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Original Article

Fresh cassava peel in dairy cattle diet: Effects on milk production, hygienic quality of raw milk and somatic cell counts

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Abstract

Hydrogen cyanide in cassava, which is a cyanogenic plant, was reported to be transformed into the non-toxic thiocyanate (SCN^-) in cows and is partly eliminated via the milk. Milk SCN^- is used in the antimicrobial function of the lactoperoxidase system (LPS) in milk while lactoperoxidase activity was reported to be useful as a possible indicator of somatic cell counts (SCC). We investigated the effects on the hygienic quality of raw milk and milk SCC through supplementation of fresh cassava peel (FCP) in the diets of dairy cattle. Twenty-four Holstein-Friesian crossbred, lactating dairy cows were blocked by lactation first and then stratified random balanced based on days in milk, milk yield, and body weight into three groups of 8 cows each. The treatment groups were: 1) cows fed a control diet of 6.49 kgDM/d of 21% crude protein concentrate together with *ad libitum* corn silage; and 2) and 3) cows were fed the same control diet supplemented with top-dressed 500 and 1000 g/d of FCP, respectively. Dietary treatments had no effect on nutrient intake, weight change, milk yield or composition. The raw milk exhibited increased SCN^- concentration and decreased SCC. FCP supplementation greatly increased the efficiency of the antibacterial activity of the LPS. The results demonstrated that increasing milk SCN^- maintained the hygienic quality of the raw milk by supplementing the diets of dairy cattle with FCP. Since there was also a commensurate reduction in the SCC, it may be possible to use FCP supplementation as an alternative measure to prevent mastitis in lactating dairy cows.

Keywords: fresh cassava peel, milk composition, hygienic quality, lactoperoxidase system, somatic cell count

1. Introduction

Milk thiocyanate (SCN^-) has been reported as an active agent in the lactoperoxidase (LP) system (LPS) with an antimicrobial function (Zapico *et al.,* 1991), which can be used to increase the shelf-life of raw milk stored at ambient temperature (Claesson, 1994). In the dairy industry of some tropical regions, certain chemicals have been tried in milk samples to activate the LPS in a combination with SCN^- and a peroxide donor as milk preservatives, but these additives are prohibited for toxicological reasons in Thailand. Therefore, alternative feed sources containing hydrogen cyanide (HCN) have been investigated to improve the hygienic quality (HQ) of milk. The HCN in the cyanogenic plant cassava was reported to be transformed into the non-toxic SCN^- by the enzyme rhodanase that in turn activates the cyanide ion which combines with thiosulfate ($S_2O_3^{2-}$) yielding SCN^- and is partly eliminated via the milk (Soto-Blanco & Gorniak, 2003). As milk LP catalyses in the presence of hydrogen peroxide (H_2O_2), SCN^- is oxidized to yield hypothiocyanite ($OSCN^-$)

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and hypothiocyanous acid (HOSCN; Shin *et al.,* 2001) and the iodide ion is oxidized to yield hypoiodite and hypoiodous acid (Bosch *et al.,* 2000), which has potent antibacterial properties.

Fresh cassava peel (FCP) is available in large volumes as a by-product from the processing of cassava roots for starch production and has gradually become widely utilised in animal feeds in Thailand, such as poultry feed. FCP is rich in cyanogenic glycoside and the levels of hydrocyanic acid are in the range of 364.2-814.7 ppm (Tewe & Lyayi, 1989). It was reported that cassava peel from a cassava starch processing factory was suitable for feedstuff. According to Claesson (1994), supplementing dairy cows with cassava hay (CH) resulted in a milk SCN^- level of 19.5 ppm; however, fewer effects were reported for cassava supplementation in dairy cattle diets on the microbial population in raw milk (Buaphan, 2003; Punthanara *et al.,* 2009). The research on cassava peel as a feed for lactating dairy cattle is also very limited. Particularly, there is no published information on *in vivo* research into the influence of the LPS on the HQ of raw milk for the extension of shelf-life through FCP supplementation in dairy cattle diets. Since it was reported that LP activity may be potentially used as an indicator of SCC, this research was undertaken to evaluate the effects of FCP supplementation in dairy cattle diet on the HQ of raw milk. The second focus of the research was to investigate the influence of the dietary supplementation with FCP on the levels of SCC and the relationship with the incidence of mastitis.

2. Materials and Methods

2.1 Experimental design and treatments

Twenty-four Holstein Friesian crossbred (>87.5% HF crossbred) early-mid lactating dairy cows that averaged 90±11 days in milk (DIM), 13.2±0.9 kg of milk, and 428±20 kg body weight (BW) were blocked by lactation number and then stratified random balanced based on DIM, milk yield, and BW into three groups of 8 cows each. All cows were housed in individual tie stalls (2.5×3 m) and the barn was under a tiled roof. On a dry-matter basis, the cows were individually fed 6.49 kgDM/d of 21% crude protein commercial concentrate daily together with *ad libitum* corn silage (the control diet) and had free access to clean water. The treatments of the three groups were: 1) the control diet; and 2) and 3) were the control diet supplemented with top-dressed with 500 and 1000 g/d of FCP, respectively. The experiment lasted for 40 days (d): a 10-d adjustment period followed by the 30-d measurement period. Live weights (LW) were recorded at the start and end of the experiment to calculate LW change (LWC).

