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Original Article

Extension of raw milk quality through supplementation of hydrocyanic acid from fresh cassava peel in dairy cattle diet

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Abstract

The objective of this study was to evaluate the effects on the extension of raw milk quality through supplementation of hydrocyanic acid (HCN) levels from fresh cassava peel (FCPe) in dairy cattle diet by increasing the milk thiocyanate (SCN^-) concentration and lactoperoxidase (LP) activity. The sample was twenty-four Holstein Friesian crossbred lactating dairy cows, averaging 87±31 days in milk (DIM), 13.4±2.9 kg of milk and 397±52 kg body weight (BW). All cows were fed the control diet with 6.5 kg/d of 21% crude protein (CP) concentrate and *ad libitum* grass silage (GS). The treatments groups were as follows: 1) the control diet for the 1st group, the 2nd group received the control diet supplemented with 400 g/d of FCPe (75 ppm HCN) and the 3rd group received the control diet supplemented with 800 g/d of FCPe (150 ppm HCN). The results showed that 800 g/h/d FCPe enhanced the efficiency of LP activity in raw milk to reduce total bacterial count (TBC) and coliform count (CC); therefore, 400 g/h/d FCPe can be used in the concentrate for lactating dairy cows.

Keywords: fresh cassava peel, hydrocyanic acid, milk thiocyanate, lactoperoxidase activity, dairy cow's diet

1. Introduction

The quality of raw milk preservation during transportation is an exceedingly important factor for milk products because some dairy cattle farms are in areas remote from the milk collection centers, especially for those small holdings which require a lengthy duration for transportation and mechanical refrigeration is either unavailable or economically prohibitive. Without refrigeration, milk can only be transported a distance of up to 20 km, after which it begins to deteriorate, beginning the process of acidification (Bennett, 2001). The dispersed nature of milk production across diverse farm operations, difficulties with collection, poor handling systems and inadequate transportation and refrigeration systems all create considerable challenges to the extension of the keeping quality (KQ) of milk during storage in several developing countries (Seifu, Buys, & Donkins, 2005). This is especially the case for smallholder dairy farmers in Thailand where cooling facilities and poor handling systems are often unavailable in rural areas which lack appropriate facilities, including the refrigeration required to maintain milk quality. In the past, raw milk was stored in unrefrigerated conditions and transported in bulk collections to a collection center. This method of collection would often result in delays between milking and the final point of delivery at a dairy processing plant of may be more than six hours (h) which had consider-

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able negative effects on the quality of the raw milk. These issues contributed to a higher frequency of milk being rejected by the dairy factories as the product would not be acceptable to consumers (Barabas, 1994). Under these circumstances, producers sought their own methods of preservation for raw milk in order to address these KQ issues. However, the subsequent addition of chemicals, such as antibiotics and formaldehyde, represented more risks than benefits to public health. In previous studies, the addition of small quantities of thiocyanate (SCN^-) and/or hydrogen peroxide (H_2O_2) to raw milk was used to stimulate the natural antibacterial function present in milk known as the Lactoperoxidase system (LPs) and to significantly extend the shelf-life of raw milk. Although LPs was not expected to present risks to consumers, the subsequent direct addition of milk preservative chemicals represented potential risks to the LPs, especially the use of H_2O_2 which still requires significant expertise on the part of the users.

The LPs has been reported as an approved method for raw milk preservation by inhibiting microorganism growth as an alternative to expensive artificial cooling systems and frequently poor standards of hygiene, particularly in some developing tropical countries (Food and Agriculture Organization of the United Nations [FAO]/World Health Organization [WHO], 2005). LPs technology could become appropriate for farmers, by using the dietary sources of SCN^-; glucosinolates and cyanogenic glycosides (CG) (linamarin and lotaustralin), and CG is found in cassava (Wolfson & Sumner, 1993). Fresh cassava peel (FCPe), an agro-industrial by-product of starch production from cassava root is widely used as feedstuff for ruminants in Thailand because it is not expensive. Cyanogenic glycosides are disintegrated to hydrocyanic acid (HCN) after cassava tissues are destroyed. Cyanide toxin in cyanogenic plants, is transformed into non-toxic SCN^- by the enzyme rhodanese action (Reiter & Härnulv, 1984) in the liver and kidneys of animals (Drakhshan Vaziri & Aminlari, 2004). Thiocyanate is eliminated partly via the milk (Soto-Blanco & Górniak, 2003). Milk SCN^- which is used in the LPs, is a milk protein with antimicrobial functions (Zapico, Gaya, De Paz, Nuñez, & Medina, 1991). As milk LP catalyses, in the presence of H_2O_2, the oxidation of SCN yields hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN) (Shin, Hayasawa, & Lonnerdal, 2001) and iodide ion (I^-) oxidation yields hypoiodite (OI^-) and hypoiodous acid (HOI) (Bosch, van Doorne, & de Vries, 2000), which has potent antibacterial properties in reducing the multiplication of bacteria by damaging the cell membranes and inhibiting the activity of many cytoplasmic enzymes (Haddadin, Ibrahim, & Robinson, 1996). As a result, the quality of raw milk is safeguarded by the LPs. Thus, for dairy farmers with small holdings, the conclusions of this research will help to develop an understanding of how activating LPs during transportation between farms and dairy plants is a superior method for the extension of the KQ of milk during storage and should assist in reducing the incidence of resorting to chemical additives to achieve the same result. The present study was undertaken to examine the effectiveness of KQ by reducing the microbial growth present in milk to enhance the activity of the LPs by increasing milk SCN^- via supplementing the FCPe in dairy cows' diet.

