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Periodic Study with Selected Mineral Elements Levels of Cow and Goat Milk Samples Obtained from Two Animal Markets in Benin City and Estimated Daily Intake of Studied Heavy Metals

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Abstract
This work entailed periodic investigations of raw goat and cow milk samples collected from Eyan and Technical College Road Animal Markets in Benin City, Nigeria; for levels of Ca, Fe, Zn, Cu, Co, Cr, Cd and Pb. The collection of samples was carried out on a weekly basis for a period of four weeks. Another portion of the work was concerned with the estimation of daily intake (EDI) of the examined heavy metals through milk consumption. Determinations of the examined mineral elements were carried out in accordance with standard methods. Significantly, atomic absorption spectrophotometry using buck scientific model 210VGP atomic absorption spectrophotometer was used for Ca, Fe, Zn, Cu, Co, Cr, Cd, and Pb Levels determinations. Estimation of daily intake (EDI) of the examined heavy metals through milk consumption was by calculation. Data generated were statistically evaluated using International Business Machine (IBM) Statistical Package for Social Sciences (SPSS). Results indicate variations in the levels of the examined mineral elements with respect to both sampling period and location. The observed variations were statistically significant ($P<0.05$). Also discernable from results, was that in the studied goat milk samples, Ca with a mean total value of 127.802mg/kg occurred highest, while Pb with a mean total value of 0.005mg/kg was the lowest occurring of the examined mineral elements. Similar trend of result was obtained for the studied cow milk samples. Results from the portion of work on the estimation of daily intake of the examined heavy metals through milk consumption, indicate that with respect to goat milk, EDI Value of 17.694µg/kg/daily consumption, obtained for Zn in males within the studied age bracket (1-5years) was the highest; while the EDI of 0.009µg/kg/daily consumption, recorded for Pb in both male and female within the studied age group bracket (17 years and above) was the lowest. Similar pattern of EDI results was obtained for the EDI values of the examined heavy metals through cow milk consumption. The occurrence of toxicologically relevant lead and cadmium in all the studied milk samples, is an indication of the need for measure that would minimize the exposure of the animals to contaminated foliage and water, to be put in place.

Keywords: Milk, Nutrients, Spectrophotometer, Exposure

1.0 INTRODUCTION

Milk, a complex colloidal system [1] and a secreted fluid of the mammary glands of female mammals [2] is consumed by persons of different ages. However, [3] posited that infants and the elderly which constitute the vulnerable age group consumed more milk and dairy products. Milk possesses remarkable nutritional quality. Notably, [2] remarked that milk contains nearly all the nutrients necessary to sustain life. Milk and milk products are among the main sources of minerals [4]. With particular respect to animals, it is pertinent to mention that the compositional chemistry of milk secreted by their mammary glands would amongst other factors like breed, source, season of harvest and the health condition of the animal, depend on the amount and the constituents of the foliage the animal is fed with. It is also imperative to emphasize, that the quality of water available to the animal for consumption would be relevant in the overall quality of the milk obtained from such animal.

In this work, raw goat and cow milk samples were used as the materials for investigation, being the two most common milk species consumed in Nigeria. There is primary link between the quality of raw milk and its source. Therefore, xenobiotic materials especially contaminants such as heavy metals and pesticides within the environment the animal producing the milk is reared, as well as portion of drugs and their metabolite, arising from medications administered to the animals, could be present in some milk samples and products marketed to consumers. The toxicological effects of these contaminants make their presence in milk an aspect of research interest. Rezaei *et al.*

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in [5] posited that the safety of dairy products decreases with increasing concentration of toxic compounds and environmental pollutants, especially heavy metals.

Dietary sources are significant means of human exposure to various mineral elements [6]. Therefore, milk which in the feeding habits of so many persons is a common diet, could presently, be a major source of exposure of concerned consumers to different mineral elements. Hence the concern of this study primarily, was to investigate the levels of calcium (Ca), Iron (Fe), Zinc (Zn), Copper (Cu), Cobalt, (Co), Chromium (Cr), Cadmium (Cd) and lead (Pb) in raw goat and cow milk samples obtained from two animal markets in Benin city, Nigeria. The estimated daily intake of the studied heavy metals was also determined. Furthermore, the authors considered it necessary to carry out periodic studies of the levels of the aforementioned mineral elements in raw goat and cow milk samples obtained from the studied animal markets. Worthy of note is that in addition to continuous sales and restocking, the goats and cows found in Benin City, are left to graze in different pasture fields. Also, there could be drugs administered to the animals. Therefore, research of this nature that is concerned with periodic studies is imperative. The four weeks study duration represented short term study period.

Several authors [7-11] had variously examined some mineral elements content of milk elsewhere. With respect to goat and cow milk obtained in Benin City, literature reports on the levels of the mineral elements investigated in this work are scarce if in existence. In particular, works on periodic study of the examined mineral elements using goat and cow milk samples are yet to be found. It would also appear that in relations to the goat and cow milk obtained in Benin City, information on the estimation of the daily intake of the examined mineral elements by different age group, based on milk consumption, is non-existence. Findings from this work will fill the existing gap in knowledge even if partly.

Atomic absorption spectrophotometry would be used to investigate the levels of Ca, Fe, Zn, Cu, Co, Cr, Cd and Pb in the studied milk samples. Additionally, the data which this work will generate, would be subjected to statistical analysis, including the analysis of variance (ANOVA). The statistical evaluation of the data would be carried out using the International Business Machine (IBM) Statistical Package for Social Sciences (SPSS).

