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Effects of Cassava Hay Supplementation on Antibacterial Activity of the Lactoperoxidase System in Raw Milk of Dairy Cows

Sureewan Punthanara[1]*, Pornsri Chairatanayuth[2], Pravee Vijchulata[2]
Somjit Surapat[3], Uthai Kuntho[4]and Wonganan Narongwanichakarn[5]

ABSTRACT

Sixteen multiparous cows in early-to-mid lactation (82.4±8.6 day in milk, DIM) were used in a completely randomized block design with repeated measurement to evaluate the effects of cassava hay (CH) supplementation on antibacterial activity of the lactoperoxidase system in raw milk. The cows were blocked into four groups based on DIM and previous milk yield. Each group was randomly fed dietary treatment twice daily for four months. Treatment diets were: T1) concentrate (control); T2) concentrate+1 kgCH/head/day; T3) concentrate+2 kgCH/head/day; and T4) concentrate+3 kgCH/head/day. The concentrate for each treatment was formulated to provide together with CH, a daily intake of 1,830-1,940 gm protein. The cows received 25 kg/head/day of ruzi grass silage. After a 14-d adaptation period, daily milk yield was recorded and the raw milk of the cows was collected every month and analyzed for lactoperoxidase and thiocyanate concentration, standard plate, coliform, psychrotrophic and thermophilic counts. Milk yield and milk lactoperoxidase concentrations were not affected by treatments ($P>0.05$). Thiocyanate concentrations were higher ($P<0.01$) for T2, T3 and T4 than for T1. Standard plate, coliform, psychrotrophic and thermophilic counts were lower in T2, T3 and T4 compared to T1. The results showed that supplementation of cassava hay to dairy cows increased the efficiency of antibacterial activity of the lactoperoxidase system in raw milk.

Key words: cassava hay, thiocyanate, lactoperoxidase system, bacterial count, milk yield

INTRODUCTION

Cassava hay has been used as protein source for dairy cattle feed. All parts of cassava foliage contain high levels of crude protein; 32.3, 14.6, 8.9 and 24.9 % for the leaves, stem, branches and whole upper part of cassava crop hay, respectively. Consumption rates for the whole upper part of cassava crop hay of 11.2 kg/cow/day or 3.20% of live weight have been advocated (Wanapat *et al*., 1997). Cassava, however, contains cyanogenic glycosides in the form of linamarin (95%) and lotaustralin (5%). The amount of cyanogenic glycosides varies with the part of the plant, maturity, variety and environmental conditions such as soil, moisture, temperature, etc. (Okigbo, 2004). Cassava leaves have the highest cyanogenic glycoside levels (White *et al*., 1998).

1 Royal Chitralada Projects, Dusit Palace, Bangkok 10303, Thailand.
2 Department of Animal Science, Kasetsart University, Bangkok 10900, Thailand.
3 Kasetsart University Dairy Center, Kasetsart University, Bangkok 10900, Thailand.
4 Department of Animal Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.
5 National Institute of Animal Health, Kasetklang, Bangkhen, Bangkok 10900, Thailand.
* Corresponding author, e-mail: kunwow@yahoo.com

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These cyanogens yield cyanide following hydrolysis (Keresztessy *et al*., 2001; Siritunga and Sayre, 2003). Cyanogenesis is initiated in cassava when the plant tissue is damaged. Rupture of the vacuole releases linamarin which is hydrolyzed by linamarase, a cell wall-associated β-glycosidase (McMahon *et al*., 1995). Hydrolysis of linamarin yields an unusable hydroxynitrile intermediate acetone cyanohydrin, plus glucose. Acetone cyanohydrin spontaneously decomposes to acetone and cyanide at pH >5.0 or temperature >35°C and can be broken down enzymatically by hydroxynitrile lyase (White *et al*., 1998). Cyanide has severe cytotoxic effects due to its inhibition of cytochrome oxidase in the mitochondrial respiratory chain (Drakhshan Vaziri and Aminlari, 2004). In cattle, a cyanide concentration of 1,000 ppm or above in forage (DM basis) is dangerous and usually will cause death (Larson, 2006). The drying process can decrease the cyanide concentration in cassava leaves to a safe level for ruminants. Sun-drying for 2-3 days eliminates more than 90% of the cyanide and enhances the palatability and long-term storage (Wanapat, 2003).

