Asian-Aust. J. Anim. Sci.
Vol. 24, No. 4 : 517 - 524
April 2011

www.ajas.info

# Cassava in Lactating Sow Diets: I. Effects on Milk Composition and Quality

**A. Jupamatta*, U. Kanto[1,2] and C. Tirawattanawanich[3]**

Agricultural Research and Development Program, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand

**ABSTRACT :** The effect on sow milk of variable levels of cassava in lactating sow diets was analyzed in an attempt to explain the beneficial effects reported by producers of including cassava as a basal feed. Twenty crossbred lactating sows were randomly assigned to five dietary treatments. The treatments were: i) broken rice (BR) as the basal feed (BR100), ii) 50% of BR replaced with cassava chip meal (CCM) (CM50), iii) 75% of BR replaced with CCM (CM75), iv) CCM as the basal feed (CM100), and v) dried boiled cassava chips (CCB) as the basal feed (CB100). The hydrocyanide (HCN) content of CCB was reduced to be intermediate between HCN in the no cassava (BR100) and the 50% cassava (CB50) diets. Hydrocyanide was 0.54, 3.24, 4.41, 5.43 and 1.77 ppm in the BR100, CM50, CM75, CM100 and CB100 diets, respectively. Increasing cassava did not affect feed intake ($p>0.05$), but increased HCN intake ($p<0.01$). Milk composition was analyzed for protein, fat, lactose, solids not fat (SNF) and total solids (TS). Milk quality was analyzed for total microbes, coliform bacteria, thiocyanate ($SCN^-$), lactoperoxidase (LPO), and glutathione peroxidase (GPx) activity. At farrowing, sow milk composition was not affected by experimental diets ($p>0.05$), but milk $SCN^-$ increased as the intake of HCN increased in sows diets ($p<0.01$), $r^2 = 0.96$. At mid-lactation (day 14), milk composition was not affected ($p>0.05$). The milk quality levels of $SCN^-$ were 9.4, 10.3, 10.5, 11.6 and 9.1 ppm for the BR100, CM50, CM75, CM100 and CB100 diets, respectively ($p = 0.01$). The LPO contents were 16.41, 42.13, 51.42, 53.94 and 22.81 unit/L, respectively ($p = 0.03$). There was no GPx activity found in sow milk. When BR was replaced with cassava meal, total microbes and coliforms were reduced 78% and 87%, respectively, by the influences of HCN. The reported beneficial effects of cassava chip meal as a basal feed in lactating sow diets is manifested by improved performance of suckling pigs. This is due to beneficial, non-toxic levels of HCN in the diets. Besides passing HCN to suckling pigs in the form of $SCN^-$, sow milk may also benefit suckling pigs with the observed (day 14) increase in lactoperoxidase content and reduction in coliform bacteria. (**Key Words :** Cassava, Sow Milk, Hydrocyanide, Thiocyanate, Lactoperoxidase)

## INTRODUCTION

Cassava root meal (*Manihot esculent,* Crantz.) is a low-cost basal feed ingredient in the tropics. It has a number of advantages for animal feeding including a high content of soft starch which rapidly absorbs water and is highly digestible in the upper digestive tract of animals. Cassava contains a natural content of lactic acid bacteria and yeast, a minimum mycotoxin contamination and a low, non-toxic content of hydrocyanide (HCN) which promotes good health in animals (Reas, 1996; Kanto and Juttupornpong, 2005; Promthong, 2005; Kanto, 2006).

The HCN content in cassava, after entering the animal, is converted to thiocyanate ($SCN^-$), an antioxidant that stimulates immunity (Utarak, 2008). Buaphan (2003) showed that the increasing of cassava levels in total mixed ration diets of dairy cows significantly increased $SCN^-$ content and lactoperoxidase activity of the cow milk and inhibited bacteria in the milk. The higher $SCN^-$ content could activate the lactoperoxidase system (LPO-system) resulting in the production of unstable intermediates, which undergo a series processes affecting all microorganisms in milk and inhibiting bacteria metabolism. The

* Corresponding Author : A. Jupamatta. Tel: +66-34-352035, Fax: +66-34-352035, E-mail: Amphol_sci.tru@hotmail.com

[1] Department of Animal Science, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand.

[2] Animal Nutrition Research and Development Center, Suwanvajokkasikit Animal Research and Development Institute, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand.

[3] Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, 10900, Thailand.

Received June 11, 2010; Accepted September 2, 2010

lactoperoxidase system consists of $SCN^-$, lactoperoxidase and $H_2O_2$. Lactoperoxidase reacts with $SCN^-$ to form hypothiocyanate ($OSCN^-$), a strong oxidizing intermediate, having potent antibacterial activity (Thomas et al., 1994; Welk et al., 2009).

Presently, cassava meal is widely used as a basal feed ingredient in animal feeds, including breeding pigs in Thailand. The substitution of cassava for broken rice in breeding pig diets has shown a number of advantages, especially in health improvement of both the sows and the piglets under practical field conditions that allowed the minimum to no use of antibiotics or chemotherapeutics, an important benefit for food animal safety (Kanto and Juttupornpong, 2005; 2007). However, there are limited studies to explain this improvement phenomenon. Therefore, the purpose of this study was to investigate the effects of variable levels of cassava in lactating sow diets on milk nutrient composition and milk quality, which could be part of the explanation for the beneficial effect on suckling piglet's health previously mentioned.

