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ESTIMATION OF SOME HEAVY METALS IN BOVINE MILK IN ASSIUT GOVERNORATE.

(With 3 Tables and 1 Figure)

By

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(Received at 16/3/1999)

قياس مستوى بعض المعادن الثقيلة فى ماشية اللبن بمحافظة أسيوط

إيناس البرنس ، أحمد عبد الباقى شرقاوى

ارتبطت الألبان ومنتجاتها منذ قديم الأزل بحياة الإنسان والحيوان لما تتمتع بــه مــن قيمــة غذائية عالية وهبها الله إياها، وبالرغم من هذا فقد تكون سببا فى إحداث الأمــراض سـواءً لتلوثها بيولوجياً أو كيميائياً. وتعتبر المعادن الثقيلة واحدة من أهم الملوثات الكيميائيــة التــى تنتشر على نطاق واسع فى البيئة فى الآونة الأخيرة لذا فأن تواجدها فى الألبــان يعــد مــن الموضوعات التى تلاقى اهتماما كبيرا من قبل المهتمين بسلامتها، ولما كانت الحيوانات أكثر تعرضا من الإنسان فأنه يتعين وجوب الاهتمام بفحص الأغذية ذات الأصل الحيوانى لتقديــر تركيزات هذه العناصر الثقيلة لما تتميز به من أثر تراكمى حتى يمكن أن يتفادى أخطارهــا قبل وصولها الى المستهلك. لذا فقد استهدفت هذه الدراسة تقدير كمى للعناصر السامة وهمــا الرصاص والكادميوم بالإضافة الى ثلاثة من العناصر الأساسية والتى عند زيادتها يكون لــها تأثير ضار وهى الحديد والنحاس والزنك فى ألبان كل من الأبقار والجاموس كما أن نقصــها يلعب دورا هاما فى وظائف الجسم المختلفة. ولهذا تم أخذ ٤٠ عينة ألبان (٢٠ عينــة مــن مزارع الأبقار و٢٠ عينة من مزارع الجاموس) بواقع ٥ عينات من كل مزرعة من مــزارع أسيوط المختبرة وتم أجراء الفحوص الطبيعية كما تم قياس مســتوى كــل مــن الرصــاص والكادميوم والحديد والنحاس والزنك باستخدام جهاز الامتصاص الذرى. وقد أوضحت النتائج أن متوسط كل من الرصاص والكادميوم والحديد والنحاس والزنك فى عينات ألبــان الأبقــار المفحوصة هى ٢٤. ٠، ٠١٧. ٠، ٤٢٨. ٠، ٥٩٢. ٠ و ٠٦٠. ٢ جزء فى المليون بينمــا كان المتوسط ٤٤٧. ٠، ٠١٩. ٠، ٣٢٢. ٠، ٨٢٥. ٠ و ٣٣٧. ٢ فى ألبان الجاموس علــى التوالى. وقد لوحظ أن متوسط كل من الرصاص والكادميوم قد فاق المعدلات المسموح بــها عالميا فى بعض مزارع الألبان بينما كانت فى حدود المسموح به بالنسبة للحديــد والنحــاس والزنك. مما سبق يتضح أن هناك نوعا من التلوث بكل من الرصــاص والكــادميوم الــذى يستوجب دراسة مصادره والعمل بكافة الوسائل على إيقافه تجنبا لمخاطره.

SUMMARY

The present study estimates the levels of some heavy metals in milk samples collected from different lactating cow's and buffalo's farms in Assiut Governorate. These samples were examined physically for flavor and color. Lead and cadmium as non essential toxic elements as well as iron, copper and zinc as essential elements were estimated by using the atomic absorption spectrophotometer. The analytical results indicated that the mean values of lead, cadmium, iron, copper and zinc in milk of investigated animals were 0.240, 0.017, 0.428, 0.592 and 2.060 ppm in cow's milk and 0.447, 0.019, 0.322, 0.825 and 2.337 ppm in buffalo's milk, respectively. The results also revealed a significant increase only in copper content in the examined buffalo's milk compared with cow's milk. The importance of public health and sources of milk contamination by heavy metals were discussed.

