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# Determination of metals in fresh cow's milk under different feeding regime in Wolaita zone, southern Ethiopia

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## Abstract

Milk is an important food in the human diet, both in its original form and in various dairy products. Feed is one of the sources of accumulation of heavy metals in milk and determining these elements in milk under different feed types is vital. Thus, this study was conducted to determine some selected metals in the fresh milk of two cow groups: grass-eaters; and "frushka" (wheat bran) and grass-eaters in Wolaita Sodo Zuriya woreda, southern Ethiopia. Eighteen milk samples were collected from both cow groups in three representative kebeles. Before the sample analysis, the working procedure was optimized, and the determination of metals was made by flame atomic absorption spectrometry. The percentage recoveries of the metals were in the range of 83.3% to 118%. The ranges of the concentrations (in mg/l) of the metals on a dry weight basis are magnesium (Mg) (130.72 – 164.26), calcium (Ca) (627.95 – 847.70), iron (Fe) (1.43 – 2.40) and Zinc (Zn) (2.85 – 5.53). Lead (Pb) and cadmium (Cd) were below the detection limit. There is a significant difference in the mean concentrations of the detected metals between the milk samples from three kebeles and under two feeding regimes. According to the findings, fresh milk is rich in calcium and magnesium and has better zinc and iron content. In most cases, the metal concentration in milk obtained from "frushka" and grass-eating cows is greater than that of grass-only eaters.

**Keywords**: Acid digestion, flame atomic absorption spectroscopy, Fresh cow's milk, Metals

## Introduction

Milk is an essential food in the human diet, both in its original form and in various dairy products. It provides energy and nutrients necessary for young children's growth and mental development (Neumann et al, 2002). Milk is an almost complete food product in the human diet since it contains all macronutrients (such as proteins, lipids, and carbohydrates) and all

micronutrients (elements, vitamins, and enzymes). This is particularly true in the case of early childhood because milk (human, cow, or formulated milk) is the only source of nutrients during the first months of a baby's life, and the diet of growing children contains a high proportion of milk and milk products (Anetta et al., 2012; IDFA, 2008). Milk is an ideal source of important elements such as Ca, K, P, Mn, Cu, Fe, Se, and Zn and even heavy metals can be found (Amah et al,2021; Sikiric et al, 2003).

Metals are present in extremely small amounts in uncontaminated milk. However, metal contamination may occur at several stages during dairy processing, including factory doors, plant equipment, catering operations, ceramic or enameled utensils, metal containers, and dairy water (Teshele, 2018). Still, their contents may be significantly altered through manufacturing and packaging, as well as metals that may be contaminated from different feeds and environments, such as Pb, Cd, Cr, Ni, and Co (Mawia and Yahya, 2018).

Animal feeds, heavy metals, mycotoxins, dioxins, and similar pollutants are considered of great concern to public health due to their toxic effects on humans and wildlife (Amponsah, 2014; Singh et al., 2010). Despite the essential benefits of consuming milk, milk contamination from moderate agricultural practices, industrial pollutants in the environment, animal feeding systems, and sewage sludge in agriculture increase and require urgent attention (Patra et al., 2008).

Trace elements in cow's milk are interesting because of their essential or toxic nature. For instance, Cr and Mn are crucial but may become contaminated at higher levels, while As, Pb, Hg, and Cd are poisonous and can be cumulative (Martino, 2000). The presence of these metals leads to metabolic disorders causing health problems (Licata, 2004). The plant feeding system is one of the sources of accumulation of these elements. Ingestion of contaminated feed and water was considered the primary source of metal residues in secreted milk (Frodello et al., 2002). Attention has been focused on milk as it is almost the perfect single foodstuff, and lactating cattle may have been exposed to high quantities of toxic metals in the environment by air, water, and the ingestion of polluted feeds. Fortunately, these animals act as a very efficient biological filter against heavy metal contamination. It is valid when the animals are grazing near motorways and roads with heavy car traffic (Raghu, 2013).

