



Hazardous Effects of Lead Intoxication on Health Status, Rumen Functions, Hematological and Serum Biochemical Parameters in Egyptian Ossimi Sheep

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Abstract | Toxic heavy metals particularly Lead (Pb) have a hazardous impact on animal health and productivity because of their ill-degradability and bio-accumulation for long periods. This study carried out on 53 Ossimi sheep belonging to Giza Governorate, including 13 sheep considered as control (kept in a private farm) and 40 sheep grazed on an area polluted with lead. Drinking water, animal blood and rumen fluid samples were collected from all sheep. Complete blood, rumen and serum constituents were analyzed. Iron, copper, zinc, oxidant and antioxidant markers were evaluated and Pb levels in Drinking water, rumen fluid and serum were investigated to show the impact of Pb on those parameters. Physical examination revealed significant disturbance in health status of Pb-exposed sheep. Rumen fluid examination showed significant increase in rumen pH, significant decrease in rumen ammonia-nitrogen, TVFAs, AST, ALT, GGT, Ca and Ph. Blood constituent revealed significant alteration as significant decrease in RBCs count, Hb%, pcv %, MCHC and TLC as well as impaired hepatic and renal function and significant decrease of antioxidant markers. These alterations associated with strong positive correlation between these altered parameters and lead level in drinking water, rumen fluid and blood samples which markedly increased more than permissible limits; these results should be put in consideration in interpretation of affected animals' status and during treatment and control of lead exposed sheep cases as well as consumption of such animals' meat and offal is not recommended.

Keywords | Sheep, Lead, Blood, Rumen, Water, Antioxidants

Received | October 12, 2020; **Accepted** | October 20, 2020; **Published** | December 05, 2020

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Citation | Oraby MI, Baraka TA, Rakha GH (2021). Hazardous effects of lead intoxication on health status, rumen functions, hematological and serum biochemical parameters in egyptian ossimi sheep. *Adv. Anim. Vet. Sci.* 9(1): 48-54.

DOI | <http://dx.doi.org/10.17582/journal.aavs/2021/9.1.48.54>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

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INTRODUCTION

Environmental pollution is a major conundrum facing animal industry and of all living organisms at different trophic and systematic levels (Ferrante et al., 2017). In recent years, water contamination by toxic heavy metals such as lead (Pb) has accelerated dramatically due to natural and industrial sources (Masindi and Muedi, 2018) and subsequently, these metals reach to plants and animals and dramatically affect human health through food chain (Miedico et al., 2016). Toxic heavy metals particularly Pb have a hazardous impact on animal health and productivity

because of their ill-degradability and bio-accumulation for long periods (Milam et al., 2017).

Grazing sheep, the most abundant ruminant livestock species is one of agriculture pillars in Egypt as it can convert low-quality roughages into meat and milk for human consumption in addition to producing wool and hides (Morsy et al., 2020). Water sources for grazing sheep are irrigation canals which contaminated with discharge of industrial, agricultural and municipal wastewaters which contain several types of hazards especially heavy metals such as lead, cadmium, nickel and mercury which are considered

the most toxic for soil, plants and animals (Park and Shin, 2006).

There is a correlation between the concentration of a heavy metal (Pb) in a natural environment and its cumulative effect on the liver, kidney and blood parameters in sheep grazing in the polluted areas. The use of leaded petrol has served a continuous source of lead accumulation in sheep which may pose serious health hazards for the consumers (Abeed et al., 2019).

Ionic Pb can also affect rumen metabolism by forming lipid soluble organometallic compounds that inhibit the growth and respiration of micro-organisms (Chaudhary et al., 2006).