2.2 Fresh cassava peel (FCP) collection

The FCP was obtained every day in the morning from Sanguan Wongse Industries Co., LTD., Nakhon Ratchasima in Thailand and divided daily into two equal meals at 07:00 and 15:00 for fed to the experimental cows. Some of the FCP was immersed immediately into an ice slurry to reduce the cyanide activity for an analysis of the cyanide content by the pyridine-pyrazolone method (O'Brien, 1991) at the Cassava and Starch Technology Research Unit; Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Bangkok, Thailand.

2.3 Laboratory analyses

Feeds offered and residues after eating were weighed for individual cows for two consecutive days weekly to calculate the dry matter intake (DMI). Feed samples were pooled to make representative samples for proximate (AOAC, 1995) and detergent analyses (Van Soest *et al.,* 1991). Net energy for lactation of feeds was calculated according to the equations of National Research Council (NRC) (2001).

Milk yields were recorded daily for each cow. Milk samples were collected at each milking for two consecutive days weekly, twice per day (at 05:00 and 16:00 h). After collection, milk was immediately analysed for composition including fat, protein, lactose, solid-not-fat, and total solids using the MilkoScan™ FT2 (Foss Hillerøed, Denmark). In addition the milk was analyzed for SCN^- concentration (Cosby & Sumner, 1945) and LP activity (Isobe *et al.,* 2009). The SCC was analyzed using the Fossomatic 5000 Basic®. HQ testing was also carried out. The pH was measured using a pH meter (MP 220 pH Meter, Mettler-Toledo Gombh, CH-9603 Schwerzenbach, Switzerland). The calibration was based on the method of Bradley *et al.* (1993). Titratable acidity (%TA) was carried out according to AOAC (2000) procedures and the alcohol precipitation procedure was performed (Barrett *et al.,* 1999). Portions of milk samples were taken to examine the microbiological properties including the methylene blue reduction test (MBRT) (International Dairy Federation [IDF], 1989), total viable count (TVC) (Petrifilm™ aerobic count plate) and *Escherichia coli* and coliform count (CC) (Petrifilm™ coliforms count plate) with small adjustments, and the psychrotrophic count and thermophilic count (Frank *et al.,* 1992).

2.4 Statistical analysis

All data were statistically analysed as randomized complete block design using the ANOVA procedure of SAS software (SAS, 2002). The relationships of HCN in dairy cattle feed intake and milk quality parameters were determined with simple linear regression, coefficient of correlation (r), and coefficient of determination (r^2).

3. Results and Discussion

3.1 Feed chemical composition, animal nutrient intake, and live weight change

Supplementary feeding with FCP at 500 and 1000 g/h/d yielded an increased intake of HCN of 70 and 140 ppm/d, respectively, as the level of FCP supplementation in treatment diets increased (Table 1). The levels of cyanide in the FCP were potentially toxic. It was reported that HCN concentrations above 600 ppm in forage are toxic for cattle and are usually lethal at 1000 ppm and above (Larson, 2006). However, these levels of HCN derived from FCP ingestion are safe for dairy cows. The overall nutrient intakes and LWC were not significantly different over the course of the trial due to dietary supplementation with FCP (Table 2). This sug-

Table 1. Effect of fresh cassava peel supplementation on nutrient intake and growth performance of dairy cows.

Item	Control	500 g/d FCPe[1]	1000 g/d FCPe[2]	SEM	P-value
Dry matter intake, kg/d					
Concentrate	6.49	6.49	6.49	-	-
Corn silage	6.98	6.72	6.81	0.31	0.381
Fresh cassava peel	0	0.12	0.25	-	-
Total	13.47	13.21	13.30	0.33	0.255
Crude protein intake, g/d					
Concentrate	1375	1375	1375	-	-
Corn silage	591	569	576	20.10	0.333
Fresh cassava peel	0	1.25	2.50	-	-
Total	1966	1945	1954	15.01	0.264
NEL_P[3], Mcal/d					
Concentrate	10.19	10.19	10.19	-	-
Corn silage	8.93	8.60	8.72	0.11	0.288
Fresh cassava peel	0	0.12	0.24	-	-
Total	19.12	18.91	19.15	0.15	0.225
Live weight change					
Initial live weight, kg	425	431	428	28.23	0.844
Final live weight, kg	426	429	425	25.15	0.791
Live weight change, g/d	33.33	-66.67	-100.00	53.32	0.650

SEM, standard error of mean
[1] The control concentrate supplemented 500 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 70 ppm HCN (DM basis) by calculation
[2] The control concentrate supplemented 1000 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 140 ppm HCN (DM basis) by calculation
[3] NEL_P, net energy for lactation at the production level

gested that the negative live weight gain among the treatments in the current study was in agreement with NRC (2001). This may be the result of a negative energy balance which often occurs in dairy cows during early lactation (i.e. overlapped early-mid lactation). Therefore, the ingestion of fresh or processed cassava based diets caused reduced growth rates in sheep and goats (Tewe, 1983). The animals also had increased serum and urinary levels of SCN^- which is a continuous cause of depletion of sulphur containing amino acids. The SCN^- also inhibits the intra-thyroidal uptake of iodine which causes an increase in secretion of thyroid stimulating hormone and causes a reduction in thyroxine level which is necessary for growth.

