Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.7 No.12, 2015
www.iiste.org

Milking Time Effect on Nutrients Level of Free Range Indigenous and Zero Grazed Exotic Cow Milk on Calcium, Magnesium, Potassium and Zinc in Kajiado County, Kenya

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Abstract
The purpose of this study was to determine whether the different milking time in a cow affects the nutrients levels in the milk. Milk is considered as balanced diet food with nutrients playing an important role in the body of a human being like calcium build and maintain strong bones and teeth, magnesiumhelps in muscular functions, potassium helps in blood pressure control, muscle and nerve function while zinc stimulates the activity of enzymes in the body, supports immune system, synthesize DNA and in wound healing.Nutrients levels were determined using analytical methodsAAS for analysis of calcium, magnesium and zinc and AES for analysis of potassium. Cow milk obtained in the morning and in the evening had different levels of nutrients in free range indigenous and zero grazed exotic cows. The results showed that in free range indigenous cow milk, nutrients are higher in the morning than in the evening except magnesium while in zero grazed exotic cow milk, the nutrients analysed are higher in the evening milk than the morning milk.Cow milk obtained in the morning from free range indigenous cows and that from evening from zero grazed exotic cows is the best to be taken.
Keywords: AAS, AES, Cow, milk, nutrients levels,

1.Introduction

Milk is a secretion from the mammary glands of female mammals that is considered to be a balance diet food. The milk is designed by the nature only for the offspring of like kind during the first few months of growth. Milk has high moisture content with adequate amount of calories, proteins, fats, a good source of vitamins like A, B_2, B_{12}, D, K and minerals like calcium, magnesium, potassium and phosphorus whereby they are the major mineral nutrients while minor minerals nutrients are zinc, manganese and copper. The nutritional value of milk makes its inclusion in the diet very important forming an integral part of the diet (Kresser, 2012; Jacob, 2015; Pehrsson et al., 2000). The study of nutrients composition in cow milk provides information of how these nutrients are essential for human health. In nutritional, immunology hygiene, economic and psychological terms, the feeding of the young ones of a species with the milk of the mother must always be considered preferable to that of any other milk (Jost, 2002; Stringleman and Scrimgeour, 2015).

Infants and children are recommended to take cow milk in their diet, although even other people also take it either fresh or in its other forms like powdered, pasteurized, mala and yoghourt(Wilson, 1943). Consumption of cow milk is important and beneficial to human in order to avoid malnutrition especially in children who are vulnerable in such a case of nutrients deficiency. Calcium and phosphorus are necessary for the bone development otherwise if they become inadequate in the body weak bone and rickets results in children (Brody, 1999). Magnesium helps in muscular functions, potassium helps in muscle and nerve function and blood pressure control. Proteins are necessary for growth and development whereby when it is insufficient, diseases like kwashiorkor results. Vitamins on the other hand are necessary for boosting immunity, brain functions and red blood cell production (Fox, 1995; Goff, 2010).

The major consumers of cow milk are infants and children below five years although in the recent past, the change in the lifestyle has necessitated the consumption of cow milk hence resulting to its high demand for fresh cow milk and its products. Cow milk is considered to be balance diet, with easier digestibility and has faster absorption in the body system. There are several factors that affect the nutrients level in cow milk such as age, breed of a cow, weather, lactating period, milking intervals and types of feeds, mental and physical state of the cow. This implies that the nutrients level in the cow milk keeps varying on varying these factors. This variation has resulted to deficiency in vitamin K and D and also iron that has been reported in infants especially those who exclusively rely on fresh cow milk (Goff, 2010; Baker and Greer, 2010). When cow is being milked, there is a progressive increase in fat content during the milking intervals. The first drawn milk contains about 1% of fat and the stripping contains over 10% of fat. The morning milk has a lower fat content than that of the evening due to the milking interval being longer at night than day especially when the milking interval is not equal (Jacob, 2015). The purpose of this study was to determine the effect of milking time on levels of calcium, magnesium, potassium

and zinc nutrients in the cow milk of free range indigenous and zero grazed exotic cows in Kajiado County in Kenya. The information obtained would be very useful in guiding dairy cow farmers on the best time to milk them in order to get high nutrients level. It is mainly focused in assisting milk consumers especially children, infants recovering and elderly people to know the best milk to be consumed in terms of the level of the nutrients. The information obtained assists in curbing the malnutrition problems that arise in the areas where the main food taken is cow milk. The study made recommendations on the best time to obtain cow milk with high level ofnutrients.

