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Original Article

Effects of dietary levels of fresh cassava pulp in dairy cattle diet on productive performance and keeping quality of raw milk

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Abstract

This research aimed to study the effects of supplementation at three levels of fresh cassava pulp (FCP) in the concentrate diet of dairy cows: 1) 0 kg/d, 2) 3.5 kg/d (35 ppm hydrogen cyanide [HCN]), and 3) 7.0 kg/d (70 ppm HCN). The effects studied were productive performance, milk yield, milk composition, and milk quality. Twenty-four Holstein Friesian crossbred lactating dairy cows were blocked by lactation first and then stratified random balanced by days in milk, milk yields, and body weight. Cows were assigned into randomized complete block design and divided into three groups of 8 cows each. All cows were individually fed 21% crude protein concentrate together with *ad libitum* fresh grass and FCP according to the supplemented treatments. The results showed that the total HCN content intake increased when the level of FCP supplementation was increased. The supplementation of FCT at either 3.5 or 7.0 kg/d FCP had no negative effects on milk yield and milk composition. Milk thiocyanate concentration, lactoperoxidase (LP) activity, and the keeping quality of milk were increased by supplementation of FCP at 7.0 kg/d and decreased the somatic cell counts, standard plate counts, coliform counts, psychrotrophic counts, and thermophilic counts. The present study suggested that supplementation of FCP at either 3.5 or 7.0 kg/d may be associated primarily with increased efficiency of antibacterial activity of the LP system in raw milk thus improving the milk keeping quality, In addition, the supplementation at either 3.5 or 7.0 kg/d of FCP possibly decreased the incidence of mastitis in lactating dairy cows.

Keywords: fresh cassava pulp, lactoperoxidase activity, keeping quality, microbial counts, lactating dairy cows

1. Introduction

It is well recognised that, as an alternative to cooling, an effective method of preserving raw milk quality is through the stimulation of the milk's natural lactoperoxidase (LP) system (LPs) (Barabas, 1995). The thiocyanate (SCN^-) present in raw milk is used in the LPs as an effective antimicrobial agent (Zapico *et al.*, 1991). In the LPs, the major components are LP, SCN^-, and hydrogen peroxide (H_2O_2). As milk LP catalyses, SCN^- is oxidised by hydrogen peroxide (H_2O_2) to yield hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN; Shin *et al.*, 2001) and the oxidation of iodide ion (I^-) to yield hypoiodite (OI^-) and hypoiodous acid (HOI; Bosch *et al.*, 2000). These products are effective in inhibiting bacterial growth activity (Wolfson and Sumner, 1993). Previous studies have shown that the SCN^- or SCN^- or both combined with H_2O_2 additives activated the LPs in raw milk *in vitro* (Dajanta *et al.*, 2008) and in cow's milk *in vivo*

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in Thailand (Buaphan, 2003; Punthanara *et al.*, 2009; Srinetra, 2001) to achieve antimicrobial action.

Utilization of the LPs by adding sodium thiocyanate (NaSCN) and H_2O_2 has become recognized as useful as an alternative milk preservation technology which retards the deterioration of raw milk quality and was proven through a trustworthy, quantitative method of testing and reliable results (FAO/WHO, 2005). However, in Thailand, farmers are prohibited from using NaSCN and H_2O_2 as additives for raw milk preservation. In seeking to solve this problem, an alternative way to enhance the storage stability of raw milk has been continually developed and applied. The method makes use of a naturally occurring enzyme present in the antimicrobial system in milk known as LPs. The utilization of the hydrogen cyanide (HCN) content of cassava (*Manihotesculenta*, Cranzt) in ruminant diets has been found to potentially extend the quality of raw milk during storage by increasing the milk SCN^- levels. Cassava is rich in cyanogenic glycosides (Wolfson & Sumner, 1993) that are hydrolysed to yield HCN (Siritunga & Sayre, 2003). HCN is transformed to the non-toxic SCN^- by the action of rhodanese in the liver and kidneys of animals (Drakhshan Vaziri & Aminlari, 2004). The SCN^- is partly eliminated (minor elimination) via the milk (Soto-Blanco & Gorniak, 2003). Consequently, it is important to establish a specific optimum inclusion rate for HCN in order to identify an appropriate supplementation ratio for fresh cassava pulp (FCP) in the diet of dairy cows which will maximize the beneficial effects that impact the productive performance and microorganism growth in the interests of extending raw milk quality during storage. The objective of this present research was to examine how to enhance the activity of the LPs by increasing milk SCN^- via the supplementation of HCN levels from FCP in the diet of lactating dairy cows.

2. Materials and Methods

2.1 Animals and dietary treatments

Twenty-four Holstein Friesian crossbred (>87.5% crossbred) lactating dairy cows, averaging 50±27 days in milk (DIM), 13.6±3.5 kg of milk, and 394±40 kg body weight (BW), were blocked into three groups of 8 cows each. All cows were fed 7 kg/d of 21% crude protein (CP) concentrate with fresh grass (FG). The treatments were 1) the control concentrate together with *ad libitum* FG), 2) the control concentrate supplemented with 3.5 kg/d of FCP of top-dressed together with *ad libitum* FG, and 3) the control concentrate supplemented with 7.0 kg/d of FCP of top-dressed together with *ad libitum* FG. The experiment lasted for 44 d with the first 2 periods (14 d) as the adjustment period followed by 30 d (6 periods of 5 d) as the measurement period.

2.2 Fresh cassava pulp collection

The FCP samples were collected once per period from Korat Flour Industry Co., Ltd., Nakhon Ratchasima, Thailand. The FCP samples were divided into 3 parts. The first part was fed to the experimental cows. The second part was dried at 60 °C for 48 h and ground for proximate analysis and detergent analyses. The third part was sealed in an airtight plastic bag and immersed immediately in ice slurry to reduce the cyanide content activity during transport for analysis of cyanide content by Pyridine Pyrazorone method (O'Brien, 1991) at the Cassava and Starch Technology Research Unit, Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Bangkok, Thailand.