Key words:*Lead, Cadmium, Iron, Copper, Zinc, Cow's & Buffalo's milk, Assiut.*

INTRODUCTION

Nowadays, a great deal is directed towards the environmental pollution as it leads to an increased interest in contamination of milk, water and foodstuffs which represent an important part of human and animal diet (Crossman, 1981). Heavy metals are among the most dangerous forms of pollutants, that have a tendency to accumulate in living tissues and organs (Antoniou et al., 1989). The amount of different heavy metals in ambient atmosphere have been increased with the development of human civilization and exploitation of geological resources as mining and fossil fuel development. An additional concern about their concentrations in domestic and industrial waste products are indestructible (Sharma et al., 1982).

Although many of these metals are essential for health, the excessive exposure and mobilization may be hazardous (Protasowicki, 1992). This is complicated even further if there is a very narrow range between the concentration at which the metal is considered essential and considered toxic (Higham and Tomkins, 1993).

Attention has been focused on milk as it is nearly almost the perfect single foodstuff especially for children. However, lactating cattle may be exposed to high quantities of toxic metals in the environment by air, water and ingestion of polluted feed. Fortunately, these animals act as a very efficient biological filter against heavy metal contamination. Where, it is valid when the animals are grazing near motor ways and roads with heavy car traffic (Carl, 1991). Moreover, the toxic effect of a number of non-essential metallic elements such as lead and cadmium are among the most deleterious agents to the biological life (Rico et al., 1989 and Wojciechowska et al., 1992).

Lead (Pb) is one of the most important pollutants in our environment which accumulates in the body due to its low rate of elimination. Its biological half-life in bones is about 27 years as stated by Shibamoto and Bjeldanes (1993). Chronic lead poisoning is characterized by anemia, muscular pains, changes in arterial elasticity resulting in hypertension, toxic effects on reproductive system and lead nephropathy (Goldfrank et al., 1990, Tsafaris and Alexaki, 1992, Goyer, 1993 and Hense et al., 1993). Also, its potential carcinogenic nature has been reported by Zawurska and Medras (1988). Strong lead neurotoxicant effect on the central nervous system is more common in children and newly born and postnatal exposed animals than in adults (Kim et al., 1990, Bresseler and Goldstein, 1991 and Silbergeld, 1992).

On the other hand, Djurie et al. (1971) noticed that Pb concentration in milk samples ranged from 1.6 to 1.9 ppm. In cattle, milk Pb levels ranged from 0.028 to 0.030 ppm and may be elevated to as high as 2.26 ppm in severely poisoned cows (Blood and Radostits, 1989). In subsequent studies, its level in analyzed milk samples was < 0.020 to 0.130 ppm (Watling and Haines, 1990 and Mitrovic et al., 1992). Shehata and Saad (1992) revealed that milk Pb levels were 0.019 ± 0.002 ppm in cows and 0.245 ± 0.016 ppm in buffaloes. While, Muller et al. (1993) showed higher Pb contents of 6.4 and 6.8 µg/l in farm and market milk, respectively, or 1.51 ppm in farm milk in Sharkia Governorate (Tork, 1994). Hygienic standards of the contents of foreign substances in food state that 0.1 ppm is the maximum Pb content in milk (Bartik and Piskac, 1981). The provisional weekly intake of Pb in food must be not exceed than 0.005 mg/kg body weight as recommended by FAO (1989). However, Carl (1991) postulated that the acceptable limits ranged from 0.05 to 0.2 ppm.

