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Full Length Research Paper

Determination of copper, zinc, lead and some biochemical parameters in fresh cow milk from different locations in Niger State, Nigeria

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Soil and water pollution of milk products by heavy metals is a common fact in Northern Nigeria. In the present study, atomic absorption spectrometry (AAS) was used for the quantitative determination of copper, zinc and lead level while some biochemical constituents analysed in fresh cow milk collected from Dangana, Mukugi and Gulu localities of Niger State, Nigeria were determined using standard methods. The results showed milk from Mukugi with the highest Copper content (0.59 ± 0.01 mg/L) while Gulu samples had the lowest (0.56 ± 0.02 mg/L). Zinc levels were highest (0.40 ± 0.03) mg/L in Gulu milk but lowest (0.25 ± 0.03 mg/L) in Mukugi samples. Lead was detected in milk from the three locations with the highest (0.63 ± 0.24 mg/L) obtained from Dangana area. Cholesterol and total fat levels were highest (15.00 ± 6.27 mg/ml and 4.08 ± 0.10 mg% respectively) in Mukugi area. While a significant ($P<0.05$) difference existed in Cholesterol and fat values, reducing sugars and protein levels were not significantly ($P>0.05$) varied between milk samples from the studied locations. The detection of lead, high cholesterol and fat levels in some samples necessitates further monitoring in other areas and some caution in the rampant consumption of such milk.

Key words: Copper, zinc, lead, biochemical parameters, cow milk.

INTRODUCTION

The world-wide contamination of milk with undesirable substances via animal feeds, heavy metals, mycotoxins, dioxins and similar pollutants is considered to be of great concern to public health due to their toxic effects on humans and wildlife. Recent report according to Semaghiul et al. (2008) indicated that good quality measurements are essential to control and often play a vital role in maintaining products and process quality, both in manufacturing, trade and in research. Milk products are very important human nutrients and their consumption has increased in recent years. It is known as an excellent source of calcium, magnesium and zinc and hence supply very small amount of Fe and Cu. The consumption of cow milk is very popular in Northern part of Nigeria among the Hausa and Fulani because of its medicinal and dietary properties (Drewnowski and Fulgoni, 2008). Consumption of cow milk in particular is associated with beneficial health effects beyond its pure nutritional value. Several reports indicated that dairy products could serve as vehicles for other functional ingredients, such as phytosterols (as cholesterol replacement), fatty acids (as omega-3 acids) and various kinds of probiotic bacteria (Mattila-Sandholm et al., 2002).

Studies equally showed that low-fat milk consumption could reduce risk of arterial hypertension, coronary heart disease, colorectal cancer, and obesity (Agrawal et al., 2003; Magjeed, 2005; Shabo et al., 2005). Despite these essential benefits derived from consuming cow milk, the

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prevalent cases of contaminated milk arising from modern agricultural practices, industrial pollutants in the environment, animal feeds and use of sewage sludge in agriculture is increasing and therefore requires urgent attention (Kira and Maihara, 2007; Semaghiul et al., 2008). For instance, the toxic metal content of milk and dairy products is due to several factors – in particular – environmental conditions. As a result of soil and water pollution by heavy metals that exposes man and grazing animals to health risks, it becomes necessary to determine and monitor the levels of toxic metals in milk, because they can significantly influence the human health. Many studies indicate the presence of heavy metals in milk. Lead, copper and zinc residues in milk are of particular concern because milk is largely consumed by infants and children (Licata et al., 2004; Caggiano et al., 2005; Zheng et al., 2007; Tajkarimi et al., 2008). These heavy metals are responsible for many pernicious effects on human health as saturnisme (lead contamination), immune-depression and skin diseases (zinc and copper contamination) (Llobet et al., 2003).

Apart from these, cholesterol and other biochemical parameters are also important and necessary constituents required by humans. Cholesterol is an important component for the manufacture of bile acids, steroid hormones, and fat-soluble vitamins including vitamin A, D, E, and K (Chilliard and Ferlay, 2004; Shingfield et al., 2008). But, their presence in a high level in milk most especially serum cholesterol is an indicator for diseases such as heart diseases. Therefore, the objective of this study was to determine the level of heavy metals, cholesterol, reducing sugars, protein and fat contents in cow milk collected in three different locations in Niger state, Nigeria.

MATERIALS AND METHODS

Sample collection

Fresh milk samples were collected from ten cows at different locations each namely Dangana, Mukugi, and Gulu of Niger state during the morning milking directly into sterile screw bottles to avoid potential contamination due to metallic containers. Cow nipples were also sterilized with cotton diluted with ethanol prior to milking. The samples were then transported to the laboratory and immediately analysed for the heavy metals and biochemical parameters. Three replicate determinations were carried out on each sample.