## MATERIALS AND METHODS

### Experimental materials and procedure

Twenty crossbred lactating sows were randomly assigned to five dietary treatments. The treatments were: i) broken rice (*Oryza sativa*) (BR) as the basal feed (BR100), ii) 50% of BR replaced with cassava chip meal (CCM) (CM50), iii) 75% of BR replaced with CCM (CM75), iv) CCM as the basal feed (CM100), and v) dried boiled cassava chips (CCB) as the basal feed (CB100). Cassava chip meal used in this experiment was prime quality clean cassava roots with no stems or woody parts. Roots were chipped into small pieces before sundrying. The cassava chips in the CCB had hydrocyanide (HCN) content reduced by soaking in water for 4 h, boiled for 1 h and sun dried for 3 d before mixing in the experimental diet. This followed the method of Egbe and Mbome (2006) except for soaking. Cassava chips were soaked in water for 4 h instead of 3 h to remove more HCN. The resulting HCN in the boiled cassava chip diet (CCB) was intermediate between HCN in the no cassava (BR100) and the 50% cassava (CB50) diets. Experimental diets were isocaloric and isonitrogenous and formulated to NRC (1998) recommendations as shown in Table 1.

The sows were targeted to be fed 3 kg/d one week before farrowing and increased to 6 kg/d two weeks after farrowing. Daily feeding times were 07.00, 09.00, 15.00 and 17.00 h. The sows were individually kept with their litters of suckling pigs in 1.7×2.0 m metal farrowing crates

**Table 1.** Feed ingredients and chemical composition of experimental diets

| Items | Experimental diets[1] | | | | |
|---|---|---|---|---|---|
| | BR100 | CM50 | CM75 | CM100 | CB100 |
| Feed ingredients (%) | | | | | |
| Broken rice | 57.48 | 25.71 | 12.11 | - | - |
| Cassava chips meal | - | 25.71 | 36.30 | 45.86 | 45.86 |
| Rice bran | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Full fat soybean | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Soybean meal 44% | 23.42 | 28.03 | 30.03 | 31.77 | 31.77 |
| Rice bran oil | - | 1.55 | 2.60 | 3.46 | 3.46 |
| Dicalcium phosphate (P18%) | 3.08 | 3.03 | 3.00 | 2.98 | 2.98 |
| Limestone | 0.18 | 0.12 | 0.10 | 0.08 | 0.08 |
| Salt | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Vitamin/mineral premix | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Chemical composition by analysis (% as feed basis) | | | | | |
| Dry matter | 89.57 | 90.04 | 90.22 | 91.91 | 91.35 |
| Gross energy (Kcal/kg) | 4,016 | 4,083 | 4,136 | 4,183 | 4,112 |
| Crude protein (CP) | 18.21 | 18.23 | 18.10 | 18.16 | 18.20 |
| Ether extract | 2.84 | 4.40 | 5.30 | 6.08 | 6.10 |
| Crude fiber | 3.72 | 4.65 | 5.00 | 5.35 | 5.30 |
| Calcium | 0.92 | 0.93 | 0.93 | 0.94 | 0.91 |
| Total phosphorus | 0.71 | 0.70 | 0.71 | 0.69 | 0.72 |
| Hydrocyanide (ppm) | 0.54 | 3.24 | 4.41 | 5.43 | 1.77 |

[1] BR100 = 100% Broken rice (BR), CM50 = 50% BR+50% cassava chip meal (CCM), CM75 = 25% BR+75% CCM, CM100 = 100% CCM and CB100 = 100% boiled-dried CCM.

[2] Provided per kg of diet: Vitamin A, 20,000 IU; Vitamin $D_3$, 2,500 IU; Vitamin E, 28 g; Vitamin $K_3$, 4 mg; Thiamin, 2 mg; Riboflavin, 6 mg; Vitamin $B_6$, 3 mg; Vitamin $B_{12}$, 20 μg, D-pantothenic acid, 18 mg; Nicotinic acid, 25 mg; Folic acid, 1.3 mg; Biotin, 0.25 mg; choline, 1 g; Mg, 4 mg; Cu, 25 mg; Mn, 60 mg; Fe, 100 mg; Zn, 100 mg; Co, 1 mg; I, 2 mg; Se, 0.15 mg.

with feed and water provided *ad libitum*. Sows were crossbred Large White×Danish Landrace in their second to fourth parities and had normal body condition and mammary development.

Milk samples were collected on days 1 and 14 of lactation by first activating milk ejection with 1 ml of oxytocin injected intramuscularly in the neck. Prior to milking the mammary gland was cleaned with 75% ethanol (Moore et al., 2000). Two milk samples from each sow were hand collected in 20 ml vials. One sample was prepared for the milk composition analysis by adding 0.02 mg of sodium benzoate per 20 ml of milk. The other sample had no sodium benzoate added and was stored at 5°C for immediate bacterial analysis (Anekaviean, 1982).