Cyanide is transformed to the non-toxic thiocyanate by the action of rhodanese, an enzyme widely distributed in nature. The liver and kidneys of animals are the major source of rhodanese, where it participates in cyanide detoxification (Drakhshan Vaziri and Aminlari, 2004). Thiocyante is eliminated mainly via the urine but also via the milk, tears and saliva (Soto-Blanco and Górniak, 2003).

There are two major dietary sources of thiocyanate; glucosinolates and cyanogenic glycosides. Vegetables in the genus *Brassica* (family Cruciferae), such as cabbage, kale, brussel sprouts and turnips, are rich glucosinolates which are hydrolyzed to thiocyanate. The cyanogenic glycosides are found in cassava, sweet potatoes, maize, millet, sugar cane, peas, beans and the kernel of various fruits (Wolfson and Sumner, 1993). When cyanogenic plants are crushed or otherwise injured, cyanide is liberated through the action of endogenous plant enzymes. Cyanogenic glycosides are also degraded by rumen microbial enzymes and are rapidly absorbed from rumen (Majak and Cheng, 1984), which in a reaction with thiocysteine or thiosulfate (metabolic product of sulfur amino acids) is detoxified by conversion into thiocyanate. The latter reaction is catalyzed by the enzyme rhodanese (Reiter and Härnulv, 1984).

Milk thiocyanate is used in the lactoperoxidase system, a natural antimicrobial system present in raw milk (Zapico *et al*., 1991). The lactoperoxidase system consists of three components: lactoperoxidase, thiocyanate and hydrogen peroxide (Reiter and Härnulv, 1984). Lactoperoxidase is found in the mammary, saliva and lachrymal glands of mammals and in their respective secretions, such as milk, saliva and tears. Lactoperoxidase has the ability to catalyze the oxidation of thiocyanate by hydrogen peroxide with the production of the antibacterial hypothiocyanite ($OSCN^-$) and other intermediates (Modi *et al*., 1991) These products have a broad spectrum of antibacterial effects to reduce bacterial growth by damaging the cell membranes and inhibiting the activity of many cytoplasmic enzymes (Haddadin *et al*., 1996). The hypothiocyanite can be produced by two different pathways (Wolfson and Sumner, 1993).

$$2SCN^- + H_2O_2 + 2H^+ \rightarrow (SCN)_2 + 2H_2O$$
$$(SCN)_2 + H_2O \rightarrow HOSCN + SCN + H^+$$
$$HOSCN \leftrightarrow H^+ + OSCN^-$$

or

$$SCN^- + H_2O_2 \rightarrow OSCN^- + H_2O$$

The antimicrobial agents of the lactoperoxidase system in milk cause inhibition of various spoilage and pathogenic organisms, thus enhancing the microbiological quality of milk (Seifu *et al*., 2005). There are many different

groups of bacteria that show varying degrees of sensitivity to the lactoperoxidase system (Seifu *et al*., 2005). Gram-positive, catalase-negative bacteria, such as *Streptococci* and *Lactobacilli* are generally inhibited but not killed by the lactoperoxidase system (Oram and Reiter, 1966). These organisms can be self-inhibitory under aerobic conditions in milk in the presence of lactoperoxidase and thiocyanate provided they generate the third component of the lactoperoxidase system, namely hydrogen peroxide (Reiter and Härnulv, 1984). However, Gram-negative, catalase-positive organisms, such as *Pseudomonas*, coliform, *Salmonellae* and *Shigellae*, are not only inhibited by the lactoperoxidase system but, depending on the pH of the medium, temperature, incubation time, cell density and particular electron donor, may be killed provided hydrogen peroxide is supplied exogenously, either chemically, enzymatically or by hydrogen peroxide-producing bacteria (Björck *et al*., 1975). This difference in sensitivity to the lactoperoxidase system can probably be explained by the difference in cell wall structure and the different barrier properties. The inner membrane of Gram-negative bacteria appears to be more extensively damaged by lactoperoxidase treatment than is that of Gram-positive species (Marshall and Reiter, 1980; Reiter and Härnulv, 1984).

