LEVELS OF NATURALLY OCCURRING THIOCYNATE LEVELS AND KEEPING QUALITY OF COW MILK UNDER DIFFERENT REARING SYSTEMS IN SELECTED AREAS OF TANZANIA

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SUMMARY

Milk from a total of 80 smallholder farms comprising of traditional herds were studied in Coastal and Kilimanjaro regions. From the Coastal region, out of 40 dairy keepers, 20 were traditional cattle keepers from Lugoba ward, 15 small scale cattle keepers and 5 large scale farms were from Kibaha and Chalinze. From Kilimanjaro highlands, out of 40 dairy keepers, 7 were traditional cattle keepers from Boma Ng'ombe and KIA areas, 30 were small scale dairy cattle keepers of Nronga, Losaa, Kalali and Foo villages and 3 large scale farms located in the Kilimanjaro lowlands (Kafoi, Kilari & Sabuko). Milk from Magadu farm, SUA farm and LITI farm, in Morogoro were analysed for their thiocyante content. Feed samples used on the farms were analysed for cynogenics (glucosinalate) contents. The potential keeping quality of milk was taken as time required for pH to change from 6.7 to 6.5. Results showed that cyanogenics content in different forage feeds was low (0.016 - 1.7 mg/kg) compared to concentrate mixtures (2.66 – 3.06 mg/kg). The mean ± standard deviation of thiocyanate (SCN) found in milk from traditional cattle grazing natural pastures was 3.49 ± 1.28 and zero grazed smallholder dairy cattle in Kilimanjaro was 4.57 ± 1.96 mg/kg. The milk from the Coastal region had SCN level of 7.13 ± 2.86 in the traditional cattle and 5.70 ± 2.35 mg/l in the small-holder dairy cattle and the differences were significant ($p<0.05$). Total plate count of the raw within 3 hours from milking was an average of 895,888 c.f.u/ml. (range 24,000 – 3.8 million). SCN content of milk from Ayrshire, Hoisten Friesian and Crossbred cows fed the same diet at Magadu Farm were 4.14, 3.37 and 3.78 mg/kg with a shelf life of milk of 8.0, 3.5 and 8.0 hours respectively at 24-28°C. SCN content of milk from Aryshire cows from Magadu, SUA farm and LITI farm was 4.42, 4.88 and 3.18 mg/kg respectively. The corresponding shelf life of 8, 13 and 9 hours was not significantly ($p>0.05$) related to SCN levels. It is concluded that, there was slight relationship between concentration of cyanogenics in animal feeds and SCN excreted in raw milk but the latter did not influence the bacteriological quality or its

potential shelf life as only 8% of the variation in TPC of milk could be ascribed to the SCN content of the milk.

INTRODUCTION

The activation of the lactoperoxidase system (LPS) naturally found in milk has been found to be effective in extending the shelf life of milk by 6-12 hours (Sarkar and Misra, 1994) depending on the ambient temperature and the bacteriological quality of milk by inhibiting the growth of milk spoilage microorganisms (IDF, 1988). Studies by Björck *et al.*, (1975) revealed that raw milk might be preserved for 7-8 hours at 30^0C due to the action of LPS enzyme, which requires 8 mg/l H_2O_2, and 15 mg/l thiocyanate (SCN) for its activation (Björck, 1978). The natural concentration of SCN in milk is about 3.2 - 4.6 mg/l and may vary from 1 - 10 mg/l depending on the type of feed consumed by lactating cows (Siha and Singhal, 1993). Addition of 10 mg/l of SCN is therefore necessary to render LPS effective. The recommended concentration of LPS in milk is low compared with that occurring in human saliva (50 - 300 mg/l) or human gastric juice (40 - 50 mg/l). Hence the LPS system has been approved by FAO/WHO Codex Alimentarius commission for use in the temporary preservation of milk where for technical or economic reasons cooling of milk cannot be practiced (Kurwijila, 1990; Mwaikambo *et al.*, 2004). While the recommended LPS in milk has been shown to be toxicologically safe, some countries, including many in Africa, still object to its commercial application for several reasons; the biggest one being the fear that without knowing the level of naturally occurring SCN in milk the prescribed levels of 15 mg/l could be exceeded unintentionally if the naturally occurring SCN content are already high. For example, Siha and Singhal (1993) found that the SCN content of milk samples collected in an Indian village where feeding of mustard cake was a common practice was found to be 9.91 mg/l. Such milk would require addition of 5.09 mg/l to bring the SCN level 15 mg/l required in the activation of LPS instead of the recommended 10 mg/l. Furthermore, since the SCN level in milk is part of the natural antibacterial system which prevents micro-organisms from multiplying during the initial 2-3 h after milking, the extent to which the level of naturally occurring SCN influences the potential keeping quality of milk is not clear. This study was therefore carried out in order to investigate the content of cyanogenics in forage and concentrate feeds used to feed lactating cows in three different production systems, their influence on SCN content in milk and the influence this may have on potential keeping quality of raw milk at room temperature.