2.0 MATERIALS AND METHODS

2.1 Sample Collection

The goat and cow milk samples used in this work were obtained from Eyan and Technical College Road Animal Markets in Benin City. The collection of samples was carried out during the dry season. Milk samples obtained from the respective lactating animals were collected in sterile plastic containers. Immediately after collection, the tightly corked plastic containers containing the milk were placed in cooler containing ice block and transported to the laboratory for analysis. On arrival in the laboratory, prior to conducting samples digestion and investigation of the subsequent digests for the examined minerals elements, the samples were stored in refrigerator wherein a temperature of 4^0C was maintained. Samples digestion and subsequent determination of the examined mineral elements were carried out within twenty-four (24) hours of samples arrival in the laboratory.

2.2 Sample Digestion

Homogenized sample was used in triplicate digests production, after all the glassware used have been thoroughly washed, followed by further cleaning with freshly prepared 10% HNO_3 solution and a subsequent cleaning with deionized distilled water. Sample digestion was by wet digestion procedure, involving the use of concentrated tri-acid (HNO_3-H_2SO_4-$HClO_4$) as digestion mixture; this is an adaptation of the method described by [12]. The tri-acid mixture (HNO_3-H_2SO_4-$HClO_4$) used was obtained by mixing concentrated HNO_3, H_2SO_4 and $HClO_4$ in 10:1:4 ratio [12] and allowed to cool.

The digestion was carried out by measuring 10ml of the homogenized milk into a 300ml digestion flask. Thereafter, 20ml concentrated H_2SO_4 was added and swirled properly. The digestion flask with a glass funnel place in its neck was lowered unto the surface of a thermostatically controlled hot plate. Subsequently, the temperature of the hot plate was gradually increased to 145^0C, and the heat was maintained at this temperature for 30 minutes. After which the digestion flask was moved away from the hot plate and allowed to cool to room temperature. Thereafter 10ml tri_acid mixture (HNO_3-H_2SO_4-$HClO_4$) was added to the cooled digestion flask and swirled; then, the digestion flask and its contents were heated again. With respect to the latter heat treatment of the digestion flask and its contents that was again carried out using a thermostatically controlled hot plate, the temperature setting was gradually increased to 200^0C. Subsequently, heating at this temperature was continued for a further 1 hour during which a clear solution was obtained.

The digestion flask and its contents were allowed to cool to room temperature, and subsequently, the content of the digestion flask was transferred to a 100 ml volumetric flask and made up to the 100ml mark with deionized distilled water. During the addition of the deionized distilled water, intermittently, the 100 ml volumetric flask was shaken to ensure proper mixing of its contents. Reagents

blank was prepared for each batch of digests. The preparation of the reagent blank was carried out in accordance with the procedures used for samples digestion and subsequent volume make-up.

2.3 Measurements

The quantifications of Cu, Cd, Co, Zn, Fe, Ca, Cr and Pb contents of the various diluted samples digests were carried out using atomic absorption spectrophotometer (Buck scientific model 210 VGP). The equipment was operated in accordance with the instructions provided by its manufacturer. The analysis of the examined mineral elements in the various diluted samples digests preceded operations, wherein series of suitable standards of each of the examined mineral elements were investigated, using atomic absorption spectrophotometer that was thereafter, used in examining the various diluted samples digests. Values obtained from the respective standards investigations for the individual mineral elements examined, were used to produce the corresponding calibration curves.

2.4 Quality control

Chemicals: All chemicals used were of analytical grade.

Measurements: Replicate measurements of at least three determinations were carried out.

Limit of detection: The determination of the limit of detection (LOD) was carried out in accordance with the method described by [13]. This entailed digestion of replicate blanks in a manner similar to that used for the various milk samples. Thereafter, the levels of the examined mineral elements in the blanks digest were investigated. The same atomic absorption spectrophotometer (Buck scientific model 210 VGP) was used for the examination of Ca, Fe, Zn, Cu, Co, Cr, Cd and Pb in both the blanks and samples digests. Similar running conditions with respect to equipment operations were maintained in all cases. The values of the limit of detection for the individual examined mineral elements in the study were recorded.

In accordance with the method described by [13], the percentage recovery for the individual examined mineral element was obtained, by spiking known concentrations of standard solution of the respective examined mineral elements in the studied milk samples. Subsequently, measurements were made both for the spiked and the non-spiked milk samples, in line with the procedures described in this work, for the determinations of the various examined mineral elements. This was done so as to calculate the percentage recovery for the examined mineral elements in the studied milk samples. Equation 1 was used to calculate the percentage recovery.

Triplicate determinations were carried out and the average of the replicate measurements was used as the final concentration. Calculation of the percentage recovery in the spiked milk samples was done in accordance with the equation given by [13] as stated below:

$$\%\ Recovery = \frac{conc.\ in\ spiked\ milk - conc.\ in\ non\ spiked\ milk \times 100}{spiked\ amount} \quad (1)$$

2.5 Estimation of daily intake of the examined heavy metals through milk consumption

Primary data were used to calculate the estimated daily intake (EDI) values of the examined heavy metals through the consumption of milk. The calculations were based on the adaptation of the recommended procedure by [14]. Data collection and subsequent calculation of the ,p]amount of daily consumption of milk for different age groups were in accordance with description of [13]. This entailed the use of a food frequency questionnaire for male and female, in which five age groups used in calculating the estimated daily intake values were specified.