The most widely recommended industrial application of the lactoperoxidase system in food production is in the dairy industry for the preservation of raw milk during storage and/or transportation to processing plants. The International Dairy Federation has published a guideline for the use of the lactoperoxidase system by adding NaSCN and H_2O_2 for the preservation of raw milk especially in the absence of refrigeration (IDF, 1988). In Thailand, farmers are not allowed to add NaSCN and H_2O_2 for the preservation of raw milk. So enhancing the activity of the lactoperoxidase system by increasing milk thiocyanate via cassava hay supplementation in dairy cow feed was the objective of this present work.

MATERIALS AND METHODS

Cows and dietary treatment

Sixteen multiparous cows in early-to-mid lactation, averaging 82.4(±8.6) DIM, were used in a completely randomized block design with repeated measurement. Cows were blocked into four groups base on DIM and previous milk yield (average 12 kg/cow/day), and the cows within blocks were randomly assigned to treatment diets for four months. The treatment diets were: T1) concentrate (control); T2) concentrate+1 kgCH/head/day; T3) concentrate+2 kgCH/head/day; and T4) concentrate+3 kgCH/head/day (Table 1). The diets were balanced to provide equal crude protein intake/day.

The concentrates were given to all cows at the rate of 6 kg/head/day (based on the pre-experimental milk yield record at a 2:1 ration). The concentrates and cassava hay were provided in two equal parts during the morning and afternoon milking. In addition, all cows were provided with ruzi grass silage at the rate of 25 kg/head/day. The silage was equally subdivided and provided after each milking.

Cassava hay used in this experiment was made from the whole upper part of the cassava crop (at 10-15 cm above the ground) harvested at three months maturity for the first harvest, then chopped and sun-dried for two days. Thereafter, harvesting occurred after every two months maturity, with subsequent processing as for the first harvest.

Sampling and chemical analysis

Cows were milked twice daily. During the four months of the experiment, after a 14-day adaptation period, milk yields were recorded after milking, and milk samples were collected from the morning milk once a month and analyzed for:

Table 1 Composition and calculated nutrients of the concentrates and intake of the supplemental treatments.

Ingredient (% by weight, kg)	T1	T2	T3	T4
Corn	28.9	36.9	40.9	52.9
Rice bran	12.0	15.0	15.0	16.0
Coconut meal	20.0	13.0	7.0	0
Wheat bran	15.0	10.0	12.0	4.0
Palm meal	0	10.0	16.0	22.0
Soy bean meal	21.0	12.0	6.0	2.0
Sulfur	0.1	0.1	0.1	0.1
Vitamin and mineral premix*	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0
% Protein	19.39	16.21	13.09	10.31
% TDN	76.64	76.68	76.28	77.26
Concentrate intake (kg)/head/day	6	6	6	6
Cassava hay intake (kg)/head/day	0	1	2	3
Silage intake (kg)/head/day	25	25	25	25
Protein intake (kg)/head/day	1.83	1.86	1.89	1.94

* Composition of vitamin and mineral premix: NaCl = 17.163%, Ca_2PO_4 (P14) = 25.744%, S = 1.716%, MnO_2 = 1.716%, Mg_2SO_4 = 8.581%, ZnO = 0.858%, $CuSO_4$ = 0.429%, Na_2SeO_3 = 0.086%, Co_2SO_4 = 0.069%, KI = 0.009%, Vitamin complex = 42.907% and Vitamin E 50 = 0.721%

thiocyanate (Cosby and Sumner, 1945) and lactoperoxidase (Shindler *et al*., 1976) concentration; standard plate (Houghtby *et al*., 1992); coliform (Christen *et al*., 1992); and psychrotrophic and thermophilic counts (Frank *et al*., 1992). Concentrate, ruzi grass silage and cassava hay samples were also collected once a month and analyzed for: DM, ash, CP (AOAC, 1990), NDF, ADF (Van Soest *et al*., 1991) and cyanide content (O'Brien *et al*., 1994).