**Experimental diets analysis**

Feed samples were collected when diets were mixed, and analyzed for dry matter, ether extract, crude fiber and ash (proximate analysis), crude protein (Kjeldahl method), calcium (titration analysis), gross energy (bomb calorimeter), phosphorus content (Vanado-Molybdate colorimetric method). The HCN content in cassava and the experimental diets were determined by alkaline titration (AOAC, 1980; Khajarern, 1980). The HCN content in prime sundried cassava chips (CCM) was 8.479 ppm which was more than the 3.4 ppm in cassava chip meal reported by Boonnop et al. (2009). The HCN content of boiled cassava chips (CCB) was 2.144 ppm before grinding. Feed ingredients and chemical compositions of the experimental diets are shown in Table 1.

**Milk composition and quality analysis**

The milk samples were analyzed for protein, fat, lactose, solids not fat (SNF) and total solids (TS) by using a Milko Scan B 133 (N Foss Electric, Denmark) milk analyzer. Total microbial count was measured by the technique of Houghtby et al. (1992) with $10^{-3}$ to $10^{-7}$ dilutions. Coliform bacterial count, with $10^{-1}$ to $10^{-3}$ dilutions was determined by the Christen et al. (1992) technique. The $SCN^-$ concentration was determined by a modified method of Cosby and Sumner (1945). The percentage of trichloroacetic acid added before centrifuging was increased from 40% to 60% to allow better protein separation. The $SCN^-$ was measured at 460 nm absorbance with a GENESYS 20, spectrophotometer (Thermo Spectronics Corp., USA). The lactoperoxidase content (LPO) in sow milk was analyzed by the methods of Shindler et al. (1976) and Kuma and Bhatia (1999). Glutathione peroxidase activity (GPx-activity) was determined by the methods of Chen et al. (2000) and Stagsted (2006). Both parameters were measured at 412 and 360 nm absorbance, respectively, using the GENESYS 20 spectrophotometer.

**Statistical analysis**

Response variables were analyzed by GLM procedures of SAS (2003) using a completely randomized design. Significance of differences in mean values among dietary treatments was reported with p-values of the F test. Means were separated using the Duncan's test, with levels of significant set at $p<0.05$ and $p<0.01$, when p-values were significant ($p<0.05$). The relationships of HCN sow intake and milk quality parameters were determined with simple linear regression, coefficient of correlation (r) and coefficient of determination ($r^2$).

## RESULTS AND DISCUSSION

**Feed and hydrocyanide intake**

The intake of sows was 4.7 to 5.1 kg/d for the first two weeks of lactation (6.0 kg/d targeted) which is in the range of 4.3 to 6.0 kg/d recommended by NRC (1998). Feed intake was unaffected by the levels of cassava in the diets ($p>0.05$). However, hydrocyanide (HCN) increased with increased levels of untreated cassava both pre- and post-farrowing ($p<0.01$). The HCN in the boiled cassava chip diet (CCB) was intermediate between HCN in the no cassava (BR100) and the 50% cassava (CB50) diets. Sows fed the CM100 diet had the highest HCN intake per day compared to the other diets (Table 2). This level of HCN

**Table 2.** Average daily feed intake and hydrocyanide intake per day of sows

| Items | Experimental diets[1] | | | | | SEM[2] | p-value[3] |
|---|---|---|---|---|---|---|---|
| | BR100 | CM50 | CM75 | CM100 | CB100 | | |
| 7 d before farrowing | | | | | | | |
| Average daily feed intake (kg/d) | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | - | - |
| Hydrocyanide (mg/d) | 1.63$^{e}$ | 9.71$^{c}$ | 13.24$^{b}$ | 16.30$^{a}$ | 5.31$^{d}$ | 0.32 | <0.001 |
| Lactating period (14 d) | | | | | | | |
| Average daily feed intake (kg/d) | 5.09 | 5.02 | 4.90 | 4.85 | 4.73 | 0.10 | 0.858 |
| Hydrocyanide (mg/d) | 2.77$^{e}$ | 16.26$^{c}$ | 21.63$^{b}$ | 26.37$^{a}$ | 8.36$^{d}$ | 2.00 | <0.001 |

[1] BR100 = 100% Broken rice (BR), CM50 = 50% BR+50% cassava chip meal (CCM), CM75 = 25% BR+75% CCM, CM100 = 100% CCM and CB100 = 100% boiled-dried CCM.

[2] SEM = Standard error of mean. [3] p value = Significance of the F test of the GLM analysis.

[abcde] Means in the same row with different superscripts are different ($p<0.01$).