The calculation of the estimated daily intake (EDI) values of the examined heavy metals through the consumption of milk was in accordance with the formula of [13] as shown in equation 2:

$$EDI = \frac{milk\ intake\ \left(\frac{kg}{day}\right) \times Heavy\ metal\ content\ in\ milk\ \left(\frac{ug}{kg}\right)}{average\ individual\ weight\ (kg)} \quad (2)$$

2.6 Data analysis

Data generated in this work were statistically analysed, using both arithmetic means and standard deviation, which represent aspect of descriptive statistical evaluation of data. Also, relation between examined variables in this study were statistically evaluated, using one-way analysis of variance (ANOVA). Confidence interval was set at $p<0.05$. International Business Machine (IBM) Statistical package for Social Sciences (SPSS) was used in statistical evaluation of data.

3.0 RESULTS AND DISCUSSION

The results of the work done with respect to the examined mineral elements viz: Ca, Fe, Zn, Cu, Co, Cr, Cd, and Pb in the respective goat milk samples collected on weekly basis over a period of four weeks from two animal markets in Benin City, are presented in Table 1. The two animal markets from where the various goat milk samples were collected are Eyan animal market and Technical College Road animal market. Cow milk samples were also obtained from the same location as reported above for goat

milk samples collection. Again, the same periodic pattern of sample collection was maintained. The levels of the examined mineral elements viz: Ca, Fe, Zn, Cu, Co, Cr, Cd and Pb in the various cow milk samples studied are presented in Table 2 below. Statistical analysis indicates statistical significant differences ($p<0.05$) with respect to the weekly reported values of the respective examined mineral elements, as well as between the respective examined mineral elements in the milk of the two studied animal species. There was however no significant difference ($p<0.05$) between values obtained for the respective examined mineral elements in the milk samples collected from the two studied locations.

Results of the estimated daily intake of the examined heavy metals among goat milk consumers are presented in Table 3 below. In Table 4 also below, the results of estimated daily intake of the examined heavy metals among cow milk consumers are presented.

It is discernible from the results presented in Table 1, that the examined nutritional and toxicologically relevant mineral elements occurred in all the goat milk samples studied. Calcium with a reported mean total of 127.802mg/kg has the highest level of occurrence among the examined mineral elements. In descending order, Zn with a mean total of 3.062mg/kg was next in abundance to Ca. The mean total for other examined mineral elements were: Fe (0.628mg/kg), Cu (0.078mg/kg), Co (0.073mg/kg), Cd (0.036mg/kg), Cr (0.025mg/kg) and Pb (0.005mg/kg). Assuming bioavailability, goat milk will furnish its consumers with the examined mineral elements.

It is further discernible from the results presented in Table 1, that on weekly basis, the respective values obtained for the examined mineral elements varied. The observed variations were statistically significant ($P < 0.05$) and were noticed in the milk samples collected from both sampling locations. The remarked variations in the weekly reported values of the respective examined minerals elements however, tended not to follow a definite pattern. Additionally, results presented in Table 1, revealed that the mean values obtained for Ca, Fe, Zn, Cu, Co and Cr in goat milk samples collected from Eyan animal market, were higher than the corresponding values for the same elements in goat milk samples collected from Technical college road animal market. Differences in the feeding habits of the lactating goats even if partly, accounted for this observation. Also, if differences exist in the compositional chemistry of the feed and the water the animals consumed in the different locations, then, there should be variation in the quality indices of the milk samples collected from the different locations. It is imperative to mention that Technical college road animal market is located in ancient residential area in Benin City. Here, the foliage available to the goats which are normally localised in the market area, are grown on over exploited soil, that has over the years, suffered significant erosion of the top soil portions. It does appear that the nutritional value of the foliage grown on this soil have been adversely affected by the soil condition. On the other hand, Eyan animal market is located in the developing part of Benin City, with nearby forest areas from where probably, foliage richer in the examined mineral elements were obtained. Mean value of 0.005mg/kg was obtained for Pb in milk samples collected from both sampling locations. With respect to Cd determinations, the mean value of 0.035mg/kg obtained for Cd in goat milk samples collected from Eyan animal market, was lower than the corresponding value of 0.036mg/kg obtained as Cd value in goat milk samples collected from Technical college road animal market. This observation is ascribed to contaminations arising from run offs of domestic wastes, agricultural wastes and other environmental factors.

Table 1: Selected Mineral Elements (mg/kg) Levels in Goat Milk Samples Collected from Two Animal Markets in Benin City- A Four Week Study Period