MATERIALS AND METHOD

Sample Collection

Different animal feed samples were taken from 40 farm holdings in Kilimanjaro highlands as follows: Traditional cattle keepers from Boma Ng'ombe and KIA areas (7), small scale dairy cattle keepers from Nnronga, Losaa, Kalali and Foo villages (30) and large scale commercial farms located in the Kilimanjaro lowlands which were Kafoi Estate, Kilari farm and Sabuko farm (3). In the Coastal region, feed samples were taken from 40 dairy keepers as follows: Traditional cattle keepers from Lugoba ward (20), small scale cattle keepers from Kibaha and Chalinze (15), and large scale dairy farms namely Ruvu dairy, FreshFood, Khalid's, Mwapachu and Reki Enterprises farms (5). Additionally, feed mixtures from the Magadu, SUA LITI farms in Morogoro were sampled and determined for their cyanogenics content. Milk from 18 lactating cows of different breeds from Magadu, SUA and LITI were determined for breed effect on SCN concentration. Breeds of cattle used in the study were Holstein Friesian (HF), Ayrshire and Crossbred (F_1 + Ayrshire). Milk samples were collected twice per day in a two-week period during which the cows were under similar feeding and management practices (Table 1).

Table 1. Farms and cattle breeds type and feed types in Morogoro

FARM	Cattle Breed	Types of Feeds
MAGADU	Holstein-Friesian (H). Ayrshire (A).	Banana leaves, Brachia, water + concentrates (Maize bran, cotton seed cake, Maclick^R , limestone, Salt , NaCl)
SUA FARM	1. Holstein Friesian 2. Ayrshire 3. Cross (F1 + Ayrshire)	Natural pasture + concentrates (Maize bran, cotton seed manned-born meal limestone + water).
LITI FARM	1. Ayrshire	Natural pasture + Concentrates (Sunflower cake, Maize bran, Maclick^R Bones common salt and lime.

Sampling Technique

Cows were milked twice daily at 6.00 am and 3.00 pm and the yield per day was recorded on each collection day. Aliquots of morning and evening milking were sampled from each cow. Two samples each 300 ml were put into two different sterile bottles, preserved in ice-cooled boxes. The samples for chemical analysis were preserved with potassium dichromate (0.5 ml 4% solution in 0.25 l of milk) taken to Sokoine University of Agriculture

Laboratory and stored under refrigeration waiting for analysis (IDF, 1981; 1990). Feeds were sampled according to method by AOAC (1990). Amount of 500 g was taken from each farm from thoroughly mixed feeds. The samples were dried in the oven at 60°C for 48 h, ground to pass a 7 mm diameter sieve and mixed thoroughly. From each portion 25 g was taken for laboratory analysis.

Laboratory analysis

Determination of cyanide content in feeds

The Björck, *et al.*, (1975) method for cyanide determination content in the feed was used.

The oven method by Egan *et al.*, (1981) was used to determine the moisture content in feed where about 25 g of feed samples (duplicates) were dried overnight at 105°C in a drying oven to constant weight. The dried samples were cooled in the desiccators and the final weight recorded.

Calculations of cyanogens levels based on dry concentrate mixture/kg

The formula based on the assumption that the CN^- has strong preference for the extraction medium compared to the dry matter of the sample.

Determine the moisture

$$CN = C\left(\frac{b + a \times M.C/100}{a}\right) \times 0.02605 \times \left(\frac{1 - M.C/100}{100}\right) \times D.M$$

Units = µg CN^-/kg dry conc. mixture

Where:

a = Weight of sub-sample for extraction = 25 g
b = Volume of added extraction medium = 100 ml
c = n Moles / tube
M.C = Moisture content
D.M = Dry matter content

Determination of SCN in milk

The FAO (1999) method of SCN determination was used. It involved the removal of milk proteins by precipitation with 20% TCA followed by filtering using Whitman No 40 filter paper. The supernatant waa Philips PU8620 UV/NIR spectrophotometer (Pyne Unicam, Ltd. UK) and the amount of SCN in the samples determined by comparison with standard curve from known concentration.