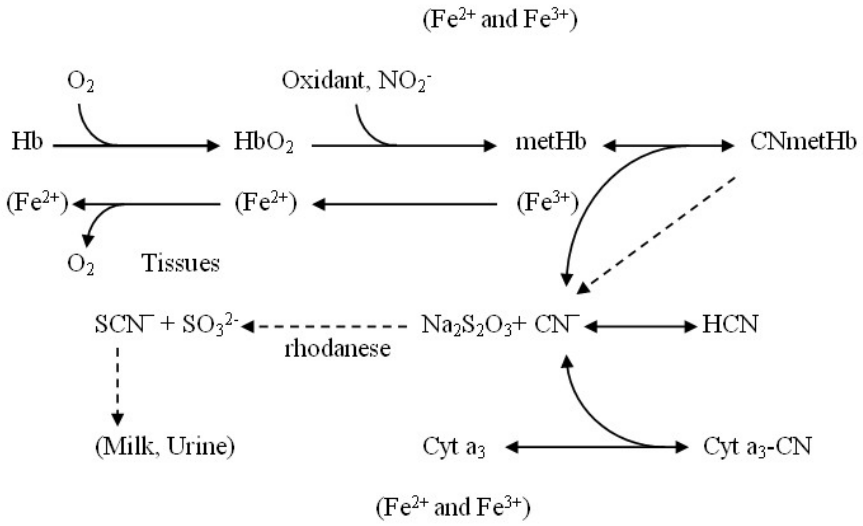


**Figure 1.** Neutralizations of hydrogen cyanide in animal, – Displacement of $CN^-$ from Cyt $a_3$, - - Conversion of $CN^-$ to $SCN^-$, Hb = Hemoglobin, metHb = Methemoglobin, CNmetHb = Cyanomethemoglobin, HCN = Hydrogen cyanide, $CN^-$ = Cyanide ion, SCN = Thiocyanate, Cyt $a_3$ = Cytochome $a_3$ (Adapted from USAMRICD, 2010).

did not reach 50 mg/d, the level considered toxic by Khajarern (1999).

Even though cyanide is toxic to animals, field experiences and the animal production industries in Thailand have never observed HCN toxicity from cassava chip meal (CCM). Good CCM that was cleaned, chipped (small pieces) and sundried 3 to 4 d until the moisture content was less than 13% had HCN contents of 22 to 30 ppm reported by Khajarern (1999), 15 to 50 ppm reported by Poungpeach (2003) and 18.46 to 25.60 ppm by Ehiagbonare et al. (2009).

The low HCN content in cassava chip meal is diluted even more in mixed diets. The low HCN in mixed diets can be neutralized by hydrolysis in the stomach resulting in free HCN, and $H^+$+$CN^-$. There are many mechanisms of animal HCN neutralization, but one main pathway occurs in the blood before entering the cells and the other is detoxification of HCN in the cells as show in Figure 1 (USAMRICD, 2010). To protect HCN from entering cells (Displacement of $CN^-$ from cytochome oxidase (Cyt $a_3$), $CN^-$ combines with $Fe^{3+}$ in the methemoglobin (metHb) which is more stable than $Fe^{3+}$ in Cyt $a_3$ resulting to cyanomethemoglobin (CNmetHb) and reduced free cyanides in the blood. The detoxification of HCN (Conversion of $CN^-$ to $SCN^-$) inside the cells is catalyzed by enzymes which are highly activated by rhodanese that in turn activates $CN^-$ to combine with thiosulfate ($S_2O_3^=$) yielding thiocyanate ($SCN^-$). The $SCN^-$ is a less toxic compound (7 times lower than HCN) that is then excreted in urine and secreted in milk, as measured in this experiment. Moreover, animals controlled the equilibrium of HCN and $SCN^-$ at a ratio of 1:1,000 by the peroxidase system (Chutharatana, 2004).

**Composition and quality of sow colostrum**

There were no treatment differences in colostrum composition which agrees with Darragh and Moughan (1998) and Verley and Wiseman (2001) who reported sow colostrum to contain 10.50% to 15.14% protein, 4.0% to 6.0% fat, 3.40% to 3.80% SNF and 21.50% to 25.00% TS. Sow colostrum increased in $SCN^-$ content with increasing levels of untreated cassava ($p = 0.002$). However the levels of $SCN^-$ in the colostrum of sows on diets CM50 was not different ($p>0.05$) from CB100 and BR100 but had similarly low concentration of HCN (Table 3). There was no apparent activity of LPO and GPx in sow milk at parturition ($p>0.05$). This agree with Shin et al. (2000) reported low peroxidase enzyme content in human colostrum (0.004% of protein) and Klebanoff et al. (1966) showed no LPO activity until after five days. For sow colostrum, total microbial content or coliform content were not affected by experimental diets, ranging from 5.33 to 5.38 $\log_{10}$ cfu/ml and from 2.05 to 2.27 $\log_{10}$ cfu/ml, respectively. The total microbial contents agree with those of Leesmith (2004) for medium quality human milk ranging from $2.0\times10^5$ to $2.0\times10^6$ cfu/ml (5.30 to 6.30 $\log_{10}$ cfu/ml).

**Composition and quality of sow milk**

At mid-lactation (day 14), dietary level of cassava had no effects on sow milk composition (Table 4). These results are similar to those of Jackson et al. (1995), Auldist et al. (2000) and Mavromichalis (2006) who reported that sow milk contained 5.00 to 5.50% protein, 7.10 to 9.13% fat and 5.00 to 5.60% lactose.