Cadmium (Cd) is also considered one of the dangerous metal contaminants of food and drinks due to its wide distribution and numerous industrial highly-valued uses in modern technology. It was presumed to be a possible source of environmental pollution through galvanized pipes and effluents from electroplating works and geological deposits (Dwivedi et al., 1997 and Melgar et al., 1997). Man and animal are exposed to Cd via air, water and food of plant or animal origin (Antoniou et al., 1989). Although, Cd is vitally absent from the human body at birth, it is highly cumulative poison with a biologic half-life of 20-30 years causing renal failure (Gracey and Collins, 1992 and Manahan, 1992). Anemia is a common manifestation of chronic cadmium toxicity due to its antagonism to copper and iron. In animals, chronic oral feeding of subtoxic doses of Cd salts leads to immunotoxic effects, reduce neutrophil, macrophage and lymphocyte functions (Beisel, 1982). It is a potent and effective carcinogen in rodents and recently has been accepted by the International Agency for Research on Cancer as category 1(human) carcinogen (IARC, 1994) based primarily on activity as pulmonary carcinogen (Stayner et al., 1992). It also plays a significant role in the cause of hypertension and osteomalacia (Friberg et al., 1986). In addition, Cd has been shown to affect the disposition and excretion of copper and zinc in growing and adult animals (Mahaffey et al., 1981 and Wesenberg et al., 1981). As Cd is ubiquitous in nature, all food is exposed to and contains it (Watson, 1993). Its presence in human food chain including milk (Cowie and Swinburne, 1977; Mertz 1989; Galal, 1990; Mitrovic et al., 1992, Protasowicki, 1992; Kirova, 1993; Galal-Gorchev, 1993; Muller et al., 1993; Jeng et al., 1994, Tork 1994 and Dwivedi et al., 1997). FAO (1989) recommended that, the provisional weekly intake of Cd in food is 0.007 mg/kg body weight. The maximum acceptable limits for Cd in milk ranged from 0.005-0.05 μg/kg in different countries as recorded by Carl (1991).

In addition to the previous toxic elements that may contaminate milk, there are a large number of essential elements usually measured in ppm or μg per liter and referred as trace elements e.g. iron, copper and zinc. Iron (Fe) is necessary to all living cells and body fluids and play an important role in regulation of vital cell processes (WHO, 1972). Relatively high concentrations present in most grains, nuts and meat (Yeshajahu, 1994). This element becomes toxic at sufficient high intake leading to health hazards as hepatocellular damage and fibrosis (Gossel and Bricker, 1990 and Doyle et al., 1993). The maximum permissible limit of Fe in food daily intake is 0.8 mg/kg body weight according to the Egyptian Organization of Standardization (1993). Copper (Cu) also is an essential element for all plants and animals, it is widely distributed and occurred in food in many chemical forms which affects its availability to the animals (Watson, 1993). Interest in the biological significance, that both deficiency and excess of Cu in the mammalian system result in serious effects (Hostynek et al., 1993). Abnormally high liver Cu levels lead to various diseases include Mediterranean anemia, hemochromatosis, cirrhosis, yellow atrophy of liver and Wilson's disease "hepatolenticular degeneration" (Underwood, 1977). In addition, its content in milk varies with the species, stage of lactation and Cu intake of dairy animal, although milk is considered the poorest source of copper (not more than 0.3 ppm) as recorded by Underwood (1977) or less than 0.1 ppm as reported by Harrison (1993). Ibrahim et al. (1984) recorded Cu levels of 0.136 and 0.132 ppm in cow's and buffalo's milk, respectively in Assiut Governorate. While, CAC (1984) stated that Cu intake from food and drinks not exceed 0.5 ppm/day.

Zinc (Zn) is essential for biological functions as protein synthesis and carbohydrate metabolism. A number of diseases and dermatological conditions are attributable to Zn deficiency. Consequently, in extreme cases, alopecia, acrodermatitis and enteropathica are most serious manifestation and is lethal if not treated successfully (Miyata et al., 1986). Moreover, Zn level in lactating animals or in milk was previously estimated by many authors (Abdel-Maged and Oheme, 1990, Citek et al., 1994 and Fayed, 1997). The maximum load of Zn was 300-1000 μg/kg body weight from all foods and drinks (CAC, 1984).

As milk is life food for both newly born animals as well as for human consumption and because of the deleterious effects of some heavy metals on animal and human health, therefore, this study aimed to investigate the environmental pollution with some heavy metals in bovine milk from different livestock and to suggest a protocol for prevention of this pollution in Assiut Governorate.

MATERIAL and METHODS

Collection of samples:

A total of 40 random samples of cow's and buffalo's milk (20 each) were obtained from 8 different governmental dairy farms in Assiut (5 samples from each farm). The collected samples were dispatched to the laboratory without delay in ice-pack containers.

Physical examination:

The samples were examined physically for color and flavor (APHA., 1985).