Progressive exposure of sheep to Pb would affect their metabolism of the Pb. The increase in Pb in rumen contents following exposure to Pb for 6 but not 9 weeks provided evidence of residues in the rumen, which may have been sequestered and passed through the rumen orifice after 6 weeks. Extended exposure reduced the digestion of long particles, which probably caused the increased buffering of rumen contents observed when alkali was added. The retardation of long particle digestion supports a toxic effect on rumen micro-organisms. DM content of the long particles was increased following long exposure to Pb, suggesting damage to acetogenic bacteria which digest the epidermal cell walls and allow moisture to be absorbed by the particle. However, despite these effects on rumen function, the duration of exposure to Pb did not affect its absorption or retention, suggesting that there were no protective mechanisms activated in the gastrointestinal tract (Phillips, et al., 2011).

Pb released into the environment via the smelting of other metals, the burning of fossil fuels, the incineration of waste materials, and the use of phosphate and sewage sludge fertilizers (Bampidis V.A. et al., 2013). Entrance and absorption of Pb take place via skin, respiratory and alimentary routes (Godt et al., 2006). Pb exposure has been speculated to affect numerous body systems especially hepatobiliary and renal system (Gunnarsson et al., 2003) causing carcinogenicity, nephrotoxicity, osteoporosis, neurotoxicity, genotoxicity, teratogenicity and endocrine and reproductive effects (European Food Safety Authority, 2009). Pb was found to be antagonist with micronutrients especially copper, zinc and iron (NRC, 2007) which are essential for all biochemical processes in the body, they are part of several enzymes, such as superoxide dismutase and glutathione peroxidase which are essential components of the antioxidant defense against oxidative stress by reactive oxygen species so protect tissues from damage (Evans and Halliwell, 2001).

Oxidative stress is the consequence of an imbalance between oxidants and antioxidants in which oxidant activity exceeds the neutralizing capacity of antioxidants (Lykkesfeldt and Svendsen, 2007). Oxidative damage positively associated with lead level and occurs even at low levels of exposure to Pb. Pb stimulate the production of free radical's reactive oxygen species (ROS) which in turn cause RBCs damage and lipid peroxidation (Ercal et al., 2001; Wang et al., 2014). Antioxidant - enzymatic and non-enzymatic- deployed in face of elevated lipid peroxidation activity to scavenge ROS to protect body cells (Pietro Celi, 2011). Studies on heavy metals pollution especially Pb were applied under experimental conditions (Stoev et al., 2003), and investigation of its effect on farm animals especially grazing sheep under natural practical condition rarely conducted (El-Sharkawy et al., 2008). Therefore, this study aimed to investigate the effect of water pollution with Pb on health status, rumen function, hematological parameters and serum constituents including hepatic, renal function tests and oxidant-antioxidant status of grazing sheep as well as correlation between Pb level and all parameters.

MATERIALS AND METHODS

ETHICAL APPROVAL

The current study was approved by Veterinary Medicine Cairo University Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Cairo University, Egypt (VET CU 16072020202).

ANIMALS AND CLINICAL EXAMINATION

In Giza Governorate, a total number of 53 Ossimi sheep aging between 3-5 years with average weight 35-45 Kg were divided into two groups: 13 sheep raised in non-polluted grazing area (control group) and 40 sheep raised on grazing in rural polluted areas (exposed group). History of flocks concerning water sources, nutrition and management were recorded before the animals being clinically examined. All animals were subjected for careful clinical investigation including general body condition, inspection of visible mucous membranes of eye and examination of the skin and wool coat. Examination extended for other vital signs of health including pulse, respiratory rates, body temperature and examination of ruminal motility.