To recommend the source of cow feed with the best quality milk, determining common metals in milk from cows under different feeding systems is vital (Ajai, 2012). A complete profile of essential and non-essential metals must be available for nutritionists and consumers. A lot of

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work has been done on metal determination in foodstuffs, including milk (e.g. Tassew et al., 2014; Jebessa, 2014). However, data regarding these elements is still lacking in developing countries like Ethiopia, and the presence of essential and toxic metals in milk was not assessed in the study area, particularly in relation to cow feeding habits. It is necessary to determine and monitor the levels of toxic metals in milk, such as lead and cadmium, because they can significantly influence human and animal health. Therefore, this research was conducted on the determination of the levels of selected metals in fresh milk in Wolaita Sodo Zuria Woreda.

## Materials and methods

### Description of the study area

The study was conducted on selected kebels namely Dalbo Wogane, Dalbo Atewaro, and Zalla Shasha in Sodo Zuria Woreda, Wolaita Zone, Southern Ethiopia. The study area is located between 037°35'30" - 037°58'36"E and 06°57'20" - 07°04'31"N and the elevation of ranges between 1473 to 2873 meters above sea level (m.a.s.l). The area has a bimodal rainfall pattern and about 31 and 39% fall during autumn (March - May) and summer (June - August) seasons respectively. Sodo Zuria Woreda is one of the administrative districts of wolaita zone in the southern nation nationalities and people's region (SNNPR). It is bounded by Damot Gale woreda in the north, Ofa woreda in the south, Humbo Woreda in the east, and Bayra koysha Woreda in the west. The study area (sodo zuria woreda) has two major Agro-ecological zones, Weyna dega (midland) and Dega (highland) (WZFEDD, 2015).

### Sample collection and preparation

#### Sample collection

The selection of sample collection areas was made mainly based on accessibility for sampling and potential areas for milk production. The samples were collected from sampling sites of Sodo Zuria Woreda: Namely Dalbo Wogane, Dallbo Atewaro, and Zalla Shasha Kebeles. The triplicate representative samples were taken randomly from each cow group (grass eaters, and “frushka’ and grass eaters) from three kebeles to collect 18 samples (3x2x3=18). The uncontrolled conditions (amount of feed offered, feeding duration, and time) to take as a formal experiment and the small sample size were the limitations of the study, though dairy producers

were informed not to provide other feed types before sampling. All samples were collected in nitric acid-washed polyethylene containers and immediately transported to the laboratory. Then, the samples were stored in the refrigerator before sample preparation, digestion, and analysis (Chandrama et al., 2014).

The sample preparation technique used for this study was acid digestion (including microwave energy). Milk samples were prepared to carry out Spectroscopic analysis using the optimized procedure as described. An aliquot of each milk sample was weighed into a Teflon digestion vessel. To determine the mentioned metals, all milk samples were prepared according to the following procedure: exactly 5 grams of finely powdered milk of each Sample was weighed in Polytetrafluoroethylene vessels and dissolved in 5 mL of concentrated nitric acid (HNO3), followed by 2 mL $H_2O_2$. After that, samples were digested in the microwave oven. This process was set in a closed system, so the sample decomposition had no contact with external surroundings, thereby reducing the risk of contamination.

Instruments and apparatus

The instruments that were used in this study for the experimentation were: volumetric flasks (1000 mL, 100 mL), conical flasks (1000 mL, 500 mL, 250 mL, and 25 mL), measuring cylinders (5 mL, 10 mL, 25 mL, and 50 mL), and electronic balance, watching glass, glass rod, pipette, dropper, and oven. The glassware was kept in a clean place to avoid contamination. Flame atomic absorption spectrometry (FAAS) was used for the determination of the analyte metals in the milk samples. A computer (data recorder), AAS equipped with a deuterium background corrector, and hollow cathode lamps with air-acetylene flame were also used as types of equipment.

Reagents and chemicals

The analytical grade reagents were used to clean the glass wares and digest the milk samples throughout this work. The analytical grade chemicals used in this study were nitric acid (69-72), H2O2 (30%, Blulux Laboratories), Ca $(NO_3)2.4\ H_2O$, Zn $(NO_3)2.6H_2O$, Pb $(NO_3)2$, Cd $(NO_3)2.4\ H_2O$ and distilled water.

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## Experimental procedures

### Cleaning apparatus

All glass and plastic wares were washed with tap water and kept overnight in a 10 % (v/v) nitric acid solution (Girma and Meareg, 2017). Afterward, it was rinsed thoroughly with ultra-pure water and dried in the oven. The glassware was held in a clean place to avoid contamination.

