DETERMINATION OF UREA AND HEAVY METAL RESIDUES IN RAW MILK AT KALIOBIA GOVERNORATE

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ABSTRACT

Forty samples of raw milk were collected from farm and market at Kaliobia Governorate. The samples were submitted for analysis calorimetrically by using spectrophotometer to determine the levels of added urea concentration and using Atomic Absorption Spectrophotometer to determine the levels of some heavy metals as lead, cadmium, arsenic and mercury. The obtained results revealed that the mean value of urea concentration in farm raw milk samples was 13.61± 1.129 mg/dl, while the average value of market raw milk was 18.57 ± 1.135 mg/dl. From the obtained results we noticed that all the samples got from the farm were within the normal values (< 20 mg/dl) while about 5% of raw milk obtained from the market was above the permissible limit(< 20 mg/dl). So this milk is considered healthy and safe for human consumption.

The mean values of lead, cadmium, arsenic and mercury levels found in farm raw milk were 0.129 ± 0.0144 ppm, 0.044 ± 0.009 ppm, 0.0004± 0.0033 ppm and 0.0095 ± 0.0045 ppm respectively, while the level of them in market raw milk samples were 0.267 ± 0.0484 ppm, 0.055 ± 0.007 ppm, 0.0007 ± 0.0038 ppm and 0.0134 ± 0.0057 ppm. From the obtained results we observed that lead in both farm and market raw milk were above the permissible limits of Egyptian Organization for Standardization and Quality Control 2001.

From obtained results farm and market raw milk urea levels are considered healthy and safe for human consumption referring the normal level of milk urea nitrogen (< 20 mg/dl) and only one sample from market raw milk was excess of 20 mg/dl judged abnormal. On the other hand non of examined samples contained cadmium, arsenic and mercury beyond the maximum permissible limits, while lead level exceed that limits.

Therefore, a regular monitoring of heavy metals and urea levels in raw milk in the governorate is recommended in order to establish the true contribution of milk and milk products to the dietary intake of urea and heavy metals also the repeated estimation of urea and heavy metals in milk must be conducted regularly to recognize its possible health hazards effects, its bioaccumulation during chronic exposure, its mobilization and secretion in milk.

INTRODUCTION

Milk is considered as nearly a complete food in that it's a good source of protein, fat, and major minerals **(Enb, et al., 2009)**.

Raw milk is a common health drink consumed by all age groups. The complements and proteins in milk are ideal in quality and balance to satisfy human amino acid requirement. The increasing adulteration of milk by urea as a nitrogen compound has been very common in developing countries. It was pointed out that urea was commonly added to increase both shelf life and also milk solids non fat (SNF) value or its total nitrogen content. Urea is a normal constituent of milk and comprises part of the non protein nitrogen (NPN) normally found in milk **(Ferguson 2000)**. When urea was added the milk looked thick and concentrated giving a feeling of rich milk while, actually it was law in fat and SNF and was poisonous due to presence of excess urea **(FAD 2005 and Renny et al., 2005)**.

A recent Indian council of medical research (ICMR) suggested that urea adulterated item has a cancerous effect on human system and can lead to gradual impairment of body functions **(Dean 1985 and Fox 1992)**.

Cow milk contains some major elements such as calcium, potassium, phosphorous, and magnesium in addition to sodium, chloride and a wide range of micro elements and even heavy metals. Increase in industrial and agricultural processes have resulted in increased concentration of metals in the air, water and soil. These metals are taken by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters also accumulate such metals in their tissues and secreted milk **(Yahaya, et al., 2010)**. This ever increasing pollution has given rise to concern on the intake of harmful metals in humans. Metals enter the human body through inhalation, ingestion or absorption through the skin **(Ogabiela, et al., 2010 and Ahmed, 2002)**.

In recent times, the amount of metals in cow milk is widely studied, particularly in industrialized and polluted areas of the developed and the non developing countries of the world since animals grazed freely on open fields are considered as bio-indicators of environmental pollution **(Korenekovg, et al., 2002 and Li et al., 2009)**. The toxicity of metals depends on a number of factors: the particular metal in question, dose absorbed and the age of the person concern. For instance, children are vulnerable to the effect of lead exposure because they absorb several times the percentage ingested compared to adults and because their brain is plastic and even brief exposure may influence developmental processes **(Samara and Richard, 2009)**. Lead, cadmium and mercury residue in milk are therefore of great concern because milk is largely consumed by infants and children. Food safety is issue that will remain at the fore front of congressional and consumer group agendas. The presence of pollutants facilitated their entry into the food chain and they increasing the possibility of those having toxic effects on human and animals. How ever, agricultural activities as the use of fertilizers and the irrigation of fields can also be important source of environment contamination food chain and eventually of food products consumed by humans **(Casarett and**

Doull 2000; Ajmal et al., 2003, and Anwaar and Hafez 2006).