SAMPLES AND LABORATORY INVESTIGATIONS

Blood samples were collected by puncture of jugular vein and divided into two portions. First portion collected on EDTA tubes for hematological examination including total erythrocytic count (RBCs), hemoglobin content (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total leucocytic count (TLC) using automated hema-

tology analyzer and the second portion collected in plain tubes for serum separation. Separated serum used for estimation of total protein, albumin, Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Gamma Glutamyl Transpeptidase (GGT), Blood Urea Nitrogen (BUN), Creatinine, calcium (Ca) and inorganic phosphorous using specific kits produced by SPECTRUM Co., Egypt, according to the method described by (Young and Friedman, 2001). Globulin, A/G ratio and BUN/Creatinine ratio were calculated. Rumen fluid samples were collected from each animal via stomach tube and suction pump; immediately examined for color, odor, consistency, pH and protozoal activity according to method described by (Alonso, 1979). Total volatile fatty acids (TVFAs) estimated by steam distillation method as described by (Eadie et al., 1967). Rumen NH₃-N was determined calorimetrically using specific kits produced by SPECTRUM Co., Egypt according to the method described by (Chaney and Marbach, 1962). Activity of AST, ALT and GGT and levels of calcium and phosphorus were determined in clear supernatant after separation of rumen fluid according to (Young and Friedman, 2001) using specific kits produced by SPECTRUM Co., Egypt. Water samples (50 ml) of drinking water were collected (six water samples from polluted area and one from non-polluted area). The technique of water sampling was conducted according to the recommendation of American Public Health Association (APHA, 2012). Pb level was estimated in water, rumen fluid and blood samples by Flame Atomic Absorption Spectrometer (model SensAA, GBC, Australia) in Micro Analytical Center, Faculty of Science, Cairo University, Egypt. All glass tubes and plastic containers used in this study for mineral estimation were cleaned twice with diluted HNO₃, then rinsed with deionized distilled water twice and then air-dried before using.

STATISTICAL ANALYSIS

Normality, mean values, standard errors (SE), Correlation coefficient and significance of correlation were calculated using (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Independent samples T-test was applied to make comparison between the mean values of groups to check the significance. The results were represented as means \pm SE and P value \leq 0.05 and \leq 0.01 considered significant.

RESULTS AND DISCUSSION

Regarding history and clinical examination, the most obvious observations are depressed appetite, illthriftiness, weakness, depression and rough wool. Disturbance in health status of these sheep in the form of emaciation, dullness, debility and other anemic changes especially pale mucous membranes could be attributed to the high level of heavy

metals especially Pb. These findings agree with (Ali, 2005) and (Bayoumi et al., 2013) who recorded similar results. Vital signs examination showed mild increase in respiratory rate which agreed with (Ali, 2005), moreover mild increase in pulse. In spite of non-significant mild increase in respiratory and pulse rate, physical examination revealed normal CH sound of lung and normal heart beats (70-90/min.). This may be attributed to normocytic normochromic anemia which reflected by significant decrease in RBCs count, Hb% and pcv % recorded in such animals. In spite of previous obtained data including decrease of body condition scores, pale mucous membrane, slight increase of pulse and respiratory rate that were recorded in grazed sheep, some owners neglect or give no attention to that changes in clinical signs specially it appears only with pushing sheep to walk or to run with effort. Owners when notice these signs kept the sheep in door and prevent them from grazing. Owners may be tried to treat these sheep while others get rid of them by selling or slaughtering. So, we could not obtain any sheep aged more than five years in this study.

Hematology showed significant decrease in RBCs count, Hb%, pcv %, MCHC and TLC, along with non-significant decrease in MCV and MCH in exposed group compared to control group, moreover blood Pb concentrations recorded a significant negative correlation with RBCs count, Hb%, pcv % and TLC as showed in Table 1. This finding agrees with (Bayoumi et al., 2013) and (Abeed et al., 2019).