S/N	Mineral Elements	Goat Milk Samples (Location and Period)												
		Eyan Animal Market						Technical College Road Animal Market						
		WK 1	WK 2	WK 3	WK 4	Mean	Range	WK 1	WK 2	WK 3	WK 4	Mean	Range	Mean Total
1	Ca	136.118±18.451	128.704±4.565	125.611±12.194	131.862±7.019	130.574	125.611-136.118	118.964±17.341	126.357±5.118	130.081±12.622	124.718±9.394	125.030	118.964-130.081	127.802
2	Fe	0.658±0.162	0.596±0.121	1.574±0.091	0.762±0.078	0.648	0.574-0.762	0.429±0.116	0.683±0.214	0.573±0.089	0.742±0.266	0.607	0.429-0.742	0.628
3	Zn	3.113±0.526	2.774±0.851	3.515±0.286	2.955±1.028	3.0089	2.774-3515	3.291±0.775	3.108±1.002	2.964±0.095	2.775±0.418	3.035	2.775-3.291	3.062
4	Cu	1.010±0.038	0.079±0.014	0.063±0.048	0.092±0.026	0.084	0.0631-0.102	0.091±0.018	0.086±0.005	0.043±0.12	0.064±0.009	0.071	0.043-0.091	0.078
5	Co	0.056±0.018	0.0079±0.025	0.093±0.031	0.084±0.022	0.078	0.056-0.093	0.064±0.012	0.071±0.014	0.049±0.022	0.088±0.030	0.068	0.049-0.088	0.073

S/N	Mineral Elements	Goat Milk Samples (Location and Period)												
		Eyan Animal Market						Technical College Road Animal Market						
		WK 1	WK 2	WK 3	WK 4	Mean	Range	WK 1	WK 2	WK 3	WK 4	Mean	Range	Mean Total
6	Cr	0.022± 0.002	0.038± 0.001	0.019± 0.005	0.029± 0.003	0.027	0.019-0.038	0.018± 0.005	0.025± 0.008	0.016± 0.003	0.028± 0.006	0.022	0.016-0.028	0.025
7	Cd	0.038± 0.004	0.027± 0.001	0.044± 0.002	0.031± 0.001	0.035	0.027-0.044	0.051± 0.018	0.024± 0.006	0.0029±0.003	0.038± 0.001	0.036	0.024-0.051	0.036
8	Pb	0.006± 0.003	0.04± 0.001	0.004± 0.002	0.005± 0.001	0.005	0.004-0.006	0.005± 0.002	0.003± 0.001	0.004± 0.002	0.006± 0.004	0.005	0.003-0.006	0.005

Table 2: Selected Mineral Elements (mg/kg) Levels in Cow Milk Samples Collected from Two Animal Markets in Benin City- A Four Week Study Period

S/N	Mineral Elements	Cow Milk Samples (Location and Period)												
		Eyan Animal Market						Technical College Road Animal Market						
		WK 1	WK 2	WK 3	WK 4	Mean	Range	WK 1	WK 2	WK 3	WK 4	Mean	Range	Mean Total
1	Ca	117.488 ± 15.136	125.171 ± 8.192	104.951 ± 26.438	122.718 ± 11.554	117.582	104.951 - 125.171	126.811 ± 17.314	121.572 ± 6.114	119.135 ± 10.466	124.783 ± 21.911	123.075	119.135 - 126.811	120.329
2	Fe	0.576± 0.150	0.628± 0.094	0.519± 0.047	0.472± 0.136	0.549	0.472-0.628	0.381± 0.079	0.577± 0.048	0.651± 0.108	0.426± 0.127	0.509	0.381-0.651	0.529
3	Zn	2.941± 0.863	2.753± 0.116	3.118± 0.537	2.988± 0.742	2.950	2.753-3.118	2.651± 0.411	2.846± 0.215	2.394± 0.962	2.507± 0.098	2.600	2.394-2.846	2.775
4	Cu	0.095± 0.016	0.128± 0.051	0.109± 0.023	0.086± 0.044	0.105	0.086-0.128	0.104± 0.012	0.073± 0.026	0.124± 0.083	0.102± 0.046	0.101	0.073-0.124	0.103
5	Co	0.082± 0.014	0.069± 0.021	0.105± 0.096	0.093± 0.018	0.087	0.069-0.105	0.116± 0.052	0.091± 0.024	0.078± 0.036	0.102± 0.019	0.97	0.078-0.116	0.092
6	Cr	0.058± 0.013	0.021± 0.001	0.047± 0.006	0.066± 0.009	0.048	0.021-0.066	0.064± 0.008	0.039± 0.011	0.051± 0.018	0.046± 0.003	0.050	0.039-0.064	0.049
7	Cd	0.042± 0.008	0.069± 0.002	0.025± 0.001	0.061± 0.005	0.049	0.025-0.069	0.035± 0.006	0.052± 0.04	0.031± 0.009	0.061± 0.003	0.045	0.031-0.061	0.047
8	Pb	0.016± 0.008	0.022± 0.003	0.011± 0.006	0.018± 0.001	0.017	0.011-0.022	0.019± 0.003	0.011± 0.005	0.015± 0.007	0.010± 0.002	0.014	0.010-0.019	0.016

Table 2 above indicated that Ca, Fe, Zn, Cu, Co, Cr, Cd and Pb were present in all the cow milk samples studied in this work. With respect to the studied cow milk samples, the highest occurring among the examined mineral elements was Ca, with a mean total of 120.329mg/kg. Next to Ca in terms of abundance of the examined mineral elements was Zn, with a mean total of 2.775mg/kg. Regarding the other examined mineral elements in cow milk and arranged in decreasing order of abundance, their mean total values were Fe (0.529mg/kg), Cu (0.103mg/kg), Co (0.092mg/kg), Cr (0.049mg/kg), Cd (0.047mg/kg) and Pb (0.016mg/kg).