2.3 Measurements, sample collection, and chemical analysis

Residual feeds were weighed for two consecutive days weekly. Feed samples were taken and dried at 60 °C for 48 h. At the end of the experimental period, feed samples were composited and subsamples were taken for further chemical analysis and proximate analysis. Crude protein content was determined by the Kjeldahl method (procedure 928.08, AOAC, 1998). Ash content was determined by burning in a muffle furnace (procedure 942.05; AOAC, 1995). The ether extract was determined by using petroleum ether in a Soxtec System (procedure 948.15, AOAC, 1998). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using the modified method for a fiber analyzer Van Soest *et al.* (1991). Chemical analysis was expressed on the basis of final dry mater (DM).

Cows were milked twice daily and milk yields were recorded daily for each cow. Milk samples (morning and evening) were collected at each milking for two consecutive days weekly. The milk composition was analyzed using the FOSS MilkoScan™ FT2 infrared automatic analyzer (Fourier Transform Infrared Spectroscopy) (FOSS Analytical; DK-3400 Hillerøed, Denmark) at The Center for Scientific and Technological Equipment Building (F1) at Suranaree University of Technology (SUT). Also analyzed were the SCN^- concentration (Codex Alimentarius Commission (CAC GL 13/91) (CAC, 1991b), LP activity (Isobe *et al.*, 2009), and somatic cell count (SCC) using a Fossomatic 5000 Basic. The keeping quality (KQ) test using the alcohol test (AL) was described by Barrett *et al.* (1999) wherein 75% alcohol was added to neutralized milk samples. The milk clotting was noted and referred to as the end of shelf life. One portion of the milk samples was stored at 25 °C and another at 30 °C for examination of microbiological properties including the standard plate count (SPC) (Houghtby *et al.*, 1992), coliform count (CC) (Christen *et al.*, 1992), psychrotrophic and thermosphilic counts (Frank *et al.*, 1992) with minor modifycations, and incubated at 0, 2, 4, 6, 8, 10, and 12 h to count the colony-forming units (CFUs)/ml of milk.

2.4 Statistical analysis

Measurements of productive performance, milk yield, milk composition, SCC and KQ of milk were analyzed by analysis of variance (ANOVA) in a randomized complete block design (RCBD) using the statistical analysis system (SAS, 2002) and treatment means were compared using Duncan's new multiple range test (DMRT). SPC, CC, psychrotrophic and thermophilic counts were analyzed by 2 x 3 factorial ANOVA in a RCBD (2 temperatures of 25 and 30 °C x 3 HCN levels by 3 levels of FCP supplementation with 8 replicates per run). Temperature, HCN levels, and their interacttion were included in the model as fixed factor effects.

When the interaction between temperature and HCN levels was significant, orthogonal polynomial contrasts were performed to determine linear, quadratic, and cubic responses to the temperature within HCN levels. When the main effect of HCN levels was significant, an orthogonal polynomial contrast was performed to determine overall linear, quadratic, and cubic responses to temperature. Significant differences among treatments were assessed by DMRT. A significant level of $P<0.05$ was used.

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 feeds (control concentrate (21% CP), FCP and FG) were in the range reported as in the case of SUT (Thanh & Suksombat, 2015). Large variations in chemical composition of feeds reflect the differences in breeds, harvesting processes, soil types, fertilizer applications, season, and location.

The FCP was the source of HCN and the FCP was rather high in HCN (72.20 mg/kg dry solid) and low in CP (2.18%). In contrast, the supplementation of FCP was chosen to increase the total HCN content intake. The average HCN content of FCP was 72.20±1.64 ppm (DM basis). At 3.5 and 7.0 kg/h/d of FCP the increase in HCN of was 35 and 70 ppm/d. These levels of HCN derived from FCP ingestion are safe for dairy cows. According to Larson (2006), the risk of HCN poisoning in forage was at 600-1000 ppm (DM basis), or above that could cause death in the cattle. The results of this study indicated that at supplemental levels of 3.5 and 7.0 kg/d of FCP there is no negative impact from the HCN content for lactating dairy cows.

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

Item	Concentrate[1]	Fresh cassava pulp	Fresh grass
	---------- % of DM ----------		
Dry matter	91.03	21.29	12.22
Ash	9.59	12.38	12.76
Crude protein	21.12	2.18	10.39
Ether extract	3.02	2.16	1.23
Crude fiber	12.64	12.69	37.82
Neutral detergent fiber	43.98	61.62	63.38
Neutral detergent insoluble N	1.44	0.30	0.25
Acid detergent fiber	21.92	15.79	35.25
Acid detergent insoluble N	0.82	0.30	0.46
Acid detergent lignin	6.32	5.61	2.82
TDN_{1x}(%)[2]	61.91	52.32	54.90
DE_{1x} (Mcal/kg)[3]	2.95	2.31	2.52
DE_P (Mcal/kg)[4]	2.87	2.38	2.56
ME_P (Mcal/kg)[5]	2.45	1.96	2.14
NE_{LP} (Mcal/kg)[6]	1.53	1.19	1.31
Cyanide content (mg/kg dry solid)	-	72.20 $\pm$ 1.64	-

[1] Contained (as DM basis): 4% cassava 27.5% cassava distillers dried meal, 16% soybean meal (solvent extract), 2% corn gluten feed, 8% rice bran A, 8% cassava ethanol, 6% molasses, 24% palm kernel meal, 2.5% urea and 1.6% mineral (dicalcium phosphate), 0.4% vitamin mineral mix and 0.02% covatak 570. Mineral and vitamin mix: provided per kg of concentrate including 2,000,000 IU Vit. A; 640,000 IU Vit. D3; 64,000 IU Vit. E; 160 g Ca; 99 g S; 80 g P; 16 g Fe; 16 g Mn; 12 g Zn; 3 g Cu; 0.2 g I; 0.05 g Co; 0.05 g Se.