2. Materials and Methods

Two experiments were conducted to determine the effects of the HCN level from FCPe supplementation in the diet of dairy cattle on their productive performance *in vivo*; and to evaluate the effects of two different temperatures and three different HCN levels from FCPe supplementation on raw milk samples *in vitro* assay.

Experiment 1 (Exp. 1) was to determine the effect of the HCN level from FCPe supplementation in the diet of dairy cattle on nutrient intake, productive performance, milk yield and composition, SCN^- concentration and LP activity in raw milk.

Experiment 2 (Exp. 2) was to examine whether the HCN level from FCPe supplementation in the diet of dairy cattle decreases bacterial counts as an effective treatment to extend raw milk quality during storages. This part of the experiment was conducted as a 2x3 factorial in a randomized complete block design (RCRD), where Factor A was temperature (25°C vs 30°C) and Factor B were the different levels of HCN from FCPe supplementation in the diet of dairy cattle (0 ppm HCN (0 g/h/d FCPe), 75 ppm HCN (400 g/h/d FCPe) and 150 ppm HCN (800 g/h/d FCPe)) with eight replicates per run using intervals of 0, 2, 4, 6, 8, 10, and 12 hrs for incubation time.

2.1 Experimental design and treatments

Twenty-four Holstein Friesian crossbred (early-mid) lactating dairy cows, averaging 87±31 DIM, 13.4±2.9 kg of milk and 397±52 kg BW, were blocked by lactation first and then stratified random balanced based on DIM, milk yield and BW. All cows were fed with 6.5 kg/d of 21% CP concentrate with *ad libitum* grass silage (GS) (Hybrid Napier; *Pennisetum purpureum x Pennisetum americanum*). Three treatments were as follows: the control diet; the control diet supplemented with 400 and 800 g/d FCPe. The experiment lasted for eight weeks, including the first two weeks as an adaptation period, followed by six weeks for measurement period. All cows were weighed at the start and end of the experiment.

2.2 Fresh cassava peel collection

The FCPe was obtained from Korat Flour Industry CO., LTD., Nakhon Ratchasima, Thailand. The FCPe samples were divided into two parts: the first part was fed to the experimental cows; the second part was used for proximate and detergent analyses and the analysis of cyanide content was conducted by the Pyridine Pyrazorone method (O'Brien,

Taylor, & Poulter, 1991) at Cassava and Starch Technology Research Unit (CSTRU), Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Bangkok, Thailand.

2.3 Laboratory analyses

Feeds offered and residues were weighed for two consecutive days weekly. Feed samples were taken and dried at 60°C for 48 hrs. At the end of the experimental period, feed samples were composited and subsamples were taken for further chemical analysis. Samples were ground through a 1 mm screen and subjected to proximate analysis. CP was determined by Kjeldahl method (Association of Official Analytical Chemists International [AOAC], 1998). Ether extract was determined by using petroleum ether in a Soxtec System (AOAC, 1998). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined using the method described by Van Soest, Robertson, and Lewis (1991), adapted for Fiber Analyzer. Chemical analysis was expressed on the basis of final DM.

Cows were milked twice daily at 05:00 and 15:00 and milk yields were recorded daily for each cow. Milk samples were collected on two consecutive days of each period. After collection, milk samples were divided into two main portions with one stored at 4°C until an analysis for composition using a Foss MilkoScanTM FT2 infrared automatic analyzer (DK-3400 Hillerøed, Denmark) at the Center for Scientific and Technological Equipment Building at SUT. The concentration of SCN^- was determined using the method of Codex Alimentarius Commission (CAC/GL 13-1991). LP activity was determined as described by Isobe, Morimoto, Nakamura, Yamasaki, and Yoshimura. (2009). The second portion of milk samples was also separated into two portions with one stored at 25°C and the other at 30°C and then incubated at 0 to 12 hrs for examination of microbiological properties including standard plate (SP) (Houghtby, Maturin, & Koenig, 1992) and coliform count (CC) (Christen, Davidson, McAllister, & Roth, 1992).

2.4 Statistical analysis

All the data of the performances of the dairy cows were statistically analyzed as RCBD using ANOVA procedure of SAS (SAS, 2002), except that TBC and CC were analyzed separately as 2x3 factorial in RCBD (2 different temperatures x 3 HCN levels from supplementing FCPe levels with eight replicates per run) with temperature, HCN levels from supplementing FCPe levels and their interaction included in the

Table 1. Chemical composition (%DM) of experimental feeds.