It is further discernible from Table 2, that with respect to the weekly reported values of the individual examined mineral elements in the studied cow milk samples, variation occurred, which as it was with the studied goat milk, was statistically significant ($P< 0.05$). The remarked variations when holistically compared vis-à-vis the respective values for all the examined mineral elements, were noted not to follow a definite pattern.

The non-conformity of the weekly reported values of the examined mineral elements in both the studied goat and cow milk samples to a definite pattern, can even if partly, be adduced to market forces (turnover rates) with respect to the supplied and sales of these animals in the respective sampled markets in Benin City. It is imperative to mention that the goats and cows are brought from different parts of northern Nigeria to the markets in Benin City. Thus, there is continuous change in the makeup of the animals found in the markets in Benin City, due to continuous sales and restocking. Therefore, the carryover influences arising from the respective places of origin of the animals and the effects of their stay in Benin City, depending on the period of their stay, appeared to be determinant factors in the overall results obtained; as well as affecting the pattern of the results reported in this work.

Findings from this study also indicated that with respect to the values obtained for the examined mineral elements in the studied cow milk samples, the mean values obtained for Fe, Zn, Cu, Cd and Pb in the samples collected from Eyan animal market were higher than the corresponding values obtained for the same elements in cow milk samples collected from Technical College Road animal market. On the other hand, the values obtained for Ca, Co and Cr in cow milk samples collected from Eyan animals were lower than the corresponding values obtained

for the same mineral elements, in cow milk samples collected from Technical College Road animal market. Again, the feeding habits of the cows are considered as factors responsible for the pattern of the results obtained.

The apparent difference in the pattern of results reported for the examined minerals in the studied goat milk samples vis-à-vis the sampling locations and those of the cow milk samples with respect to the same sampling location, is ascribed to the fact that unlike goats that are usually localized in the market area, the cows are allowed to move to areas outside the market environment. The occurrence levels of the examined mineral elements in the foliage they fed on in the various areas they are reared, as well as the quality of water they drank, appeared to have contributed to the overall observed pattern of results reported in this work. Additionally, on mass-to-mass bases, cows should consume more foliages than goats. Also, on liter-to-liter bases, cows should consume more water than goats. The amount of the examined mineral elements consumed as well as the quantity utilized by the difference animal species in various biochemical processes, could also be determinant factors in their levels present in the studied milk samples.

The occurrence of the examined mineral elements and their levels of occurrence in the studied goat and cow milk samples are further ascribed to varying factors such as: feed, water, environmental conditions the lactating animals were exposed to and drugs administered to the lactating animals, from which the milk samples were obtained. Time of administration of such drugs before sample collection, as well as the quantity and type of drug administered, could also be relevant factors affecting the results obtained in this study. Furthermore, the health status of the referenced animals, as well as their species could have even if partly, contributed to the overall results reported in this study. Worthy of note is that results indicate that the mean total values for Ca, Fe and Zn in the studied goat milk were higher than those of the same examined mineral elements in the cow milk samples investigated. On the other hand, the mean total values reported for Cu, Co, Cr, Cd and Pb in the studied cow milk samples were higher than the corresponding values obtained for the same examined mineral elements in the investigated goat milk samples. Causative factors attributable to this include animal type, breed, health status, the type and quantity of drugs administered to the animals, as well as the time the drugs were administered. Additionally, the feeding habits of the respective animal types could also have contributed to this observation.

It is nutritionally desirable that mineral elements such as Ca, Zn, Fe, Cu, Co and Cr occurred in goat and cow milk samples obtained in Benin City. This obviously, is an indication that consumptions of such milk types could help meet the body requirements for these dietary relevant mineral elements, assuming high bioavailability. Dibie and Ukhun in [6] posited that although abundance is important, nutritionally speaking, bioavailability is also relevant, as the absorption or metabolic utilization of some mineral elements can be interfered by the presence of some other chemical substance in the food. Significantly, [15] posited that phytic acid, gossypol, oxalic acid and glycosinolates dietary fibres, are among the antimineral substances. Additionally, [16] earlier, opined that oxalates, phylates and polyphenols or tannins, when present in excessive amounts, may interfere with the absorption of iron.

The mineral element copper as noted by [16], facilitates the absorption of iron. Also, some other authors viz: [17], then [18] remarked that Cu is needed for skin and blood vessels strength, in addition to being essential for the production of myelin, hemoglobin and normal functioning of the enzyme systems. Nonetheless, [19] and [20] cautioned that excessive intake of Cu may lead to immunity disorders, dermatitis, impaired nervous system, as well as gastrointestinal and neurological problems. In this work, findings indicate that the mean value of Cu in the studied goat milk samples was 0.078mg/kg (Table 1) with a range of 0.043-0.102mg/kg. For the studied cow milk samples, the obtained mean value for Cu was 0.103mg/kg (Table 2) and the range was 0.073-0.128mg/kg. Institutions and individuals have variously, proposed limits for Cu in milk. Significantly, IDF in [21] proposed maximum limit of 0.01µg/g for Cu in milk. On the other hand, [22] proposed the range of 0.1-0.9µg/g for Cu in milk. Comparing the respective values obtained for Cu in the various goat and cow milk samples studied in this work with the range proposed by [22], they were either below the range or within the range. This is both nutritionally and toxicologically interesting. It is important to mention however, that the respective mean values obtained for Cu in the goat and cow milk samples studied in this work were higher than the 0.068µg/g reported for milk by [13]. On the other hand, the value of 1.451µg/g reported for Cu in milk samples obtained in Egypt by [23] and 0.914µg/g reported by [24] for Cu in milk samples obtained in Croatia, are higher than the values reported in this study.