2. Materials and methods

2.1 Determination of calcium, magnesium,potassium and zinc

2.1.1 Preparation of stock solutions

Suitable standard solutions of Ca, Mg and Zn of various concentrations were run in AAS instrument while K concentration was run in AES. The absorbance values obtained were used to draw the calibration curves. The stock solutions were stored in different plastic bottles which were placed in a cool dry place in the laboratory.

2.1.2 Working standard solutions

Respective aliquots of the standard stock solutions for each element were taken in series of 100ml volumetric flasks. These were diluted to the mark using distilled water and transferred into plastic sample bottles. During each analysis, fresh working standards were prepared. Different concentration ranges were prepared for each element (Baker and Greer, 2010).

2.1.3 Sample preparation

5ml of the milk sample was placed in test-tube and be shaken vigorously to ensure complete mixing, and then digested using nitric acid. It was transferred to the muffle furnace for ashing at 550^0C for one hour. Each digested sample was then transferred to 50ml volumetric flask and diluted to volume with distilled-deionized water(Baker, R. D. and Greer, 2010).

2.1.4 Equipment for analysis

An atomic absorption spectrometer AAS (Shimadzu-AA-630-12) and atomic emission spectrometer AES (PTF 7, Jenways) were used in this analysis. The instruments conditions were that AAS used air/acetylene fuel and AES used butane/air fuel and was fitted with filters for potassium for its analysis.

2.1.5 Total elements determination

Ca, Mg and Zn were analyzed using AAS. The right operating conditions for AAS were set. The sample and each of the standards were aspirated. The standards were aspirated to give a linear relationship at different concentrations. The distilled-deionized water was aspirated to zero the instrument.

After every three samples, a standard working solution was aspirated to check on the stability and the reproducibility of the instrument. This was repeated for the sample digests and the analysis was done in triplicate. An aliquot of about 10ml of the prepared cow milk samples was put in a test tube and then run in the AAS machine. K was analysed using AES. The samples and the working standards were prepared (as given earlier). Potassium was analyzed by fitting its filter. The meter reading scale was calibrated using 1000ppm working standards the maximum scale and he minimum scale was set using distilled-deionized water. The working standards of different concentrations were aspirated to obtain a linear range. An aliquot of about 10ml of the prepared milk samples was put in a test tube and then be run in the AES.

A calibration curve for each element and a graph of mean absorbance against concentration of the standards was plotted. In all cases the graphs were found to be linear.

2.2 Data Collection:

The curve of absorbance versus concentration of the stock solution was obtained first that was then used to determine the nutrients levels in the cow milk. The means of the nutrients concentration were calculated and the values obtained were expressed as parts per million (ppm) incalcium, magnesium, potassiumand zinc.

2.3 Data Analysis:

The null hypothesis being tested is that there is no significant difference in the levels of nutrients in the cow milk that was milked in the morning and that was milked in the evening.Results obtained in this study were expressed as mean of concentration levels(mean ± S.D) of three replicates. Theconcentrations of milk components were compared with the standard values using one-sample t-test.When differences between two means werecompared between the morning and the evening, paired t-test was used. The mean and the S.D of each milk sample were used to compute the calculated p-value. Differences between the critical p-value and calculated p-values of the concentrations were computed.

3. Results and Discussions

The researcher ensured that milking interval was twelve hours in both breeds of the cow. This time interval gave equal time to the cow milk nutrients to be able to distribute themselves between the two milking times; morning

and evening.

3.1 Calcium

Calcium is essential for living organisms, particularly in cellphysiology, where there is movement of the calcium ion into and out of the cytoplasm functions as a signal for many cellular processes. As a major material used in mineralization of bone, teeth and shells, calcium is the most abundant metal by mass in many animals. Calcium is an important component of a healthy diet and a mineral necessary for life. The National Osteoporosis Foundation says, "Calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life." Approximately 99 percent of the body's calcium is stored in the bones and teeth. Calcium-rich foods include milk and dairy products whereby it is classified as major nutrients. The body require high level of calcium making daily requirement to be high (Table 2).