3.2 Milk production

Milk yield and composition for the entire period were not affected by the supplementation of FCP at 500 and 1000 g/d (Table 2). This was partly due to the lack of significant differences in the results found for overall nutrient intakes among the groups, and thus no remarkable changes occurred for milk yield and composition among the treatments. Equally, no undesirable side-effects were reported largely because the components are naturally present in milk. Indeed, the decrease in milk production reported in some studies was associated with a depression in DMI and diet digestibility due to disturbances in rumen function which was possibly caused by a high level of HCN intake from FCP. The nutrient intakes of this study were in agreement with most literature reports that showed few effects of the concentration and level of HCN concentration from FCP supplement (i.e. total HCN concentration on a dry matter basis) in this study was below 250 ppm. Discrepancies among the studies on the effects of cassava supplementation in diets on milk yield and composition for the entire period was possibly due to nutrient digestibility and particularly fiber (M. Khunkaew, unpublished observation) and other factors, such as the balance of nutrients provided from the concentrates (Punthanara *et al.*, 2009), the form and amount of cassava, differences in the experiments, and varying experimental durations.

Table 2. Effect of fresh cassava peel supplementation on milk yield, milk composition, thiocyanate concentration, lactoperoxidase activity, and milk somatic cell count.

Item	Control	500 g/d FCPe[1]	1000 g/d FCPe[2]	SEM	P-value
Milk yield, kg/d	13.32	13.27	12.93	0.31	0.431
3.5% FCM[3], kg/d	14.64	14.95	14.29	0.32	0.417
Fat, g/d	548	568	536	31	0.271
Protein, g/d	374	381	350	22	0.597
Lactose, g/d	587	591	560	28	0.718
Solid-not-fat, g/d	1093	1097	1075	27	0.613
Total solid, g/d	1641	1665	1611	35	0.536
Milk composition (g/100 g of raw milk)					
Fat	4.11	4.28	4.15	0.16	0.215
Protein	2.81	2.87	2.71	0.09	0.439
Lactose	4.41	4.45	4.33	0.10	0.327
Solid-not-fat	8.21	8.27	8.31	0.07	0.323
Total solid	12.32	12.55	12.46	0.23	0.539
Thiocyanate, ppm	6.84^c	10.3^b	13.8^a	1.12	0.001
LP activity, U/ml	5.87^b	6.87^{ab}	6.96^a	0.36	0.030
Milk somatic cell, $x10^3$ cells/ml	287.6^a	92^b	75^c	5.20	0.034

SEM, standard error of mean
[a, b] Means within a row with different superscripts are significant different ($p<0.05$)
[1] The control concentrate supplemented 500 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 70 ppm HCN (DM basis) by calculation
[2] The control concentrate supplemented 1000 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 140 ppm HCN (DM basis) by calculation
[3] FCM, fat-corrected milk; 3.5% FCM = (0.432 x kg of milk) + (16.216 x kg of milk fat)

3.3 Thiocyanate concentration, lactoperoxidase activity, and milk somatic cells

The diets supplemented with FCP resulted in greater concentrations of milk SCN^- than the control diet. These results were in agreement with other studies (Buaphan, 2003; Petlum *et al.,* 2012; Punthanara *et al.,* 2009), which reported similar increases in milk SCN^- with increased levels of cassava included in the diets of dairy cows. Modified diets containing HCN resulted in marked alterations in the concentration of milk SCN^- which was correlated with the level of FCP supplemented in the feed. A gradual increase in milk SCN^- was observed with increased HCN from the FCP. The regression model indicated that 99% of the SCN^- in cow's milk was positively correlated as a result of the total HCN intake in the diets (r^2=0.99) (P<0.001) (Table 3). This effect relies on the high level of HCN in FCP to alter the SCN^- through the cyanide detoxification of dietary HCN by its conversion to SCN^- in the liver and kidney of animals by the rhodanese action (Drakhshan Vaziri & Aminlari, 2004) and partly through elimination in the milk (Soto-Blanco and Górniak, 2003) which resulted in the observed increases of the milk SCN^-. Similarly, Jupamatta *et al.* (2011) demonstrated that the regression model results between SCN^- and HCN (r^2=0.85, P=0.004) in dietary cassava was also positively correlated during a study of effects on milk quality in the case of lactating sows.

