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BIOCHEMICAL AND HEMATOLOGICAL PROFILE IN SHEEP AND GOATS EXPOSED TO POLLUTED UNDERGROUND WATER IN EL-FAYOM GOVERNORATE

Arafa, M.M.; Sahar A. Aly ;Mona M.A.Heta and Darwish.A.S.

Animal Health Research Institute, Dokki-Cairo.

ABSTRACT

The aim of this investigation is to determine the effect of trace elements and heavy metals pollutant of the under ground water on the health status of sheep and goat of both sexes at El-Fayom Governorate. This study was conducted on 50 adult sheep and goat (25 each)of both sexes drinking polluted underground water at El-Fayom Governorate ad-libitum, together with twenty adult sheep and goat (10 each) drinking fresh tap water to be used as control. Water samples were collected from both fresh tap water and polluted under ground water for determination of Ca, Mg, Na, Cl, Cu, Fe, Zn, Pb, Cd, Sulphate and Nitrite. Chemical analysis of polluted water revealed significant increase in Nitrite and all heavy metals compared to fresh water. The body weight of sheep and goat drinking polluted water was significantly decreased and they showed emaciation, anorexia associated with roughness of hairs. Blood samples were collected from each animal for biochemical and hematological investigations. Sheep and goats which were drinking polluted ground water recorded significant increase in serum Ca, Mg, Na, Cl, lead, cadmium and nitrite levels with significant decrease in, Ph, Cu, Iron and zinc levels. Also, there were a marked decrease in serum total protein, albumin and A/G ratio was observed while globulin and glucose levels were significantly

increased. AST activity, ALT activity, urea and creatinine were highly elevated compared to control group. The hematological profile showed significant decrease in Hb, PCV, with low red blood cells and total white cells count compared to values recorded in control animals.

Therefore a great deal of attention must be given to analysis of under ground water before allowing rural populations to use it as a water source for man and animals.

INTRODUCTION

Water pollution is defined as the presence of unwanted substances beyond the acceptable levels which considered hazardous for health or aesthetics. These substances may include organic matter, heavy metals, minerals, sediment biological contaminants, and toxic chemicals ***(Crompton, 1998)***. Environmental pollution associated with heavy metals has global concern over many decades. These metals are natural components of the environment but high rate of industrialization has been responsible for their wider diffusion and dispersal in the environment ***(Miranda et al., 2005)***.

Heavy metals and other trace elements have been considered as dangerous substances, causing serious health hazards to human and animals, through progressive irreversible accumulation in their bodies as a result of repeated consumption of small amounts of these elements (***Raghib et al., 2003*** and ***Igado et al., 2008***). Among the most dangerous metallic ions are copper, mercury, lead, cadmium and antimony which are toxic to many forms of life ***(Mekawy, 1996).*** For example Long term chronic exposure to cadmium has been associated with anaemia, osteomalacia and cardiovascular disease especially hypertension

(Bersenyi et al., 2003). It damages the proximal tubules of nephrons, leading first to leakage of low molecular weight proteins and essential ions like calcium into the urine, and then with progression over time to kidney failure *(Satrug et al.,2000)*. Even the loss of calcium caused by the adverse effect of cadmium on the kidney can lead to weakening of the bones *(Okoye and Ugwu,2010)*. Lead is considered as one of the most hazards and cumulative environmental pollutants that affect all biological systems through exposure from air, water and food sources ***Khalaf Allah and Abd EL-aal 1999 and Amal et al., 2008***).Lead poisoning may affect numerous organs, and is associated with number of morphological, biochemical and physiological changes, including kidney dysfunction, abnormal glucose metabolism, as well as nervous, immune, reproductive, cardiovascular disturbance, impairment of liver function, and hematological disorders ***(Elayat and Bakheat,2010).***

Khalaf et al.,(1994) declared that the permissible limits of copper, zinc, cadmium, iron and lead in drinking water must not exceed 2.4,1.5,0.01, 0,3and0.01ppm respectively.

Eventually, the increase in the levels of heavy metals than the permissible limits exerts toxic effect on the animal body which will be clearly seen on blood constituents and enzymatic activities, ***Erdogan et al., (2002).***

The aim of the present work is to determine the biochemical and hematological changes in blood profile of sheep and goats due to drinking underground water polluted with heavy metals at El-Fayom Governorate. Also its effect on the levels of some serum macro and micro elements in the investigated sheep and goats was studied.