Statistical analysis

Milk yield, standard plate, coliform, psychrotrophic, thermophilic counts, milk thiocyanate and lactoperoxidase concentrations were statistically analyzed using a completely randomized block design with repeated measurement and treatment means were compared using Tukey (SAS, 2002). The statistical model is described by Equation 1:

$$Y_{ijk} = \mu + \rho_i + A_j + \delta_{ij} + T_k + AT_{jk} + \varepsilon_{ijk} \quad (1)$$

where, Y_{ijk} = Response variable
μ = Overall mean
ρ_i = Effect of i^{th} block (i = 1, 2, 3, 4)
A_j = Effect of j^{th} cassava supplementation level (j = 1, 2, 3, 4)
T_k = Effect of k^{th} month (k = 1, 2, 3, 4)
AT_{jk} = Effect of j^{th} cassava supplementation level at k^{th} month
δ_{ij} = Random error from i^{th} block at j^{th} cassava supplementation level
ε_{ijk} = Random error from i^{th} block, j^{th} cassava supplementation level at k^{th} month

RESULTS AND DISCUSSIONS

Chemical composition and cyanide content in the concentrate, silage and cassava hay

Chemical compositions of the

concentrates, silage and cassava hay are presented in Table 2. The average cyanide content of cassava hay was 112.29 ppm (DM basis). This level is safe for dairy cows. According to Larson (2006), the levels of cyanide in forage that were potentially toxic and should not be used as the only source of roughage were 600-1000 ppm (DM basis), and the levels of cyanide that could cause death in the cattle were 1,000 ppm HCN or above.

Milk yield

The supplementation of cassava hay did not affect milk yield. The daily milk production of cows fed T1 (control) or supplemented with cassava hay in T2 to T4 (Table 3) varied from an average of 12.32 to 13.04 kg (P>0.05). This was partly due to the limited amount of roughage and the balance of nutrients from the concentrates provided.

Lactoperoxidase concentrations in raw milk

Concentrations of lactoperoxidase in the raw milk of all cows (varying from 10.09-12.05 unit/ml) as shown in Table 4 were not significantly different (P>0.05). The normal range of lactoperoxidase concentration in cow's milk reported by Gothefors and Marklund (1975) was 1.2 to 19.4 units/ml. According to Marshall *et al.* (1986), a lactoperoxidase concentration at 1.44 unit/ml was sufficient to catalyze lactoperoxidase system activity. Hence, the concentrations observed in this study were sufficient to provide effective antibacterial activity.

Thiocyanate concentrations in raw milk

Thiocyanate concentrations of raw milk from the cows supplemented with cassava hay were higher than those from the cows fed control feed (without cassava hay). The concentrations of thiocyanate in raw milk tended to increase (P<0.05) as supplementation of cassava hay increased. Time did not affect the thiocyanate concentrations in the milk (Table 5). Mean concentration of thiocyanate in the raw milk from

Table 2 Chemical composition and cyanide concentration in the concentrates, silage and cassava hay.

Items	Concentrates				Silage	Cassava hay
	T1	T2	T3	T4		
	% DM					
DM	91.30	91.08	90.89	90.74	40.19	88.02
Ash	7.71	7.52	7.07	7.05	10.88	9.97
CP	19.22	16.12	12.96	10.26	8.35	23.60
NDF	23.03	22.18	20.95	21.07	69.92	34.12
ADF	17.13	16.98	16.48	16.46	52.51	26.85
Cyanide (ppm)	0	0	0	0	0	112.29

Table 3 Milk yield of cows supplemented with different levels of cassava hay.

Treatments	Milk yield (kg/day)				
	1st Month	2nd Month	3rd Month	4th Month	Means
T1	12.55	12.88	12.18	11.80	12.35
T2	12.75	12.53	12.10	12.78	12.54
T3	12.10	12.68	12.40	12.10	12.32
T4	13.30	13.45	12.70	12.70	13.04
SE	0.97	1.15	1.28	1.18	1.04

T1=control; T2=concentrate+1 kgCH/day; T3=concentrate+2 kgCH/day; and T4=concentrate+ 3 kgCH/day.