The difference in LP activity in milk from cows with a diet supplemented by FCP 500 g/d (Table 3) was not significant and certainly not as pronounced as the difference in HCN intakes in diets (Table 4). However, linear regression showed that 76% of the increase in milk LP activity could be attributed to HCN intakes in the diets (r^2=0.76) (Table 6). LP activity in the milk increased significantly after dietary supplementation of FCP at 1000 g/d compared with that of the control diet. These results suggested that the intakes of HCN in the diets imparted the SCN^- and may be responsible for the activation of the LP in cow's milk, but there is no direct evidence to corroborate the finding that higher levels of SCN^- in milk always results in increased milk LP, although LP has SCN^- as a substrate form in animal milk. The supplementation of FCP 500 g/d did not produce a significant effect on LP. Similarly, Punthatnara *et al.* (2009) stated that cows fed treatment diets supplemented with different levels of CH also produced unaltered LP in milk despite increased SCN^- compared with the untreated diet. However, the overall result is in agreement with Buaphan (2003) which found that increasing the level of both SCN^- and LP in milk were significant in relation to dairy cows fed cassava chips in total mixed rations compared with those fed corn diets. The variations in LP may be because the exhibition of LP activity is

Table 3. Regression analysis of raw milk quality parameters on cyanide content intake of fresh cassava peel supplementation of diets.

Parameters	Simple linear regression	r^2	r	p-value
HCN (X): Milk SCN^- (Y)	0.046X+6.833	0.99	0.99	<0.001
HCN (X): Milk LP activity (Y)	0.007X+5.984	0.76	0.87	0.047
HCN (X): Milk SCC (Y)	-1.446X+258.5	0.80	0.89	0.009
HCN (X): Milk TVC (Y)	$-1541X+2.14x10^4$	0.77	0.87	0.045
HCN (X): Milk *E. coli* and CC (Y)	$-7.562X+1.51x10^3$	0.83	0.91	0.027
HCN (X): Milk Psychrotrophic count (Y)	$-24.14X+1.22x10^4$	0.86	0.93	0.025
HCN (X): Milk Thermophilic count (Y)	-0.316X+109.9	0.83	0.91	0.027

r^2, simple correlation coefficient; r, simple coefficient of determination; SCN^-, thiocyanate; LP, lactoperoxidase; SCC, somatic cells count; TVC, total viable count; *E. coli*, *Escherichia coli*; CC, coliform count; h, hour

Table 4. Chemical composition (% DM) of the experimental feeds (mean±SE).

Item	Concentrate[1]	Fresh cassava peel	Corn silage
	% of DM		
Dry matter	92.85±0.07	24.71±0.31	27.55±0.42
Ash	7.16±0.54	18.17±1.15	9.37±0.47
Crude protein	21.18±0.44	1.04±0.21	8.47±0.17
Ether extract	3.20±0.54	1.21±0.44	1.53±0.19
Crude fiber	12.16±0.74	10.21±0.14	38.48±1.07
Neutral detergent fiber	38.73±0.14	74.01±0.29	65.44±1.74
Acid detergent fiber	24.44±1.31	18.37±0.87	44.26±0.77
Acid detergent lignin	9.78±0.72	7.72±0.34	4.44±0.87
Neutral detergent insoluble, N	1.29±0.01	0.30±0.11	0.24±0.02
Acid detergent insoluble, N	0.45±0.02	0.20±0.07	0.21±0.02
TDN_{1x} (%)[2]	69.11±0.95	41.50±2.56	50.08±1.61
DE_{1x} (Mcal/kg)[3]	2.95±0.10	1.73±0.04	2.53±0.11
DE_P (Mcal/kgDM)[4]	2.77±0.04	1.87±0.03	2.49±0.07
ME_P (Mcal/kgDM)[5]	2.41±0.07	1.58±0.04	2.09±0.11
NEL_P (Mcal/kgDM)[6]	1.57±0.04	0.99±0.04	1.28±0.07
Cyanide content (mg/kg dry solid)	-	588.94±4.70	-

[1] Contained (as DM basis): Control concentrate (0%; without 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, Mineral and vitamin mix. The second and third group (supplementing FCPe at 500 and 1000 g/h/d respectively)

[2] Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF-7 (NRC, 2001)

[3] Digestible energy, DE_{1X}(Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100)x4.2]+[(tdCP/100)x5.6]+[(FA/100)x9.4]-0.3

[4] DE_P (Mcal/kgDM) = DE_{1X} x Discount (NRC, 2001)

[5] Metabolisable energy, ME_p = [1.01 x (DE_p)-0.45] + [0.0046 x (EE-3)] (NRC, 2001)

[6] Net energy for lactation, NEL_P = ([0.703 x MEp (Mcal/kg)]-0.19) + ([(0.097 x MEp + 0.19)/97] x [EE-3])

[2,3,4,5,6] Calculated using published values of NRC (2001)

a cyclic pattern with alternating peaks and troughs throughout lactation and that large variations were observed between and within cows (Fonteh *et al.,* 2002) and between breeds and seasons (Fonteh, 2006). Moreover, the factors regulating its expression and activity are currently not well understood (Fragoso *et al.,* 2009). It has been suggested that the observed increase in LP from cows with diets supplemented with FCP is a clear indicator of increased SCN^- directly related to the HCN of the ingested FCP. Indeed, more research is required in order to accurately identify the role of residual HCN in cassava on milk SCN^- and its significance in any activation of the LPs which occurs.