Item	Concentrate[1]	Fresh cassava peel	Grass silage
		% of DM	
DM	90.65	25.81	28.54
Ash	9.16	23.44	8.63
CP	20.48	1.19	6.51
EE	2.90	1.91	1.83
CF	13.67	11.26	36.28
NDF	44.03	70.60	61.51
ADF	16.43	16.37	57.06
ADL	7.10	7.02	5.58
NDIN	1.29	0.33	0.17
ADIN	0.45	0.24	0.11
TDN_{1x} (%)[2]	61.82	36.50	55.98
DE_{1x} (Mcal/kg)[3]	2.95	1.63	2.53
DE_p (Mcal/kgDM)[4]	2.88	1.96	2.55
ME_p (Mcal/kgDM)[5]	2.45	1.53	2.12
NE_{LP}[§] (Mcal/kgDM)[6]	1.54	0.89	1.30
Cyanide content (mg/kg dry solid)	-	670.14±1.30	-

DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, NFC: non fiber carbohydrate = 100 - (%NDF + %CP + %Fat + %Ash), NDIN: neutral detergent insoluble nitrogen, ADIN: acid detergent insoluble nitrogen. [§]Calculated using published values of NRC (2001) as follows: [1] Contained (as DM basis): the control concentrate for the first group (without fresh cassava peel supplement (FCPe)) = 18% cassava, 10% rice bran A, 6% molasses, 20% palm kernel meal, 12% soybean meal, 12% bush bean, 17% cassava ethanol, 2.5% urea, 1.6% dicalcium phosphate, 0.4 % premix, 0.02% covatak 570 and 0.5% fat powder. The second and third group (supplementing FCPe at 400 and 800 g/h/d respectively). [2] TDN_{1X} (%), total digestible nutrient = tdNFC + tdCP + (tdFA x 25.25) + tdNDF–7. [3] DE_{1X} (Mcal/kg), digestible energy = [(tdNFC/100)×4.2]+[(tdNDF/100)×4.2]×[(tdCP/100)×5.6]+[(FA/100)×9.4]–0.3. [4] DE_p (Mcal/kg) = {[(TDN_{1X} –[(0.18 × TDN_{1X}) –10.3]) × Intake]/TDN_{1X}} × DE_{1X}. [5] ME_p (Mcal/kg), metabolisable energy at production level= [1.01 x (DE_p) –0.45] + [0.0046 x (EE–3)]. [6] NE_{LP}(Mcal/kg), net energy for lactation = [0.703 × ME_p] –0.19, (EE < 3%). (see before) = [0.703 × ME_p] –0.19) + [(0.097 × ME_p)/97] × [(EE –30], (EE > 3%).

model as fixed factor effects. When the interaction between temperature and HCN levels from supplementing FCPe levels was significant, orthogonal polynomial contrasts were performed to determine linear, quadratic and cubic responses to the temperature within HCN levels from supplementing FCPe levels. When the main effect of HCN levels from supplementing FCPe levels was significant, orthogonal polynomial contrasts were performed to determine overall linear, quadratic and cubic responses to temperature. Significant differences among treatments were assessed by Duncan's multiple range test. Overall differences between treatment means were considered to be significant at $P<0.05$. Data are expressed as mean±SEM, which represents the pooled SEM for the model.

3. Results and Discussion

3.1 Feed chemical composition

The chemical compositions of the feeds are presented in Table 1. The analyzed values of chemical composition of the 21% CP concentrate, GS and FCPe are within the range widely reported in the case of the SUT dairy farm by other research studies. Variations in chemical composition and energy values reflect the differences in breeds, the age of harvesting processes, soil types, weather, and seasons. Fresh cassava peel was used as a source of HCN due to high amounts of HCN content (670.14±1.30 ppm HCN). The HCN content of FCPe in this study was in agreement with the literature reported of Tewe & Lyayi (1989) that the HCN content of fresh peel was 364.2 to 814.7 ppm. Tangkawanit, Banterng, Samahadthai, and Susri (2014) reported that cassavas in different areas had different levels of cyanide content, which may involve minerals in soils, season and cultivation practices.

3.2 Animal performance and nutrient intake

The foremost consideration to supplement FCPe in dairy cow feeding is the potential adverse effects of CG which yield high HCN content from FCPe supplementation on feed intake and lactation performance. However, ruminant animals can neutralize the harmful effects of HCN through the activities of rumen microbial enzymes and by absorption from rumen (Majak & Cheng, 1984), cassava can be utilized more efficiently, HCN toxicity is considered to be a limiting issue in using high level of HCN from cassavas in the diets of ruminants. Larson (2006) reported that HCN levels above 600 ppm in forage are toxic for cattle and usually lethal at 1,000 ppm and above. Supplementary feeding with FCPe at 400 and 800 g/d yields an increased intake of HCN levels of 75 and 150 ppm/d respectively. These levels of HCN derived from FCPe ingestion are safe for dairy cows. Intakes of DM in the current study were in agreement with the literature reported that showed greater cytotoxic effects of HCN content from cassava supplement when HCN was at 2 mg/kg BW in cattle and sheep (lethal dose) (Majak & Cheng, 1987). Research on feeding concentrates containing cassava peel, in particular, revealed that the pattern of FCPe to lactating dairy cows is very limited. Therefore, these results showed that there was no significantly different effects found on DM, CP and NE_{LP} intakes, final live weight and live weight change (LWC) of the cows as a result of HCN content from the dietary supplementation with FCPe in the study (Table 2). Supplementing FCPe did not affect the lactating dairy cows in the experiment as the animals were apparently healthy over the course of the trial. This result is supported by Wanapat, Puramongkonand, and Siphuak (2000), the dairy cows' diet with supplemented increased from cassava hay intakes did not have an effect on overall intake.