Table 4 Concentrations of lactoperoxidase in raw milk of cows supplemented with different levels of cassava hay.

Treatments	Concentration of lactoperoxidase (unit/ml)				
	1st Month	2nd Month	3rd Month	4th Month	Means
T1	10.67	10.09	11.32	10.15	10.56
T2	11.15	10.93	10.89	10.74	10.93
T3	11.38	11.08	11.41	11.36	11.31
T4	11.10	12.05	11.21	11.89	11.56
SE	0.39	0.47	0.42	0.43	0.38

T1=control; T2=concentrate+1 kgCH/day; T3=concentrate+2 kgCH/day; and T4=concentrate+ 3 kgCH/day

Table 5 Concentrations of thiocyanate in raw milk of cows supplemented with different levels of cassava hay.

Treatments	Concentration of thiocyanate (ppm)				
	1st Month	2nd Month	3rd Month	4th Month	Means
T1	7.11 [b]	6.94 [b]	6.67 [c]	7.23 [c]	6.99 [d]
T2	14.39 [a]	13.24 [a]	13.22 [b]	13.43 [b]	13.57 [c]
T3	14.95 [a]	14.69 [a]	14.75 [ab]	15.18 [ab]	14.89 [b]
T4	15.99 [a]	15.74 [a]	15.82 [a]	16.08 [a]	15.90 [a]
SE	0.50	0.45	0.44	0.43	0.49

T1=control; T2=concentrate+1 kgCH/day; T3=concentrate+2 kgCH/day; and T4=concentrate+ 3 kgCH/day

[abc] Means in the same column with different superscripts are different ($P<0.05$).

the cows fed control feed in this study was 6.99 ppm. This value was higher than the average thiocyanate concentration in raw milk reported at 5 ppm in the study of Lambert (2001). However, it was within the 1-10 ppm range of thiocyanate concentrations of bovine milk described by Reiter and Härnulv (1984). Milk thiocyanate concentrations vary with breed, species and type of feed (Wolfson and Sumner, 1993). Cows in natural pastures containing clover produced milk with up to 15 ppm of thiocyanate (Zapico *et al*., 1991). The increase in the milk thiocyanate concentration was due to the detoxification of cyanide in cassava hay by the enzyme rhodanese (Reiter and Härnulv, 1984; Drakhshan Vaziri and Aminlari, 2004).

Milk thiocyanate is used in the lactoperoxidase system (Zapico *et al*., 1991). A level of 10-15 ppm of thiocyanate was required to achieve an optimal antibacterial effect in the lactoperoxidase system of milk (Reiter and Härnulv, 1984; Björck, 1978 and Dahlberg *et al*., 1984). Therefore, supplementation of cassava hay at 1 kg/cow/day was sufficient to produce the milk thiocyanate (mean=13.57 ppm) for the activity of the lactoperoxidase system in raw milk (Table 5).

Bacterial counts in raw milk

Compared to the control (T1), standard plate, coliform, psychrotrophic and thermophilic counts in raw milk of cows supplemented with cassava hay for all intervals tended to be lower throughout the experiment (Tables 6-9). In particular, the supplementation of cassava hay at 2 (T3) and 3 (T4) kg/head/day caused a decrease in bacterial counts when compared to those of T1 ($P<0.05$) for all collection periods. These declines in the bacterial count were related to the level of cassava hay supplementation. When cassava hay in the feed increased, the bacterial load tended to

Table 6 The effects of cassava hay supplementation on the total plate count of raw milk.