The second and third group (supplementing FCPu at 3.5 and 7.0 kg/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%) = [0.703 × ME_P] -0.19) + [(0.097 × ME_P)/97] × [(EE -30], (EE > 3%)

3.2 Animal nutrient intake, live weight change, milk yield and milk composition

Cows supplemented with FCP yielded greater total DM, CP and net energy for lactation (NE_{LP}) intakes than those fed the control diets (Table 2). However, the results showed that treatments had no effects (P>0.05) on live weight change of the animals (Table 2). The cows with diets supplemented with FCP had greater DM intake due to the higher DM concentration in the FCP supplemented group compared with the control. This result was supported by Suksombat *et al.* (2006) which found that feeding concentrates containing cassava pulp at 35 to 45% DM to lactating dairy cows had no effect on DM intake, milk yield and milk composition. Ukanwoko and Ibeawuchi (2014) recommended reducing the level of substitution of cassava with high HCN concentrates in the diet in order to avoid low nutrient intake and milk production of ruminants. In general, these results suggest that the effect of HCN content from FCP on DM intake varies with the amount, part or type of cassava with differing levels of cyanogenic glycoside depending on its origin. This may be as a result of variation in minerals in soils, season, and cultivation practices (Tangkawanit *et al.*, 2014). The negative live weight gain among the treatments in the current study was in agreement with National Research Council (NRC) (2001). This may be the result of negative energy balance, which often occurs in dairy cows during early lactation.

Supplementation of FCP at 3.5 and 7.0 kg/h/d had no effect on milk yield. Unchanged milk yield was also reported in the studies of Petlum *et al.* (2012), Lunsin *et al.* (2012), and Ukanwoko and Ibeawuchi (2014). In contrast to CP intake, no remarkable changes were found for milk yield and compositions among the treatments (P>0.05) (Table 3). However, the milk yields increased linearly with 400 g/day cassava hay (CH) with a slight reduction at 500 g/day of CH in lactating goat diets (Dung *et al.*, 2010). Discrepancies among studies on the effect of cassava supplementation on milk yield of dairy cows might be due to the level added and limitation of the amount of roughage and concentrates provided as balance for the nutrients.

Table 2. Effects of dietary levels of fresh cassava pulp on nutrient intakes, crude protein and net energy for lactation at the production level (NE_{LP}) and live weight change.

Item	Control[1]	35 ppm HCN[2]	70 ppm HCN[3]	SEM	P-value
Dry Matter, kg/cow/d					
Concentrate	6.13	6.13	6.13	-	-
Fresh grass	5.27	5.32	5.25	0.02	0.118
Fresh cassava pulp	0	0.48	0.96	-	-
Total	11.40^{c}	11.93^{b}	12.34^{a}	0.02	<0.001
Crude Protein, g/cow/d					
Concentrate	1295	1295	1295	-	-
Fresh grass	548	553	545	14.23	0.273
Fresh cassava pulp	0	10.46	20.93	-	-
Total	1842^{b}	1858^{a}	1861^{a}	14.26	0.005
NE_{LP}, Mcal/cow/d					
Concentrate	9.38	9.38	9.38	-	-
Fresh grass	6.90	6.97	6.88	0.03	0.102
Fresh cassava pulp	0	0.57	1.14	-	-
Total	16.28^{c}	16.92^{b}	17.40^{a}	0.03	<0.001
Live weight change					
Initial live weight, kg	393	396	394	22.42	0.995
Final live weight, kg	385	390	389	22.84	0.987
Live weight gain, g/d	-250	-192	-154	58.47	0.517

SEM: standard error of mean, HCN: hydrogen cyanide, NE_{LP}: net energy for lactation at production level

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

[1] control: 7.0 kg/d concentrate without fresh cassava pulp supplement together with *ad libitum* fresh grass

[2] control concentrate plus 3.5 kg/h/d of fresh cassava pulp together with *ad libitum* fresh grass with approximately 35 ppm HCN (dry matter basis) by calculation

[3] control concentrate plus 7.0 kg/h/d of fresh cassava pulp together with *ad libitum* fresh grass with approximately 70 ppm HCN (dry matter basis) by calculation

Table 3. Effects of dietary levels of fresh cassava pulp on milk yield, milk composition, thiocyanate concentration, lactoperoxidase activity and somatic cell count.

Item	Control[1]	35 ppm HCN[2]	70 ppm HCN[3]	SEM	P-value
Yield					
Milk, kg/d	12.87	13.61	14.28	1.11	0.672
3.5% FCM[4], kg/d	12.89	13.45	14.28	0.86	0.627
Fat, g/d	452	467	500	33	0.615
Protein, g/d	408	438	461	33	0.496
Lactose, g/d	604	642	683	59	0.465
Solid-not-fat, g/d	1002	1176	1244	86	0.463
Total solid, g/d	1553	1644	1744	99	0.569
Composition (g/100 g of raw milk)					
Fat	3.51	3.44	3.50	0.15	0.932
Protein	3.17	3.22	3.23	0.05	0.726
Lactose	4.69	4.72	4.78	0.07	0.156
Solid-not-fat	8.56	8.64	8.71	0.12	0.221
Total solid	12.07	12.08	12.21	0.21	0.888
Thiocyanate (ppm)	7.67^{c}	8.20^{b}	8.68^{a}	0.14	<0.001
LP activity (U/ml)	4.37^{b}	4.66^{ab}	4.92^{a}	0.11	0.011
SCC (x10^3 cells/ml)	302.3^{a}	68.0^{b}	62.2^{b}	48	0.012