Cows supplemented with 400 and 800 g/d FCPe, no remarkable changes were found for milk yield and compositions (Table 3). Unchanged milk yield was reported in the study supplementing cassava hay in dairy cattle' diet (Punthanara *et al.*, 2009); whereas milk composition which was agreed with previous study using ensiled cassava foliage (Petlum *et al.*, 2012). Discrepancies among studies on the effect of cassava supplement on milk yield and composition from dairy cows might be due to the differences in types of feed (particularly fiber), a portion of cassava, HCN level and the experimental duration.

Neither the distribution of net energy intake nor rumen degradable protein (RDP) nor rumen undegradable protein (RUP) intakes (Table 4) were affected by 0, 400 and 800 g/d FCPe. All groups of cows had a considerable supply of NE_{LP}, but the milk yields were lower than would have been predicted from NE_{LP} intakes. The respective intakes of 16.27, 16.49, and 17.01 Mcal daily by the control, 75 and 150 ppm HCN, in theory, should have been able to produce approximately 13.3, 14.4 and 13.9 kg milk/d. A lower milk yield than that would be expected from the NE_{LP} available which can be attributed to the probable underestimates of NE_{LM} for dairy cows in the tropics. Since the dairy cows in the tropics are fed lower quality feeds than cows in the United States, the use of the equation suggested by the Nutrient Requirements of Dairy Cattle (NRC, 2001) might be inappropriate. The Australian Agricultural Council (AAC, 1990) recommended that dairy cattle consuming feeds containing energy lower than 10 MJ ME/kg DM (2.39 Mcal ME/kgDM) needed more energy for maintenance. The present study used a net energy for maintenance value of 0.080 Mcal/kg $BW^{0.75}$ for predicting NE_{LM}. If the hypothesis by AAC (1990) is true, we can assume that the average net energy values of milk and LWC are unaffected by the quality of feeds, as in the case of NE_{LM}, the average net energy for maintenance value of 0.081 Mcal/kg $BW^{0.75}$ should be used in this study. Therefore, feeds containing a true protein are needed to increase RDP supply. However, optimum levels of RDP to provide the proper balance of NH_3-N and true protein are not well defined. With poorer-quality forages, which are common in the tropics, undegradable dietary protein generally stimulates milk yield. Therefore, feeds containing a high bypass protein are needed to increase RUP supply.

Table 2. Effects of hydrocyanic acid levels from fresh cassava peel supplementation in the diet of dairy cattle on nutrient intake and productive performance.

Item	Control[1] HCN[2]	75 ppm HCN[3]	150 ppm	SEM	P-value
DM, kg/cow/d					
Concentrate	5.86	5.86	5.86	-	-
Grass silage	5.59	5.68	6.00	0.22	0.327
Fresh cassava peel	0	0.11	0.22	-	-
Total	11.45	11.65	12.08	0.22	0.113
CP, g/cow/d					
Concentrate	1200	1200	1200	-	-
Grass silage	364	370	391	14.20	0.326
Fresh cassava peel	0	1.31	2.62	-	-
Total	1564	1571	1594	14.17	0.276
NE_{LP}, Mcal/cow/d					
Concentrate	9.0	9.0	9.0	-	-
Grass silage	7.27	7.39	7.82	0.28	0.328
Fresh cassava peel	0	0.10	0.20	-	-
Total	16.27	16.49	17.01	0.28	0.165
Live weight change					
Initial live weight, kg	395	401	398	23.33	0.984
Final live weight, kg	380	382	383	21.51	0.995
Live weight change, g/d	-500	-629	-509	156.24	0.810

SEM: standard error of the mean, HCN: hydrocyanic acid, DM: dry matter, CP: crude protein, NE_{LP}: net energy for lactation at production level. [1] The control: 6.5 kg/d concentrate without fresh cassava peel supplement together with *ad libitum* grass silage. [2] The control concentrate supplemented with 400 g/d fresh cassava peel together with *ad libitum* grass silage with approximately 75 ppm HCN (DM basis) by calculation. [3] The control concentrate supplemented with 800 g/d fresh cassava peel together with *ad libitum* grass silage with approximately 150 ppm HCN (DM basis) by calculation.

Table 3. Effects of hydrocyanic acid levels from fresh cassava peel supplementation in the diet of dairy cattle on milk production and composition, thiocyanate concentration and lactoperoxidase activity in raw milk.