Lead, cadmium, arsenic, mercury are considered the most important hazardous pollutants in our environment and are distributed widely in all classes of natural foods of animal origin specially milk and milk products **(Marine and Ayele 2002)**. Direct metal contaminated may occur at several stages during dairy processing e.g plant equipments .catering operations, ceramic, enameled utensils, metals containers, and water used for dairy production **(Reilly 1991)**. Lead is considered one of the most important pollutants in environment transport and distribution of lead from stationary or mobile source mainly via air although large amounts points of such discharge **(WHO 1977)**. Lead is an accumulative poison it has a continuous effect on haemobiotic system long term exposures of lead reduces function of kidney liver also cause encephalopathy resulting in ataxia, coma and convulsions **(Ukhan et al., 1990; Carl 1991 Shehata and Nagah 1992)**. Cadmium is well recognized as one of major toxic elements even low levels cause in accumulation in tissues which toxicity manifested by a variety of syndromes and effects including renal dysfunction **(Robards and Worsfold 1991)** hypertensions **(Piperakia 1985)** moreover it may induce prostate cancer bone changes and slight anemia **(WHO 1980)**. The main source of arsenic toxicity is drinking water contaminated by natural geological sources. Current risk assessment spaced on the recognized carcinogenicity of arsenic, but neurotoxic risks have been overlooked **(Dakeishi et al., 2006)**. The more common sources of arsenic have been the various pesticide compounds used on crops and live stock and preparations used as herbicides, together with containers for these materials that have not been probably destroyed. **(Clark and Clark, 1975)**. Mercury is a toxic and hazardous metal that occurs naturally in earth's crust. Through consumptions of mercury in food, the populations of many areas, particularly in the developing world, have been confronted with catastrophic outbreaks of mercury-induced diseases and mortality **(Tchounwou et al., 2003)**.

Therefore the present work was planned to detect quantitatively any additions of urea to fresh raw milk and some heavy metal residues in farm and market milk to ensure the availability of milk for consumption.

Material and Methods

Collection of samples :

A total 40 samples of farm bulk milk (20) and market raw milk (20) the farm samples were collected weekly for 10 weeks (2 samples/week) from private farms in Kaleubia Governorate, market raw milk sample were collected from different shopping sites, vendors and groceries samples ware labeled and kept in an ice box then transferred to laboratory for chemical analysis.

Determination of urea :

These are on the classical spectrophotometric method recommended by **(Bector et al., 1998)**.

Determination of heavy metals :

The level of lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were detected in raw milk according to **Medina et al. (1986)** using Atomic Absorption Spectrophotometer model

2380, USA, after the samples were prepared according to **Tsoumbaris and Papadopoulou (1994)**.

Statistical analysis:

The results were calculated according **(SPSS, 1993)**.

RESULTS AND DISCUSSION

Chemical adulteration of milk by addition urea as a nitrogen compound is mostly problem of public health. It was noticed from the result (table1) that mean urea concentration in farm milk was 13.61±1.128mg/dl with minimum concentration of 9.60mg/dl and maximum concentration of 19.00 mg/dl these results lie around normal level of milk urea nitrogen (MUN) measured as nitrogen in urea (10-16mg/dl) as mentioned by **(Jonker et al., 1998; Ferguson, 2000; Kohn, 2000; Payne, 2001; Jonker et al., 2002 and Abdallah et al. 2008)**. Where the mean level of urea market milk was 18.57 ± 1.135 mg/dl with minimum concentration of 14.30 mg/dl and maximum concentration of 25.60 mg/dl according to Ontario Dairy Hard Improvement (DHI) **(Calberry, 2003)**, values excess of 20mg/dl are considered abnormal. **Bector et al. (1998)** recorded higher result 53.36mg/dl on contrary none of milk samples examined by **Sethi (2005)** had any added urea detected.

Major problem associated with excess urea happens when Kidneys begin to work overtime get rid the body from excess urea, they also excrete large amounts of minerals, and the most important of their components is calcium. The high concentration of calcium mixed with uric acid in the kidneys forms kidney stones and the body becomes depleted of calcium and draws it out of the bones rendering them weak, the ending result can be osteoporosis **(Atukorale, 1979)**.