Treatment	Total Plate Count (CFU/ml)				
	1st Month	2nd Month	3rd Month	4th Month	Means
T1	$2.59\times10^{5\ a}$	$2.93\times10^{5\ a}$	$2.23\times10^{5\ a}$	$2.16\times10^{5\ a}$	$2.54\times10^{5\ a}$
T2	$1.91\times10^{5\ ab}$	$1.75\times10^{5\ b}$	$1.57\times10^{5\ b}$	$1.37\times10^{5\ ab}$	$1.70\times10^{5\ b}$
T3	$1.40\times10^{5\ bc}$	$1.77\times10^{5\ b}$	$1.36\times10^{5\ bc}$	$1.13\times10^{5\ b}$	$1.45\times10^{5\ b}$
T4	$1.25\times10^{5\ c}$	$1.66\times10^{5\ b}$	$1.20\times10^{5\ c}$	$1.02\times10^{5\ b}$	$1.29\times10^{5\ b}$
SE	1.0995	1.1281	1.0660	1.1655	1.1675

T1=control; T2=concentrate+1 kgCH/day; T3=concentrate+2 kgCH/day; and T4=concentrate+ 3 kgCH/day

[abc] Means in the same column with different superscripts are different (P<0.05).

Table 7 The effects of cassava hay supplementation on the coliform count of raw milk.

Treatment	Coliform Count (CFU/ml)				
	1st Month	2nd Month	3rd Month	4th Month	Means
T1	$1.32\times10^{3\ a}$	$1.36\times10^{3\ a}$	$1.41\times10^{3\ a}$	$1.23\times10^{3\ a}$	$1.35\times10^{3\ a}$
T2	$1.08\times10^{3\ ab}$	$1.03\times10^{3\ b}$	$9.49\times10^{2\ b}$	$9.90\times10^{2\ b}$	$1.02\times10^{3\ b}$
T3	$9.52\times10^{2\ bc}$	$9.17\times10^{2\ b}$	$9.01\times10^{2\ b}$	$9.65\times10^{2\ b}$	$9.37\times10^{2\ b}$
T4	$7.93\times10^{2\ c}$	$9.29\times10^{2\ b}$	$8.17\times10^{2\ b}$	$8.17\times10^{2\ b}$	$8.25\times10^{2\ b}$
SE	1.0494	1.0531	1.0866	1.0641	1.0494

T1=control; T2=concentrate+1 kgCH/day; T3=concentrate+2 kgCH/day; and T4=concentrate+ 3 kgCH/day

[abc] Means in the same column with different superscripts are different (P<0.05).

Table 8 The effects of cassava hay supplementation on the psychrotrophic count of raw milk.

Treatment	Psychrotrophic Count (CFU/ml)				
	1st Month	2nd Month	3rd Month	4th Month	Means
T1	$1.32\times10^{4\ a}$	$1.35\times10^{4\ a}$	$1.29\times10^{4\ a}$	$1.32\times10^{4\ a}$	$1.32\times10^{4\ a}$
T2	$1.12\times10^{4\ ab}$	$1.11\times10^{4\ ab}$	$1.11\times10^{4\ ab}$	$1.08\times10^{4\ ab}$	$1.11\times10^{4\ ab}$
T3	$1.01\times10^{4\ b}$	$1.01\times10^{4\ b}$	$9.64\times10^{3\ b}$	$9.72\times10^{3\ b}$	$9.91\times10^{3\ b}$
T4	$9.50\times10^{3\ b}$	$9.48\times10^{3\ b}$	$9.43\times10^{3\ b}$	$9.08\times10^{3\ b}$	$9.38\times10^{3\ b}$
SE	1.0489	1.0630	1.0413	1.0604	1.0486

T1=control; T2=concentrate+1 kgCH/day; T3=concentrate+2 kgCH/day; and T4=concentrate+ 3 kgCH/day

[ab] Means in the same column with different superscripts are different (P<0.05).

Table 9 The effects of cassava hay supplementation on the thermophilic count of raw milk.