The SCC decreased significantly throughout the experiment with increasing FCP supplementation. In the case of LP activity in the milk, the highest LP was, to some extent, associated with extremely decreased SCC. The correlation between LP and SCC in the milk and the use of LP activity as an indicator of mastitis has been studied previously (Isobe *et al.,* 2011). Cows supplemented with ensiled cassava foliage were shown to be significantly affected with increased levels of milk SCN^- and decreased SCC (Petlum *et al.,* 2012). Lowering the number of SCC has been recommended to prevent mastitis which is an infectious disease in cows. LP is a protein found in milk with beneficial properties due to its antimicrobial function. It inhibits a broad spectrum of Gram-positive and Gram-negative bacteria (Mark *et al.,* 2001) and inhibits the growth of pathogens such as *Streptococcus uberis* (Marshall *et al.,* 1986), *E. coli* O157:H7 and *Staphylococcus aureus* ATCC 25923 (Seifu *et al.,* 2004) which is the main cause of mastitis. Thus, its activity has been shown to inhibit various bacterial invasions of the teat canal by a variety of bacterial sources. Shakeel-ur-Rehman and Farkye (2003) stated that since the level of LP in milk increases with mastitic infection, LP activity may be used as a possible index for mastitis. While a decrease in SCC along with an increase in LP activity was not directly proven to be a result of dietary supplementation in this study, increased levels of SCN^- and LP were coincident with a reduction in SCC in the milk of cows with diets supplemented with FCP at both 500 and 1000 g/d and it is reasonable to conclude a cause and effect correlation. However, the regression analysis showed that 80% of the decrease in SCC could be attributed to HCN intakes in diets (Table 6). The supplementation of FCP may activate the LPS components to boost the effectiveness of its antimicrobial ability in raw milk by enhancing SCN^-. This occurs through the increased intake of HCN which is transformed into milk SCN^-, a key component used in the LPS (Zapico *et al.,* 1991), given that LP appears to be constitutively present in milk (Fonteh *et al.,* 2002). SCN^- plays an important role in LPs and has inhibitory effects on the micro-organisms contained in the milk (Saidu, 2004). The results of this study suggest that the SCC may decrease by increasing the HCN and milk SCN^- via the supplementation of feed with FCP.

3.4 Quality stability of milk (pH, titratable acidity, and alcohol test)

The values for HQ in the control diet milk were maintained up to 6 h. However, the milk from cows supplemented with FCP returned improved HQ values up to 10 h (Table 5). The *in vitro* HQ procedure provides a useful assessment and reliable results for the extent of the shelf-life of milk, particularly acid production of lactic acid bacteria used in the fermentation of milk. The results also indicated that the values of the milk pH and %TA for cows with diets supplemented with FCP were similar to those of milk from the control diet, but exhibited an increase in HQ up to 10 h which was significantly longer than the milk from cows on the control diet at just 6 h. With increased bacterial contamination, the presence of bacteria converts more lactose (sugars) into lactic acid and the resulting increase in acidity lowers the pH level. These two values are indicators of the quality of milk (Chaiprasop, 2001). Milk from cows with diets supplemented with FCP at 500 g/d showed a detectable quantity of LP activity enzyme at 6.87 U/ml; however, it was not significantly higher in the treatments groups over cows on the control diet. An increase in HQ from 6 to 10 h was seen in the milk from cows with diets supplemented with FCP which could imply that the entire LPS was activated in the raw milk which presumably led to the observed increase in HQ. Mark *et al.* (2001) suggested that the LPS was responsible for the

Table 5. Effect of fresh cassava peel supplementation on the keeping quality of raw cow's milk during storage.

Item	Control	500 g/d FCPe[1]	1000 g/d FCPe[2]	SEM	P-value
pH[3]					
0 h	6.69	6.77	6.79	0.070	0.077
2 h	6.65	6.71	6.72	0.050	0.147
4 h	6.62	6.68	6.69	0.071	0.079
6 h	6.60	6.65	6.67	0.058	0.148
8 h	6.53	6.63	6.65	0.048	0.084
10 h	6.21	6.53	6.56	0.122	0.136
% Titratable acidity[4]					
0 h	0.15	0.15	0.15	0.001	0.295
2 h	0.16	0.15	0.15	0.004	0.179
4 h	0.17	0.15	0.15	0.007	0.208
6 h	0.18	0.16	0.15	0.011	0.143
8 h	0.20	0.17	0.16	0.014	0.135
10 h	0.23	0.18	0.17	0.022	0.491
Alcohol test					
0 h	N*	N	N	-	-
2 h	N	N	N	-	-
4 h	N	N	N	-	-
6 h	P*	N	N	-	-
8 h	-	N	N	-	-
10 h	-	P	P	-	-