MATERIAL AND METHODS

Animals:

The present study was conducted on fifty sheep and goat 2-3 years old of both sexes (25 each) their weights ranged between 25 and 50 kg at El-Fayom Governorate, and were drinking polluted underground water ad libitum. They were suffering from emaciation, anorexia, roughness of the coat, loss of weight and high mortality rate reached about 40%.Twenty adult sheep and goats (10 each) 2-3 years old their weights ranged between 25 and 50 kg kept on fresh tap water were used as control group.

Samples:

1- Water Sampling:

Five water samples were collected from each of the fresh tap water and artesian wells in different localities at El-Fayom Governorate were used for chemical analysis of calcium, magnesium using test kits supplied from Bio-Merieux (Bains/France), sodium was determined using flamphotometer (Corning 400) and chloride was determined using chloride analyzer Model 925.cupper, iron, zinc, lead and cadmium were determined using UNICAM 969 Atomic Absorption Spectrophotometer. The pH was estimated using pH meter while nitrites, sulphates and total solids were analyzed according to ***Fresenius et al., (1988).***

2- Blood Sampling:

From each investigated animals, one blood sample was collected by the jugular vein puncture and divided into 2 portions. The first one was placed in small labeled dry and clean vials containing EDTA as anticoagulant for hematological examination according to the method described by ***Jain (1986).*** While, the second portion was left without anticoagulant in a centrifuge tube and allowed to clot at room temperature, then centrifuged to separate clear serum. The collected sera were kept at -20°C till used for determination of serum glucose total protein, albumin, AST activity, ALT activity, urea, creatinine, calcium, phosphorus and magnesium, spectrophotometrically by using standardized test-kits supplied from Bio-Merieux (Bains / France).

Blood serum sodium and potassium were estimated using flamphotometer (Corning 400). Blood serum chloride was determined by chloride analyzer Model 925.Blood serum cupper, iron, zinc, lead and cadmium were determined by using Atomic absorption spectrophotometer (Perkin Elmer Model 2380 USA) in Soil and Irrigation Department, Faculty of Agriculture, Assiut University. Blood serum globulin and albumin globulin ratio were calculated mathematically.

Statistical analysis:

The obtained data was analyzed statistically using t-test according to ***Selvin (1996).***

RESULTS

The obtained results were shown in tables (1- 4). Table (1) showed chemical analysis of polluted water which revealed significant increase in calcium, magnesium, sodium, potassium, chloride, copper, iron, zinc, lead, cadmium, total solids and nitrite compared to fresh tap water (Control One).

Table (1): Chemical Analysis of fresh tap water and under ground water samples related to their permissible limits according to ***WHO (2004).***

Parameters	Fresh water	Under ground water	Permissible limit
pH	6.9± 0.01	8.04± 0.5*	>8.0
Ca (mg/ l)	24.50±1.1	42.25±2.5**	50.0
Mg (mg/ l)	13.75±2.2	47.85±3.8**	30.0
Na (meq/l)	16.50±3.8	29.2±4.1**	20.0
Cl(mg/ l)	4.05±7.8	7.305±10.5**	5.0
Cu (mg/ l)	0.18±0.02	0.50±0.09*	1.0
Fe(mg/ l)	0. 338±0.01	0.552±0.03*	0.3
Zn (mg/ l)	0.09±0.3	0.16±0.01**	3.0
Pb (mg/ l)	0.056±0.02	0.095±0.03**	0.05
Cd (mg/ l)	0.002±0.01	0.005±0.01**	0.003
Total Solid (mg/l)	99.2±3.5	151.0±1.2**	120
Nitrite (mg/l)	0.000±0.01	0.248±0.031**	0.2
Sulphate (mg/l)	0.420±0.02	0.510±0.03	500

Data expressed as mean ± SE.

* Significantly different from control at ($P < 0.05$)

** Significantly different from control at ($P < 0.01$)

Results of chemical analysis of serum presented in Tables 2 and 3 showed that, sheep and goats which were drinking polluted ground water recorded significant increase in serum Ca, Mg, Na, Cl, Lead, Cadmium and Nitrite levels and significant decrease in, P, Cu, Iron and Zinc levels. Also, there were a marked decrease in serum total protein, albumin and A/G ratio while globulin and glucose levels were significantly increased. AST activity, ALT activity, urea and creatinine exhibited a highly significant increase compared to control group.