**Table 3.** Composition and quality of sow colostrums milk on day 1

| Items | Experimental diets[1] | | | | | SEM[2] | p-value[3] |
|---|---|---|---|---|---|---|---|
| | BR100 | CM50 | CM75 | CM100 | CB100 | | |
| Nutrient composition (%) | | | | | | | |
| Protein | 10.33 | 9.42 | 8.04 | 9.87 | 7.94 | 0.36 | 0.101 |
| Fat | 4.42 | 5.36 | 5.01 | 4.61 | 5.77 | 0.18 | 0.177 |
| Lactose | 3.70 | 3.85 | 4.20 | 3.95 | 4.12 | 0.07 | 0.195 |
| Solid not fat | 14.62 | 13.62 | 12.90 | 12.26 | 12.77 | 0.29 | 0.159 |
| Total solid | 19.04 | 18.98 | 18.07 | 18.87 | 18.54 | 0.13 | 0.103 |
| Quality of sow milk | | | | | | | |
| $SCN^{-}$[4] (ppm) | $10.40^{b}$ | $13.32^{ab}$ | $16.12^{a}$ | $17.50^{a}$ | $10.82^{b}$ | 0.79 | 0.002 |
| LPO[5] (unit/L) | - | - | - | - | - | - | - |
| GPx-activity[6] (OD/min) | - | - | - | - | - | - | - |
| Total microbial ($log_{10}$cfu/ml) | 5.38 | 5.33 | 5.38 | 5.34 | 5.36 | 0.07 | 0.708 |
| Coliform ($log_{10}$cfu/ml) | 2.27 | 2.05 | 2.19 | 2.20 | 2.24 | 0.13 | 0.512 |

[1] BR100 = 100% Broken rice (BR), CM50 = 50% BR+50% cassava chip meal (CCM), CM75 = 25% BR+75% CCM, CM100 = 100% CCM and CB100 = 100% boiled-dried CCM.
[2] SEM = Standard error of mean. [3] p value = Significance of the F test of the GLM analysis.
[4] $SCN^{-}$ = Thiocyanate. [5] LPO = Lactoperoxidase.
[6] GPx activity = Glutathioneperoxidase activity. Where - is show, there was no activity even though parameters were analyzed.
[a, b] Means in the same row with different superscripts are different ($p<0.01$).

Milk $SCN^{-}$ on day 14 was lower than on day 1. The $SCN^{-}$ content in sow milk at mid-lactation ranged from 9.08 to 11.62 ppm. As the level of untreated cassava increased in the diets, the level of $SCN^{-}$ in the milk increased ($p = 0.01$). However, the differences in $SCN^{-}$ (Table 4) are not as pronounced as the differences in HCN content (Table 2). Nevertheless, regression indicated that 85% of $SCN^{-}$ in sow milk resulted from HCN in the diet ($r^2 = 0.85$ from Table 5). Dietary HCN is converted to $SCN^{-}$ in the liver and excreted in the urine as well as in other body secretions, including milk. Although the effects of dietary cassava on milk quality of lactating sows has not been reported previously, the results of this study are in good agreement with Kanchanapreuttipong et al. (1999) and Buaphan (2003). They demonstrated that the level of $SCN^{-}$ in dairy cow milk was significantly related to the level of cassava in concentrate diets ($SCN^{-}$ ranging from 11.19 to 11.60 ppm) compared to corn concentrate diets ($SCN^{-}$ = 7.19 ppm).

**Table 4.** Composition and quality of sow mature milk on day 14

| Items | Experimental diets[1] | | | | | SEM[2] | p-value[3] |
|---|---|---|---|---|---|---|---|
| | BR100 | CM50 | CM75 | CM100 | CB100 | | |
| Nutrient composition (%) | | | | | | | |
| Protein | 4.53 | 4.79 | 4.90 | 4.95 | 4.99 | 0.10 | 0.594 |
| Fat | 8.60 | 8.01 | 8.08 | 7.59 | 8.43 | 0.20 | 0.663 |
| Lactose | 5.74 | 5.70 | 5.62 | 5.67 | 5.34 | 0.07 | 0.491 |
| Solid not fat | 10.97 | 11.19 | 11.22 | 11.33 | 11.02 | 0.07 | 0.580 |
| Total solid | 19.58 | 19.20 | 19.30 | 18.92 | 19.45 | 0.22 | 0.925 |
| Quality of sow milk | | | | | | | |
| $SCN^{-}$[4] (ppm) | $9.40^{b}$ | $10.25^{ab}$ | $10.50^{ab}$ | $11.62^{a}$ | $9.08^{b}$ | 0.27 | 0.010 |
| LPO[5] (unit/L) | $16.41^{d}$ | $42.13^{cd}$ | $51.42^{c}$ | $53.95^{c}$ | $22.81^{d}$ | 4.91 | 0.026 |
| GPx-activity[6] (OD/min) | - | - | - | - | - | - | - |
| Total microbial ($log_{10}$cfu/ml) | 5.43 | 5.24 | 5.27 | 4.93 | 5.39 | 0.29 | 0.309 |
| Coliform ($log_{10}$cfu/ml) | 2.22 | 1.84 | 1.79 | 1.82 | 2.15 | 0.15 | 0.058 |

[1] BR100 = 100% Broken rice (BR), CM50 = 50% BR+50% cassava chip meal (CCM), CM75 = 25% BR+75% CCM, CM100 = 100% CCM and CB100 = 100% boiled-dried CCM.
[2] SEM = Standard error of mean. [3] p value = Significance of the F test of the GLM analysis.
[4] $SCN^{-}$ = Thiocyanate. [5] LPO = Lactoperoxidase.
[6] GPx activity = Glutathioneperoxidase activity. Where - is show, there was no activity even though parameters were analyzed.
[ab] Means in the same row with different superscripts are different ($p<0.01$).
[cd] Means in the same row with different superscripts are different ($p<0.05$).