Physical examination of rumen fluid revealed that physical characters were whitish green to brownish color according to type of ration, watery to sticky in consistency according to drinking state accompanied with aromatic to offensive odor as well as the protozoa were microscopically low in number and motility in grazed sheep compared to control ones. This may indicate that a lower supply of lead had significant negative influence on the production of protozoa in the rumen fluids of sheep leading to decrease in feed intake and consequently decrease in rumen contraction and this agree with (Sviatko and zelenak, 2000), also chemical examination of rumen fluid revealed significant increase in pH associated with significant decrease in ammonia-nitrogen, TVFAs, AST, ALT, GGT, Ca and Ph in exposed group compared to control group along with a significant negative correlation with ammonia-nitrogen, TVFAs, AST, ALT, GGT, Ca and Ph and a significant positive correlation with pH as shown in Table 2. Significant decrease in rumen ammonia nitrogen in grazed sheep compared to control group may be attributed to decrease in urease activity due to high level of lead leading to reducing the rate of ammonia nitrogen release from dietary urea and this urea accumulated in the rumen and not converted to ammonia,

Table 1: Mean values ±S.E. of RBCs, PCV, Hb, MCV, MCH, MCHC and TLCs in both control and exposed sheep and correlation with blood Pb.

	Control sheep	Exposed sheep	blood Pb
RBCs(×10 ⁶)	10.93 ± 0.18	9.95 ± 0.07**	-.458**
PCV%	29.85 ± 0.60	24.80 ± 0.26**	-.723**
Hb%	9.94 ± 0.13	8.17 ± 0.09**	-.691**
MCV(fl)	27.33 ± 0.39	24.99 ± 0.33**	-.498**
MCH(pg)	9.11 ± 0.10	8.23 ± 0.11**	-.462**
MCHC (%)	33.37 ± 0.33	33.08 ± 0.44	-.043
TLC(×10 ³)	7.10 ± 0.15	5.53 ± 0.10**	-.557**

* (P ≤ 0.05)

** (P ≤ 0.01)

Table 2: Mean values ±S.E. of Rumen PH, TVFA, ammonia N, AST, ALT, GGT, Ca and Ph in both control and exposed sheep and correlation with rumen Pb.

	Control	Exposed	rumen Pb
Rumen PH	6.50 ± 0.07	7.10 ± 0.02**	+.637**
TVFA	56.05 ± 0.34	39.74 ± 0.73**	-.668**
ammonia N	5.25 ± 0.06	4.17 ± 0.05**	-.693**
AST (IU/L)	0.25 ± 0.01	0.19 ± 0.00**	-.645**
ALT (IU/L)	0.51 ± 0.01	0.40 ± 0.00**	-.697**
GGT (IU/L)	0.12 ± 0.00	0.10 ± 0.00**	-.411**
Ca (mg/dl)	1.45 ± 0.04	0.88 ± 0.01**	-.786**
Ph (mg/dl)	6.54 ± 0.06	5.05 ± 0.06**	-.775**

* (P ≤ 0.05)

** (P ≤ 0.01)

this agree with (Faix et al., 2005), (Faixova et al., 2006) and (Phillips, et al., 2011). Significant decrease in rumen TVFAs in grazed sheep compared to control group may be attributed to high level of Pb which cause damage to rumen microbiota and decrease fermentation, this prove decrease in energy intake leading to poor body condition scores, emaciation and weakness and this finding in agreement with (Sviatko and Zelenak, 2000; Faix et al., 2005; Faixova et al., 2006; Phillips, et al., 2011; Mousa, 2014). Significant increase in ruminal pH of grazed sheep compared to control group may be attributed to decrease of volatile fatty acids, drinking drainage water which mainly of more alkaline pH or accumulation of urea in rumen due to inhibition of urease activity as recorded by Faixova et al. (2006). These results indicate that long term exposure to pollutants in drainage areas can alter the ruminal parameters of sheep and these finding agreed with Sviatko and Zelenák (2000). Published papers on effect of lead on rumen functions under natural conditions are very scare but presence of correlation between lead level and rumen parameters may suggest these all changes occurred.

Serum biochemical estimation showed significant decrease in total protein, albumin, globulin, calcium and inorganic phosphorus along with significant increase in AST, ALT,

GGT, BUN and creatinine in exposed group compared to control. Furthermore, blood Pb was significantly correlated in a negative mode with serum total protein, albumin, globulin, calcium and inorganic phosphorus and a significant positive correlation with AST, ALT, GGT, BUN and creatinine as showed in Table 3. These results agreed with the literatures (Bayoumi et al., 2013; Abeer et al., 2019).