The current results in table 4 cleared, the hematological alterations which indicate the anemic status of sheep and goats that drinking polluted under ground water. This status was presented by significant decrease in the level of Hb, PCV with low red blood cells and total white cells count compared to control group.

Table (2): Serum macro and micro-elements of sheep and goats subjected to polluted water and that drinking fresh water.

Parameters	Sheep		Goat	
	Fresh water	Polluted water	Fresh water	Polluted water
Ca(mg%)	7.9± 0.30	11.30±0.50**	8.9±0.28	10.4±0.30**
P(mg%)	5.60 ± 0.20	4.26±0.01*	5.80±0.18	5.10±0.2*
Mg(mg%)	1.80±0.03	2.30± 0.30*	1.5±0.3	2.40±0.15*
Na(Meq/L)	137.75±1.4	142.50 ± 2.3*	133.50±2.30	139.0±3.0*
K(Meq/L)	4.20± 0.90	4.79±1.1*	4.80±0.09	5.20±0.90
Cl (mg%)	94.25 ± 1.70	138.50±3.60**	100.0±2.1	142.0±1.70**
Cu(µ g%)	78.20±3.1	68.70± 2.40**	81.30±2.4	65.20±1.70**
Fe(µ g%)	102.25±7.10	66.50± 6.50**	159.30±4.1	100.0±3.20**
Zn(µ g%)	69.0±3.10	58.50 ± 2.20*	73.4±1.60	55.30±3.10*
Pb(µ g%)	17.52± 1.90	36.70±4.50**	15.5 ±2.1	34.20±1.90**
Cd(µ g%)	15.10± 3.20	27.0±5.60**	17.40±1.43	30.10±1.50**
Nitrite(mg%)	0.00	0.52±0.02**	0.00	0.52±0.02**

Data expressed as mean + SE.

* Significantly different from control at ($P < 0.05$)

** Significantly different from control at ($P < 0.01$).

Table (3): Serum biochemical constituents of sheep and goats subjected to polluted water and that drinking fresh water.

Parameters	Sheep		Goat	
	Fresh water	Polluted water	Fresh water	Polluted water
Glucose	60.90±	86.5±1.20*	75.30±1.20	112.0±2.10*
Total protein	7.90±1.30	6.95±1.10*	8.10±1.1	7.50±0.9*
Albumin	3.85±0.30	2.75±0.5*	3.80±0.50	2.90±0.85*
Globulin	4.0±0.10	4.2±0.01	4.30±0.5	4.60±0.72
A/G ratio	0.95±0.01	0.65±0.02*	0.88±0.01	0.63±0.02*
AST activity	48.82±1.70	98.25±4.50**	40.0±1.30	54.0±3.1**
ALT activity	36.35±1.50	88.75±3.8**	35.0±1.5	70.0±2.90**
Urea	30.5±2.11	52.1±3.1**	25.0±0.9	44.0±1.80**
Creatinine	0.71±0.05	2.10±0.34**	0.43±0.03	1.20±0.08*

Data expressed as mean + SE.

* Significantly different from control at ($P < 0.05$)

** Significantly different from control at ($P < 0.01$)

Table (4): Hematological profile of sheep and goats subjected to polluted water and that drinking fresh water.

Parameters	Sheep		Goat	
	Fresh water	Polluted water	Fresh water	Polluted water
RBCs / 10^6 µl	5.43±0.12	3.15±1.20*	5.10±0.20	3.02±0.68*
Hb (g/dl)	10.90±1.30	7.15±1.10**	10.10±0.88	6.50±0.90*
PCV(%)	31.85±0.30	24.05±0.5*	31.04±0.50	22.90±0.85**
TLC/10^3 µl	8.90±0.10	4.5±0.01**	9.30±0.5	4.10±0.72*

Data expressed as mean + SE.