### Optimization of the working procedure

Before the sample analysis, the working procedure was optimized to give maximum signal strength by adjusting the parameters such as wavelength, slit width, lamp current, and sample energy for each element. The optimized microwave digestion procedure was selected depending on the clearness of digests, minimum digestion time, minimum reagent volume, absence of undigested milk samples, simplicity, and low heating temperature (Bell et al, 2004).

### Sample digestion

The fresh milk samples were oven dried at 65°C until they reached dry mass. The dry samples were ground to powder using watching glass and glass rods. After that, 5g of the finely powdered milk sample was weighed and taken into a 500 mL pyrex conical flask, and then 5 mL of 69-72% $HNO_3$ was added, followed by 2 mL 70% $HClO_4$. The digestion flask was heated at a temperature of $90^oC$, and on heating, brown fumes appeared, the temperature was then gradually increased to $120^oC$ and an extra brown stink evolved, and all the organic matrix of milk was destroyed, and the clear solution was formed with the elements left. After digestion, the mixture was allowed to cool for a few minutes and then filtered into a 25mL conical flask using Watchman filter paper to remove any suspended residues. The digestions of the blank reagent samples were also performed in parallel with the milk samples, keeping all the digestion parameters the same. Finally, the mixture volume was adjusted to 25 mL using distilled water and used for analysis (Kebbeku, 2003).

Instrumental calibration and determination of selected metals by FAAS

Instrumental calibration

Calibration of the instrument was done with the standard solutions prepared before the determinations of metals. The stock solution of each mineral was used to prepare a working standard solution for the calibration curve. So, the stock solutions of Ca, Mg, Fe, Zn, Pb, and Cd were used for these purposes. The standards were prepared from the 100 mg/L of the elements which were prepared prior by taking 1 mL from the stock standard solutions containing 1000 mg element/L, in 2 % $HNO_3$, of the metals. The absorbance of the working standard solutions was measured, and the calibration curves for each of the analyte metals were constructed.

Determination of selected metals by FAAS

The metal contents of milk obtained from two cow feeding regimes (grass eaters and frushka eaters) were compared. Then the difference in metal content between the milk obtained from two cow groups was determined. Some metals analyzed in this study were Ca, Mg, Fe, Zn, Pb, and Cd. These metals were determined by reading their absorbance at specific wave lengths using flame atomic absorption spectrometry (FAAS). An atomic absorption spectrometer equipped with a deuterium arc background corrector and standard air acetylene burner system was used. A hollow cathode lamp was used for each metal (Ca, Mg, Fe, Zn, Pb, and Cd) operated at the manufacturer's recommended condition. The acetylene and airflow rates were managed to ensure suitable flame conditions. The burner height was adjusted for optimum sensitivity, and the nebulizer uptake rate was optimized to provide an optimum absorbance signal in conventional sample aspiration.

Method of performance and method of validation

Method Detection Limit

The method of detection limit (MDL) is the minor measurable concentration of analyte that is statistically different from the blank. It is based on the detection limit of the instrument. Seven blank samples were digested following the same procedure as the samples, and each metal concentration was determined. The standard deviation for each element was calculated from the seven blank measurements. The standard deviation was multiplied by three to give MDL (Miller, 2005; Minaleshshewa, 2007).

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MDL = 3 x SD ---------- (1)

Where SD is the standard deviation of the blank solution

Method Validation

Validation is the process of evaluating a method's performance and demonstrating that it meets a particular requirement. It shows what the method is capable of delivering, particularly at low concentrations. Some method validation is a recovery test. This was measured properly and revealed in the current study. Finally, the recovery formula was used to calculate the percentage of recovery (Mitra and Brukh, 2003).

$$\% \text{ Recovery} = \frac{Amount\,after\,spike - Amount\,before\,spike}{Amount\ added} x100 \quad \text{---------- (2)}$$

Statistical analysis

One-way variance analysis (ANOVA) and a t-test were widely used statistical methods to compare group means. The least significant difference (LSD) method was used to analyze the presence or absence of significant differences in the mean concentration of each metal between the milk samples.

