

Original Research

Assessing Lead (Pb) Residues in Lohi Sheep and its Impact on Hematological and Biochemical Parameters

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Abstract

Pb, a major environmental pollutant, can bio-accumulate in different tissues of food animals. The present study was accomplished to investigate the presence of Pb residues in various tissues of Lohi sheep ($n = 360$) and its deleterious effects. The blood and tissue samples (kidney, liver, and muscles) were collected from a local slaughterhouse for 6 weeks and analyzed through flame atomic absorption spectrophotometry. The Pb concentration was determined to be maximum in kidney samples, followed by liver, serum, and muscle. In our analysis, 82.78% of muscle samples showed Pb concentrations below the maximum permissible limit (1 mg/kg) by the Australia New Zealand Food Authority (ANZFA). While values of serum ALT, AST, ALP, and urea were within normal range. In all the samples, RBC, TLC, PCV, and Hb values were significantly lower than control ($P < 0.05$). Based on the above results, Lohi sheep seems to be quite resistant to the deleterious effects of Pb; however, its edible offal and lean muscle could pose a serious risk to public health.

Keywords: Pb, hematology, tissues, Lohi sheep, slaughterhouse

Introduction

Pb is a bluish-gray heavy metal naturally found in the earth's crust in small traces. The effluents originating from recycling plants of lead-based batteries are an emerging source of lead toxicity – especially in developing countries like Pakistan [1]. Various herbal products and medicinal plants have been found to be a

source of lead accumulation in animals and humans [2]. Lead is disseminated from natural reservoirs to biological systems via water, forage, and supplements. The contaminated forage, concentrates, air, dust, insecticides, fertilizers, household wastes, and industrial effluents are sources of heavy metal pollution.

Pb has well-known hazardous effects on human and animal health as it accumulates in the body via the food chain [3]. It can seriously affect the hematopoietic, renal, reproductive, nervous, and hepatic tissues to produce metabolic interactions with enzyme systems

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[4]. Pb is considered the major environmental pollutant that has been a leading cause of accidental poisoning in domestic animals. Meanwhile, lead poisoning has been well documented in domestic animals from contaminated forage and supplements. In ruminants, the acidic environment of the fore-stomach enhances its absorption and thus marks it as extremely toxic. The acute Pb poisoning proved to be life-threatening in animals, but chronic cases are producing severe intoxication in the human population [1].

Animal products are a major source of Pb transmission from animals to humans, and the deficiency of essential elements in the human body enhances Pb absorption [5]. According to reports, animals with blood Pb concentration of ≥ 0.20 $\mu\text{g/ml}$ may remain asymptomatic, but their products like milk and meat cause intoxication in humans. It has been reported that the milk and meat of animals grazing in contaminated areas have higher Pb concentrations than others [6]. Among domestic animals, sheep exhibit more chances of ingestion of Pb due to grazing of herbage very close to the ground surface. More likely, it can ingest contaminated soil at rates exceeding the recommended safety threshold limit. Sheep have been found to excrete more Pb (and cadmium) compared to other animals [7]. Lohi is one of the best sheep breeds of Pakistan which is famous for rapid growth and good quality meat. Its general body color is white with a large reddish-brown head [8].

The higher Pb concentrations in drinking water, soil, sewage water, agricultural products, and animal products have been analyzed from various Districts of Pakistan [9]. Pakistani phosphate rocks contain higher levels of Pb and are being used for the production of phosphorus-based fertilizers, detergents, acids, and other products of common use. A higher Pb level in meat and milk from different areas of Pakistan has also been reported [10], but there is no report on the hematological and biochemical effects of Pb in small ruminants – especially indigenous sheep of Pakistan.

Pb concentrations above permissible levels have been reported in many regions of Pakistan. The transfer of Pb from water and the food chain has been declared to be a serious threat for animals and humans [11]. There was dire need to unveil the consequences of Pb residues in local breeds in order to denote changes in the natural environment of Pakistan. This paper describes the Pb residues in various tissues and its deleterious effects in Lohi sheep.

