sulfur (PELFUR) on rumen ecology and microorganisms in Thai native beef cattle.

Items	FCR 15 g/kg BW		FCR 20 g/kg BW		SEM	P-value		
	PELFUR 15	PELFUR 30	PELFUR 15	PELFUR 30		F	P	F x P
Rumen ecology								
Ruminal pH								
0 h post feeding	6.84	6.84	6.87	6.80	0.09	0.94	0.48	0.51
4 h post feeding	6.55	6.71	6.45	6.68	0.17	0.70	0.27	0.82
Mean	6.70	6.78	6.66	6.75	0.10	0.72	0.42	0.97
Ruminal temperature, ◦C								
0 h post feeding	39.30	39.00	39.15	39.25	0.30	0.87	0.74	0.51
4 h post feeding	38.70	38.58	38.68	39.05	0.29	0.45	0.67	0.40
Mean	39.00	38.79	38.91	39.15	0.17	0.42	0.94	0.20
NH_3-N concentration, mg/dL								
0 h post feeding	16.46	15.51	16.62	15.47	0.66	0.93	0.14	0.89
4 h post feeding	19.78	18.78	19.00	18.81	1.11	0.88	0.73	0.86
Mean	17.92	17.14	17.81	17.14	0.49	0.91	0.16	0.91
Ruminal microbes, cell/mL								
Protozoa$\times10^6$								
0 h post feeding	8.25	8.63	8.25	8.75	0.41	0.88	0.31	0.88
4 h post feeding	10.75	10.61	10.63	10.34	0.50	0.70	0.68	0.88
Mean	9.50	9.62	9.44	9.54	0.37	0.86	0.77	0.99
Bacteria$\times10^8$								
0 h post feeding	32.66[a]	35.38[b]	37.31[c]	41.63[d]	0.20	<0.01	<0.01	<0.01
4 h post feeding	34.95[a]	37.95[b]	38.34[b]	43.41[c]	0.13	<0.01	<0.01	<0.01
Mean	33.81[a]	36.66[b]	37.83[c]	42.52[d]	0.11	<0.01	<0.01	<0.01

[a,b,c]Means in the same row with different superscripts differ ($p < 0.05$). F: p-value level of fresh cassava root. P: p-value level of sulfur in pellet. SEM: standard error of mean. FCR 15, fresh

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cassava root at 15 g/kg BW; FCR 20, fresh cassava root at 20 g/kg BW; PELFUR 15, pellet containing high sulfur at 15 g/kg; PELFUR 30, pellet containing high sulfur at 30 g/kg

Table 4. Feeding of fresh cassava root (FCR) with pellet containing high sulfur (PELFUR) in Thai native beef cattle on blood urea-nitrogen and blood thiocyanate.

Items	FCR 15 g/kg BW		FCR 20 g/kg BW		SEM	P-value		
	PELFUR 15	PELFUR 30	PELFUR 15	PELFUR 30		F	P	F x P
Blood parameters								
Blood urea-nitrogen, mg/dL								
0 h post feeding	12.50	12.75	13.00	13.50	0.59	0.31	0.54	0.84
4 h post feeding	14.13	14.75	15.25	15.50	0.86	0.30	0.62	0.83
Mean	13.31	13.75	14.13	14.50	0.63	0.24	0.53	0.96
Blood thiocyanate, mg/dL								
0 h post feeding	11.88[a]	13.13[b]	16.88[c]	19.88[d]	0.39	<0.01	<0.01	<0.01
4 h post feeding	15.75[a]	17.50[b]	21.00[c]	24.50[d]	0.31	<0.01	<0.01	<0.01
Mean	13.81[a]	17.19[b]	17.06[b]	22.19[c]	0.27	<0.01	<0.01	<0.01

[a,b]Means in the same row with different superscripts differ ($p < 0.05$). F: p-value level of fresh cassava root. P: p-value level of sulfur in pellet. SEM: standard error of mean. FCR 15, fresh cassava root at 15 g/kg BW; FCR 20, fresh cassava root at 20 g/kg BW; PELFUR 15, pellet containing high sulfur at 15 g/kg; PELFUR 30, pellet containing high sulfur at 30 g/kg

Table 5. Ruminal volatile fatty acid (VFA) profiles in Thai native beef cattle fed fresh cassava root (FCR) with pellet containing high sulfur (PELFUR).

Items	FCR-15 g/kg BW		FCR-20 g/kg BW		SEM	P-value		
	PELFER 15	PELFUR 30	PELFUR 15	PELFUR 30		F	P	F x P
Total VFA, mmol/L								
0 h post feeding	100.79	102.49	104.96	107.13	0.32	<0.01	<0.01	0.47
4 h post feeding	103.84	106.25	110.05	112.21	0.34	<0.01	<0.01	0.71
Mean	102.31	104.37	107.51	109.67	0.25	<0.01	<0.01	0.83
VFA profiles, mol/100 mol								
Acetic acid								
0 h post feeding	62.61	63.34	61.83	62.91	0.64	0.36	0.18	0.79
4 h post feeding	63.62	63.58	63.15	62.61	0.36	0.07	0.43	0.50
Mean	63.46	63.12	62.49	62.76	0.43	0.15	0.93	0.49
Propionic acid								
0 h post feeding	27.39	27.55	27.99	27.42	0.42	0.60	0.64	0.41
4 h post feeding	27.76	28.18	28.85	29.27	0.17	<0.01	<0.05	0.99
Mean	27.66	27.79	28.42	28.35	0.29	0.06	0.92	0.73
Butyric acid								
0 h post feeding	10.00	9.11	10.19	9.67	0.48	0.45	0.17	0.70
4 h post feeding	8.61	8.23	8.00	8.11	0.36	0.33	0.72	0.50
Mean	8.67	9.31	9.09	8.89	0.31	0.99	0.50	0.21
Acetic/propionic acid ratio	2.29	2.28	2.20	2.22	0.03	0.06	0.90	0.67

[a,b]Means in the same row with different superscripts differ ($p < 0.05$). F: p-value level of fresh cassava root. P: p-value level of sulfur in pellet. SEM: standard error of mean. FCR 15, fresh cassava root at 15 g/kg BW; FCR 20, fresh cassava root at 20 g/kg BW; PELFUR 15, pellet containing high sulfur at 15 g/kg; PELFUR 30, pellet containing high sulfur at 30 g/kg

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Table 6. Effects of fresh cassava root (FCR) with pellet containing high sulfur (PELFUR) on N utilization of Thai native beef cattle.

Items	FCR 15 g/kg BW		FCR 20 g/kg BW		SEM	P-value		
	PELFUR 15	PELFUR 30	PELFUR 15	PELFUR 30		F	P	F x P
Nitrogen (N) balance, g/d								
N intake	46.31	45.61	49.46	48.94	1.76	0.09	0.74	0.96
N fecal	15.24	13.73	14.26	14.21	0.94	0.80	0.42	0.45
N urine	6.92	5.67	7.10	4.49	1.39	0.73	0.19	0.63
N absorption	31.07	31.89	35.19	34.73	2.23	0.14	0.94	0.78
N retained	24.15	26.22	28.09	30.24	2.58	0.15	0.43	0.99
N retained/N intake	0.51	0.57	0.57	0.62	0.04	0.25	0.19	0.92

F: p-value level of fresh cassava root. P: p-value level of sulfur in pellet. SEM: standard error of mean. FCR 15, fresh cassava root at 15 g/kg BW; FCR 20, fresh cassava root at 20 g/kg BW; PELFUR 15, pellet containing high sulfur at 15 g/kg; PELFUR 30, pellet containing high sulfur at 30 g/kg