Cobalt as reported in this work occurred in the investigated goat and cow milk samples. It is discernible from results obtained, that the mean Co level in the studied goat milk samples was 0.073mg/kg (Table I) and the range was 0.049-0.093mg/kg. Findings also indicate that in the studied cow milk samples, mean value for Co was 0.092mg/kg, the range of occurrence was 0.069-0.116mg/kg. Cobalt level as reported by [25] in milk samples obtained from India, was 0.19µg/g. Also, some

other workers viz: [26] reported that mean Co level in milk samples obtained from Korea was 0.006µg/g.

Furthermore, in Spain, [27] noted that the mean Co level in the milk samples they investigated was 0.005µg/g. In this work, the respective values of Co level in both the goat and cow milk samples investigated, as well as the corresponding mean levels of Co obtained, were lower than what [25] reported for milk samples obtained from India; but higher than the values reported by [26] for milk samples obtained from Korea; as well as that reported for milk samples obtained from Spain by [27]. Cobalt is one of the micro-nutrient mineral elements. Significantly, it is a constituent of vitamin B12.

However, [28] posited that when cobalt is present in excess, it could disturb the reproductive system and the thyroid glands. Also, according to [29] classification, Co is mentioned as a possible carcinogenic substance. Therefore, the occurrence of Co in goat and cow milk would be nutritionally desirable to their consumers, to the extent that contributions from other dietary sources do not cause Co level in their body to exceed the threshold limit.

There is great concern that the examined toxicologically relevant Cd and Pb occurred in all the studied goat and cow milk samples. The implication of this is that marketed goat and cow milk in Benin City, appeared relevant sources of consumers exposure to these toxicologically relevant mineral elements. Swarup *et al.* in [30] posited that Pb is one of the most toxic heavy metals. With respect to Cd, [31] opined that Cd toxicity in humans may lead to kidney failure, as well as to liver and skeletal disorders. Therefore, there is need to put in place more stringent measures that would help check further increase in the level of these toxic metals, especially with respect to the feed and water goats and cows in Benin City are fed with.

In earlier work done by [32], they posited that heavy metals (cadmium, chromium, lead and nickel) concentrations in milk obtained from Pakistan were higher than the concentrations considered suitable for human consumption. Also, Malhat *et al.* in [23] reported lead value of 4.404µg/g and cadmium value of 0.288µ/g in cow milk found in Egypt. In this work, the mean total levels reported for lead and cadmium in cow milk were 0.016mg/kg and 0.047mg/kg respectively. It is imperative to note that among the various weekly values reported for lead and cadmium in this work, no level as high as the levels reported by [23] was recorded. The observed differences in results of both works are ascribed to variations in the levels of urbanization, industrialization, feed type and drugs that cows reared in both regions were exposed to. Another literature report of Pb level in milk samples obtained in Egypt was by [33]. According to these authors, the milk samples they investigated had Pb mean level of 0.327µg/g. Again, the mean total value of 0.016mg/kg reported for Pb in milk samples investigated in this work is lower. When the value of 0.327µg/g reported for milk samples in Egypt by [33] is compared with the reported Pb value of 4.404µg/g in milk samples also obtained in Egypt by [23], the respective values are different, with that of [23] that was a more recent study, higher. This observation is in accord with the remark of [30] that the levels of Pb in milk and milk products, is increasing day by day. Of concern is that if the trend is left unchecked, lead toxicity especially the ones arising from milk consumption could be on the increase. Holistic comparison of the works of [33] and [23] would also require consideration of the regions of Egypt samples were collected from, as well as the season of sampling and the health status of the lactating cows that provided the milk samples.

It is further discernible from the work of [23] that the level of lead was higher than cadmium level in the cow milk samples they worked on. The reverse was the case in this study. The primary levels of individual metals in the materials, the cows consumed, as well as the weather conditions the various cows were exposed to, could be respond for this. It would appear therefore, that by controlling the mineral elements levels in the materials cows are exposed to, safer levels of mineral elements would be found in cow milk.

In goat milk samples from different districts of Punjab province, [13] reported that Pb levels occurred within the range of 0.007µg/g and 0.021µg/g (mean = 0.013µg/g). In the same studied locations with respect to cow milk, [13] reported that Pb occurred in the range of 0.014µg/g to 0.033µg/g (mean = 0.022µg/g). In the present study, the range of values of Pb in the studied goat milk samples was 0.003mg/kg to 0.006mg/kg (mean = 0.005mg/kg). For cow milk samples, Pb occurred in the range of 0.010mg/kg to 0.022mg/kg (mean = 0.016mg/kg). The respective reported mean values of Pb in goat milk and cow milk in this work, are lower than the corresponding values reported by [13] in their work on goat and cow milk from different districts of Punjab province. However, the reported mean level of 0.012µg/g for Pb in cow milk samples obtained from Iran by [34] is lower than the corresponding value we obtained in our work. Additionally, Pb level of 0.004µg/g reported by [26] in milk from Korea is equally lower than our value. Contributory factors to the observed variations in Pb levels with respect to the various studies should include differences in the levels of industrialization, as well as urbanization, particular in the areas the cows are kept and more importantly, controlled measures existing in the various studied locations meant to ensure that the animals fed on less contaminated feeds.