Treatment	Thermophilic Count (CFU/ml)				
	1st Month	2nd Month	3rd Month	4th Month	Means
T1	121.25 [a]	120.00 [a]	122.50 [a]	123.75 [a]	121.88 [a]
T2	93.75 [b]	93.75 [b]	97.50 [b]	95.00 [b]	95.00 [b]
T3	80.00 [bc]	81.25 [bc]	88.75 [bc]	78.75 [bc]	82.19 [c]
T4	71.25 [c]	72.50 [c]	76.25 [c]	68.75 [c]	72.19 [c]
SE	4.72	3.75	3.99	4.38	3.85

T1=control; T2=concentrate+1 kgCH/day; T3=concentrate+2 kgCH/day; and T4=concentrate+ 3 kgCH/day

[abc] Means in the same column with different superscripts are different (P<0.05).

decrease. The collection period did not affect microbial load and no interaction with the treatments was observed.

The supplementation of cassava hay in the dairy cow feed caused the milk thiocyanate concentrations to increase. The increaseing of thiocyanate concentration in raw milk could enhance the efficiency of antibacterial activity of the lactoperoxidase system. According to Reiter *et al*. (1976), the bactericidal effect of the lactoperoxidase system against *E. coli* increased when the thiocyanate concentration rose from 0.015 to 0.15 mM. Kamau *et al*. (1990) reported that the addition of thiocyanate and hydrogen peroxide in milk decreased the number *of Listeria monocytogenes* and *Staphylococcus aureus* below those in untreated milk or milk treated with hydrogen peroxide alone. The activation of the antibacterial lactoperoxidase system in raw milk, by increasing thiocyanate concentration, together with hydrogen peroxide resulted in a substantial reduction in the bacteria flora and prevented the growth of psychrotrophic bacteria for up to five days (Björck *et al*., 1975), decreasing the number of *Salmonella* in the acidified (pH 5.3) raw milk (Wray and McLaren, 1987). The activation also reduced standard plate, coliform and psychrotrophic counts below those of untreated milk (Zajac *et al*., 1983) and untreated goat's milk in ambient temperature at 7 hrs of storage (Nigussie and Seifu, 2008).

The results in this study agreed with Buaphan (2003), who reported that increasing the content of cassava chips in total mixed rations caused an increase in the concentration of thiocyanate in milk with a subsequent decrease in total plate and coliform counts in the milk. Similarly, Srinetra (2001) demonstrated that supplementation of cassava hay at the rate of 0.5, 1.0 and 1.5 kg/day, increased methylene blue reduction time from 2.65 hrs in the control (0 kg/day) to 2.77, 3.18 and 4.05 hrs, respectively.

Although, the bacterial counts in the raw milk of cows supplemented with cassava hay tended to decrease, the supplementation of cassava hay at 1 kg/head/day (T2) did not produce a consistent decline in the bacterial load. The bacterial counts were not significantly different from those of the control. The reduction percentage of total plate, coliform, psychrotrophic and thermophilic counts in raw milk from T2, when compared to the control were 33.07, 24.44, 15.91 and 22.05, respectively. The bacterial loads in the raw milk of cows supplemented with cassava hay at 2 kg/head/day (T3) showed a significant and consistent decline from those of the control. The reduction percentage of total plate, coliform, psychrotrophic and thermophilic counts in raw milk from T3 when compared to the control were 42.91, 30.59, 24.92 and 32.56, respectively. In T4 (supplemented with cassava hay at 3 kg/head/day), the bacterial numbers also declined ($P<0.05$) in a pattern similar to T3. The percentage of reduction of total plate, coliform, psychrotrophic and thermophilic counts in raw milk from T4 when compared to the control were 49.21, 38.89, 28.94 and 40.77, respectively. Hence, the supplementation of cassava hay at 2 kg/head/day to dairy cows was a suitable level to increase the antibacterial efficiency of the lactoperoxidase system in raw milk.

CONCLUSION

The cyanide content in cassava hay was at a safe level and the cassava hay could be used as a supplement for dairy cows with no harm. While the milk lactoperoxidase concentrations were not affected, thiocyanate concentrations were observed to increase in the raw milk of cows supplemented with cassava hay. As a result, bacterial counts in the raw milk tended to decrease when the supplementation of cassava hay in feed increased. This is believed to be due to an improvement in the efficiency of the antibacterial activity of the lactoperoxidase system in raw milk.

According to this experiment, 2 kg of cassava hay/ head/day was the recommended supplemental level in lactating cows.

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