SEM: standard error of mean, HCN: hydrogen cyanide, LP: Lactoperoxidase, SCC: somatic cell counts

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

[1] control: 7.0 kg/d concentrate without fresh cassava pulp supplement together with *ad libitum* fresh grass

[2] control concentrate plus 3.5 kg/h/d of fresh cassava pulp together with *ad libitum* fresh grass with approximately 35 ppm HCN (dry matter basis) by calculation

[3] control concentrate plus 7.0 kg/h/d of fresh cassava pulp together with *ad libitum* fresh grass with approximately 70 ppm HCN (dry matter basis) by calculation

[4] FCM = fat-corrected milk: 3.5% FCM = (0.432 x milk (kg)) + (16.216 x fat (kg))

In the current study, supplementing the diets of dairy cows with FCP had no effect on milk composition which agreed with previous studies when cows were fed cassava leaf silage (Modesto *et al.,* 2009). In contrast, lactating goats fed diets supplemented with CH (Dung *et al.,* 2010) had greater milk fat, protein, and total solids (TS) compared to the control. Recent research also reported a reduction of TS and lactose when lactating goats were supplemented cassava peel with 30% DM cassava leaf meal (Ukanwoko & Ibeawuchi, 2014); however, no effect was found on milk fat and protein. Therefore, the decrease in milk fat in some previous studies was affected by lower DM and NE_{LP} intake, nutrient digestibility, and particularly fiber (Khunkaew, 2009 unpublished data).

3.3 Thiocyanate concentration, lactoperoxidase activity, somatic cell counts, and alcohol test

The SCN^- concentration increased throughout as FCP supplementation increased, whereas a significant increase in LP activity was detected in 7.0 kg/h/d of FCP (4.92 U/ml). In the current study, diets supplemented with FCP resulted in marked alterations in milk SCN^- concentration and yield relative to the diet without added FCP (Table 3). SCN^- is derived from glucosinolates and the detoxification of the cyanogenic glycosides which are presented in the feed (Althaus *et al.,* 2001). Feeding high HCN is typically associated with a production of SCN^- in milk (Punthanara *et al.,* 2009). These possible changes rely on the high level of HCN in cassava to alter the SCN^- levels. In the present study, SCN^- was significantly increased as FCP supplementation increased. This is supported by Buaphan *et al.* (2003), Petlum *et al.* (2012), and Punthanara *et al.* (2009) whereby increasing the total HCN intake in the diet of dairy cows increased milk SCN^- concentration. Milk SCN^- concentration ranged from 0.1 to 15 mg/kg in cows (Perraudin, 1991), and its concentration varies with animal species (de Wit & Van Hooydonk, 1996), breed and lactation cycle (Zapico *et al.,* 1991), and season of the year (Dabur *et al.,* 1996). A gradual increase of enzyme activity was observed with increased FCP supplementation. A value of 4.37 U/ml was measured for control but for samples taken throughout with increasing application of FCP, enzyme activity continued to increase reaching values of 4.66 and 4.92 U/ml for 3.5 kg/h/d and 7.0 kg/h/d FCP supplementation, respectively. In contrast, for dairy cows fed a treatment diet supplemented with cassava

hay (1, 2, 3 kg/h/d), the enzyme activity was not affected by the treatment (Punthatnara *et al.,* 2009). Mee *et al.* (1994) reported that the SCN^- concentration required for activation of the LPs is between 10-15 ppm to achieve an optimal antibacterial activity of the LPs in milk. Although the results of supplementation of FCP produced slightly less milk SCN^- (mean=8.20 to 8.68 ppm) 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 activity of the LPs (Marshall *et al.*, 1986).

A decrease of SCC alongside an increase of SCN^- concentration led to decreased SCC in the milk of cows whose diet was supplemented with FCP at 3.5 (35 ppm HCN) and 7.0 kg/h/d (70 ppm HCN). Similarly, Petlum *et al.* (2012) reported that SCC was significantly decreased as milk SCN^- increased with the level of ensiled cassava foliage supplementation, and suggested that the decrease in SCC could result in a reduction in mastitis which is an inflammation of udder tissue that leads to a reduction in milk quality and quantity from lactating cows. These results suggest that the number of somatic cells may decrease as the content of HCN increases via supplementation of feed with FCP.

The benefit of supplementation of FCP was demonstrated by extending the KQ of cows' milk compared to the control. The alcohol stability test for KQ (h) of milk has been recommended as a useful indicator due to its reliability and consistent results (Barrett *et al.,* 1999). Milk from cows with diets supplemented with 7.0 kg/h/d FCP demonstrated increased LP activity, resulting in improvement of the KQ values in milk at 37 ºC for up to 8 h (Figure 1). This result has confirmed that FCP supplementation can improve the LP activity to extend the KQ parameter similar to those reported by Mark *et al.* (2001) and Dajanta *et al.* (2008). Indeed, Dajanta *et al.* (2008) revealed that the LPs affects the KQ of raw milk and thus can be manipulated to extend its shelf life. It is therefore possible to utilize stimulation of the LPs as an effective, alternative method of preserving raw milk quality during storage and transportation in locations where cooling facilities are unavailable or inadequate.

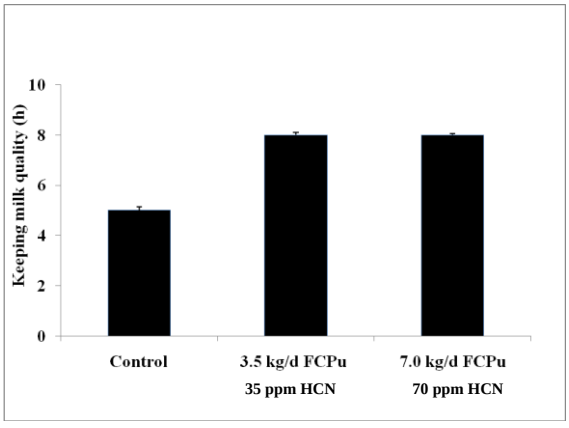

Figure 1. Effects of dietary levels of fresh cassava pulp in concentrate diet of dairy cows on keeping quality (h) of raw cow's milk samples at 37 ºC.