The Codex Alimentarius Commission in [35] maximum permissible limit for Pb in milk is 0.02µg/g. With respect to the results presented in this work, what is deducible is that the levels of Pb in all the studied goat milk samples were within safe limits. For the investigated cow milk samples, the values of Pb were also within safe limit, except for cow milk samples collected from Eyan animal market in week 2 of the studied period. This obviously is worrisome. In particular, it is a strong indication that there is need to ameliorate the levels of Pb both in the environment and in the feeds provided for animals, whose milk or carcass could be consumed by humans.

It is also discernible from this work, that for the investigated goat milk samples, mean Cd level was 0.036mg/kg with a range of 0.024 – 0.051mg/kg (Table 1); then 0.047mg/kg as mean Cd value and a range of 0.025 – 0.069mg/kg for cow milk. Erub *et al.* in [36] reported that in bovine milk samples from Egypt, mean Cd level was 0.086µg/g. Also, [8] reported mean Cd level in milk from Nigeria to be 0.131µg/g. Apparently the mean levels of Cd reported both for goat and cow milk in this work, are lower than the reports of the aforementioned researchers. It is particularly interesting to note the obvious difference between the mean Cd level reported by [8] and the ones reported in this study, even when the researchers investigated milk samples obtained from Nigeria. It should however be emphasized that while [8] worked on milk samples collected from cows grazed around Challawa industrial estate of Kano (northern Nigeria), in this work, the milk samples investigated were collected from animal markets located in Benin City (southern Nigeria), around residential areas. It would appear therefore, that sampling location even within the same country among other factors, could affect the mean Cd level of milk samples.

Interestingly low Cd level of 0.004µg/g was reported by [37] for milk obtained from Poland. Additionally, Bilanžić *et al*. in [24] reported even lower Cd level of 0.003µg/g for milk obtained from Croatia. The reported low values of Cd occurrence in studied milk samples obtained from these countries, is suggestive of very low if any Cd toxicity due to consumption of milk obtained from the aforementioned countries. Cumulative sources of consumers exposure to toxicologically relevant mineral elements are however very important in overall assessment of food risks. Evidently, whether for goat or cow milk the mean Cd levels reported in this work are greater than those reported by [37] and [24] for milk obtained from Poland and Croatia. This should be of concern to the appropriate authorities, if toxicity arising from consumption of Cd contaminated foods is to be kept at a minimum in Benin City, Nigeria.

It is deducible from the reports of several authors [8-37] that even among respective mineral elements examined in this work, variations exist in their specific reported levels of occurrence in milk. Significantly, varying values were obtained for milk samples collected from different regions. The peculiarity of regional practices and conditions, including animal feeding habits, breed, medication and health status; in addition to the environmental conditions the animals were exposed to, it would appear, influenced the various reported values.

Table 3: Estimated Daily Intake (EDI) of The Examined Heavy Metals Among Goat Milk Consumers in Benin City, Based on The Average of Four Weeks Studies.

Age group bracket (years)	Gender (M/F)	No of consumers	Average weight (kg)	Average goat milk intake (kg/day)	Examined Heavy Metals (µg/kg/daily consumption)						
					Fe	Zn	Cu	Co	Cr	Cd	Pb
1-5	Male	24	18.69	0.108	3.629	17.694	0.451	0.422	0.144	0.208	0.0209
	Female	28	16.21	0.093	3.603	17.567	0.448	0.419	0.143	0.207	0.029
6-8	Male	20	31.46	0.148	2.954	14.405	0.367	0.343	0.118	0.169	0.024
	Female	25	26.95	0.126	2.936	14.316	0.365	0.341	0.117	0.168	0.023
9-12	Male	30	45.86	0.115	1.574	7.678	0.196	0.183	0.063	0.90	0.013
	Female	23	41.55	0.098	1.481	7.222	0.184	0.172	0.059	0.085	0.012
13-16	Male	38	54.79	0.149	1.708	8.327	0.212	0.199	0.068	0.098	0.014
	Female	27	52.86	0.135	1.604	7.859	0.199	0.186	0.064	0.092	0.013
17 and above	Male	36	68.93	0.128	1.166	5.686	0.145	0.136	0.046	0.067	0.009
	Female	50	65.18	0.114	1.098	5.355	0.136	0.128	0.044	0.063	0.009

M = Male; F = Female

Table 4: Estimated Daily Intake (EDI) of The Examined Heavy Metals Among Cow Milk Consumers in Benin City, Based on the Average of Four Weeks Studies.

Age group bracket (years)	Gender (M/F)	No of consumers	Average weight (kg)	Average cow milk intake (kg/day)	Examined Heavy Metals (µg/kg/daily consumption)						
					Fe	Zn	Cu	Co	Cr	Cd	Pb
1-5	Male	38	21.85	0.496	12.008	62.993	2.338	2.088	1.112	1.067	0.363
	Female	42	17.34	0.421	12.844	67.375	2.501	2.234	1.190	1.141	0.388
6-8	Male	29	32.19	0.539	8.857	46.466	1.735	1.540	0.820	0.787	0.268
	Female	25	28.64	0.464	8.570	44.958	1.669	1.491	0.794	0.761	0.259
9-12	Male	26	46.73	0.490	8.547	29.298	1.080	0.965	0.514	0.493	0.168
	Female	24	42.55	0.432	5.371	28.174	1.046	0.934	0.497	0.477	0.162
13-16	Male	21	56.09	0.486	4.584	24.044	0.892	0.797	0.425	0.407	0.139
	Female	25	54.86	0.475	4.580	24.027	0.892	0.797	0.424	0.407	0.139
17 and above	Male	68	69.49	0.264	2.010	10.543	0.391	0.350	0.186	0.179	0.061
	Female	75	66.88	0.193	1.527	8.008	0.265	0.265	0.141	0.136	0.046