Determination of heavy metals

Microwave system was used for acid digestion of all the samples. Samples were dried at 70°C in a forced stove until dry weight was obtained. 0.3 g of the sample was measured into a clean 250 ml dry Pyrex digestion flask. 10 ml of 65% nitric acid was added, followed by the addition of 3.0 ml of 30% hydrogen peroxide. The digestion flask was heated gently until frothing subsided. The sample was then heated to dryness, dissolved in 30 ml deionized and filter with No. 42 Whatman filter paper. The solution was made up to volume in a 100 ml flask and stored in a special container ready for analysis (AOAC, 2000).

Sample analysis of heavy metal content

Calibration curves were prepared for the three metals using absorbance at 283.3 nm (Pb), 324.8 nm (Cu) and 213.9 nm (Zn) from "atomic absorption spectrophotometer" (Model 9190 Pye Unicam, U.K). The actual concentration for each metal in the samples was hence obtained by extrapolation from the standard curves (AOAC, 2000).

Determination of biochemical parameters

Cholesterol, reducing sugars, protein and fat content were determined according to the method described by Association of Official Analytical Chemists (AOAC, 2000) and Amadi et al. (2004).

Cholesterol

Cholesterol was determined using a modified form of Liebermanns-Burchard's Method (Amadi et al., 2004). This method is based on the dehydration of cholesterol followed by coupling of two such molecules to yield biocholestadiene in the presence of chloroform, acetic acid and acetic anhydride absorbs at 650 nm. A blank of chloroform and ilca's reagent (1:4) was used.

Reducing sugars

The dinitrosalicylic acid (DNS) method was used (Amadi et al., 2004). This involves sample oxidation with 1.5 M tetraoxosulphate (vi) acid neutralization with 10% Sodium hydroxide and the addition of 3,5-dinitrosalicylic acid (DNS) with absorbance being taken at 540 nm.

Proteins

Total proteins were determined by the Micro-kjeldahl method which is based on quantification of nitrogen. A factor of 6.38 was used to obtain the final values since the samples were milk (AOAC, 2000).

Fats

This was done mainly by the gravimetric method (AOAC, 2000) 10 g milk sample was weighed into a Mojonnier extraction tube. The lipid contained in the sample was exhaustively extracted using petroleum ether (40 to 60°C) for 3 h. The extractant (petroleum ether) was distilled off and the flask reweighed. The percentage lipid was then calculated.

Statistical analysis

The statistical analysis was done using ANOVA. There were no significant difference in the concentration of copper and zinc in the investigated samples, $P>0.05$. The values are mean±standard error

Table 1. Heavy metal concentration in fresh milk from three different locations in Niger State.

Location	Lead mg/L	Copper mg/L	Zinc mg/L
A	0.63 ± 0.24	0.57 ± 0.01	0.35 ± 0.18
B	0.21 ± 0.13	0.59 ± 0.01	0.25 ± 0.03
C	0.16 ± 0.13	0.56 ± 0.02	0.40 ± 0.03

n = number of samples = 10. A = Dangana; B = Mukugi; C = Gu.

Table 2. Cholesterol, reducing sugar, protein and fat content in fresh milk from three different locations in Niger state.

Location	Cholesterol (mg/ml)	Reducing sugar (mg/ml)	Protein (%)	Fat (%)
A	14.00 ± 5.71	1.03 ± 0.15	3.14 ± 0.38	3.70 ± 0.08
B	15.00 ± 6.27	1.15 ± 0.07	2.76 ±0.42	4.08 ± 0.10
C	8.00 ± 1.27	1.01 ± 0.13	2.34 ± 0.26	3.55 ± 0.13

n = number of sample = 10. A = Dangana; B = Mukugi; C = Gulu.

of mean of four replicates.

RESULTS

Table 1 represents the results for the heavy metal concentration in fresh milk from three different locations in Niger state. The mean concentration of lead, copper and zinc in the milk sample collected from Dangana village were 0.63 ± 0.24 ,0.57 ± 0.01 and 0.35 ± 0.18 mg/L while the mean concentration of lead, copper and zinc in Mukugi were as follow 0.21 ± 0.13, 0.59 ± 0.01 and 0.25 ± 0.03 mg/L. Finally the mean concentration of lead, copper and Zinc in Gulu were in the following order 0.16 ± 0.13, 0.56 ± 0.02, 0.40 ± 0.03 mg/L. Milk from Dangana contained the level of lead while there were no significant difference in the concentration of copper and zinc in the sample collected from Mukugi and Gulu. The results obtained from the biochemical parameters determined are presented in Table 2 and it showed that the cholesterol value was high in the milk sample collected from Dangana and Mukugi.