**Table 5.** Regression of sow milk quality parameters on hydrocyanide (HCN) content of diets

| Items | Parameters | Simple linear regression | $r^2$ | r | p-value |
|---|---|---|---|---|---|
| Day 1 | HCN (X) : Milk $SCN^-$ (Y) | Y = 8.813+0.522X | 0.96 | 0.98 | 0.004 |
| Day 14 | HCN (X) : Milk $SCN^-$ (Y) | Y = 8.712+0.096X | 0.85 | 0 .92 | 0.027 |
| | HCN (X) : LPO (Y) | Y = 10.994+1.743X | 0.97 | 0.99 | 0.002 |
| | HCN (X) : Total microbial (Y) | Y = 5.529-0.018X | 0.78 | 0.88 | 0.048 |
| | HCN (X) : Coliform (Y) | Y = 2.262-0.020X | 0.87 | 0.93 | 0.021 |

$r^2$ = Simple correlation coefficient, r = Simple coefficient of determination and p value = Significance of the F test of the GLM analysis.
The regression of total microbial and coliform at day 1 were not significant (p>0.05).
HCN = Hydrocyanide intake of sows, $SCN^-$ = Thiocyanate, LPO = Lactoperoxidase.

Lactoperoxidase content in sow milk increased as HCN levels from cassava increased (p = 0.03) (Table 4). Linear regression showed that 97% of LPO in sow milk resulted from HCN in the diet ($r^2$ = 0.97 from Table 5). Since $SCN^-$ is a substrate for LPO activity in animal milk, higher levels of $SCN^-$ in milk always result in higher LPO activity in the milk (Tahboub et al., 2005). The results are in agreement with Buaphan (2003) who showed that LPO in milk of dairy cows fed cassava diets was higher (p<0.05) than those on corn diets. The high metabolic activity of sows during mid lactation produces a higher level of $H_2O_2$ in the milk. This causes a higher LPO activity in the milk than in the colostrum (Klebanoff et al., 1966). The minute amount of HCN intake is detoxified by a liver mitochondrial enzyme, rhodanese that activates HCN to combine with thiosulfate, $S_2O_3^=$, yielding $SCN^-$ that is excreted into animal excreta and body secretions. The $SCN^-$ in milk plays a key role in LPO-system and has inhibitory effects on the microorganisms contained in the milk (Chang and Wood, 1971; Saidu, 2004).

There was no GPx activity found in either colostrum or mid-lactation sow milk (Tables 3 and 4). In several reports on the activity of GPx in human milk, GPx were determined by an automated modification of the enzymatic coupled assay. This method has been developed for GPx in erythrocyte that contains mainly cellular GPx types. Debski et al. (1987) reported that GPx-dependent peroxidase activity was approximately one-third of the total peroxidase activity and found to be similar in human and bovine milk. In addition, Bhattacharya et al. (1988) and Abbe and Friel (2000) showed that GPx-activity in human milk was 16.6 U/mg and 73 to 86 mU/ml, respectively. However, those assays did not consider GPx form in milk that mainly contains extra-cellular GPx type. This is a lower rate constant of glutathione (GSH) and the reaction appears to be up to10-fold slower than the cellular GPx type (Chen et al., 2000). Because there were no reports and specific methods to determine GPx activity in sow milk, this study used the recent methods of Chen et al. (2000) and Stagsted (2006). The results showed no activity of GPx in sow milk which agree with Stagsted (2006) who reported that both GSH and GPx-activity were absent in bovine milk. Moreover, Fox and Kelly (2006) reported that milk contains a low level of indigenous GPx. More than 90% of GPx is the extra-cellular type which has no known enzymatic function in milk, in which it binds 30% of total selenium, an important trace element in the diet. And also the level of GPx in milk varies with the species (human>carprine> bovine). Since this study used only the one method of Chen et al. (2000), future research should examine other methods such as the coupled GPx-dependent peroxidase assay in milk.

When comparing experimental diets, there were no differences in total microbial content of mid-lactation sow milk (p = 0.31 from Table 4). However, regression showed that 78% of the decrease in total microbial content could be attributed to HCN in the diet ($r^2$ = 0.78 from Table 5). Similarly, 87% of the decrease in coliform content could be attributed to HCN in the diet ($r^2$ = 0.87 from Table 5). Losendahl et al. (2000) reported that bacteria in raw mature milk could be killed by the LPO found in milk. The activities of LPO in a system, together with $SCN^-$ and $H_2O_2$, catalyze the peroxidation of $SCN^-$ to make any one of the intermediate antimicrobials hypothiocyanite ($OSCN^-$), thiocyanogen or hypothiocyanous acid. All of these intermediates are unstable, the $OSCN^-$ is the main factor in the LPO-system that affects to all microbial groups. The antibacterial mechanism is related to the oxidation of vital sulfhydryl-containing metabolic enzymes by $OSCN^-$. The oxidation of -SH groups in the bacterial cytoplasmic membrane results in loss of the ability to transport glucose and also in leaking of potassium ions, amino acids and peptides (Reiter and Harnulv, 1984; Thomas et al., 1994; Welk et al., 2009). Gram negative bacteria such as coliform bacteria and salmonella are not only more readily inhibited by LPO, but also are killed if sufficient $H_2O_2$ is provided chemically, enzymatically or by $H_2O_2$ producing microorganism. Although mammalian cells can be damaged by LPO-derived oxidants, a 10-fold higher concentration of oxidant is required to kill them compared to *Streptococcus viridians*. Moreover, microbial structure is contained within the periplasm, which lacks GSH reducing agents that play a protective role in eukaryotic cells (Eastvold, 2005).