**Results and discussion**

Optimization for digestion of milk sample

The optimum digestion procedure that was chosen was the one that required 2 hours for complete digestion of 5 g of powdered milk sample with 5 mL of 69-72 % HNO3 and 2 mL of 70 % HClO4 (Table 1). However, the other tested procedures have some limitations. They required a somewhat large reagent volume and longer digestion time. In addition, the result in the formation of cloudy and colored solutions (Chandravanshi and Feleke, 2005; Bell et al., 2004). The reagent blank was also prepared and digested with the same procedure at the sample.

Table 1. Different conditions tested for optimizations of the digestion procedure for 5 g of the milk powder sample.

| Trial | Reagent used | Volume ratio | Temp ($^0$c) | Digestion time (min) | Observation |
|---|---|---|---|---|---|
| 1 | $HNO_3$: $HClO_4$ | 3:2 | 65 | 20 | Pale yellow solution |
| 2 | $HNO_3$: $HClO_4$ | 3:2 | 70 | 40 | Pale yellow solution |
| 3 | $HNO_3$: $HClO_4$ | 4:2 | 90 | 70 | Slight Pale yellow solution |
| 4 | $HNO_3$: $HClO_4$ | 4:2 | 100 | 90 | Slight pale yellow solution |
| 5 | $HNO_3$: $HClO_4$ | 5:2** | 120** | 120** | Clear colorless solution |
| 6 | $HNO_3$: $HClO_4$ | 5:2 | 150 | 130 | Colorless solution |
| 7 | $HNO_3$: $HClO_4$ | 5:2 | 180 | 140 | Colorless |
| 8 | $HNO_3$: $HClO_4$ | 5:2 | 200 | 150 | Cloudy suspension |

** Indicates an optimum condition

Calibration of instrument

Calibration of the instrument was done with the standard solutions prepared before the determinations of metals were done. The standard solutions were made from 100 mg/L elements that were previously prepared by taking 1 mL from stock standard solutions containing 1000 mg/L elements in 2% HNO3. The correlation coefficients of the elements were determined using prepared standards versus their corresponding absorbance. The standards, their corresponding correlation coefficients, and the equation for the calibration curve are given in Table 2.

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Table 2. Working Standard solution, correlation coefficient, and calibration curve equation for determination of metal using FAAS

| Metals | Working standard solutions in mg/L | Correlation coefficient of Calibration curve | Equation for calibration curve |
|---|---|---|---|
| Mg | 1, 3, 5, 7, 10 | 0.997 | $Y = 0.0903x + 0.1127$ |
| Ca | 0.05, 0.1, 0.15, 0.2, 0.25 | 0.997 | $Y = 0.0556x - 0.0013$ |
| Fe | 1, 2.5, 5, 8, 10 | 0.998 | $Y = 0.0284x - 0.0046$ |
| Zn | 0.05, 0.5, 1, 1.5, 3 | 0.997 | $Y = 0.2971x + 0.0288$ |
| Pb | 0.5, 1, 1.5, 2, 2.5 | 0.997 | $Y = 0.0178x - 0.0035$ |
| Cd | 0.1, 1, 1.5, 2, 2.5 | 0.996 | $Y = 0.2921x + 0.0565$ |

Method of performance and method of validation

Method detection limit

The determinative procedures involve digesting and diluting the blank solutions and then analyzing the concentration of each element in the samples under the optimized procedure (Table 3).

Table 3. Method detection limit (in mg/L) of metals

| Metals | Mg | Ca | Fe | Zn | Pb | Cd |
|---|---|---|---|---|---|---|
| MDL | 0.063 | 0.0600 | 0.0420 | 0.0075 | 0.015 | 0.003 |
| IDL | 0.001 | 0.0100 | 0.0300 | 0.0050 | 0.100 | 0.005 |

MDL = Method Detection Limit IDL = Instrumental detection limit

Method validation

The Recovery of the method ranged from 83.3 % to 118 %. The good Recovery for most nutrients indicates that the digestion method which was used for sample preparation is precise and reliable (Welz and Sperling, 1999). As presented, the recovery of metals in this study was good. This indicates that the digestion method used for sample preparation is precise and reliable. The results include six metals (Mg, Ca, Fe, Zn, Pb, and Cd) of milk samples from two cow groups that were presented in Table 4.