Estimation of metals:

The level of some heavy metals as lead and cadmium (non essential and toxic elements), iron, copper and zinc (essential elements) was estimated by using the atomic absorption spectrophotometer AAS (Buck Model 210 VGP, USA). The absorption and concentration were recorded directly from the digital scale of AAS in ppm. Standard procedures were used to estimate lead, cadmium, iron, copper and zinc in milk. All glassware, pipette tips and plastic ware were rinsed with 25 % HNO_3 to avoid metal contamination.

(a) For lead and cadmium determination, 5 ml milk were used. Duplicate milk samples were treated with concentrated nitric acid and perchloric acid (2:1), samples were brought to a constant volume and determination of lead and cadmium were carried out according to Agemain et al. (1980) by using AAS (Buck Model 210 VGP, USA).

(b) Milk copper and zinc were measured by using AAS according to Parker et al. (1968).

Statistical analysis:

The obtained data were statistically analyzed according to Snedecor and Cochran (1974) using student's "t" test. The obtained values in this study were compared with the permissible acceptable limits of both lead and cadmium (0.02 and 0.002 ppm), respectively (Carl, 1991), and the normal levels of iron (0.8 ppm) by Egyptian Organization of Standardization (1993), copper (0.3 ppm) by Forstner and Prosi (1979) and zinc (3.0 ppm) by Underwood (1977).

RESULTS

The physical examination (color and flavor) revealed that all examined milk samples were within the normal limits. Results of analysis of cow's and buffalo's milk samples for Pb, Cd, Fe, Cu and Zn were outlined in Tables 1, 2, 3 and Fig. 1.

Table 1: Levels (Mean ± S.E.M) of some metals in cow's milk/ppm.

Farms No.	Lead	Cadmium	Iron	Copper	Zinc
1	0.384 ± 0.106[a]	0.031 ± 0.001[b]	0.623 ± 0.066	0.861 ± 0.209	2.319 ± 0.841
2	0.246 ± 0.074[a]	0.004 ± 0.001[c]	0.422 ± 0.071[a]	0.409 ± 0.074	2.636 ± 1.376
3	0.208 ± 0.091	0.022 ± 0.003[a,c,d]	0.226 ± 0.061[b,c]	0.537 ± 0.205	1.323 ± 0.232[a]
4	0.123 ± 0.038	0.011 ± 0.001[b,c,d,e]	0.440 ± 0.083[a]	0.563 ± 0.157	1.962 ± 0.527

a: Significant difference from the permissible acceptable limits or normal level at $p < 0.05$.

b: Significant difference from the permissible acceptable limits or normal level at $p < 0.001$.

c: Significant difference of farm 2, 3, 4 from farm 1 at $p < 0.05$.

d: Significant difference of farm 3, 4 from farm 2 at $p < 0.001$.

e: Significant difference of farm 4 from farm 3 at $p < 0.05$.

Table 2: Levels (Mean ± S.E.M) of some metals in buffalo's milk/ppm.

Farms No.	Lead	Cadmium	Iron	Copper	Zinc
1	0.309 ± 0.056[a]	0.023 ± 0.005[a]	0.253 ± 0.050[b]	0.691 ± 0.230	1.393 ± 0.402[a]
2	0.119 ± 0.007[b,c]	0.018 ± 0.001[b]	0.181 ± 0.060[b]	0.949 ± 0.334	2.906 ± 0.805
3	0.630 ± 0.041[b,c]	0.023 ± 0.009	0.487 ± 0.107[a]	0.794 ± 0.206	1.730 ± 0.615
4	0.730 ± 0.092[a,c]	0.014 ± 0.001[b]	0.368 ± 0.139[a]	0.868 ± 0.216	3.319 ± 0.980

a: Significant difference from the permissible acceptable limits or normal level at $p < 0.05$.

b: Significant difference from the permissible acceptable limits or normal level at $p < 0.001$.

c: Significant difference of farm 2, 3, 4 from farm 1 at $p < 0.05$.

Table 3: Mean values (± S.E.M) of some metals in cow's and Buffalo's milk.