In conclusion, cassava chip meal in lactating sow diets

improves sow milk quality by the increasing thiocyanate and lactoperoxidase content and by reducing total microbes and coliform bacteria content while maintaining sow milk composition on day 14 of lactation. Cassava chip meal functions as a low-level, non-toxic source of HCN that improves animal health and milk quality of sows. It should be considered a necessary component in animal diets to at least 50% of the basal energy feed in the diet.

## ACKNOWLEDGMENTS

The authors wish to thank Animal Nutrition Research and Development Center, Suwanvajokkasikit Animal Research and Development Institute (SARDI), Kasetsart University, and Thai Tapioca Development Institute (TTDI) for funding support of the project, Thepsatri Rajabhat University for allowance of the author to conduct the study. Special thanks go to Dr. David G. St. Louis, SARDI, Kasetsart University, for his guidance and improvement of this report.

## REFERENCES

Abbe, M. R. L. and J. K. Friel. 2000. Superoxide dismutase and glutathione peroxidase content of human milk from mothers of premature and full-term infants during the first 3 months of lactation. J. Pediatr Gastroenterol. Nutr. 31(3):270-274.

Anekaviean, T. 1982. Milk science. Department of Animal Science, Faculty of Agriculture. Kasetsart University, Bangkok, Thailand.

AOAC. 1980. Official method of analysis. 13th Ed. Association of Official Analytical Chemists, Washington, DC.

Auldist, D. E., D. Carlson, L. Morrish, C. M. Wakeford and R. H. King. 2000. The influence of suckling interval on milk production of sows. J. Anim. Sci. 78:2026-2031.

Bhattacharya, I. D., M. F. Picciano and J. A. Milner. 1988. Characteristics of human milk glutathione peroxidase. Biol. Trace Elem. Res. 18:59-70.

Boonnop, K., M. Wanapat, N. Nontaso and S. Wanapat. 2009. Enriching nutritive value of cassava root by yeast fermentation. Sci. Agric. (Piracicaba, Braz.) 66(5):629-633.

Buaphan, S. 2003. Effects of increasing content of cassava chips in total mixed rations on somatic cell counts, microorganisms, aflatoxins and peroxidase activity in raw milk of cows. Ms. Thesis, Kasetsart University, Bangkok, Thailand.

Chang, J. and J. L. Wood. 1971. The importance of glutathione in human disease. J. Biol. Chem. 249:4346-4349.

Chen, J. H., L. M. Mansson and B. A. Kesson. 2000. Optimisation of a coupled enzymatic assay of glutathione peroxidase activity in bovine milk and whey. Int. Dairy J. 10:347-351.

Christen, G. L., P. M. Davidson, J. S. McAllister and L. A. Roth. 1992. Coliform and other indicator bacteria. In: Standard and Method for the Examination of Dairy Products. 16th Ed. (Ed. R. J. Marshell). Port City Press, Port City. pp. 247-269.

Chutharatana, A. 2004. Cyanide Introduction. Department of Primary Industries and Mines, Ministry of Industry of Thailand, Bangkok, Thailand.

Cosby, E. L. and J. B. Sumner. 1945. Rhodanese. Arch. Biochem. 7:457-460.

Darragh, A. J. and P. J. Moughan. 1998. The composition of colostrums and milk. In: The Lactating Sow. (Ed. M. W. A. Verstegen, P. J. Moughan and J. W. Schrama). Wageningen Pers, Wageningen. pp. 3-21.

Debski, B., M. F. Picciano and J. A. Milner. 1987. Selenium content and distribution of human, cow and goat milk. J. Nutr. 117:1091-1097.

Eastvold, J. S. 2005. Hypothiocyanous acid: An Overiew. Free Radical and Radiation Biology Program. The University of Iowa. Iowa City.

Ehiagbonare, J. E., S. A. Enabulele, B. B. Babatunde and R. Adjarhore. 2009. Effect of cassava effluent on Okada denizens. Sci. Res. Essay. 4(4):310-313.

Egbe, T. A. and I. L. Mbome. 2006. The effects of processing techniques in reducing cyanogen levels during the production of some Cameroonian cassava foods. J. Food Compost. Anal. 19:354-363.

Fox, P. F. and A. L. Kelly. 2006. Indigenous enzyme in milk: overview and historical aspects-part 2. Int. Dairy J. 16:517-532.

Houghtby, G. A., L. A. Maturin and E. K. Koenig. 1992. Microbiological count methods. In: Standard and Method for the Examination of Dairy Products. 16th Ed. (Ed. R. J. Marshell). Port City Press, Port City. pp. 247-269.

Jackson, J. R., W. L. Hurley, R. A. Easter, A. H. Jensen and J. Odle. 1995. Effects of induced or delayed parturition and supplemental dietary fat on colostrum and milk composition in sows. J. Anim. Sci. 73:1906-1913.