Item	Control[1] HCN[2]	75 ppm HCN[3]	150 ppm	SEM	P-value
Milk yield, kg/d	11.32	11.82	12.53	0.83	0.593
3.5% FCM[†], kg/d	12.16	12.53	13.41	0.80	0.247
Fat, g/d	481	459	558	36	0.147
Protein, g/d	308	327	338	20	0.577
Lactose, g/d	486	520	545	50	0.628
Solid-not-fat, g/d	873	930	971	68	0.483
Total solid, g/d	1354	1389	1529	95	0.336
Composition (g/100 g of raw milk)					
Fat	4.25	3.88	4.45	0.18	0.095
Protein	2.72	2.77	2.70	0.06	0.679
Lactose	4.29	4.40	4.35	0.08	0.308
Solid-not-fat	7.71	7.87	7.75	0.08	0.223
Total solid	11.96	11.75	12.20	0.23	0.329
Thiocyanate (ppm)	8.63^{b}	9.19^{b}	9.67^{a}	0.16	0.001
LP activity (U/mL)	4.87^{b}	5.25^{ab}	5.66^{a}	0.16	0.008

SEM: standard error of the mean, HCN = hydrocyanic acid. [†] FCM = fat-corrected milk: 3.5% FCM = (0.432 x milk (kg)) + (16.216 x fat (kg)). [1] The control: 6.5 kg/d concentrate without fresh cassava peel supplement together with *ad libitum* grass silage. [2] The control concentrate supplemented with 400 g/d fresh cassava peel together with *ad libitum* grass silage with approximately 75 ppm HCN (DM basis) by calculation. [3] The control concentrate supplemented with 800 g/d fresh cassava peel together with *ad libitum* grass silage with approximately 150 ppm HCN (DM basis) by calculation. [a, b] Means within a row with different superscripts are significantly different ($P<0.05$).

Table 4. Effects of hydrocyanic acid levels from fresh cassava peel supplementation in the diet of dairy cattle on net energy intake, the supply of rumen degradable protein and rumen undegradable protein.

Item	Control[1] HCN[2]	75 ppm HCN[3]	150 ppm	SEM	P-value
NE_{LP} intake[4] (Mcal/d)	16.27	16.49	17.01	0.28	0.165
NE_{LM}[5] (Mcal/d)	6.97	7.03	7.00	0.30	0.993
NE_{LG}[6] (Mcal/d)	-1.24	-1.56	-1.26	0.11	0.361
NE_{LL}[7] (Mcal/d)	8.25	8.32	9.42	0.57	0.292
NE_{LR}[8] (Mcal/d)	13.98	13.79	15.17	0.80	0.503
Efficiency	0.86	0.84	0.89	0.02	0.301
RDP_{req} intake[9] (g/d)	1047	1060	1091	16.31	0.157
RDP_{sup}[10] (g/d)	872	875	883	5.10	0.256
Deficit/surplus	-175	-185	-208	11.22	0.117
RUP_{req}[11] (g/ d)	894	958	931	115.79	0.924
RUP_{sup}[12] (g/d)	692	696	711	9.07	0.294
Deficit/surplus	-202	-262	-220	115.08	0.926

HCN = hydrocyanic acid. [1] The control: 6.5 kg/d concentrate without fresh cassava peel supplement together with *ad libitum* grass silage. [2] The control concentrate supplemented with 400 g/d fresh cassava peel together with *ad libitum* grass silage with approximately 75 ppm HCN (DM basis) by calculation. [3] The control concentrate supplemented with 800 g/d fresh cassava peel together with *ad libitum* grass silage with approximately 150 ppm HCN (DM basis) by calculation. [4] NE_{LP} = net energy for lactation at production level. [5] NE_{LM} = net energy requirement for maintenance = $0.08 \times LW^{0.75}$. [6] NE_{LG} = net energy requirement for gain = reserve energy × (0.64/0.75). reserve energy = see NRC (2001). [7] NE_{LL} = net energy requirement for lactation = milk yield (kg/d) × (0.0929 × %fat + 0.0547 × %CP + 0.0395 × %lactose). [8] NE_{LR} = net energy retention. Efficiency = NE_{LR}/NE_{LP} intake. [9] RDPreq = rumen degradable protein requirement = 0.15294 × TDN actual. [10] RDPsup = rumen degradable protein supply = total DM fed × 1,000 × diet CP × CP_RDP. [11] RUPreq = rumen undegradable protein requirement = total CPReq – (MP Bact + MP Endo)/diet RUPDigest. [12] RUPsup = rumen undegradable protein supply = CP Total – RDPsup.