Table 4. Recovery test for metals

| Cow groups | Metal | Unspiked concentration (mg/L) | Amount added (mg/L) | Spiked concentration (mg/L) | Recovery test (%) |
|---|---|---|---|---|---|
| Grass-eaters | Mg | 145.63 | 2 | 147.71 | 104 |
| | Ca | 683.67 | 2 | 685.89 | 111 |
| | Fe | 1.49 | 2 | 3.48 | 99.5 |
| | Zn | 3.12 | 2 | 5.13 | 100.5 |
| | Cd | ND | ND | ND | ND |
| | Pb | ND | ND | ND | ND |
| Frushka and grass-eaters | Mg | 158.82 | 1.5 | 160.59 | 118 |
| | Ca | 821.66 | 1.5 | 823.19 | 102 |
| | Fe | 2.13 | 1.5 | 3.59 | 97.3 |
| | Zn | 4.96 | 1.5 | 6.21 | 83.3 |
| | Cd | ND | ND | ND | ND |
| | Pb | ND | ND | ND | ND |

The concentration of selected metals in milk

To determine the concentration of some metals in milk samples, knowing the operating condition of the instrument is essential. Therefore, the operating condition of the instrument is given in Table 5.

Table 5**.** Instrument operating conditions for the determination of nutrients by flame atomic absorption spectrometer.

| Metals | Wave Length(nm) | Lamp current(mA) | SW(nm) | IDL | Fame source/color |
|---|---|---|---|---|---|
| Mg | 285.2 | 4.0 | 0.5 | 0.001 | $C_2H_2$ gas/blue |
| Ca | 422.7 | 2.0 | 0.7 | 0.01 | $C_2H_2$ gas/blue |
| Fe | 248.3 | 7.0 | 0.2 | 0.03 | $C_2H_2$ gas/blue |
| Zn | 213.8 | 2.0 | 0.7 | 0.005 | $C_2H_2$ gas/blue |
| Pb | 283.3 | 5.0 | 1.0 | 0.1 | $C_2H_2$ gas/blue |
| Cd | 228.9 | 2.0 | 0.7 | 0.005 | $C_2H_2$ gas/blue |

SW = Slit Width IDL = Instrumental Detection Limit

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The results determined from each sample site were listed in terms of the mean value plus/minus standard deviation of mg/100g dry weight as presented in Table 6. The result includes six metals (Mg, Ca, Fe, Zn, Pb, and Cd) in fresh cow's milk taken from two cow groups.

Table 6. Mean Concentration of metals (X ± SD, n = 3) in the milk of two groups of cows from different sites.

| Cow groups | | Concentration of metals in mg/L | | | | | |
|---|---|---|---|---|---|---|---|
| | Sites | Mg | Ca | Fe | Zn | Pb | Cd |
| | Dalbo Wogane | 130.72 ± 1.49$^{f}$ | 717.79 ± 1.61$^{a}$d | 1.80± 0.02$^{d}$ | 3.07±0.09$^{f}$ | ND | ND |
| Grass-eaters | Dalbo Atewaro | 151.03 ± 1.42$^{d}$ | 687.09 ± 1.93$^{e}$ | 1.63± 0.04$^{e}$ | 2.85±0.06$^{e}$ | ND | ND |
| | Zalla Shasha | 143.69 ± 1.64$^{e}$ | 627.95 ± 2.34$^{f}$ | 1.43± 0.02$^{f}$ | 3.18±0.0$^{d}$ | ND | ND |
| | CV (%) | 1.069 | 0.289 | 1.646 | 1.868 | ND | ND |
| | Dalbo Wogane | 164.26 ± 1.12$^{a}$ | 817.88 ± 1.71$^{c}$ | 2.04± 0.12$^{b}$ | 5.53 ± 0.07$^{a}$ | ND | ND |
| Frushka and Grass-eaters | Dalbo Atewaro | 158.30 ± 1.55$^{c}$ | 847.70 ± 1.32$^{a}$ | 2.40± 0.03$^{a}$ | 4.91 ± 0.03$^{c}$ | ND | ND |
| | Zalla Shasha | 160.76 ± 0.55$^{b}$ | 826.52 ± 1.18$^{b}$ | 1.82± 0.05$^{c}$ | 5.20 ± 0.11$^{b}$ | ND | ND |
| | CV (%) | 0.576 | 0.122 | 3.194 | 1.342 | ND | ND |
| | LSD (mg/L) | 0.820 | 2.880 | 0.073 | 0.096 | ND | ND |

ND = Not detected CV = Coefficient of variance LSD = Least significant difference

Values are mean of triplicate ± SD. Means not sharing common superscript letters in a column are significantly different at ($p<0.05$).