Cow milk produce high amount of calcium second from potassium (Table 1). In free range indigenous cow milk samples, calcium level was lower in the samples of the evening milk than that obtained in the morning (27.16ppm versus 31.11ppm) (figure 1).On the other hand, there was lower level of calcium in the milk samples obtained in the morning than the milk samples obtained in the evening from zero grazed exotic cows (29.85ppm versus 30.83ppm) (figure 2).

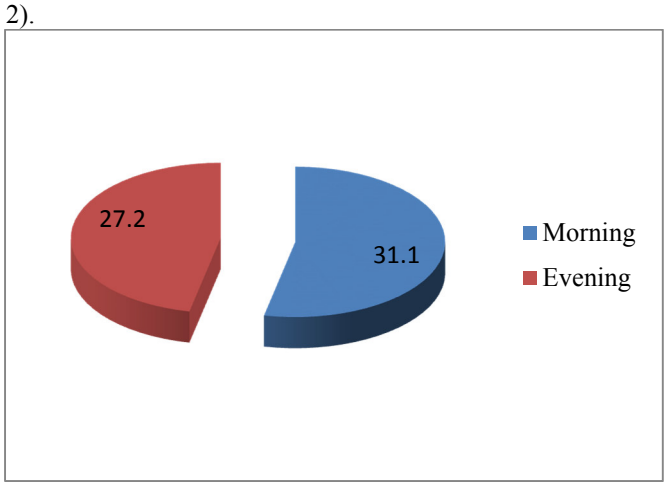

Figure 1: Level of calcium (ppm) in free range indigenous cow milk

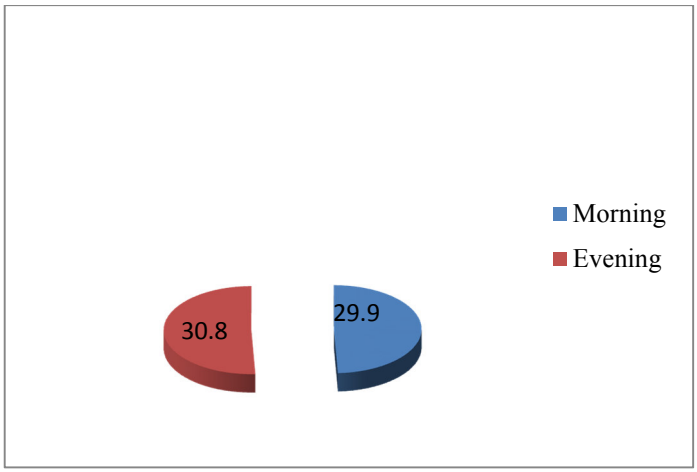

Figure 2: Level of calcium (ppm) in zero grazed exotic cow

The resting time at night for free range indigenous cows is very important due to tedious walking during the day. During this time, the nutrients are being distributed in the body in order to replace all the used up. At such time, calcium finds its way in the morning milk at higher levels. In zero grazed exotic cows the activities are almost balance thus there is neglible difference in the concentration of calcium between the two milking times.

The statistical analysis using t-test was carried out to analyse the results of this study which showed that the p-value was 0.013 in free range indigenous cow milk and 0.299 in the milk from zero grazed exotic cows. Therefore there was significance difference at 95% confidence level in calcium concentration in the milk in the morning and that obtained in the evening in free range indigenous cow milk while in zero grazed exotic cow milk, there was no significance difference in the milk that was obtained in the morning and in the evening.

In the study carried out by Shahram *et al.*, (2012), the level of calcium in the cow milk obtained in the morning and in the evening was 31.42ppm and 31.07ppm respectively, whereby the results did not agree with the results in this study. However, these results show a close range with the results obtained by Shahram.