3.3 Thiocyanate concentration and lactoperoxidase activity in raw milk

Supplementing FCPe diets resulted in a greater concentration of milk SCN^- when the cows were fed 150 ppm HCN from FCPe or 800 g/d FCPe diets compared to the control diet (Table 3). The results were in agreement with those studies (Buaphan *et al.*, 2003; Petlum *et al.*, 2012; Punthanara *et al.*, 2009), who reported similar increases in milk SCN^- with increasing cassava products in the diet of dairy cows. The high level of HCN content fed diets resulted in marked alternations in the concentration of milk SCN^- relative to the level of supplemented FCPe. These possible changes rely on the high level of HCN in FCPe to alter SCN^-. However, concentration of SCN^- in milk varies according to many factors including breeds and the lactation cycle (Zapico *et al.*, 1991), feed types (Wolfson & Sumner, 1993), and season of the year (Dabur, Srivastava, & Kapoor, 1996). Althaus, Molina, Rodriguez, and Fernandez (2001) reported that SCN^- derives from glucosinolates and the detoxification of the CG which are present in the feed. Zapico *et al.* (1991) demonstrated that the milk SCN^- of cows using natural pastures with clover can increase up to 15 ppm. They also mention possible changes which rely on the high level of HCN in FCPe to alter SCN^- concentrations. Findings indicated an increase in the milk SCN^- was due to the detoxification of cyanide in FCPe by conversion into SCN^- by enzyme rhodanese (Drakhshan Vaziri, & Aminlari, 2004).

Significant differences were obtained between the LP activity levels determined of the control and 800 g/d FCPe, whereas the value of 5.25 U/mL (400 g/d) showed no significant difference from those of 0 and 800 g/d FCPe. In contrast, for dairy cows fed diet treatments supplemented with cassava hay, the milk LP was not affected by the treatments (Punthatnara, 2009). Normally, the LP activity is ubiquitous in cow's milk; the concentration varies widely from between 1.2 up to 19.4 U/mL (Gothefors & Marklund, 1975). Although the results of the supplementation of FCPe in this present study produced slightly less SCN^- concentration in milk for the activity of the LPs, an LP activity at 1.44 U/mL was sufficient to act as a catalyst for effective stimulation of the antibacterial properties of the LPs (Marshall, Cole, & Bramley, 1986). The variations in LP also exhibited a cyclic pattern with alternating peaks and troughs throughout lactation and extremely large variations were observed between and within cows (Fonteh, Grandison, & Lewis, 2002).

3.4 Effects of temperatures and hydrocyanic acid levels from fresh cassava peel supplementation in the diet of dairy cattle on bacterial counts in raw milk

The preservation of the raw milk by LPs showed effective antimicrobial activities and promoted prolonged shelf-life of milk by delaying bacterial growth of both Gram-

negative and Gram-positive microorganisms (Naidu, 2000). This study was undertaken to further explore the possible use the HCN content from FCPe supplementation in the diet of dairy cattle to determine whether such dietary modifications could stimulate LPs to serve as an alternative method of inhibiting the microorganisms' growth for milk preservation. The basic protocols for microbial growth of TBC and CC in milk samples were used as the spoilage model which was designed for between 25 and 30°C as this was based on the average, ambient temperatures recorded for 12 months at the SUT farm (in the range of 26.18-34.20°C or approximately 30.19 ºC).

When supplementing diets with FCPe, positive effects on TBC and CC were monitored from 0 to 12 hrs in milk as shown in Tables 5 and 6, unless their initial pattern was slightly different. These tables show the overall pattern of bacterial growth during storage for the three dietary treatments, and the curves for the control diet were virtually identical, as expected. Cows supplemented with FCPe gave a similar curve, but the initial numbers of TBC and CC declined slightly which continued for approximately 4 and 6 hrs respectively, after which growth continued at lower numbers than the control diet. Although TBC and CC all increased with longer incubation times regardless of temperature as expected, clear reductions were observed in the TBC and CC with increasing FCPe supplementation relative to the control values at both temperatures. The use of FCPe as a dietary supplement led to improved KQ and thus significantly extended the shelf-life of milk by inhibiting the growth of microorganisms evaluated as TBC ($P<0.001$), and CC ($P<0.05$) during storage. The method of activating the LPs decreases the microbial growth under storage conditions, which has demonstrated the effects on KQ and thus can be used to extend the shelf-life of milk (Dajanta, Chukeatirote, & Apichartsrangkoon, 2008). These

Table 5. Effects of temperatures (25°C vs 30°C) and hydrocyanic acid (HCN) levels (0, 75 and 150 ppm HCN) from fresh cassava peel supplementation in the diet of dairy cattle on total bacterial count in raw milk at incubation time (0, 2, 4, 6, 8, 10 and 12 h) (N= 8).