SEM, standard error of mean

* N is no change of milk samples (negative)

** P is a transformation of the milk samples (positive)

[1] The control concentrate supplemented 500 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 70 ppm HCN (DM basis) by calculation

[2] The control concentrate supplemented 1000 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 140 ppm HCN (DM basis) by calculation

[3] Identified pH levels at about 6.60 to 6.90 as considered being the normal range by National Bureau of Agricultural Commodity and Food Standards (ACFS.6003) (2005)

[4] Titratable acidity (% lactic acid): samples with an acidity $\geq$0.18% recorded as rejected

improved HQ in pasteurised milk *in vitro*. Maneerate (2006) reported that the shelf life of LP-treated milk was longer (14 h) than pasteurised (12 h) and raw milk (10 h). Moreover, the increased HQ values agreed with Dajanta *et al.* (2008) who showed an active LPS was found to greatly increase the HQ and *E. coli* inoculated raw milk. This result confirmed that stimulation of the LPS can extend the HQ values.

Given the similarity between values for pH, acidity, alcohol precipitation, and MBRT between the control milk at 6 h and the supplemented FCP milk at 8 h to 10 h, it can be asserted that cows with diets supplemented with FCP produce raw milk with extended shelf-life. The present study agreed with an unpublished observation in 2001 by Srinetra who reported that feeding CH at increasing rates of 0, 0.5, 1.0, and 1.5 kg/d increased the MBRT from 2.65 h to 2.77, 3.18, and 4.05 h, respectively. However, all milk samples in the MBRT test were judged to be in a good quality range. This was possibly caused by the condition of the microbes in the raw milk used to determine short-term microbial growth (growth phase) of the bacteria. Bacteria types or contamination by white blood cells (leucocytes) can change the colour of the MBRT (Thangcharoenchai, 1995).

3.5 Microbiological properties

The five tests undertaken were the MBRT, TVC, CC, psychrotrophic count, and thermophilic count from cows with diets supplemented with FCP. The results of the MRBT presented obvious differences between the control diet and the diets supplemented with FCP (Table 6). None of the milk samples underwent decolourisation within the first 30 min. However, the samples from cows with diets supplemented with FCP were "excellent" showing no reduction of blue colour for up to 8 h. The control milk was maintained in the "good" grade from 0 to 6 h. The growth of TVC, *E. coli* and coliform, psychrotrophic count, and thermophilic count abundance decreased with FCP supplementation levels. However, the differences between the two levels of FCP supplementation at 500 and 1000 g/d were not significant. The highest values were in the control group.

The use of FCP as a dietary supplement led to improved HQ and thus, accompanied by significantly extended shelf-life of milk by inhibiting the microbial growth evaluated by the TVC ($P<0.04$), CC ($P<0.04$), psychrotrophic count ($P<0.02$), and thermophilic count ($P<0.01$). The average lower values were in the treatment groups corresponding to 500 and 1000 g/d FCP compared with the control diet. Dajanta *et al.* (2008) stated that this method of activating the LPS decreases the microbial population in raw milk under storage conditions, which has demonstrated effects on the HQ and thus can be used to extend the shelf-life of milk. These results showed the LP activity value was 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 FCP, extension of the milk HQ is believed to be due to an improvement in the efficiency of the antibacterial activity of the LPS activated in the raw milk. Regression analysis of the TVC, *E. coli* and coliform, psychrotrophic count, and thermophilic count exhibited 77%, 83%, 86%, and 83% decreases in the microbial growth, respectively, which could be attributed to HCN intakes in the diets (r^2=0.77, 0.83, 0.86, and 0.83, respectively) (Table 6). The results were in agreement with Buaphan (2003) and Punthanara *et al.* (2009) who reported a similar trend, whereby the vegetative bacteria counts in milk of cows fed cassava diets were detectably lower than those not fed cassava diets, and also in the case of sow milk (Jupamatta *et al.*, 2011). They demonstrated that the regression analysis of HCN from cassava in diet was significantly positively correlated with the corresponding decrease in total microbial count and coliform count in sow milk (Jupamatta *et al.*, 2011). LP has been shown to destroy the bacteria in raw mature milk (Losendahl *et al.*, 2000). This study suggests that it is the action of the LPS on microbial growth that gives rise to the observed increase in HQ of milk from cows supplemented with FCP compared with those fed the control diet.

Table 6. Effect of fresh cassava peel supplementation on microbiological properties in raw milk.