Distribution patterns of selected metals in milk Sample

There is a variation in metal concentration in the milk of two cow groups (Table 6). The concentration of Ca was the highest of all the metals determined in this study and ranged from 627.95 – 847.70 mg/L by dry weight. The minimum concentration of Ca (627.95 mg/L) was observed in grass eater groups at Zalla Shasha kebele, and the maximum concentration of Ca (847.70 mg/L) was observed in frushka and grass eaters at Dalbo Atewaro kebele. In contrast to calcium, the concentration of Fe was the lowest among the metals determined in this study and ranged from 1.43 – 2.40 mg/L dry weight. The minimum concentration (1.43 mg/L) was observed in grass eaters at Zalla shasha kebele, and the maximum concentration (2.40 mg/L) was

observed in frushka and grass eaters at Dalbo Atewaro kebele. In the current study, all of the determined metals were found to be higher in the milk of cows fed frushka and grass than in the milk of cows fed only grass. This shows that as feeding habits are good for cows, milk yield increases, and hence the metal content also increases. Among the metals determined, lead and cadmium were below the detection limit in all milk samples from two cow groups, and thus, they were not detected. In short, based on their concentration, the decreasing order of four metals detected and recorded from milk samples of two cow groups at different kebeles was found to be Ca > Mg > Zn > Fe. In general, in all sample sites, the calcium concentration is highest and that of iron is least in the milk of two groups of cows. Of the six metals included in the study, lead and cadmium were not detected, and the concentration range of the four metals (Mg, Ca, Fe and Zn) seen in the survey is given in Table 6.

Comparison of individual metals in milk sample

This study observed that in the milk samples of two cow groups at all sample sites, the concentrations of calcium and magnesium were much greater than the concentrations of the other metals included in the study. This revealed that all animals, including cows, are plant dependent. The content of these metals in the plant is high when compared with others.

Magnesium

The values for the level of magnesium content determined from the milk of the two cow groups were significantly different ($p<0.05$). Among the cow groups, Frushka and grass eaters have the highest magnesium content, and their concentration ranges from 158.30 – 164.26 mg/L (Table 6). The highest concentration (164.26 mg/L) was observed at Dalbo Wogane kebele (Table 6). The magnesium content of milk in other groups, namely: a Grass eater was increased from 130.72 – 151.03 mg/L (Table 8). The obtained results agree with Maja's (2003) study. However, there is a slight difference in the magnesium content of the current study and the previous one. This may be due to genetic makeup variation, feeding habits, and differences in environmental conditions. Magnesium is a very important mineral because it functions as a co-factor for many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes (Habtamu et al, 2013). Therefore, milk is also essential to maintain both the acid-alkaline balance in the body

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and the healthy functioning of nerves and muscles, including the heart, since it has a higher content of magnesium (Minaleshshewa, 2007).

Calcium

The calcium content in the milk of cows eating both frushka and grass ranged from 817.88 – 847.70 mg/L (Table 6), and this is a higher concentration than the calcium content in the milk of cows eating only grass. The highest concentration (847.70 mg/L) was recorded from Dalbo Atewaro kebele (Table 6). The calcium concentration in the milk of grass-eating animals was increased from 627.95 – to 717.79 mg/L (Table 8). The calcium content in the milk of the two groups of cows differed significantly ($p<0.05$) from one another. Tadesse (2015) reported that the calcium content of milk was 618.7 mg/L which is less than the value in this study (627.95 – 847.70 mg/L). The variation in the calcium content could be because of genetic differences among the cows, their feeding habits, differences in the breeding situation, and other practices carried out on this site. Calcium is the most abundant mineral in the body, and 99 % of the calcium is found in the bones, where it acts as an integral part of bone structure and calcium storage. The remaining 1% circulates in extracellular and intercellular fluids, where it is essential for life functions such as normal blood pressure and muscle contraction (Whitney and Rolfes, 2008).