Temperature	Level (ppm HCN)	Number of total bacterial count of hydrocyanic acid levels from fresh cassava peel supplementation in the diet of dairy cattle						
		Incubation time (h)						
		0	2	4	6	8	10	12
25ºC	**Mean**	**$1.66x10^5$**	**$2.49x10^5$**	**$3.43x10^5$**	**$4.74x10^5$**	**$4.45x10^5$**	**$7.32x10^5$**	**$1.15x10^6$**
	0	$1.99x10^{5a}$	$3.07x10^{5a}$	$4.12x10^{5a}$	$6.41x10^{5a}$	$7.18x10^{5a}$	$1.17x10^{6a}$	$1.70x10^{6a}$
	75	$1.62x10^{5b}$	$2.28x10^{5b}$	$3.14x10^{5b}$	$3.94x10^{5b}$	$3.03x10^{5b}$	$5.35x10^{5b}$	$9.61x10^{5b}$
	150	$1.38x10^{5b}$	$2.11x10^{5b}$	$3.03x10^{5b}$	$3.87x10^{5b}$	$3.13x10^{5b}$	$4.94x10^{5b}$	$7.87x10^{5b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l*
30ºC	**Mean**	**$2.41x10^5$**	**$3.13x10^5$**	**$4.01x10^5$**	**$5.40x10^5$**	**$5.08x10^5$**	**$8.33x10^5$**	**$1.34x10^6$**
	0	$2.88x10^{5a}$	$3.52x10^{5a}$	$4.72x10^{5a}$	$7.51x10^{5a}$	$8.00x10^{5a}$	$1.36x10^{6a}$	$1.94x10^{6a}$
	75	$2.32x10^{5b}$	$3.04x10^{5ab}$	$3.54x10^{5b}$	$4.24x10^{5b}$	$3.46x10^{5b}$	$5.68x10^{5b}$	$1.17x10^{6b}$
	150	$2.05x10^{5b}$	$2.82x10^{5b}$	$3.76x10^{5b}$	$4.44x10^{5b}$	$3.78x10^{5b}$	$5.82x10^{5b}$	$9.05x10^{5b}$
	Contrast	*l*	*l*	*l, q*	*l, q*	*l, q*	*l, q*	*l*
HCN level	0	$2.44x10^{5a}$	$3.29x10^{5a}$	$4.42x10^{5a}$	$6.96x10^{5a}$	$7.58x10^{5a}$	$1.76x10^{6a}$	$1.82x10^{6a}$
	75	$1.97x10^{5b}$	$2.66x10^{5b}$	$3.34x10^{5b}$	$4.10x10^{5b}$	$3.25x10^{5b}$	$5.52x10^{5b}$	$1.06x10^{5b}$
	150	$1.72x10^{5c}$	$2.47x10^{5b}$	$3.40x10^{5b}$	$4.16x10^{5b}$	$3.46x10^{5b}$	$5.33x10^{5b}$	$8.46x10^{5b}$
	Contrast	*l*	*l*	*l, q*	*l, q*	*l, q*	*l, q*	*l*
SEM								
Temperature		0.204	0.383	0.381	0.365	0.609	0.259	0.361
HCN level		0.250	0.469	0.467	0.447	0.746	0.317	0.442
Temperature x HCN level		0.354	0.664	0.660	0.632	1.055	0.449	0.625
***P*-value**								
Temperature		<0.001	<0.001	0.002	0.209	0.025	0.390	0.253
HCN level		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temperature x HCN level		0.596	0.744	0.735	0.812	0.837	0.851	0.953

SEM: standard error of the mean. HCN = hydrocyanic acid. ^{a,b,c} Means within a column within temperature or the main effect of HCN level from fresh cassava peel supplementation in the diet of dairy cattle having different superscript letters are different at $P<0.05$. *l, q, c*: Within a column, the effect HCN level from fresh cassava peel supplementation in the diet of dairy cattle for individual temperature or the main effects HCN level from fresh cassava peel supplementation in the diet of dairy cattle is linear, quadratic, and cubic, respectively, at $P<0.05$.

Table 6. Effects of temperatures (25°C vs 30°C) and hydrocyanic acid (HCN) levels (0, 75 and 150 ppm HCN) from fresh cassava peel supplementation in the diet of dairy cattle on coliform count in raw milk at incubation time (0, 2, 4, 6, 8, 10 and 12 h) (*N*= 8).