3.4 Effect of temperatures and HCN levels from fresh cassava pulp on standard plate, coliform count, and psychrotrophic and thermophilic counts

For SPC, psychrotrophic and thermophilic counts, there were no temperature x HCN level interactions at 0 through 12 h of incubation (Table 3). At 6, 8, 10, and 12 h, the SPC, psychrotrophic and thermophilic counts were not affected by temperature; however, the reaction proceeds only in the forward direction. SPC, and psychrotrophic and thermophilic counts were highest at 25 ºC and 30 ºC for the control and lowest for 35 and 70 ppm HCN at 25 ºC. Whereas, there was no response in CC due to the HCN level. The CC presented only at 2 and 4 h at both 25 ºC and 30 ºC (Table 4). After 0 to 12 h, CC was temperature dependent ($P \leq 0.001$). At all incubation times, the CC was higher at 30 ºC than 25 ºC. CC responses at 25 ºC showed linear pattern results (8, 10, and 12 h) and at 30 ºC (0 and 6 h).

Under incubation time (0 to 12 h), SPC, CC, psychrotrophic and thermophilic counts in cows' milk of cows with diets supplemented with FCP tended to be lower throughout the experiment (Tables 3-6). Although all microbial counts reached their peak at 12 h, their pattern was slightly different between 25 ºC and 30 ºC. From the beginning to the end of the incubation time, these declines in all microbial counts were related to the HCN content resulting from the level of FCP supplementation. Interestingly, as FCP levels were increased in the feed, all microbial reactions tended to continue to decrease. This supports the theory that increasing milk SCN^- concentration by supplementing cow diets with FCP can enhance the efficiency of antimicrobial activity associated with the LPs in milk. Also, the significant influence of the LPs reaction is likely to be between 2 and 8 h. These results were in agreement with recent studies (Buanphan, 2003; Punthanara *et al.,* 2009) which reported similar changes in microbial counts when increasing levels of cassava supplements were used. It has been reported there is potential for the antimicrobial effect associated with the LPs to inhibit the growth of various Gram-positive and Gram-negative bacteria (Naidu, 2000). This activity has proven to have beneficial effects for raw milk and can be used as a preservation technique during the manufacture of milk products. However, the LPs consists of three components: LP, SCN^-, and H_2O_2. The efficacy of LP antibacterial activity is known to have identical dependence in the range of concentrations of not only the amount of H_2O_2 but also the SCN^- concentration and the initial quality of raw milk (such as bacteria). LP catalyzes, in the presence of H_2O_2, the oxidation of SCN^- to yield $OSCN^-$ and HOSCN. These compounds react with the microbial sulfhydryl groups to restrain various cellular functions (Shin *et al.,* 2001). Discrepancies among studies on the antimicrobial effect of the LPs on sensitivity of bacteria strain may be explained by the differences in cell wall structure, the different barrier properties of the experiment (de Wit & van Hooydonk, 1996) and/or the type of electron donor, test media, pH, storage temperature, and length of incubation (Gay & Amar, 2005; Naidu, 2000). Thus the antimicrobial response in the milk of cows with diets containing FCP was temperature dependent for many variables. At 25 ºC and 30 ºC, all microbial counts

Table 4. Effect of temperature (25ºC vs 30ºC) and hydrogen cyanide (HCN) levels of fresh cassava pulp in the diet of dairy cattle (0, 35 and 70 ppm HCN) on total plate count in raw cow's milk at incubation time (0, 2, 4, 6, 8, 10 and 12 h) (N=8).

Temperature	**Level (ppm HCN)**	**Number of total plate count of HCN in the diet of dairy cattle (0, 35 and 70 ppm HCN)**						
		Time (h)						
		0	**2**	**4**	**6**	**8**	**10**	**12**
25ºC	**Mean**	**$1.70x10^{5B}$**	**$2.53x10^{5B}$**	**$3.47x10^{5B}$**	**$4.78x10^{5B}$**	**$4.49x10^{5B}$**	**$7.36x10^{5A}$**	**$1.15x10^{6A}$**
	0	$2.02x10^{5a}$	$3.09x10^{5a}$	$4.15x10^{5a}$	$6.43x10^{5a}$	$7.20x10^{5a}$	$1.17x10^{6a}$	$1.70x10^{6a}$
	35	$1.66x10^{5b}$	$2.33x10^{5b}$	$3.19x10^{5b}$	$4.00x10^{5b}$	$3.08x10^{5b}$	$5.40x10^{5b}$	$9.66x10^{5b}$
	70	$1.42x10^{5b}$	$2.16x10^{5b}$	$3.08x10^{5b}$	$3.92x10^{5b}$	$3.18x10^{5b}$	$4.98x10^{4b}$	$7.91x10^{5b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l*
30ºC	**Mean**	**$2.60x10^{5A}$**	**$3.33x10^{5A}$**	**$4.11x10^{5A}$**	**$5.33x10^{5A}$**	**$5.11x10^{5A}$**	**$7.80x10^{5A}$**	**$1.29x10^{6A}$**
	0	$3.03x10^{5a}$	$3.72x10^{5}$	$4.63x10^{5a}$	$6.91x10^{5a}$	$7.68x10^{5a}$	$1.22x10^{6a}$	$1.75x10^{6}$
	35	$2.48x10^{5b}$	$3.20x10^{5}$	$3.70x10^{5b}$	$4.41x10^{5b}$	$3.61x10^{5b}$	$5.84x10^{5b}$	$1.18x10^{6}$
	70	$2.29x10^{5b}$	$3.06x10^{5}$	$4.00x10^{5ab}$	$4.68x10^{5b}$	$4.02x10^{5b}$	$5.96x10^{5b}$	$9.29x10^{5}$
	Contrast	*l*	*l*	*l, q*	*l*	*l, q*	*l*	*ns*
HCN level	**Mean***	**$2.15x10^{5F}$**	**$2.93x10^{5EF}$**	**$3.79x10^{5DE}$**	**$5.05x10^{5C}$**	**$4.80x10^{5CD}$**	**$7.68x10^{5B}$**	**$1.22x10^{6A}$**
	0	$2.53x10^{5a}$	$3.41x10^{5a}$	$4.39x10^{5a}$	$6.67x10^{5a}$	$7.44x10^{5a}$	$1.20x10^{6a}$	$1.73x10^{6a}$
	35	$2.07x10^{5b}$	$2.76x10^{5b}$	$3.44x10^{5b}$	$4.19x10^{5b}$	$3.35x10^{5b}$	$5.62x10^{5b}$	$1.07x10^{6b}$
	70	$1.86x10^{5b}$	$2.61x10^{5b}$	$3.54x10^{5b}$	$4.30x10^{5b}$	$3.60x10^{5b}$	$5.47x10^{5b}$	$8.60x10^{5b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l*
SEM								
Temperature		0.633	0.392	0.403	0.368	0.581	0.241	0.360
HCN level		0.775	0.480	0.493	0.451	0.712	0.296	0.441
Temperature x HCN level		1.096	0.679	0.698	0.638	1.007	0.418	0.624
***P* value**								
Temperature		<0.001	<0.001	0.001	0.295	0.022	0.560	0.410
HCN level		<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Temperature x HCN level		0.656	0.783	0.547	0.960	0.840	0.975	0.912