Farms No.	Lead	Cadmium	Iron	Copper	Zinc
Cow's milk	0.240 ± 0.047[a]	0.017 ± 0.005[a]	0.428 ± 0.070[a]	0.592 ± 0.080[a]	2.060 ± 0.243[a]
Buffalo's milk	0.447 ± 0.122[a]	0.019 ± 0.001[b]	0.322 ± 0.058[b]	0.825 ± 0.047[b,c]	2.337 ± 0.399

a: Significant difference from the permissible acceptable limits or normal level at $p < 0.05$.

b: Significant difference from the permissible acceptable limits or normal level at $p < 0.001$.

c: Significant difference of copper level in buffalo's milk from that in cow's milk at $p < 0.05$.

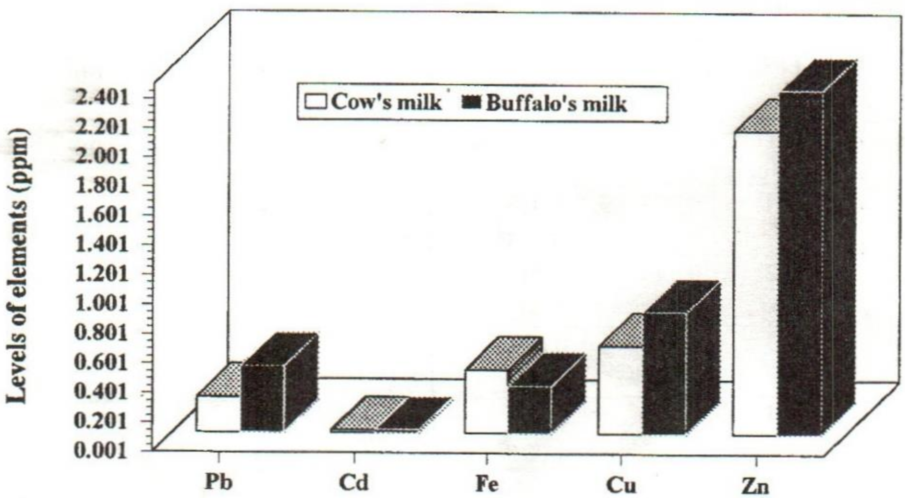

Fig. 1: Levels of some elements in cow's and buffalo's milk

DISCUSSION

As the dairy animals can be exposed to toxic elements from various sources, so it is necessary to monitor the level of lead and cadmium as toxic elements and iron, copper and zinc as essential elements in milk. Investigation on essential elements have screened mainly on whether they were present in toxic amounts, rather than on its occurrence as normal constituents.

The analytical findings of lead revealed that the mean values in cow's and buffalo's milk, outlined in Tables 1 and 2, ranged from 0.123 ± 0.038 to 0.384 ± 0.106 and 0.119 ± 0.007 to 0.730 ± 0.092 ppm, respectively. This increase of bovine milk lead level was in agreement with the previous toxicological studies recorded by Djurie et al. (1971), Tork (1994) and Fayed (1997). However, lower contents were estimated by Favretto and Marletta (1984), Blood and Radostits (1989), Watling and Haines (1990), Mitrovic et al. (1992), Shehata and Saad (1992) and Muller et al. (1993). The hygienic standards of the foreign substances in food stated that 0.1 ppm is the maximum lead content in milk (Bartik and Piskac, 1981). Blood and Radostits (1989) found that in severely poisoned cows, milk lead content was 2.26 ppm. Carl (1991) postulated that the maximum acceptable limits in different countries ranged from 0.05 to 0.2 mg/kg body weight. It has been apparent that the results in this study concede the maximum acceptable lead limits which correlated with the result obtained by El-Shreif (1991), who recorded a high lead level in all examined water sources and plants in Assiut Governorate. This may be arise from the food, as cattle are normally feed on grasses and the milk lead content will be dependent on the amounts which were present in pasture and cereals. The major source of lead in the environment resulting from the manufacture and application of alkyl lead fuel additives. Its transport and distribution from stationary or mobile sources mainly via air and probably discharged into soil and water (WHO, 1977).