3.2 Magnesium

Magnesium, an abundant mineral in the body naturally present in many foods. It is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation. Magnesium is required for energy production, oxidative phosphorylation and glycolysis. It contributes to the structural development of bone and is required for the synthesis of DNA, RNA, and the antioxidant glutathione (Maguire et al., 2013). Magnesium intake in the body is an average showing that it is a minor nutrient (Table 2).In this analysis of cow milk, magnesium was third in the level compared to the other nutrients that were analysed (Table 1).The milk samples milked in the evening from both cow breeds had higher concentration of magnesium (free range indigenous had magnesium concentration being 3.62ppm and zero grazed exotic had 5.72ppm) compared to that milked in the morning (free range indigenous had magnesium concentration 2.99ppm and zero grazed exotic had 4.49ppm) (figure 3 and figure 4).

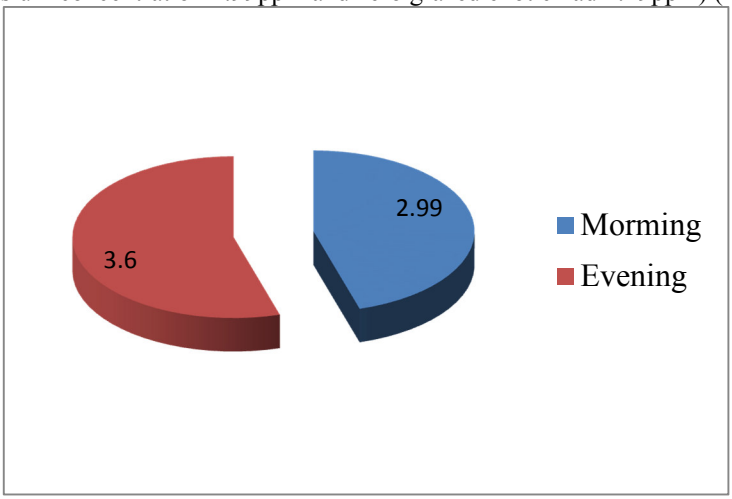

Figure 3: Level of magnesium (ppm) in free range indigenous cow milk

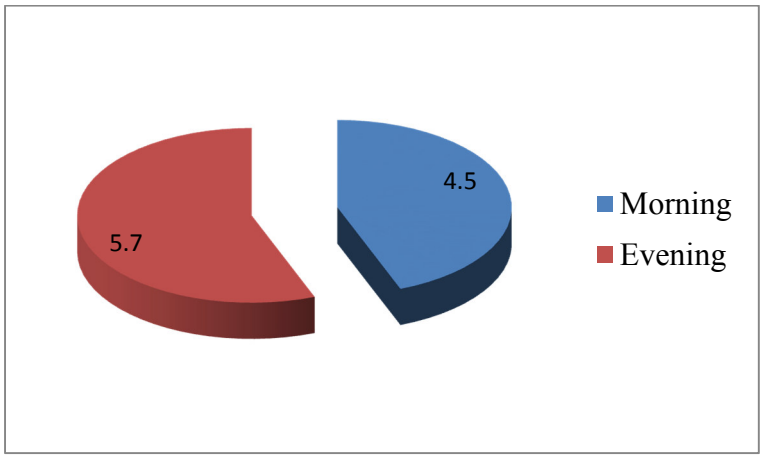

Figure 4: Level of magnesium (ppm) in zero grazed exotic cow milk

The concentration of calcium and magnesium is opposite correlated. Therefore these results reflect this observation whereby high levels of calcium in the diet resulted in decreased magnesium absorption and consequently in the milk.

The statistical analysis using t-test was carried out to analyse the results of this study which showed that the p-value was 0.051 in free range indigenous cow milk and 0.063 in the milk from zero grazed exotic cows. Therefore there was no significance difference at 95% confidence level in magnesium concentration in the milk obtained in the morning and that obtained in the evening in both breeds of the cow.

In the study carried out by Shahram *et al.*, (2012), the level of magnesium in the cow milk obtained in the morning and in the evening was 3.90ppm and 3.74ppm respectively. The results in this study were within the

range with the results obtained in the previous studies.