SEM: standard error of mean

[a,b] Means within a column within temperature or the main effect of HCN level from fresh cassava pulp supplementation in the diet of dairy cattle having different superscript letters are different at P<0.05; [A,B] Means within the same column for the main effects of temperature having different superscript letters are different at P<0.05

[A,B,C,D,E,F] Means* within the same row within time having different superscript letters are different at P<0.05;

l, q, c: Within a column, the effect of HCN level for individual temperature or the main effect of HCN level is linear, quadratic, and cubic, respectively, at P<0.05

in milk samples from cows with diets supplemented by FCP at 3.5 and 7.0 kg/h/d were reduced. However, maximum response was observed for cows with diets supplemented at the higher level of FCP at 7.0 kg/h/d. From the results, all microbial counts revealed that the difference in temperature influenced the LPs activity in the milk. Milk at 30 ºC was inhibited less than milk at 25 ºC. The results further confirmed the efficiency of lower temperature on the effectiveness of the LPs for milk retention. These results are in conformity with those obtained by other authors who also noticed bacterial inhibition by the LPs (Dajanta *et al.*, 2008; Rasbawati *et al.*, 2014). In addition, the efficacy of the LPs also persists for a limited period of time, which decreases as the ambient temperature increases. The duration of the antibacterial activity achieved by the LPs activation is inversely related to the temperature of the milk during storage (IDF, 1988). The antibacterial effect of the LPs lasts for 7 to 8, 11 to 12, 16 to 17, and 24 to 26 h, as milk is stored at 30, 25, 20 and 15 ºC, respectively (IDF, 1988).

Table 5. Effect of temperature (25ºC vs 30ºC) and hydrogen cyanide (HCN) levels of fresh cassava pulp in the diet of dairy cattle (0, 35 and 70 ppm HCN) on coliform count (CC) in cows' milk at incubation time (0, 2, 4, 6, 8, 10 and 12 h) (N=8).

Temperature	**Level (ppm HCN)**	**Number of coliform count of HCN in the diet of dairy cattle (0, 35 and 70 ppm HCN)**						
		Time (h)						
		0	**2**	**4**	**6**	**8**	**10**	**12**
25ºC	**Mean**	**$2.09x10^{3B}$**	**$2.93x10^{3B}$**	**$2.22x10^{4B}$**	**$3.43x10^{4B}$**	**$2.59x10^{5B}$**	**$4.57x10^{5B}$**	**$1.65x10^{6B}$**
	0	$2.54x10^{3}$	$3.09x10^{3}$	$2.73x10^{4}$	$5.06x10^{4}$	$3.17x10^{5a}$	$5.28x10^{5a}$	$2.88x10^{6a}$
	35	$2.28x10^{3}$	$2.91x10^{3}$	$2.01x10^{4}$	$2.73x10^{4}$	$2.34x10^{5b}$	$4.23x10^{5b}$	$1.05x10^{6b}$
	70	$1.46x10^{3}$	$2.77x10^{3}$	$1.94x10^{4}$	$2.52x10^{4}$	$2.26x10^{5b}$	$4.21x10^{5b}$	$1.02x10^{6b}$
	Contrast	*ns*	*ns*	*ns*	*ns*	*l*	*l*	*l*
30ºC	**Mean**	**$4.56x10^{3A}$**	**$6.77x10^{3A}$**	**$4.99x10^{4A}$**	**$7.65x10^{4A}$**	**$5.79x10^{5A}$**	**$1.02x10^{6A}$**	**$3.69x10^{6A}$**
	0	$5.62x10^{3a}$	$7.45x10^{3}$	$6.09x10^{4}$	$1.12x10^{5}$	$7.02x10^{5a}$	$1.17x10^{6a}$	$6.38x10^{6a}$
	35	$4.63x10^{3a}$	$6.62x10^{3}$	$4.59x10^{4}$	$6.18x10^{4}$	$5.30x10^{5b}$	$9.61x10^{5b}$	$2.39x10^{6ab}$
	70	$3.40x10^{3b}$	$6.24x10^{3}$	$4.28x10^{4}$	$5.62x10^{4}$	$5.03x10^{5b}$	$9.37x10^{5b}$	$2.28x10^{6b}$
	Contrast	*l*	*ns*	*ns*	*l*	*l, q*	*l, q*	*l, q*
HCN level	**Mean***	**$3.32x10^{3D}$**	**$4.85x10^{3D}$**	**$3.61x10^{4D}$**	**$5.54x10^{4D}$**	**$4.19x10^{5C}$**	**$7.40x10^{5B}$**	**$2.67x10^{6A}$**
	0	$4.08x10^{3a}$	$5.27x10^{3}$	$4.41x10^{4a}$	$8.11x10^{4a}$	$5.10x10^{5a}$	$8.49x10^{5a}$	$4.63x10^{6a}$
	35	$3.46x10^{3a}$	$4.77x10^{3}$	$3.30x10^{4b}$	$4.45x10^{4b}$	$3.82x10^{5b}$	$6.92x10^{5b}$	$1.72x10^{6b}$
	70	$2.43x10^{3b}$	$4.50x10^{3}$	$3.11x10^{4b}$	$4.07x10^{4b}$	$3.65x10^{5b}$	$6.79x10^{5b}$	$1.65x10^{6b}$
	Contrast	*l*	*ns*	*l*	*l*	*l, q*	*l, q*	*l, q*
SEM								
Temperature		0.269	0.316	0.252	0.275	0.463	0.206	0.253
HCN level		0.330	0.387	0.308	0.337	0.566	0.252	0.310
Temperature x HCN level		0.466	0.547	0.436	0.476	0.801	0.356	0.438
***P* value**								
Temperature		<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
HCN level		0.004	0.370	0.001	0.019	<0.001	<0.001	<0.001
Temperature x HCN level		0.468	0.708	0.483	0.557	0.086	0.188	0.022