Microbiological analysis

Total plate counts was done according to IDF 100, (1981)

Determination of shelf life of milk

The potential shelf life of milk was determined by measuring the time taken for pH to change from initial pH to pH 6.5, which was

considered to be the start of milk acidification. Immediately after milk samples (300 ml each) were collected from the farms, a sample were divided into two parts where 150 ml was put in 250 conical flask, kept at temperature 24-28^{0}C and the pH changes for each milk sample was recorded at intervals of 30
minutes. Changes in pH were monitored until the pH was below 6.50, which is the minimum pH for normal raw milk. A second sample of same milk was stored at 4 °C to be used to repeat the experiment.

Statistical analysis

The data was analysed by using the SAS (1990) statistical package. Descriptive statistics and the general linear model was used to compare the differences of results of measured parameters collected from different farms and breeds of cattle, hence the statistical model used was:

Yijk = X + Si + Tij+ eijk

Where as:

Yijk = General observation
X = General mean
Si = the effect of the feeding system on ith farm
Tij = Effect of the jth breed in the ith farm
eijk = the random error

RESULTS

Cyanogenics content in the different animal feeds

Table 2 shows that the average cyanide (CN) concentration and dry matter content in the animal feed samples from the Kilimanjaro, Coast and Morogoro regions were low. The CN concentration was higher in the concentrate mixture than the forages. The Table shows further that the feed from Magadu farm contained the highest amount of cyanogenics (about 3.06 mg/kg DM of concentrate mixture) and it was lowest in LITI farm (about 2.66 mg/kg DM).

Table 2. Cyanide content in feed samples from Kilimanjaro, Coast and Morogoro

Dry Feed samples	Source	% DM of dry feed	CN (mg /kg DM)
Forages			
Brachiaria spp	Coast / Kilimanjaro	92.2	1.56
Elephant grass	Coast / Kilimanjaro	89.5	0.3
Setaria spp	Coast / Kilimanjaro	88.5	0.4
Guatemala grass	Coast / Kilimanjaro	91.0	1.7
Banana leaves and stem	Coast / Kilimanjaro	90.7	4.5
Leucaena leaves	Coast / Kilimanjaro	89.2	40.0
Crop residues			
Rice straw	Coast / Kilimanjaro	96.0	2.8
Maize cobs and straw	Coast / Kilimanjaro	89.4	1.2

Oil cake			
Cotton seed cake	Coast / Kilimanjaro	90.6	1.9
Cereal brans			
Maize bran	Coast / Kilimanjaro	89.4	0.7
Concentrate mix	Coast / Kilimanjaro		
Sunflower cake + maize bran + common salt	Morogoro	93.0	1500
Magadu Farm: cotton seed cake concentrate mix	Morogoro	90.7	3060
SUA Farm: cotton seed cake concentrate mix	Morogoro	90.7	2970
LITI farm: Sunflower concentrate mix	Morogoro	90.8	2660

The SCN content of milk from cows of different breed under similar feeding regimen Magadu farm

Table 3 shows that the SCN content of milk of different breeds under similar feeding regime was not significantly different (P>0.05) with a coefficient of variation of 10.47%. There was no relationship between the amount of SCN in milk and the microbial counts in different farms.

Table 4. Effect of breed and potential shelf life of milk of cows fed concentrate diet containing on *2.97 mg* CN /kg) SCN at Magadu farm

Cow Breed	[SCN] in milk (mg/L) (N=18)	Shelf life at 24-28 ^{0}C in hours^{++} (n =18)
Ayrshire	4.14^a	8.0^a
Friesian	3.37^a	3.5^b
Crossbred (FI+Ayrsh.)	3.78^a	8.0^a

Within column, means followed by the different superscript differ significantly (P<0.05) $^{++}$Time taken for pH pf milk to drop from 6.7 to 6.5

Table 4 shows that different breeds (in Magadu farm) differ in secreting SCN in the milk. Ayrshire secreted more SCN in milk than the other breeds used in the experiment. Also Table 4 shows that at the same feeding regime, with the same CN content in feed, cows gave different SCN in milk, which resulted in different potential shelf life of milk to reach the pH of 6.5. There was a direct relationship between the level of SCN in milk and the potential shelf life of milk.