DISCUSSION

Routine heavy metal analyses and other biochemical parameters in dairy products are presently essential. Cow milk either fresh or fermented is a common dietary component of communities in northern Nigeria. There is an increased incidence of different diseases among the rural populace. Such problems could be associated with their dietary habits among which could be high fat intake. Also soil and water pollution by heavy metals exposes man and grazing animals to health risks. Heavy metals are widely dispersed in the environment (Coni et al., 1999). This is because of increased environmental pollution and risk factors associated with heavy metal exposure and toxicosis. Among the heavy metals studied, copper for instance is needed for proteins involved in growth, nerve function and energy release (IM, 2001). It is vital for the formation of some important proteins. It is a critical functional component of a number of essential enzymes, known as cuproenzymes. Copper is stored in appreciable amounts in the liver. It also has anti-oxidant properties and involved in the regulation of gene expression. The mean Cu concentration in the three locations are in line with those reported by other authors that had independently worked on cow milk (Tripathi et al., 1999; Kira and Maihara, 2007; Kondyli et al., 2007). There is no significant difference as the value obtained are closely related irrespective of sample location at P>0.05. These values obtained are however, higher than those reported by Semaghiul et al. (2008).

Previous studies showed that cow milk from rural areas often contained copper concentration less than 0.39 mg/L (Licata et al., 2004). However, the plausible reason for this may be due to the proximity to industrial and traffic areas which increases significantly the copper concentration in cow milk. Zinc is such a critical element in human health of which, a minor deficiency is detrimental to health. Zinc deficiency is characterized by growth retardation, loss of appetite and impaired immune function. Reports have shown that zinc deficiency in most cases causes hair loss, diarrhoea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions (Ryan-Harshman and Aldoori, 2005). Based on this fundamental fact, the mean Zn concentration in cow milk collected from Dangana was 0.35 mg/L and the Zn concentrations in the Mukugi cow milk samples was 0.25 mg/L. While cow milk collected from Gulu had the highest level of Zinc (0.40 mg/L). These concentrations were lower than those reported for goat milk 32.10 mg/L (Park, 2000). The mean Zn concentration in the analyzed fresh cow milk samples in the three locations were also lower than those reported in raw bovine milk (0.29 to 4.96 mg/L) (Licata et al., 2004). Lead was detected in milk samples from the three locations. The highest value was obtained from Dangana.

Lead concentration in the three locations ranged from 0.16 to 0.63 mg/L which is higher than those reported in literature (Caggiano et al., 2005; Tajkarimi et al., 2008) and lower than those reported by Licata et al. (2004). However, it is of particular interest to note the presence of lead (Pb) in Dangana and probably Mukugi and Gulu samples. The obtained values were not within the permissible concentrations and this could arise from run offs emanating from petroleum product pollution by heavy duty trucks and related sources. Lead is very toxic. It is a potent neurotoxin and has a cumulative effect on vital organs (Licata et al., 2004). The obtained results show how this metal is ever more frequently found in milk samples in regions with rural agrarian settings without industrial activity. The presence of Pb in milk samples from such areas could also be due to other factors such as transhumance along roads and/or motorways, fodder contamination, climatic factors, such as winds, and the use of pesticide compounds. One of the most important sources of lead contamination in milk is water, especially in more contaminated areas (Codex, 2003) so water testing should be one of the important topics for future study. Therefore, it is necessary to monitor this metal over time for better clarification of its presence in milk from the studied areas.

Fat content in fresh cow milk varied only slightly from the three locations. The values obtained were within permisible range (John et al., 2007). Milk is sold on the basis of fat content for example butter fat. Fat is a source of high energy for humans. Excessive intake of milk with high fat values are however not desirable. Skimmed milk is preferred alternative (Olson, 1998). Fresh milk cholesterol level was highest in Mukugi and lowest in Gulu samples. Elevated plasma cholesterol levels are a major factor in promoting atherosclerosis (Park, 2000). Atherosclerosis is characterized by deposition of cholesterol and cholesterol esters from plasma lipoprotein into the artery wall (Toso et al., 2002). Atherosclerosis predisposes to coronary heart disease, a major cause of death among most populace worldwide (John et al., 2007). Although no epidemiological survey exists in the three communities from where the milk samples were obtained, the Fulani milk sellers usually awkward the products in distant communities. Most unexplained deaths among the rural and sub urban populace might be traced in part to dietary habits, the long term consumption of such milk which could be a factor. Defatted milk with less cholesterol is hence preferable. Mukugi milk samples contained the highest reducing sugar. Lactose is the major milk sugar; it produces no disease conditions and is well tolerated except in people within born errors of lactose metabolism (Basnet et al., 2010).

Milk collected from Dangana had highest protein content. Although the values were low when compared with the results obtained by Chilliard and Ferlay (2004). Fresh milk is the most important source of protein to infants and individuals that are vegetarians. This protein source contains high levels of essential amino acids and is desirable for consumption (Roy, 2008).

Conclusion

The available evidence from these studies even when compared with most research conducted in Nigeria and Africa in particular suggest that milk obtained from Gulu with low cholesterol, fat and lead concentration would be more preferred for consumption than those of Dangana and Mukugi. This is because high fat and cholesterol intake are predisposing factors to artherosclerosis and Cardiac disease. The detection of lead in milk samples from such rural areas is also noteworthy. This is with a view to excluding such feedstuff as possible contaminants. A mechanism to monitor heavy metals in other food samples from the areas is necessary.

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