SEM: standard error of mean

[a,b] Means within a column within temperature or the main effect of HCN level from fresh cassava pulp supplementation in the diet of dairy cattle having different superscript letters are different at P<0.05; [A,B] Means within the same column for the main effects of temperature having different superscript letters are different at P<0.05; [A,B,C,D] Means* within the same row within time having different superscript letters are different at P<0.05

l, q, c: Within a column, the effect of HCN level for individual temperature or the main effect of HCN level is linear, quadratic, and cubic, respectively, at P<0.05

Table 6. Effect of temperature (25ºC vs 30ºC) and hydrogen cyanide (HCN) levels of fresh cassava pulp in the diet of dairy cattle (0, 35 and 70 ppm HCN) on psychrotrophic count in raw cows' milk at incubation time (0, 2, 4, 6, 8, 10 and 12 h) (N=8).

Temperature	**Level (ppm HCN)**	**Number of psychrotrophic count of HCN in the diet of dairy cattle (0, 35 and 70 ppm HCN)**						
		Time (h)						
		0	**2**	**4**	**6**	**8**	**10**	**12**
25ºC	**Mean**	**$1.14x10^{4B}$**	**$1.27x10^{4B}$**	**$1.48x10^{4B}$**	**$1.80x10^{4B}$**	**$2.37x10^{4B}$**	**$3.11x10^{4B}$**	**$3.85x10^{4B}$**
	0	$1.40x10^{4a}$	$1.54x10^{4a}$	$1.74x10^{4a}$	$2.06x10^{4a}$	$2.60x10^{4a}$	$3.46x10^{4a}$	$4.20x10^{4a}$
	35	$1.02x10^{4b}$	$1.15x10^{4b}$	$1.36x10^{4b}$	$1.70x10^{4b}$	$2.21x10^{4b}$	$2.94x10^{4b}$	$3.68x10^{4b}$
	70	$1.00x10^{4b}$	$1.14x10^{4b}$	$1.34x10^{4b}$	$1.66x10^{4b}$	$2.20x10^{4b}$	$2.93x10^{4b}$	$3.67x10^{4b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l*
30ºC	**Mean**	**$1.15x10^{4A}$**	**$1.28x10^{4A}$**	**$1.49x10^{4A}$**	**$1.81x10^{4A}$**	**$2.34x10^{4A}$**	**$3.15x10^{4A}$**	**$3.87x10^{4A}$**
	0	$1.51x10^{4a}$	$1.64x10^{4a}$	$1.85x10^{4a}$	$2.17x10^{4a}$	$2.70x10^{4a}$	$3.63x10^{4a}$	$4.47x10^{4a}$
	35	$1.12x10^{4a}$	$1.25x10^{4b}$	$1.46x10^{4b}$	$1.78x10^{4b}$	$2.31x10^{4b}$	$3.11x10^{4b}$	$3.78x10^{4b}$
	70	$1.10x10^{4b}$	$1.23x10^{4b}$	$1.44x10^{4b}$	$1.76x10^{4b}$	$2.29x10^{4b}$	$3.10x10^{4b}$	$3.76x10^{4b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l, q*
HCN level	**Mean***	**$1.15x10^{4D}$**	**$1.28x10^{4D}$**	**$1.49x10^{4D}$**	**$1.81x10^{4D}$**	**$2.34x10^{4C}$**	**$3.15x10^{4B}$**	**$3.87x10^{4A}$**
	0	$1.46x10^{4a}$	$1.59x10^{4a}$	$1.80x10^{4a}$	$2.12x10^{4a}$	$2.65x10^{4a}$	$3.54x10^{4a}$	$4.33x10^{4a}$
	35	$1.07x10^{4a}$	$1.20x10^{4b}$	$1.41x10^{4b}$	$1.73x10^{4b}$	$2.26x10^{4b}$	$3.03x10^{4b}$	$3.73x10^{4b}$
	70	$1.05x10^{4b}$	$1.18x10^{4b}$	$1.39x10^{4b}$	$1.71x10^{4b}$	$2.25x10^{4b}$	$3.02x10^{4b}$	$3.72x10^{4b}$
	Contrast	*l*	*l*	*l*	*l*	*l, q*	*l*	*l, q*
SEM								
Temperature		0.332	0.326	0.442	0.395	0.523	0.224	0.361
HCN level		0.430	0.401	0.488	0.432	0.615	0.282	0.482
Temperature x HCN level		0.626	0.617	0.665	0.658	0.711	0.471	0.673
***P* value**								
Temperature		<0.001	0.005	0.011	0.015	0.039	0.134	0.055
HCN level		0.002	0.070	0.005	0.019	0.006	<0.001	0.014
Temperature x HCN level		0.668	0.711	0.490	0.890	0.882	0.938	0.931