* Significantly different from control at ($P < 0.05$)

** Significantly different from contrbl at ($P < 0.01$)

DISCUSSION

Toxic metal is defined as that metal, which is neither essential nor has beneficial effect, but on the contrary, it displays severe toxicological symptoms even at low levels. With increasing industrialization, more and more metals are entering into the environment. These metals stay permanently because they cannot be degraded from the environment. They pass into the food and water and from food they ultimately make their passage into the tissues, ***Mariam et al., (2004)***

The affected sheep and goat showed low growth rate, emaciation, dullness, depression, unthriftiness, pale mucous membranes, rough coat and anorexia. These changes could be attributed to drinking salty water which lead to reduction in feed consumption, decrease in salivary flow and the adverse effect on ruminal microflora as a result of decreased ruminal pH. Moreover, the heavy metals especially cadmium, lead and iron exerted their toxicity on the animals as recorded by ***Meyer et al.,(1992) Radostits et al., (2000); Raghib et al., (2003) Yazar et al., (2006) and Okoye and Ibeto (2008).***

As shown in table (2) serum calcium and magnesium levels were significantly increased after drinking polluted water. Such results are previously obtained by ***Ibrahim et al., (1992) Thakeb et al.,(2004) and Mariam et al .,(2004)***, who reported that, the high dietary calcium and magnesium increased their serum levels. On the other hand the observed reduction in serum phosphorus could be attributed to the high dietary calcium, which increased excretion of fecal phosphorous and reduced its concentration in serum ***(Shalicka et al., 2002).*** Also, serum inorganic phosphorous was reduced by raised serum magnesium level and high

intake of potassium in the drinking water (***Raghib et al.,2003***). Significant increase in serum sodium noticed in sheep and goat may be due to high intake of sodium in water similar results were previously reported by ***(Satrug et al., 2000 and Yazar et al., 2006).***

The significant decrease reported in serum copper and zinc levels seen in sheep and goat could be attributed to the higher cadmium exposure and to the chemical interactions between cadmium, copper and zinc, as these metals share the ability to induce metallothionein (MT) synthesis and compete for the cation-binding thiol sites of MT, during intestinal absorption and after storage in the liver as reported by ***Goyer, (1997) Miranda et al .,(2005) and Swaileh et al.,(2009)*** also, it was probably due to the negative interaction of zinc on copper absorption and utilization, so that high dietary level of zinc in drinking water induced signs of copper deficiency as mentioned before by ***Sedki et al., (2003).***

The significant decrease in serum iron level in sheep and goat drinking polluted water compared to the control group recorded in table (2) was coincided with that reported by ***Jin et al., (2008) and Koratam et al., (2009)***) this may be due to low iron intake which indicates that sheep do not benefit from water as a source of iron . Moreover the high level of dietary zinc impaired iron metabolism both directly and indirectly. The direct effect of zinc on iron metabolism result from competition of zinc and iron for common binding sites in the intestine, while the indirect effect was through the reducing effect of zinc on iron which resulted in accumulation of tissue iron and reduced its serum level. ***(Arafa et al.,2008).*** On the other hand, ceruloplasmin which was markedly depressed in copper deficient animals is important in

oxidation of ferrous ions to be mobilized to serum, so iron deficiency may be secondary to copper deficiency ***(Mlay and Mgumia 2008 and Jin et al., 2008).***

In the present study in spite of elevated zinc level in polluted water significant decrease in serum zinc level in sheep and goat drinking this polluted water was recorded, this decrease could be attributed to hypoproteinaemia as most of plasma zinc is protein bound, competition of lead with essential element such as zinc, stimulation of urinary excretion of zinc and interfering with its reabsorption in kidney, inhibition of absorption of zinc due to lead –zinc interaction at the molecular level in the G.I.T. ***(Amal et al.,2008***). Also, excessive dietary magnesium stimulated the production of metalo-proteins which bound zinc and reduce zinc utilization in calves as recorded by ***Ibrahim et al., (1992).*** Multiple interaction of zinc and calcium also play a role in zinc deficiency as increased calcium intake was accompanied by decreased zinc absorption from intestinal mucosa and increased fecal excretion of zinc ***(EL-Debaky 1998; and Oluyemi et al., 2008).***

The significant changes in biochemical parameters seen in table (3) was coincided with that previously recorded by ***Bosel et al.,(2001) Arafa et al.,(2008) and Elayat and Bakheet (2010)*** they reported that, lead is known to inhibit many enzymatic activities and interfere with the synthesis of protein or RNA or both.