3.3 Potassium

Dairy milk is a good source of potassium and skim milk has higher level in potassium than other types of milk. Although all types of cow's milk contain comparable amounts of the nutrient, higher potassium levels are proportionally related to lower fat contents. One cup of whole milk provides 322 milligrams of potassium, or 9 per cent of the nutrient's daily value, while the same amount of either reduced-fat or low-fat milk provides about 10 per cent of the daily value for potassium. Non-fat milk is highest in potassium, supplying 382 milligrams, or 11 per cent of the daily value, per 1-cup serving. The main role of potassium in the body is to control blood pressure. Inadequate potassium intake results to symptoms such as muscle cramps, nausea,frequent urination and vomiting. Potassium is the nutrient that is found in the largest quantity in the cow milk among the mineral salts. This makes potassium to be classified as a major nutrient resulting to its dairy requirement also to be high in the body (Table 2). In this analysis, potassium was highest in nutrients levels when compared with the other nutrients that the study was carried out (Table 1). In the free range indigenous cows, the potassium level was higher in the morning milk samples than the evening milk samples (84.91ppm versus 67.26ppm) (figure 5). In the zero grazed exotic cows, the level of the potassium in the samples milked in the morning was lower than the samples milked in the evening (86.52ppm versus 96.27ppm) (figure 6).

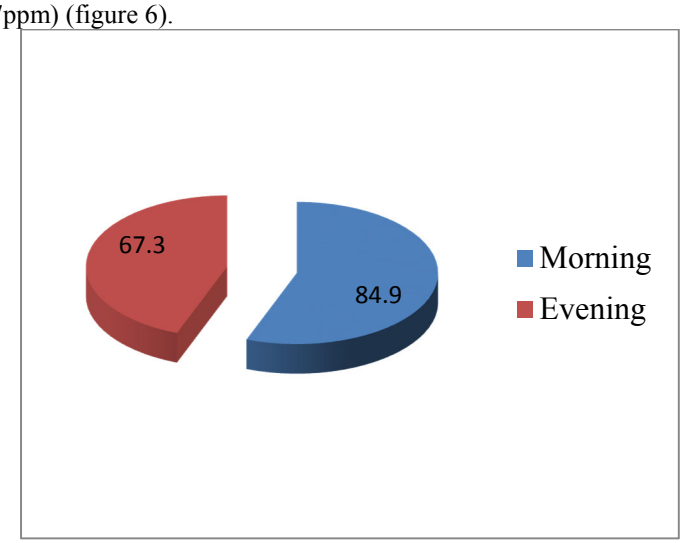

Figure 5: Level of potassium (ppm) in free range indigenous cow milk

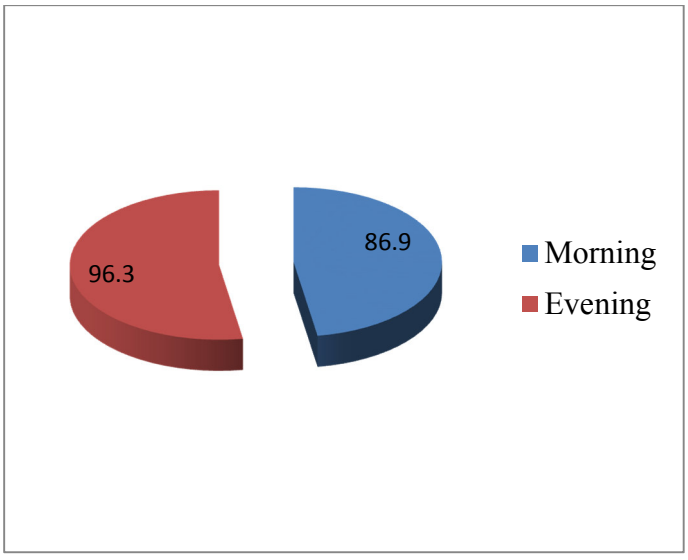

Figure 6: Level of potassium (ppm) in zero grazed exotic cow milk

These results are due to potassium being directly correlated to calcium but opposite correlated to magnesium.The statistical analysis using t-test was carried out to analyse the results of this study which showed that the p-value was 0.005 in free range indigenous cow milk and 0.124 in the milk from zero grazed exotic cows.

Therefore there was significance difference at 95% confidence level in potassium concentration in the milk in the morning and that obtained in the evening in free range indigenous cow milk while in zero grazed exotic cow milk, there was no significance difference in the milk that was obtained in the morning and in the evening.