Temperature	Level (ppm HCN)	Number of total bacterial count of hydrocyanic acid levels from fresh cassava peel supplementation in the diet of dairy cattle						
		Incubation time (h)						
		0	2	4	6	8	10	12
25°C	**Mean**	**$1.88x10^{3}$**	**$2.66x10^{3}$**	**$2.20x10^{4}$**	**$3.41x10^{4}$**	**$2.59x10^{5}$**	**$4.57x10^{5}$**	**$1.65x10^{6}$**
	0	$2.29x10^{3}$	$2.85x10^{3}$	$2.71x10^{4a}$	$5.03x10^{4a}$	$3.17x10^{5a}$	$5.28x10^{5a}$	$2.87x10^{6a}$
	75	$2.06x10^{3}$	$2.57x10^{3}$	$1.97x10^{4b}$	$2.69x10^{4b}$	$2.33x10^{5b}$	$4.21x10^{5b}$	$1.04x10^{6b}$
	150	$1.29x10^{3}$	$2.56x10^{3}$	$1.92x10^{4b}$	$2.50x10^{4b}$	$2.26x10^{5b}$	$4.20x10^{5b}$	$1.02x10^{6b}$
	Contrast	*ns*	*ns*	*l*	*l*	*l*	*l*	*l*
30°C	**Mean**	**$4.27x10^{3}$**	**$6.41x10^{3}$**	**$4.87x10^{4}$**	**$7.49x10^{4}$**	**$5.74x10^{5}$**	**$1.00x10^{6}$**	**$3.64x10^{6}$**
	0	$5.31x10^{3a}$	$7.23x10^{3a}$	$6.06x10^{4a}$	$1.11x10^{5a}$	$7.02x10^{5a}$	$1.16x10^{6a}$	$6.31x10^{6a}$
	75	$4.49x10^{3a}$	$6.21x10^{3b}$	$4.55x10^{4ab}$	$6.14x10^{4b}$	$5.30x10^{5b}$	$9.60x10^{5b}$	$2.37x10^{6b}$
	150	$3.01x10^{3b}$	$5.78x10^{3b}$	$4.00x10^{4b}$	$5.22x10^{4b}$	$4.89x10^{5b}$	$8.69x10^{5b}$	$2.15x10^{6b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l, q*
HCN level	0	$3.80x10^{3a}$	$5.04x10^{3a}$	$4.38x10^{4a}$	$8.08x10^{4a}$	$5.10x10^{5a}$	$8.49x10^{5a}$	$4.63x10^{6a}$
	75	$3.28x10^{3a}$	$4.38x10^{3b}$	$3.26x10^{4b}$	$4.41x10^{4b}$	$3.82x10^{5b}$	$6.91x10^{5b}$	$1.72x10^{6b}$
	150	$2.15x10^{3b}$	$4.18x10^{3b}$	$2.96x10^{4b}$	$3.86x10^{4b}$	$3.58x10^{5b}$	$6.45x10^{5b}$	$1.59x10^{6b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l, q*
SEM								
Temperature		0.247	0.326	0.249	0.274	0.457	0.223	0.253
HCN level		0.302	0.399	0.305	0.335	0.560	0.273	0.310
Temperature x HCN level		0.428	0.564	0.432	0.474	0.792	0.386	0.438
P-value								
Temperature		<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
HCN level		0.001	0.010	0.005	0.015	<0.001	<0.001	<0.001
Temperature x HCN level		0.323	0.582	0.342	0.501	0.052	0.057	0.017

SEM: standard error of the mean. HCN = hydrocyanic acid. [a,b] Means within a column within temperature or the main effect of HCN level from fresh cassava peel supplementation in the diet of dairy cattle having different superscript letters are different at P<0.05. *l, q, c*: Within a column, the effect of HCN level from fresh cassava peel supplementation in the diet of dairy cattle for individual temperature or the main effects of HCN level from fresh cassava peel supplementation in the diet of dairy cattle is linear, quadratic, and cubic, respectively, at P<0.05. *ns*: Within a column, the effect of HCN level from fresh cassava peel supplementation in the diet of dairy cattle for an individual temperature is not linear, quadratic or cubic, at P>0.05.

results were asserted that an LP activity value found to be 1.44 U/mL, which was sufficient to act as a catalyst for the LPs (Marshall *et al.*, 1986), thus for cows with diets supplemented by HCN content from FCPe, the extension of milk KQ is believed to be due to an improvement in the efficiency of the antibacterial activity of the LPs activated in the milk. The results are in agreement with Buaphan (2003) and Punthanara *et al.* (2009) who reported a similar trend whereby the bacterial counts in raw milk of dairy cows fed cassava diets was detectably lower than those not fed cassava diets in Thailand. Hong, Wanapat, Wachirapakorn, Pakdee, and Rowlinson (2003) concluded that cassava hay can be a good source of forage and has the ability to improve milk quality in lactating dairy cows. This study suggests that it is the action of the LPs on TBC and CC that gives rise to the observed increase in KQ of milk from cows with diets supplemented with FCPe compared with those fed the control diet. Although the LPs exert a wide range of antimicrobial activity against bacteria, the LPs alone are unable to exhibit sufficient antibacterial activity to maintain milk quality during storage for extended periods, its antimicrobial properties are controlled by the reaction of all three components; LP, SCN^- and H_2O_2 under LP catalysis (Seifu *et al.*, 2005) and the resultant generation of short-lived intermediary oxidation (hypothiocyanate; $OSCN^-$) products with antibacterial properties. The activity of the system as described above has shown that the LPs can increase storage times and extend the shelf life of milk by delaying bacterial growth. Due to these generated oxidation products react with microbial sulfhydryl groups to inhibit various cellular functions essential for microbial

metabolism (Shin *et al.*, 2001). The optimum effectiveness for LPs activation has been reported to occur if the system is not activated immediately, but delayed slightly until after the multiplication of existing bacteria has begun and when the indigenous antibacterial system still remains significantly effective. The Codex Alimentarius Commission (CAC, 1991) reported that raw milk contains various species of bacteria and that the resistance of those bacteria using the LPs is based on a microorganism's specific species and strains, temperature (Wolfson & Sumner, 1993) and the differences in cell wall structure and their different barrier properties (de Wit & van Hooydonk, 1996).

4. Conclusions

The results of the present study indicate that both at 400 and 800 g/d FCPe decreases bacterial counts for KQ of milk during storage, therefore 400 g/d FCPe (75 ppm HCN) can be used in the concentrate for dairy cows.

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