On the other hand urea is a waste product plays a very important role in up the countercurrent system in the nephrons. The countercurrent system in the nephrons allows for reabsorption of water and critical ions **(Walter, 2003)**. Excess of urea in milk by boiling decomposes to carbonic acid, acetic acid, and ammonia. Those ammonia- containing calculi might be formed by the partial fermentation of urea in the bladder also, it may convert into biuret which causes fall in blood pressure and produces strong irritation in the urinary tract **(Prout, 2003)**.

Contamination of raw milk by heavy metals is one of the major problems confronting public health. lead, cadmium, arsenic and mercury have received increasing attention due to their toxic effects. Therefore, it has become important to determine the levels of toxic elements in milk as it is considered an essential part of human diet, since the dairy animals are exposed to heavy metals from various sources as a result of modern industrialization.

The mean values of lead concentration found in farm raw milk and market raw milk were 0.129±0.0144 ppm and 0.267±0.0484ppm, respectively in (table 3) according to the permissible limits of Egyptian Organization of **Standerization and Control (2001)** we notice that lead in both types exceed that limits. This result agreed with that reported by **(Saad et al. 2001, Mohammed 2005 and Al-Ashmawy Maha et al., 2008)**.

Lead is one of the limited classes of element that can be described as purely toxic. High level of lead is particularly of great concern especially due to the fact that milk and dairy products are consumed mostly by infants and children who are uniquely susceptible to the effect of lead. Lead absorption constitutes serious risk to public health. It induces reduced cognitive development and intellectual performance in children, increased blood pressure, and cardiovascular diseases in adult as well as liver and kidney disfunctioning **(Abdallah 2011)**. Lead concentration which had been increased in dairy products may be due to polluted milk. Also milk collected from an area crossed by roads of heavy traffic or near an industrial area may be contaminated by lead. Moreover, lead contamination from leaching containers during processing, storage and marketing is an important source for lead contamination of milk **(Bayomi et al., 1999, Saad et al., 2001 and Abdallah 2011)**.

The mean levels of cadmium in farm and market raw milk were 0.044±0.009 and 0.055 ±0.007 ppm respectively (table 3) nearly similar levels those reported by **Saad et al., (2001), Mohammed (2005) and Al-Ashmawy Maha et al., (2008)**. All examined raw milk samples were below the toxic value reported by WHO, 1999 (0.62 ppm).

The source of food contamination by cadmium were identified by **WHO (1989)** as phosphate fertilizers and swage sludge used on agricultural lands and the use of cadmium plated utensils or galvanized equipment in food processing and preparation. Cadmium is well recognized as one of the major toxic elements to man and animals. Even low levels may cause in time considerable accumulation in the tissues. It act as supfhyryl group of essential metal enzymes **(Scoullos et al., 2001)**.

It is obvious from the results presented in table 3 that the mean arsenic (As) concentration in examined farm and market raw milk were 0.0004 ± 0.0033 and 0.0007 ± 0.0038 ppm. The obtained results indicated that all samples are within the APLs recorded by **WHO, 1999** (0.008 ppm) and are in accordance with that of **Vela & Hettkemper (2004) and Al-Ashmawy Maha et al., (2008)**.

Arsenic has long been a frequent cause of intoxication in farm animals, particularly in cattle. Studies on laboratory animals have demonstrated that toxicity of arsenic is dependant on its form and its oxidation state . It is generally recognized that the soluble inorganic arsenical are more toxic than the organic ones. Arsenic in food occurs as a mixture of organic and the inorganic arsenicals, which account for 75% in dairy products **(WHO, 2001)**.

Concerning mercury (Hg) concentration in farm and market raw milk (table 3), it was clear that the average concentration of mercury residues were 0.0095±0.0045 ppm and 0.0134±0.0057 ppm respectively. Our results were in accordance with those reported by **El-Malt (2001) and Al-Ashmawy Maha et al., (2008)**. The concentration of mercury in all examined samples were within the APLs (0.5 ppm) reported by **FAO/WHO (1992); EC, (2001) and EC, (2008)**.

The results obtained indicated that farm and market raw milk urea levels were considered healthy and safe for human consumption referring the normal level of milk urea nitrogen (10-16 mg/dl) and only one sample (5%) of market raw milk was judged abnormal (> 20mg/dl). On the other hand non of examined samples contained cadmium, arsenic and mercury beyond the maximum permissible limits, while lead exceed that limits.