In the study carried out by Shahram *et al.,* (2012), the level of potassium in the cow milk obtained in the morning and in the evening was 117.04ppm and 117.01ppm respectively. The results from this study were slightly lower than those of the previous study.

3.4 Zinc

Zinc is found in cells throughout the body, although its dairy requirement intake is small (Table 2). It is needed for the body's defensive (immune) system to properly work. It plays a role in cell division, cell growth, wound healing, the breakdown of carbohydrates for the senses of smell and taste. During pregnancy, infancy, and childhood the body needs zinc to grow and develop properly. High-protein foods contain high amounts of zinc while low-protein diets and vegetarian diets tend to be low in zinc. Zinc is in most multivitamin and mineral supplements. These supplements may contain zinc gluconate, zinc sulphate, or zinc acetate. Deficiency in zinc shows symptoms such as, skin sores and wounds that take a long time to heal.

In the milk, zinc is classified among the minor nutrients. Zinc is the nutrient in the lowest quantity among the mineral nutrients that were analysed (Table 1). Free range indigenous cow milk had higher level of zinc in the samples obtained in the morning (1.21ppm) than that obtained in the evening (0.95ppm) (figure7) while in zero grazed cows, milk samples had higher zinc level in the evening (1.26ppm) than in the morning (1.22ppm). In overall, zinc is highest in the cow milk of zero grazed exotic cows obtained in the evening (1.26ppm) (figure 8).

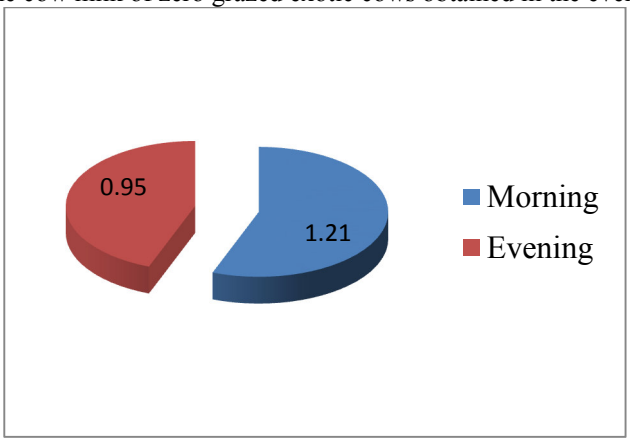

Figure 7: Level of zinc (ppm) in free range indigenous cow milk

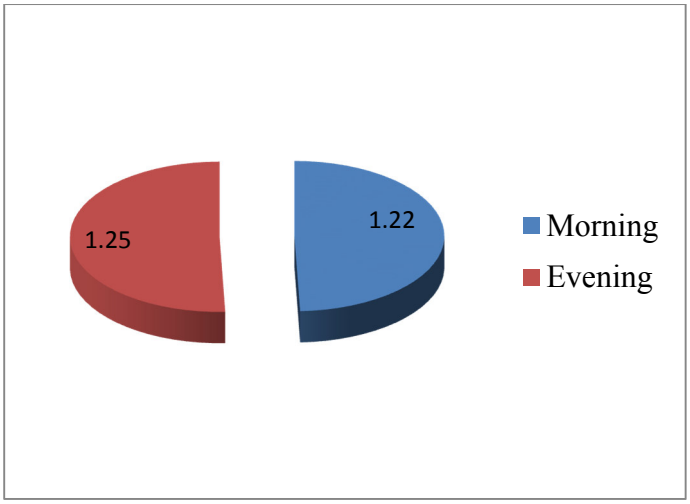

Figure 8: Level of zinc in zero grazed exotic cow milk

Zinc is a micro nutrient that it concentration will vary between the milking time due to the types of the feed eaten and the volume of these feeds. In addition, the resting time that affects the rate of absorption will results to free range indigenous cows excreting more zinc in the morning milk after a long rest compared to zero grazed that excrete more in the evening due to feeds given that has high level of zinc.