Table 3. LS Means and SE for bacterial count and SCN content of raw milk samples from Kilimanjaro and coast Regions

			Bacterial counts x 10^3 c.f.u. /ml			
Area	Production system	N	Total plate count	Thermoduri cs	Coliforms	SCN content (mg/litre)
Kilima-njaro	TRA	7	226±330^{b}	18.5 ±2.7^{a}	4.4± 1.2^{a}	3.8±1.0^{b}
	SHC	30	474±145^{b}	6.5 ±1.2^{b}	3.4±0.5^{a}	4.5±0.4^{b}
	LSC	3	100±466^{b}	7.1±3.9^{b}	1.8±1.7^{a}	5.9±1.4^{ab}
Coast	TRA	20	2011±1[illegible]1^{a}	22.5± 1.5^{a}	4.0± 0.7^{a}	7.1± 0.5^{a}
	SHC	15	536±216^{b}	7.7±1.8^{b}	2.4± 0.8^{a}	5.7± 0.6^{ab}
	LSC	5	1316±361^{ab}	5.1± 3.0^{b}	2.9± 1.3^{a}	4.8± 1.1^{ab}

Source: Field survey 2000
TRA = traditional cattle keepers, SHC = smallholder commercial dairy cattle keepers and LSC = large scale commercial producers.
Values within the columns having different superscript means the values differ significantly at $P<0.05$

Table 5. Effect of CN in concentrate feeds on SCN content and potential keeping quality of raw milk of Ayrshire cows from different farms stored at room temperature 24 - 28°C

Farm	CN in concentrate feed (µg CN/kg DM) (500g)	Level of SCN in milk (mg/L) (N= 18)	Shelf life in hours at 24-28^{0}C^{++} (n= 18)
Magadu	3.06	4.88^{a}	13.0^{b}
SUA	2.97	4.42^{b}	8.0^{a}
LITI	2.66	3.18^{c}	9.0^{a}

Note: Within column, means followed by different superscript differ significantly at ($P<0.05$).
$^{++}$ Time taken for pH pf milk to drop from 6.7 to 6.5

Effect of SCN in milk to the Total plate count and potential shelf life of raw milk

Table 5 shows that milk of the same breed from different farms differed in their potential shelf life as measured by pH change from 6.7 to 6.5. The differences were related to the content of SCN.

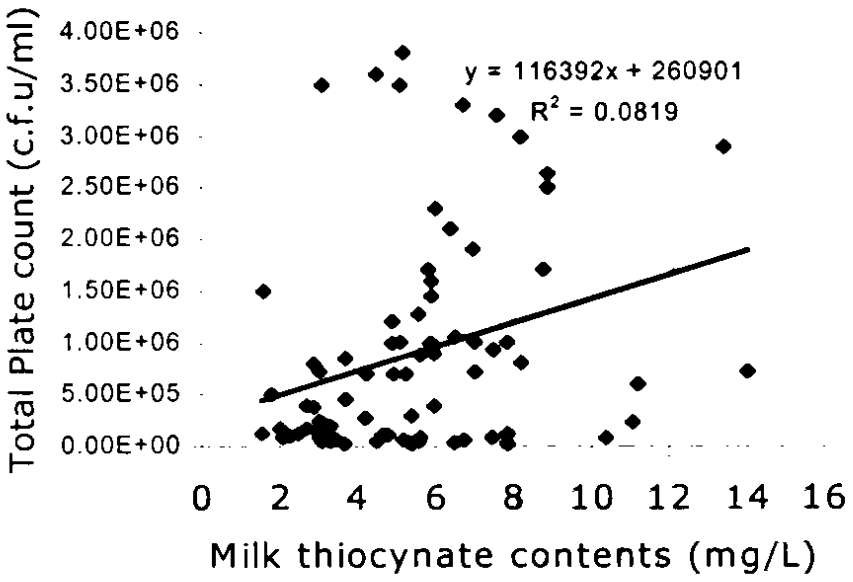

Figure 1. Effect of milk SCN content on Total Plate Count of raw milk collected within 3 hours from milking from Kilimanjaro and Coast regions

DISCUSSION

From Table 2, it was observed that cyanogens level in forage feed samples collected from Kilimanjaro and Coast regions were lower than those of concentrate. This could be due to by different factors such as seasonal variations, soils, locations and content of the feed mixtures. Similar observation was reported in India (Siha and Singhal, 1993). Concentrate mixtures contained more CN than forages and crop residues. This finding was in agreement with Siha and Singhal (1993) who reported 31.5 mg/l in white cabbage, 88 mg/l in cauliflower, 32-200 mg/l in sauerkraut, 85-500 mg/l in savo kale and less than 1 mg/l in other vegetables.