The most acceptable explanation to such increase in enzymatic activities could be attributed to the destructive effects of Pb on liver and kidney tissues and consequently liberating their intra cellular enzymes in to the blood stream (Stoev et al., 2003). This confirmed by the strong positive correlations between blood Pb with serum AST, ALT and GGT.

The decrement in serum calcium and inorganic phosphorus could be attributed to the toxic effect of ingested Pb on the mucosal epithelial cell of gastrointestinal tract causing decrease of intestinal absorption of both calcium and phosphorus (Abdel- Azeem and Hafez, 2006).

Trace element status of grazing sheep showed a significant reduction in serum iron, copper and zinc compared to control group and these results could be owed to the competi-

Table 3: Mean values ±S.E. of serum T. Protein, Albumen, Globulin, A/G Ratio, AST, ALT, GGT, BUN, Creatinine, BUN / Creat. Ca and Ph in both control and exposed sheep and correlation with blood Pb.

	Control sheep	Exposed sheep	blood Pb
T. Protein (g/dl)	7.19 ± 0.10	5.95 ± 0.06**	-.717**
Albumen (g/dl)	3.48 ± 0.03	2.72 ± 0.04**	-.684**
Globulin (g/dl)	3.70 ± 0.10	3.23 ± 0.07*	-.395**
A/G Ratio	0.95 ± 0.03	0.87 ± 0.03	-.099
AST (IU/L)	69.62 ± 1.42	100.10 ± 1.39**	+.711**
ALT(IU/L)	24.72 ± 0.79	39.98 ± 0.71**	+.805**
GGT(IU/L)	36.30 ± 1.69	72.66 ± 1.07**	+.866**
BUN (mg/dl)	21.33 ± 0.47	35.55 ± 0.41**	+.859**
Creatinine (mg/dl)	0.73 ± 0.03	1.02 ± 0.02**	+.633**
BUN / Creat.ratio	29.89 ± 1.37	35.42 ± 0.71	+.430**
Ca (mg/dl)	9.99 ± 0.12	7.68 ± 0.05**	-.830**
Ph (mg/dl)	5.98 ± 0.10	4.71 ± 0.05**	-.769**

* (P ≤ 0.05)

** (P ≤ 0.01)

Table 4: Trace elements, oxidant and antioxidants levels in both control and exposed sheep and correlation with blood Pb.

	Control sheep	Exposed sheep	Blood Pb
Fe (PPM)	1.46 ± 0.03	0.69 ± 0.04**	-.732**
Zn (PPM)	0.86 ± 0.01	0.56 ± 0.01**	-.747**
Cu (PPM)	0.20 ± 0.01	0.10 ± 0.00**	-.729**
serum MDA (nmol/ml)	0.46 ± 0.01	0.89 ± 0.01**	+.853**
SOD (U/ml)	1.39 ± 0.03	0.88 ± 0.02**	-.837**
CAT (U/l)	57.62 ± 1.15	37.32 ± 0.66**	-.759**
GSH-PX (mu/ml)	1.07 ± 0.05	0.39 ± 0.01**	-.822**
Vitamin A (µg/dl)	85.68 ± 2.32	60.83 ± 0.75**	-.717**
Vitamin E (umol/l)	24.85 ± 0.69	15.20 ± 0.28**	-.809**
Vitamin C (mg/dl)	1.38 ± 0.07	0.76 ± 0.02**	-.720**

** (P ≤ 0.01)

PPM: part per million

Table 5: Pb level in drinking water, rumen fluid and blood of both control and exposed sheep and its correlation with each other.