Item	Control	500 g/d FCPe[1]	1000 g/d FCPe[2]	SEM	P-value
MBRT[3]	Fair	Excellent	Excellent	-	-
TVC, CFU/ml	$2.51x10^{5a}$	$2.44x10^{4b}$	$1.98x10^{4b}$	0.180	0.035
E. coli and CC, CFU/ml	$1.65x10^{3a}$	$6.54x10^{2b}$	$5.17x10^{2b}$	0.487	0.040
Psychrotrophic count, CFU/ml	$1.27x10^{4a}$	$9.58x10^{3b}$	$9.03x10^{3b}$	0.305	0.021
Thermophilic count, CFU/ml	116^{a}	74^{b}	69^{b}	13	0.012

SEM, standard error of the mean; MBRT, methylene blue reduction test; TVC, total viable count; *E. coli*, *Escherichia coli*; CC, coliform count; h, hour

[1] The control concentrate supplemented 500 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 70 ppm HCN (DM basis) by calculation

[2] The control concentrate supplemented 1000 g/h/d fresh cassava peel together with *ad libitum* corn silage with approximately 140 ppm HCN (DM basis) by calculation

[3] MBRT method provides discernment of four classifications of milk quality: Class 1: The quality of milk is "Excellent" if no reduction of blue colour for up to 8 h, Class 2: The quality of milk is "Good" if milk sample shows decolourisation in 6-8 h, Class 3: The quality of milk is "Fair" if milk sample shows decolouration in 2-6 h, and Class 4: The quality of milk is "Poor or bad" if milk sample shows decolourisation in less than 2 h

[a, b] Means within a row with different superscripts are significant different ($P<0.05$)

4. Conclusions

FCP can be used as a supplement in the diets of lactating dairy cows at a level of both 500 and 1000 g/d to achieve improved HQ in raw milk and prohibit bacterial activity for milk preservation and to extend the shelf-life without affecting milk yield, milk composition or feed intake. The supplementation of FCP at 500 g/d may be insufficient to influence LP activity enough to clearly indicate an association with reduced SCC; therefore, the optimum supplementation level of 1000 g/d FCP based on corn silage is recommended for the diets of lactating dairy cows without causing any detrimental effect on milk production or cow performances.

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References

Association of Official Analytical Chemists. (1995). Official Methods of Analysis (16th ed.). Arlington, VA: Author.

Association of Official Analytical Chemists. (2000). *Official Methods of Analysis* (17th ed.). Guithersburg. MD: Author.

Barrett, N., Grandison, A. S., & Lewis, M. J. (1999). Contribution of the lactoperoxidase system to the keeping quality of pasteurised milk. *Journal of Dairy Research, 66*(1), 73-80.

Bosch, E. H., van Doorne, H., & de Vries, S. (2000). The lactoperoxidase system: The influence of iodide and the chemical and antimicrobial stability over the period of about 18 months. *Journal of Applied Microbiology, 89*, 215–224.

Bradley, R. L, Jr., Arnold, E. Jr., Barbano, D. M., Semerad, R. G., Smith, D. E., & Vines, B. K. (1993). Chemical and physical methods. In R. T. Marshall (Ed.), *Standard Methods for the Examination of Dairy Products (16th ed.)* (pp. 433-531). Washington, DC: American Public Health Association.

Buaphan, S. (2003). *Effects of increasing contents of cassava chips in to total mixed rations on somatic cell counts, microorganisms, aflatoxins and peroxidase activity in raw milk of cows* (Master thesis, Kasetsart University, Bangkok, Thailand).

Certificate Authentication Code. (2004). *Code of Hygienic Practice for Milk and Milk Products* (CAC/RCP 57–2004). Available from http://www.coReiterdexalimentarius.net/download/standards/10087/CXC_057_2004e.pdf

Chaiprasop, O. (2001). *Dairy Technology (FT468).* Bangkok, Thailand: Ramkhamhaeng University press.

Claesson, O. (1994). The use of the lactoperoxidase system. Proc. Regional workshop on raw milk handling and preservation in near East region. Rome, Italy.

Cosby, E. L., & Sumner, J. B. (1945). Rhodanese. *Archives of Biochemistry, 7*, 457-460.

Dajanta, K., Chukeatirote, E., & Apichartsrangkoon, A. (2008). Effect of lactoperoxidase system on keeping quality of raw cow`s milk in Thailand. *International Journal of Dairy Science, 3*, 112-116.

Fonteh, F. A., Grandison, A. S., & Lewis, M. J. (2002). Variations of the lactoperoxidase activity and thiocyanate content in cows' and goats' milk throughout lactation. *Journal of Dairy Research, 69*(3), 401-409.

Fonteh, F. A. (2006). Influence of breed and season on lactoperoxidase system components in cow's milk in Western Cameroon. *Livestock Research for Rural Development, 18*.

Fragoso, M. A., Torbati, A., Fregien, N., & Conner, G. E. (2009). Molecular heterogeneity and alternative splicing of human lactoperoxidase. *Archives of Biochemistry and Biophysics, 482*, 52-57.

Härnulv, B. G., & Kandasamy, C. (1982). Increasing the keeping quality of milk by activation of its lactoperoxidase system. *Milchwissenschaft, 37*(8), 454-457.

International Dairy Federation. (1989). *Modern microbiological Methods for Dairy Products*. Santander, Spain: Author.