Iron

Iron was another mineral analyzed in this study. Iron levels in milk from cows fed only grass, and frushka and grass ranged from 1.43-1.80 mg/L and 1.82-2.40 mg/L, respectively (Table 6). Frushka and grass-eaters had a higher iron content compared to only grass eaters. The highest concentration (2.40 mg/L) was recorded from Dalbo Atewaro kebele and the least concentration (1.43 mg/L) was recorded from Zalla Shasha kebele (Table 6). The concentration of iron in the milk of the two cow groups differed significantly ($p<0.05$). Lukacova, et al (2012) reported that the iron content of milk was to be 1.76 mg/L which agreed with the value in this study (1.43 – 2.40 mg/L). This significant variation in the iron content could be because of genetic differences among the cows. It is vital to many of the cell's activities. Most iron in the body is found in two proteins, hemoglobin in red blood cells and myoglobin in muscle cells. In both cases, iron helps

to carry and release oxygen. Iron deficiency is the major type of malnutrition in a developing country.

Zinc

The value of the zinc content of milk for the studied cow groups is given in Table 6. The level of zinc content in milk significantly differed ($p<0.05$) between the two groups of cows. The zinc content of milk from grass-eating cows increases from 2.85 to 3.18 mg/L, while milk from frushka-and grass-eating cows increases from 4.91 to 5.53 mg/L (Table 6). The highest concentration of zinc (5.53 mg/L) was recorded in the milk of cows eating both frushka and grass at Dalbo wogane kebele and the lowest concentration (2.85 mg/L) was recorded in the milk of cows eating only grass at Dalbo Atewaro kebele (Table 6). As shown in Table 7, the zinc contents obtained in this study were slightly less than the result of a study done by Teshale (2018) (5.59 mg/L). The possible explanation for the differences in zinc content might be differences in the environment in which the study took place and cow groups, as well as feeding habits. Zinc is a versatile trace element required as a cofactor in more than 100 enzymes that are involved in the regulation of gene expression, and it also stabilizes the cell membranes by helping to strengthen their defense against free radical attacks and the like (Whitney and Rolfes, 2008).

Lead and cadmium

The level of lead and cadmium in the milk of two cow groups at all sample sites was below the detection limit. This is due to the low concentrations of lead and cadmium in the plant. As a result, the metal content in milk is also very low because animals are plant dependent. Since lead and cadmium are cumulative poisons, very small quantities that cause no harm may become dangerous when taken constantly (Amede et al., 2004). Hence, cadmium was not detected in the milk of the two cow groups. In general, the concentration level of nutrients in the milk of the two cow groups was observed in decreasing order as; Ca > Mg > Zn > Fe while Pb and Cd.

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Table 7. Comparison on nutrients in milk of two cow groups with some literatures value

| Nutrients | The value of current study (mg/L) | | Literature value and References | |
|---|---|---|---|---|
| | Grass eaters | Frushka and Grass eaters | Value (in mg/L) | References |
| Mg | 130.72 – 151.03 | 158.30 – 164.26 | 136.02 – 196.67 | Maja (2003) |
| Ca | 627.95 – 717.79 | 817.88 – 847.70 | 618.7 | Tadesse (2015) |
| Fe | 1.43 – 1.80 | 1.82 – 2.40 | 1.76 | Lukacova et al. (2012) |
| Zn | 2.85 – 3.18 | 4.91 – 5.53 | 5.59 | Teshale (2018) |

## Conclusions

From the result of this study, we can conclude that fresh milk is rich in calcium and magnesium and has better zinc and iron content. Therefore, using milk as food is necessary when these minerals are scarce. Lead (Pb) and cadmium (Cd) were below the detection limit. There is a significant difference in the mean concentrations of the detected metals between the milk samples from three kebeles and under two feeding regimes. The metal concentration in milk obtained from "frushka" and grass-eating cows is greater than that of grass-only eaters. In the future, research can be conducted on fresh milk, which can contain more mineral content than the minerals included in the study. This needs to be in controlled conditions (amount of feed offered, feeding duration, and time) as a formal experiment and with more sample size. So, by doing this, we may satisfy the mineral needs of the people who are highly dependent on milk.

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## Availability of data and materials

Data generated during this research work can be available upon request.

## Competing interests

The authors declare that they have no competing interests.

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