	Control sheep	Exposed sheep	Water Pb	rumen Pb
Water Pb (PPM)	0.058 ± 0.023	0.91 ± 0.10**		
rumen Pb (PPM)	0.78 ± 0.04	1.80 ± 0.04**	+.956**	
blood Pb (PPM)	0.25 ± 0.02	0.80 ± 0.02**	+.877**	+.995**

** (P ≤ 0.01)

-tion of Pb with those biometals together with the reduction in appetite observed in exposed animals as showed in Table 4. These results agreed with the literatures (El-Sharkawy et al., 2008; Bayoumi et al., 2013). The decrease in zinc level may be attributed to hypoproteinaemia where most of plasma zinc is protein bound and this supported by the negative correlation recorded between serum copper, iron and zinc with blood Pb. These results confirmed

that study interaction of heavy metals with essential elements help to interpret the relation between heavy metals and the nutritional problems occur in the exposed animals. Thus, supplementation of copper, iron and zinc is essential in treatment and prevention of toxic effect of Pb.

Oxidant status of grazing sheep showed significant increase in serum Malondialdehyde (MDA) levels which in

accordance with those previously mentioned by Kanter et al. (2009) and Bayoumi et al. (2013). Such elevation could be attributed to lipid peroxidation caused by Pb. The strong positive correlations between serum and MDA with blood Pb concentrations proved the fact that MDA is a marker for lipid peroxidation caused by Pb intoxication as recorded by Deger et al. (2009) and Crnogaj et al. (2010).

Antioxidant parameters showed significant decrease in SOD, CAT, GSH-Px, Vit.A, Vit.E and Vit.C in exposed group compared to control. And also blood Pb concentrations recorded a significant negative correlation with SOD, CAT, GSH-Px, Vit.A, Vit.E and Vit.C as showed in Table 4. The obtained results agreed with those recorded by Ercal et al. (2001), Ali (2005) and Bayoumi et al. (2013).

Pb level estimation showed significant increase in polluted water, rumen fluid and blood in exposed sheep compared with control group and a significant positive correlation between Pb level in water, rumen and serum as showed in Table 5.

Polluted water revealed significant increase in Pb concentrations which exceed the corresponding values in water collected from non-polluted water and exceed the recommended permissible limits recorded by WHO (2004). The obtained results were in accordance to those reported by Ali (2005) and Bayoumi et al. (2013). The high concentrations of Pb in drainage water could be attributed mostly to the high level of pollution from continuous discharge of sewage, industrial and agriculture effluents. Agricultural wastes include a wide range of organic materials (often containing pesticides), animal wastes, sewage sludge, and phosphate fertilizer beside industrial wastes and its contents from heavy metals. This high-risk value indicated that many notices, awareness, laws, information and impressive attention should be taken to use this drainage water to plant irrigation or animal drinking rather than for human consumption.

Significant increase in ruminal lead level in grazed sheep compared to control group nearly agree with Faix et al. (2005), who recorded high Pb level in rumen wall of grazed sheep.

Mean values of blood Pb concentrations showed significant increase in exposed sheep compared to control group. Our results agreed with those recorded by (Bayoumi et al., 2013) and (Abeed et al., 2019).

CONCLUSION

Exposure of sheep to lead pollutant has adverse effect on body condition scores, rumen functions, liver and kidney

functions; reduction in antioxidants activity which enhances occurrence of infectious and non-infectious diseases. Significant alterations and correlations were detected in this study; these results should be put in consideration in interpretation of affected animals' status and during treatment and control of Pb exposed sheep cases as well as consumption of such animals' meat and offal is not recommended.

ACKNOWLEDGEMENTS

The authors wish to thank the micro analytical center, Faculty of science, Cairo University for providing facilities for analysis of samples for lead estimation and Laboratory of Rumenology, Department of m=Medicine, Faculty of Veterinary Medicine, Cairo University for analysis of rumen samples.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

Gamal Rakha designed the work and collected the data, Taher Baraka manipulate the data and results and write the manuscript, Mohamed Oraby collected and analysed the samples and made statistical analysis for results.

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