Kanchanapreuttipong, J., U. Kanto, S. Juttupornpong and W. Chowuthai. 1999. Substitution of cassava for corn in dairy concentrates diets. In: Cassava for Animal Feed Project 1999 Annual Report. Thai Tapioca Development Institute, Bangkok, Thailand.

Kanto, U. 2006. Cassava and pig health. Part 1. Asian Pork, Oct-Nov:18-20.

Kanto, U. and S. Juttupornpong. 2005. Cassava in Animal Nutrition: With Reference To Thailand Cassava. Animal Nutrition Research and Development Center, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand.

Kanto, U. and S. Juttupornpong. 2007. Cassava in Animal Nutrition: With Reference To Thailand. 2nd Ed., Animal Nutrition Research and Development Center, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand.

Khajarern, J. 1980. Manual of animal feed analysis. 1st Ed., Department of Animal Science Faculty of Agriculture, Khon Kean University, Khon Kean, Thailand.

Khajarern, S. 1999. Feeds and feeding of nonruminants. Department of Animal Science, Faculty of Agriculture, Khon Kean University, Khon Kean, Thailand.

Klebanoff, S. J., W. H. Clem and R. G. Luebke. 1966. The peroxidase thiocyanate hydrogen peroxide antimicrobial system. Biochem. Biophys. Acta. 117:63-72.

Kuma, R. and K. L. Bhatia. 1999. Standardization of method for lactoperoxidase assay in milk. Lait. J. 79:269-274.

Leesmith, J. 2004. Laboratory of microbiology. Microbiology Program, Faculty of Liberal Arts and Scince, Kasetsart

University, Kamphaeng Saen, Nakhon Pathom, Thailand.
Losendahl, K. J., H. Wang, M. Aslam, Z. Sixiang and W. L. Hurley. 2000. Antimicrobial proteins in milk. Available at: http://www.classes.ag.uiuc.edu./AnSci.308/antimi-croprot IDR. Html
Mavromichalis, I. 2006. Applied nutrition for young pigs. Cromwell Press, Trowbridgs.
Moore, M. A., R. C. Wander, Y. M. Xia, S. H. Du, J. A. Butler and P. D. Whanger. 2000. Selenium supplementation of Chinese women with habitually low selenium intake increases plasma selenium, plasma glutathione peroxidase activity, and milk selenium, but not milk glutathione peroxidase activity. J. Nutr. Biochem. 11:341-347.
National Research Council (NRC). 1998. Nutrient requirements of swine. 10th Ed. National Academy Press, Washington, DC.
Promthong, S. 2005. Comparative studies on physiological, histological and microbial properties in the digestive tract of broilers fed cassava versus corn diets. Ph.D. Thesis, Kasetsart University, Bangkok, Thailand.
Poungpeach, N. 2003. Documentary Technique of Cassava. Department of Agriculture, Ministry of Agriculture, Bangkok, Thailand.
Reas, B. P. 1996. A study on the comparative digestibility of cassava, maize, sorghum and barley in growing pigs. Ms. Thesis, University of Queensland, Queensland.
Reiter, B. and G. Harnulv. 1984. Lactoperoxidase antibacterial system: Natural occurrence, biological functions and practical application. J. Food Prot. 47:724-732.
Saidu, Y. 2004. Physicochemical features of rhodanese: A Review. Afr. J. Biotect. 3(4):370-374.
SAS Institute. Inc. 2003. SAS/STAT User's Guide. SAS Institute Inc., North Carolina.
Shin, K., M. Tomita and B. Lonnerdal. 2000. Identification of lactoperoxidase in mature human milk. J. Nutr. Biochem. 11:94-102.
Shindler, J. S., R. E. Childs and W. G. Bardsley. 1976. Peroxidase from human cervical mucus the isolation and characterisation. Eur J. Biochem. 65:325-331.
Stagsted, J. 2006. Absence of both glutathione peroxidase activity and glutathione in bovine milk. Int. Dairy J. 16:662-668.
Tahboub. Y. R., S. Alijasevic, M. P. Diamond and H. M. AbuSoud. 2005. Thiocyanate modulates the catalytic activity of mammalian peroxidases. J. Biol. Chem. 280(28):26129-26136.
Thomas, E. L., T. W. Milligan, R. E. Joyner and M. M. Jefferson. 1994. Antibacterial activity of hydrogen peroxide and the lactoperoxidase-hydrogen peroxide-thiocyanate system against oral streptococci. Infect. Immun. Feb. 529-535.
The United States Army Medical Research Institute of Chemical Defense (USAMRICD). 2010. Cyanide. Available at: http://www.gnyha.org/210/File.aspx+USAMricd+cyanide.
Utarak, T. 2008. Effect of cassava meal in swine feed on immunological system and performance of weaned pigs. Ms. Thesis, Kasetsart University, Bangkok, Thailand.
Verley, M. A. and J. Wiseman. 2001. The weaner pig: Nutrition and management. Cromwell Press, Trowbridgs.
Welk, A., C. Meller, R. Schubert, C. Schwahn, A. Kramer and H. Below. 2009. Effect of lactoperoxidase on the antimicrobial effectiveness of the thiocyanate hydrogen peroxide combination in a quantitative suspension test. BMC Microbiol. 9:134-140.