Therefore, a regular monitoring of heavy metals and urea levels in raw milk in the Governorate is recommended in order to establish the true contribution of milk and milk products to the dietary intake of urea and heavy metals also the repeated estimation of urea and heavy metals in milk must be conducted regularly to recognize its possible health hazards effects, its bioaccumulation during chronic exposure, its mobilization and secretion in milk.

(Table 1): Urea concentration values of farm and market raw milk (n=20).

types	Positive samples		Urea concentration(mg/dl)		
	No	%	minimum	maximum	mean±S.E
Farm raw milk	20	100	9.60	19.00	13.61±1.129
Market raw milk	20	100	14.30	25.60	18.57±1.135

(Table 2) : Frequency distribution of urea concentration of farm and market raw milk .

Urea concentration mg/dl of farm raw milk				Urea concentration mg/dl of market raw milk			
Within normal values		Abnormal values		Within normal values		Abnormal values	
No	%	No	%	No	%	No	%
20	100	0	0	19	95	1	5

(Table 3) : levels of heavy metals residues in farm and market raw milk n= 20 .

Types of samples	NO. of Samples	Level (ppm)	Heavy metals			
			Pb	Cd	As	Hg
Farm raw milk	20	Min	0.07	0.02	0.00	0.00
		Max.	0.19	0.09	0.003	0.04
		Mean±S.E	0.129±0.0144	0.044±0.009	0.0004±0.0033	0.0095±0.0045
Market raw milk	20	Min .	0.08	0.01	0.00	0.00
		Max.	0.50	0.1	0.003	0.05
		mean±S.E	0.267±0.0484	0.055±0.0072	0.0007±0.0038	0.0134±0.0057

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الملخص العربى

قياس اليوريا وبقايا المعادن الثقيلة فى اللبن الخام فى محافظة القليوبية

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قسم الكيمياء الحيوية والنقص الغذائى*

قسم صحة الأغذية**

تعتبر اليوريا والعناصر الثقيلة من أهم الملوثات الكيميائية للألبان والتى لها آثار ضارة على الصحة العامة، ولذلك أجرى هذا البحث للكشف الكمى والنوعى لهذه العناصر فى ألبان المزارع والألبان المتداولة فى الأسواق.

تم تجميع عدد ٤٠ عينة ٢٠ من ألبان المزارع و ٢٠ عينة من الألبان المتداولة فى الأسواق وذلك فى محافظة القليوبية.

وقد أثبتت النتائج أن متوسط تركيز اليوريا فى ألبان المزارع كانت ١٣,٦١ ± ١,١٢٩ بينما فى الألبان المتداولة فى الأسواق كانت ١٨,٥٧ ± ١,١٣٥ مجم/ديسيلتر وقد كانت تلك النسب فى المستوى المسموح به (١٠-١٦) مجم/ديسيلتر فيما عدا عينة واحدة تجاوزت الحد المسموح به ٢٠ مجم/ديسيلتر وهى تمثل نسبة ٥٪ من إجمالى عينات ألبان المتداولة فى الأسواق.

بالنسبة للعناصر الثقيلة (الرصاص - الكادميوم - الخارصين - الزنبق) أثبتت النتائج أن متوسط تركيز تلك العناصر فى ألبان المزارع كانت ٠,١٢٩ ± ٠,٠١٤ ، ٠,٠٤٤ ± ٠,٠٠٩ ، ٠,٠٠٤ ± ٠,٠٠٣٢ و ٠,٠٠٩٥ ± ٠,٠٠٥ جزء فى المليون على التوالى، بينما فى الألبان المتداولة فى الأسواق فقد كانت ٠,٢٦٧ ± ٠,٠٤٨٤ ، ٠,٠٥٥ ± ٠,٠٥٥ ، ٠,٠٠٠٧ ± ٠,٠٠٣٨ و ٠,٠١٣٤ ± ٠,٠٠٥٧ جزء فى المليون على التوالى.

وأثبتت الدراسة أن جميع الألبان المفحوصة تحتوى على هذه العناصر بنسبة أقل من المعدلات المسموح بها فيما عدا الرصاص.

وقد تم مناقشة النتائج وخطورة تلك المتبقيات على الصحة العامة وكذلك الاشتراطات الصحية الواجب اتخاذها فى إنتاج النظيفة والآمنة.