Concerning cadmium, the mean content in cow's milk was 0.017 ± 0.005 and in buffalo's milk was 0.019 ± 0.001 ppm as shown in Table 3. Higher findings were recorded by Tork (1994) and Fayed (1997), they showed a higher level of 0.113 and 0.20 ppm in the examined raw milk samples, respectively. Inversely, Favretto and Marletta (1984), Papajova and Hermonova (1986), Mitrovic et al. (1992), Kirova (1993), Muller et al. (1993), Jeng et al. (1994) and Dwivedi et al. (1997) recorded lower levels. It has been reported that the concentration of cadmium in milk at or below the limits of detection <0.002 mg/kg (Galal, 1990 and Galal-Gorchev, 1993). According to Carl (1991) the maximum acceptable limits of cadmium in milk was 0.005-0.05 mg/kg body weight. Hence, the obtained results in this study showed a slight rise above the previously recorded maximum acceptable limits. Higher milk cadmium concentration in lactating animals could be attributed to the greater ingestion of contaminated feed and water and inhalation of fumes and dusts from the industrial activities (Dwivedi et al., 1997).

The recorded mean levels of milk iron as outlined in Table 3, were 0.428 ± 0.070 and 0.322 ± 0.058 ppm in cow's and buffalo's milk, respectively. A previous study revealed that the mean contents of iron in cow's and buffalo's milk were 0.053 ± 0.034 and 0.077 ± 0.003 ppm, respectively (Shehata and Saad, 1992). The normal acceptable limits in food daily intake is 0.8 mg/kg body weight (Egyptian Organization of Standardization, 1993), therefore, the obtained results in this study considered within the normal range. This held the view reported by Abdel-Maged and Oheme (1990) as they postulated that milk is considered a poor source of iron. Depletion of iron stores in human or animal body or latent iron deficiency leading to overt iron deficiency anemia which characterized by fatigue, listlessness, anorexia, depressed growth and immunosuppression in children and young growing mammals. So, it is necessary to increase iron food intake to overcome its low level in milk.

In cow's and buffalo's milk, the mean values of copper were 0.592 ± 0.080 and 0.825 ± 0.047 ppm, respectively. These values were lower than the results intended by Fayed (1997). While, lower findings were detected by Favretto and Marletta (1984), Ibrahim et al. (1984) and Shehata and Saad (1992). It is well documented that milk is considered the poorest source of copper, not more than 0.3 ppm as reported by Underwood (1977) and Forstner and Prosi (1979) or less than 0.1 mg/kg as stated by Harrison (1993). The high level of copper in examined bovine milk may be due to the wide application of copper in agriculture and industry, therefore, it is potentially very hazardous trace element and very toxic at higher concentrations. The maximum acceptable daily load of copper ranged from 50 to 500μg/kg b.w./day (CAC, 1984).

Also, the results presented in Table 3, show that the mean contents of zinc in both examined cow's and buffalo's milk were 2.060 ± 0.243 and 2.337 ± 0.399 ppm, respectively. The obtained findings in cow's milk were in accordance with those recorded by Citek et al. (1994), while higher values were estimated by Fayed (1997) in the examined raw milk samples. Underwood (1977) showed that zinc level in normal milk was 3 ppm, however CAC (1984) recorded that 300-1000 mg/kg body weight was the maximum load of zinc in all foods and drinks. Accordingly, the reported zinc levels in this investigation not exceed the normal limit (3 ppm). On the contrary, high levels of dietary zinc may interfere with the hepatic copper storage and may compete with calcium for intestinal absorption. The antagonistic effects of zinc against iron and copper can result in suppression of hematopoiesis (Osweiler, 1996).

Finally, the comparison between the obtained results and the maximum permissible limits showed that, lead and cadmium were increased significantly, while iron and zinc decreased significantly and copper increased non significantly in both cow's and buffalo's milk. However, the copper level in buffalo's milk was significantly higher than that in cow's milk.

In conclusion, the investigation of bovine milk revealed a high concentration of lead and cadmium. It is seemly necessary to warning about the hazardous effects of these toxic elements on both newly born animals and human being in Assiut Governorate. The consuming of such milk considered as an additional source of exposure beside the direct sources of air, water and plants. Moreover, the effect on consumers may contribute through disturbing the levels of essential elements (iron, copper and zinc) in vital foods.

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