The statistical analysis using t-test was carried out to analyse the results of this study which showed that the p-value was 0.162 in free range indigenous cow milk and 0.806 in the milk from zero grazed exotic cows. Therefore there was no significance difference at 95% confidence level in zinc concentration in the milk obtained in the morning and that obtained in the evening in both breeds of the cow.

Table 1.Nutrients levels in free range and zero grazed cows milked that was milked in the morning and the evening.

Nutrient	Milking time	Free range indigenous cow milk (Mean±SD)	Zero grazed exotic cow milk (Mean±SD)
Calcium(ppm	Morning	31.1084±4.8942a	29.8514±2.2457b
	Evening	27.1558±5.97a	30.8372±2.6658c
Potassium(ppm)	Morning	84.9078±13.024a	86.5249±11.366b
	Evening	67.2635±17.94a	96.268±27.3833c
Zinc(ppm)	Morning	1.2123±0.5861b	1.2238±0.5165b
	Evening	0.9517±0.3028c	1.2595±0.533c
Magnesium(ppm)	Morning	2.9894±1.0755b	4.4944±1.4606b
	Evening	3.6150±1.1082c	5.7206±1.2065c

Mean± SD with letters (a) (attached) issignificantly different while those with letter b and c (attached) arenot significantly different ($p<0.05$) between the milking times.

Table 2: Daily reference intake (DRI) values for minerals (Compiled from USDA RDI tables).

			Children		Male				Female					
	Unit		**1-3 yr**	**4-8 yr**	**9-13 yr**	**14-18 yr**	**19-50 yr**	**above 50 yr**	**9-13 yr**	**14-18 yr**	**19-50 yr**	**above 50 yr**	**Pregnant**	**Lactating**
Minerals (Ash)														
Calcium	mg	DRI	500	800	1300	1300	1000	1200	1300	1300	1000	1200	1000	100
		UL	2500	2500	2500	2500	2500	2500	2500	2500	2500	2500	2500	2500
Magnesium	mg	DRI	65	110	240	350	350	350	240	350	320	320	350	310
		UL	80	130	350	410	400	420	350	360	350	350	350	350
Potassium	g	DRI	3	3.8	4.5	4.7	4.7	4.7	4.5	4.7	4.7	4.7	4.7	5.1
		UL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Zinc	mg	DRI	3	5	8	11	11	11	8	9	8	8	11	12
		UL	7	12	23	34	50	40	23	34	40	40	40	40

[1]DRI = USDA daily reference intake amount.
[2]UL = USDA upper limit for safety amount.
[3]ND = none determined.

4. Conclusion

The cow milk samples used in this study were obtained from two different breeds of cow with different mode of feeding. The study showed that different cow breeds produce milk that contains different nutrients levels. The milking time for both cow breeds was the same as it was done twice a day, in the morning at 6.00a. m and in the evening at 6.00 p. m to ensure that equal time is given for nutrients excretion in the cow milk. From this research study different milking time produce cow milk that has different levels of nutrients.The null hypothesis was rejected on comparing morning and evening levels of calcium, potassium, zinc and magnesium of zero grazed exotic cow milk and zinc in free range indigenous cow milk because the calculated p-value was less than the critical p-value at $p<0.05$.However null hypothesis was retained in calcium and potassium of free range indigenous cow milk because the calculated p-value was less than the critical p-value at $p<0.05$ (Table 1).

Zero grazed commercial feeds are digested faster and the nutrients released in the milk before the end of the day. The feeds eaten by free range indigenous cow are grass and shrubs from the field. They take a long time to be digested and absorption is also delayed. This makes the nutrients to take long before they are released and excreted in the cow milk.

The recommendations of the study based on the findings:

- The dairy cow farmers should balance the minerals in animals' diets, so that it may be possible to achieve the highest milk quality and quantity yield
- Free range indigenous cows dairy farmers should be encouraged to do cross breeding with exotic dairy cows in order to increase the nutritional level of milk.
- Creating awareness on cow milk consumers and dairy farmers on nutrients level variation within different milking times.

Acknowledgment
We are greatly thankful to the Department of Chemistry of Jomo Kenyatta University of Agricultureand Technology for guidance during the research work and to laboratory in Kari Muguga for the provision of laboratory space, equipment, and technical assistance to carry out this research.

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