M = Male; F = Female

The results of the estimated daily intake (EDI) of the examined heavy metals among goat milk consumers in Benin City, based on the average of a four week studies are presented in Table 3 above. Also presented above (Table 4), are the EDI values of the examined heavy metals among cow milk consumers in Benin City, based on the average of a four week studies. It is deducible from findings, that the EDI value for each of the examined heavy metals arising from cow milk consumption, was higher than the corresponding EDI value of the respective examined mineral elements, due to the consumption of goat milk. This is ascribed to the quantity of each species of milk consumed.

With respect to goat milk consumption in Benin City, findings indicate that among the studied age bracket (years), in decreasing order, the EDI values were: Zn > Fe > Cu > Co > Cd > Cr > Pb. Invariably, Zn had the highest EDI value and Pb the lowest EDI value. The EDI values obtained based on cow milk consumption in Benin City with respect to the studied age bracket (years) is herein presented in decreasing order as: Zn > Fe > Cu > Co > Cr > Cd > Pb. Comparatively, similarity exists in the pattern of the EDI values for the examined heavy metals due to goat milk consumption, and that arising from cow milk consumption. In particular, in both of them, Zn had the highest EDI value and Pb the lowest EDI value. The relative abundance of each of the examined heavy metals accounted for the order of results reported above. This explanation accounts for why in goat milk, the EDI value for Cd was higher than that of Cr, but with respect to cow milk, the EDI value for Cr was higher than that of Cd.

Comparing the EDI values for each of the examined heavy metals and the studied age bracket, findings indicate that whether for cow milk or goat milk consumption, the age bracket (1-5 years) had the highest, while the age bracket (17 years and above) had the lowest. This observation is consistent with that of [13] that is, the youngest studied age bracket having the highest EDI values for the examined mineral elements and the eldest studied age bracket having the lowest corresponding EDI values for the respective examined mineral elements. The noted remark is ascribed to the quantity of milk consumed by the various age bracket. It is pertinent to mention however, that while with respect to cow milk consumption, progressive decrease in EDI values was observed with increasing age of members of the studied age bracket (1-5 > 6-8 > 9-12 > 13-16 > 17 and above); on the other hand, it is discernible from Table 3, that the EDI values for the examined heavy metals with respect to goat milk consumption presented a pattern, which showed progressive decrease in EDI values with increasing age of members of the studied age bracket, up to age bracket (years) 9-12 years. Thereafter, for the studied age bracket (years) 13-16 years, the respective EDI value obtained for all the examined mineral elements was higher than the next in terms of younger studied age bracket (years) of 9-12 years. Feeding pattern could be responsible for this. In milk samples obtained from Punjab province, [13] reported the following highest EDI values for the mineral elements they examined: Pb (0.946µg/kg/day), Cd (0.150µg/kg/day), Co (4.330µg/kg/day), Ni (1.884µg/kg/day), and Cu (3.126µg/kg/day). These values were recorded for the youngest age bracket of 1-3 years that was used in their study. In the present study, the EDI values for similar examined mineral elements in the youngest age bracket of 1-5 years studied as results indicate are: Pb (0.029µg/kg/daily consumption), Cd (0.208µg/kg/daily consumption), Co (0.422µg/kg/daily consumption) and Cu (0.451µg/kg/daily consumption) for goat milk consumption. With respect to cow milk consumption, the obtained EDI values for the studied age bracket (years) 1-5 were: Pb (0.363µg/kg/daily consumption), Cd

(1.067µg/kg/daily consumption), Co (2.088µg/kg/daily consumption) and Cu (2.338µg/kg/daily consumption). Obviously, except for the respective EDI values obtained for Cd in both goat and cow milk in this work, which were higher than the corresponding EDI values obtained for the same mineral elements in milk sample of Punjab province by [13], the EDI values obtained for Pb, Co and Cu in both goat and cow milk in this work, were lower than the EDI values for the same mineral elements reported by [13] in their work.

The high EDI value obtained for Cd in the different species of milk samples studied in this work is worrisome. This is particularly so, considering the toxic nature of Cd. The WHO as noted by [38] suggests that 50µg daily is a maximum tolerable intake for an adult, so that ordinary diets do not present any problem. It is important to consider the fact that other dietary sources could be contributing to the overall daily intake of this highly toxic heavy metal in the feeding habits of persons living in the studied location.

4.0 CONCLUSION

The reported occurrence of the examined dietary relevant mineral elements in goat and cow milk samples found in Benin City, is nutritionally desirable. It is however of concern, that the toxicologically relevant Pb and Cd also occurred in all the studied milk samples. There is need for measures that would help ameliorate the levels of these toxic metals in the environment to be adopted, in order to minimize the exposure of goat and cows in Benin City to contaminated foliage and water. It is hoped that the respective EDI values obtained in this work, will be relevant to consumers in estimating their intake of the examined mineral elements.

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