From Table 3, the level of CN in feed was directly related to the SCN level in milk samples. Sorbo (1975) reported that in the conversion of CN of the feed to SCN in milk, sulphur containing amino acid are important hence supplementation of protein sources to the lactating cow is necessary in order to increase the SCN in milk. Also Siha and Sighal (1993) suggested that glucosinalate containing feedstuff such as cabbage, cauliflower, mustard fodder is known to increase the SCN level in milk. Since the concentrate mixtures had good source of protein it led to increased the conversion of CN in feeds to SCN in milk. Claesson (1999) reported that maximum SCN in milk should be 13.00 mg/l and that this concentration can be achieved by feeding a cow 4.2 g daily equivalent of 30 kg marrow stem kale.

The average SCN level in milk from Ayrshire, Hoisten Friesian and crossbred (Friesian +Ayrshire) cows under similar feeding regimen were found to be 4.14, 3.37 and 3.78 mg/l respectively.

This was in a close agreement with the results in the IDF(1988) where it was reported that the concentration of SCN in milk normally ranges from 2 to 7 mg/l. Although there was a slight difference in the of levels of SCN in milk of cows of the same feeding regime, Sorbo (1975) showed that genetically different breeds differ in their conversion efficiency of CN to SCN. Considering cows of the same breed at different farms, the results of SCN content in milk were significantly different ($P<0.05$). This could have been due to variation in feed ingredients as well as individuality within the breed as reported by Siha and Sighal (1993).

Results in Figure 1 as well as in Tables 4 and 5 show that the SCN levels did not influence the bacteriological quality of milk as only 8% of the variation in TPC of milk could be ascribed to the SCN content of the milk. Other factors, especially initial microbial contamination and storage temperature are more important factors. Even though some samples had SCN levels as high as 11-14 mg/l (Figure 1), the antibacterial effect requires addition of at least 10 mg/l hydrogen peroxide for it to be effective. However, results of this study show that establishing the level of SCN in milk in a given area is important in determining whether or not the prescribed level of 15 mg/l Sodium SCN would not be exceeded by adding 10 mg/l SCN in milk.

Milk samples when stored at room temperature (24 - 28° C), the pH of milk from different breeds under similar feeding regimen, showed different shelf life to reach pH of 6.5 (Table 5). Patel and Shannbhati(1993) reported the shelf life of 5 hours, Thakar and Dave (1986) reported the shelf life of milk of 4 hours and 5 hours with initial acidity of 0.16 to 0.128% Lactic acid respectively but did not show the level of SCN in their milk. Also it has been reported that low shelf life of milk could be caused by microbial contamination through dirty udder, improperly sanitized milk equipment, dirty milking environment, dirty milker and inclusion of fore milk. (Oram and Reiter, (1966) reported that presence of high levels of SCN in milk inhibits many gram positive bacteria such as lactic acid *Streptococci* and while many gram negative bacteria such as *Escherichia coli, Pseudomanas spp, salmonella spp* are killed activated LPS (Björk *et al.*, 1975; Reiter *et al.*, 1976). The slight difference in shelf life of milk between the Ayrshire breed cow and Crossbred (F1 + Ayrsh.) cow might be due to the differences in microbial contamination.

CONCLUSION

It can be concluded that most animal feed used by small-holder farmers in the study sites contained low levels of CN while concentrate mixtures contained higher level. There was direct relationship between cyanide concentration in feed and SCN

excreted in raw milk. Milk from different production systems tend to have different levels of naturally occurring SCN and should always be determined to establish average levels before application of the lactoperoxidase systems for milk preservation.

ACKNOWLEDGEMENT

This study was conducted as part of M.Sc. research for co-author Rehema Magesa support by the Norwegian Government through NORAD. The financial support is highly appreciated.

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