SEM: standard error of mea

[a,b] Means within a column within temperature or the main effect of HCN level from fresh cassava pulp supplementation in the diet of dairy cattle having different superscript letters are different at P<0.05; [A,B] Means within the same column for the main effects of temperature having different superscript letters are different at P<0.05; [A,B,C,D] Means* within the same row within time having different superscript

letters are different at P<0.05

l, q, c: Within a column, the effect of HCN level for individual temperature or the main effect of HCN level is linear, quadratic, and cubic, respectively, at P<0.05

Table 7. Effect of temperature (25ºC vs 30ºC) and hydrogen cyanide (HCN) levels of fresh cassava pulp in the diet of dairy cattle (0, 35 and 70 ppm HCN) on thermophilic count in raw cows' milk at incubation time (0, 2, 4, 6, 8, 10 and 12 h) (N=8).

Temperature	**Level (ppm HCN)**	**Number of thermophilic count of HCN in lactating dairy cows' diets (0, 35 and 70 ppm HCN)**						
		Time (h)						
		0	**2**	**4**	**6**	**8**	**10**	**12**
25ºC	**Mean**	**93.3^{B}**	**111.8^{B}**	**232.2^{B}**	**396.8^{B}**	**606.7^{B}**	**911.0^{B}**	**1560.5^{B}**
	0	110.0^{a}	133.2^{a}	250.0^{a}	418.4^{a}	688.3^{a}	959.7^{a}	1850.0^{a}
	35	93.3^{b}	103.7^{b}	228.3^{b}	393.2^{b}	573.3^{b}	893.1^{b}	1453.3^{b}
	70	78.5^{b}	98.5^{b}	218.3^{b}	378.8^{b}	558.5^{b}	878.8^{b}	1378.2^{b}
	Contrast	*l*	*l*	*l*	*l*	*l*	*l*	*l*
30ºC	**Mean**	**100.6^{A}**	**146.3^{A}**	**281.1^{A}**	**502.7^{A}**	**745.2^{A}**	**1073.6^{A}**	**1800.7^{A}**
	0	120.3^{a}	183.2^{a}	354.0^{a}	636.3^{a}	834.3^{a}	1259.0^{a}	2110.3^{a}
	35	97.8^{b}	132.3^{b}	259.3^{b}	443.3^{b}	703.3^{b}	992.3^{b}	1693.3^{b}
	70	83.8^{b}	123.5^{b}	230.1^{b}	428.5^{b}	698.0^{b}	969.5^{b}	1598.5^{b}
	Contrast	*l*	*l*	*l*	*l*	*l*	*l, q*	*l, q*
HCN level	**Mean***	**97.0^{E}**	**129.1^{E}**	**256.7^{E}**	**449.8^{D}**	**676.0^{C}**	**992.3^{B}**	**1680.3^{A}**
	0	115.2^{a}	158.2^{a}	302.0^{a}	527.4^{a}	761.3^{a}	1109.4^{a}	1980.0^{a}
	35	95.6^{b}	118.0^{b}	243.8^{b}	418.3^{b}	638.3^{b}	942.7^{b}	1573.3^{b}
	70	81.2^{b}	111.0^{b}	224.2^{b}	403.7^{b}	628.3^{b}	924.2^{b}	1488.4^{b}
	Contrast	*l*	*l*	*l*	*l*	*l*	*l, q*	*l, q*
SEM								
Temperature		0.240	0.216	0.372	0.235	0.383	0.407	0.251
HCN level		0.133	0.322	0.376	0.355	0.305	0.562	0.312
Temperature x HCN level		0.356	0.464	0.799	0.416	0.523	0.806	0.437
***P* value**								
Temperature		<0.001	<0.001	0.001	0.053	0.122	0.208	0.120
HCN level		0.002	0.002	0.001	0.003	<0.001	<0.001	<0.001
Temperature x HCN level		0.368	0.538	0.403	0.476	0.679	0.726	0.704

SEM: standard error of mean

^{a,b} Means within a column within temperature or the main effect of HCN level from fresh cassava pulp supplementation in the diet of dairy cattle having different superscript letters are different at P<0.05; ^{A,B} Means within the same column for the main effects of temperature having different superscript letters are different at P<0.05; ^{A,B,C,D,E} Means* within the same row within time having different superscript letters are different at P<0.05

l, q, c: Within a column, the effect of HCN level for individual temperature or the main effect of HCN level is linear, quadratic, and cubic, respectively, at P<0.05

4. Conclusions

From the results of this study, it has been concluded that improved preservation of raw cows' milk can be achieved through the supplementation of the diets of lactating dairy cows with FCP at 3.5 and 7.0 kg/h/d. This technique can be used to extend milk quality during storage, decrease SCC and prohibit bacterial activity which is the main cause of deterioration of milk over time. Although only the animals with diets supplemented with FCP at 7 kg/h/d achieved stimulation of LP activity, improved SCN^{-} concentration was observed in the milk from cows at both FCP supplementation levels and values for keeping quality were also similar for both. Therefore, the optimum supplementation level of 3.5 kg/h/d FCP could be used in the concentrate for diets of lactating dairy cows.

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