Impaired liver and kidney function may be attributed to the drastic conditions caused by increased hardness, high Ca and Mg levels, ***(WHO, 2003.)*** mineral and heavy metals content in the underground water ***(Amal et al .,2008 Koratam et al., 2009 and Okoye and Ugwu2010).***

In the present work the hematological picture shown in Table(4)were significantly altered .As it has been previously reported, the presence of excessive levels of lead and cadmium in the consumed water, negatively affect the hemopoietic system also, low serum iron concentration leads to anemia ***(Bosel et al., 2001 Koratam et al., 2009 and Elayat and Bakheet 2010)***.

Finally, the obtained results suggested that, nitrite and heavy metals pollution may be incriminated as causative agents of metabolic disturbances such as diabetes mellitus, liver and kidney diseases. Therefore a great deal of attention must be given to analysis of under ground water before allowing rural populations to use it as a source of drinking water for man and animals.

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التغيرات البيوكيميائية والهيماتولوجية في الأغنام والماعز المعرضة للمياه الجوفية الملوثة بمحافظة الفيوم

د/ محمود محمد عرفة- د/ سحر احمد علي د/ منى محمد علي حته- د/ اشرف شحتة درويش

معهد بحوث صحة الحيوان – الدقى – قسم الكيمياء

أجريت هذه الدراسة لتحديد الدور الذي يلعبه تلوث المياه الجوفية بالعناصر النادرة والعناصر الثقيلة علي الحالة الصحية للأغنام والماعز من كلا الجنسين في محافظة الفيوم . وقد تم إجراء هذا البحث على عدد (25) من الأغنام و (25) من الماعز التى تتراوح أعمارها ما بين2-3 سنوات بمحافظة الفيوم والتى تشرب من مياه آبار ملوثة بنسبة عاليه من المعادن الثقيلة مع وجود ارتفاع فى نسبة النيتريت بالإضافة إلى عشرة من الأغنام وعشرة من الماعز تتراوح أعمارها ما بين 2-3 سنوات وتشرب من المياه الصالحة للشرب واعتبرت كمجموعة ضابطة .

تم تجميع عدد (5) عينات عشوائية من ابار المياه بمحافظة الفيوم والمياه العذبة وتحليلها كيميائيا ولقد أظهرت النتائج وجود زيادة معنوية فى معدلات الكالسيوم – الفوسفور – الماغنسيوم – الصوديوم – البوتاسيوم – الكلوريد – النحاس – الحديد – الزنك – الرصاص – الكادميوم بالإضافة إلى نسبة النيتريت فى المياه الجوفية عنها فى المياه الصالحة للشرب.

وقد لوحظ وجود نقص فى أوزان جميع الحيوانات التى تناولت المياه الملوثة بالمعادن الثقيلة والنيتريت عن مثيلاتهاالتى تناولت مياه صالحة كما لوحظ أيضا على بعض الحيوانات هزال شديد وضعف الشهية مع خشونة فى الشعر.

أثبتت نتائج فحص مصل الدم زيادة معنوية في مستوي الكالسيوم، الماغنسيوم، الصوديوم، الكلوريد، الرصاص، الكادميوم، النيتريت مصحوبا بنقص معنوي في مستوي الفوسفور، النحاس، الحديد، الزنك. كما أظهرت النتائج أيضا انخفاض معنوى فى نسبة البروتين الكلى والألبيومن مع زيادة معنوية فى جميع نسب أنزيمات الكبد والكلية والسكر والجلوبيلين فى الحيوانات التى تشرب من المياه الملوثة مقارنة بالحيوانات التى تشرب من المياه السليمة.

وأظهر الفحص الخلوي للدم نقصا معنويا في كل من عدد الكريات الحمراء نسبة الهيموجلوبين وحجم خلايا الدم المرصوصة وكذلك نقص في العدد الكلي لكرات الدم البيضاء.

وبناء علي هذه النتائج يتضح أن تلوث المياه الجوفية بالعناصر الثقيلة أدى إلى الحالة الصحية السيئة للحيوانات مما يؤكد ضرورة الحرص علي توفير المياه الصالحة لشرب كل من الحيوان والإنسان لتجنب التأثير السلبي على الصحة و أجراء التحاليل للمياه الجوفية قبل استخدامها كمياه للشرب.