Isobe, N., Kubota, H., Yamasaki, A., & Yoshimura, Y. (2011). Lactoperoxidase activity in milk is correlated with somatic cell count in dairy cows. *Journal of Dairy Science, 94*, 3868-3874.

Isobe, N., Morimoto, K., Nakamura, J., Yamasaki, A., & Yoshimura, Y. (2009). Intramammary challenge of lipopolysaccharide stimulates secretion of lingual antimicrobial peptide into milk of dairy cows. *Journal of Dairy Science, 92*, 6042-6051.

Jupamatta, A., Kanto, U., & Tirawattanawanich, C. (2011). Cassava in Lactating Sow Diets: I. *Effects on Milk Composition and Quality, 24*(4), 517-524.

Kussendrager, K. D., & Van Hooijdonk, A. C. M. (2000). Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications. *The British Journal of Nutrition, 84*(1), 19-25.

Larson, B. (2006). Precaution when utilising sorghum/Sudan crops as cattle feed. University of Missouri Extension. College of Agriculture Food and Natural Resources, University of Missouri, Missouri.

Losendahl, K. J., Wang, H., Aslam, M., Sixiang, Z., & Hurley, W. L. (2000). Antimicrobial proteins in milk. Retrieved from http://www.classes.ag.uiuc.edu./AnSci. 308/antimi-croprot IDR.html

Maneerate, J. (2006). *Process development of mozzarella cheese made from raw milk preserved by lactoperoxidase (LP-system)* (In Thai). (Doctoral thesis, Suranaree University of Technology, Nakhon Ratchasima, Thailand).

Marks, N. E., Grandison, A. S., & Lewis, M. J. (2001). Challenge testing of the lactoperoxidase system in pasteurised milk. *Journal of Applied Microbiology, 91*, 735-741.

Marshall, V. M. E., Cole, W. M., & Bramley, A. J. (1986). Influence of the lactoperoxidase system on susceptibility of the udder to *Streptococcus ubiris* infection. *Journal of Dairy Research, 53*, 507-514

National Research Council. (2001). Nutrient Requirements of Dairy Cattle (7th ed). Washington, DC: Author.

O'Brien, G. M., Taylor, A. J., & Poulter, W. H. (1991). Improved enzymatic assay for cyanogens in fresh and processed cassava. *Journal of the Science of Food and Agriculture, 56*(3), 277-296.

Petlum, A., Boonlue, S., Thongdee, W., Patano, K., Wongchiangpheng, P., Phomphai, A., . . . Kongyen, M. (2012). Effect of ensiled cassava foliage supplementation on milk yield and milk quality of lactating dairy cows in smallholder farms. *Khon Kaen Agriculture Journal, 40*(2), 114-117.

Punthanara, S. (2009). Effects of cassava hay supplementation on antibiotic activity of the lactoperoxidase system in raw milk of dairy cows. *Kasetsart Journal, 43*, 486-496.

SAS. (2002). SAS Software User's Guide, Release 9.0 edn. Cary, NC: SAS Institute, Inc.

Saidu, Y. (2004). Physicochemical features of rhodanese: A Review. *African Journal of Biotechnology, 3*(4), 370-374.

Seifu, E., Buys, E. M., Donkin, E. F., & Petzer, I.-M. (2004). Antibacterial activity of the lactoperoxidase system against foodborne pathogens in Saanen and South African Indigenous goat milk. *Food Control, 15*, 447-452.

Shakeel-ur-Rehman & Farkye, N. Y. (2003). Lactoperoxidase. In R. Roginski, J. W. Fuquay & P. F. Fox (Eds.), *Encyclopedia of dairy science* (pp. 939-941). Amsterdam, Netherlands: Academic Press.

Shin, K., Hayasawa, H., & Lönnerdal, B. (2001). Inhibition of *Escherichia coli* respiratory enzymes by the lactoperoxidasehydrogen peroxide-thiocyanate antimicrobial system. *Journal of Applied Microbiology, 90*, 489-493.

Soto-Blanco, B., & Gornial, S. L. (2003). Milk transfer of cyanide and thiocyanate: Cyanide exposure by lactation in goats. *Veterinary Research, 34*, 213-220.

Tewe, O. O. (1983). Thyroid cassava toxicity in animals. In F. Delange & R. Ahluwalio (Eds.). *Cassava toxicity and thyroid research and public health issues, IDRC-207e* (pp. 114-118). Ottawa, Canada.

Tewe, O. O., & Lyayi, E. A. (1989). Cyanogenic glycosides. In P. R. Cheeke (Ed.), *Toxicants of Plant Origin, Glycosides Vol. 2* (pp. 43-60). Boca Raton, FL: CRC Press.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal production. *Journal of Dairy Science, 74*, 3583-3597.

Zapico, P., Gaya, P., De Paz, M., Nuñez, M., & Medina, M. (1991). Influence of breed, animal, and days of lactation on lactoperoxidase system component in goat milk. *Journal of Dairy Science, 74*, 783-787.