Materials and Methods

Selection of Animals

For this study we included adult sheep of the Lohi breed, aged 1-2 years, physically healthy, and weighing 40-50 kg were presented for slaughter at the district slaughterhouse (SH) of Jhang, Pakistan. A total of 12 sheep including (n = 8) female and (n = 4) males were randomly selected

on a first-come basis each working day. As per the law of Punjab Province, the slaughterhouse works five days weekly Thursday to Monday, for a total of (n = 60) sheep in a week and (n = 360) sheep during six weeks of study. Similarly, a total of (n = 12) sheep (8 female and 4 male) were reared at the College of Veterinary and Animal Sciences (CVAS) in Jhang for 8 weeks under optimum conditions to serve as control.

Collecting Blood and Serum Samples

After a thorough antemortem inspection, a total of (n = 360) blood samples were collected aseptically from the jugular vein of each sheep using a disposable syringe attached to a hypodermic needle. The blood samples were drawn into EDTA-coated vacutainer (purple topped) and gel-coated vacutainer (yellow topped) separately. After clotting, the blood samples in yellow-topped vacutainers were centrifuged at 3,500 rpm for 5 minutes. The clear straw-colored supernatant (serum) was collected using a pasture pipette and stored in eppendorf tubes at -20°C until analysis. The blood and serum samples were also collected from control sheep and were analyzed in the Pathology Laboratory at CVAS in Jhang, Pakistan.

Tissue Sample Collection

After slaughter, the carcass and viscera were examined for gross lesions and a total of (n = 360) samples from liver, kidney, and muscle were obtained from the selected sheep at a slaughterhouse. The tissue samples were also collected from the sheep of the control group after slaughtering. The tissue samples were frozen for further processing at CVAS, Jhang.

Detecting Pb Residues in Serum and Tissues

The serum and tissue samples were subjected to wet digestion method [12]. The digested samples were transported to the Central Hi-Tech Laboratory at the University of Agriculture in Faisalabad for Pb analysis using flame atomic absorption spectrophotometry [13]. The average of three values for absorbance was used for final quantification. The process was standardized by running the standard of known concentrations after each 20 samples.

Hemato-Biochemical Analysis

All the unclotted blood samples were subjected to hematological analysis. Blood samples were tested for total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), hemoglobin estimation (Hb), pack cell volume (PCV), and erythrocyte sedimentation rate (ESR). Blood samples were analyzed within 8 hours of collection by using an automated hematology analyzer standardized for analyzing ovine blood samples. Erythrocyte sedimentation rate and

Table 1. Lead (Pb) concentration (mean±SE) in kidney, liver, muscle, and serum of Lohi sheep.

Week	Kidney (mg/kg)	Liver (mg/kg)	Muscle (mg/kg)	Serum (mg/L)
Week 1 (n=60)	2.37±0.241 ^a	1.06±0.171 ^a	0.90±0.140 ^a	1.04±0.132 ^a
Week 2 (n=60)	1.78±0.157 ^{ab}	0.92±0.153 ^a	0.58±0.088 ^a	0.88±0.088 ^a
Week 3 (n=60)	2.35±0.489 ^{ac}	0.96±0.154 ^a	0.44±0.089 ^a	0.78±0.103 ^a
Week 4 (n=60)	1.99±0.379 ^{bd}	0.88±0.134 ^a	0.44±0.076 ^a	0.75±0.042 ^a
Week 5 (n=60)	1.56±0.230 ^{bde}	0.96±0.136 ^a	0.66±0.095 ^a	0.90±0.101 ^a
Week 6 (n=60)	1.81±0.315 ^{bdef}	0.94±0.125 ^a	0.44±0.063 ^a	0.80±0.098 ^a
Control (n=12)	1.51±0.054 ^{bdefg}	0.79±0.030 ^a	0.61±0.030 ^a	0.63±0.116 ^a

Values with different letters (a-g) within a column differ significantly ($P<0.05$).

differential leukocyte count were performed manually according to the standard procedure [14].

The serum samples were tested for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine using the commercially available kit (DiaSys, Germany).

Statistical Analysis

The data was analyzed by IBM SPSS Statistics version 21. The values of different parameters were compared by using the independent-samples Kruskal-Wallis test due to the asymmetric nature of the data [15].

Results and Discussion

Pb concentrations in kidney liver, muscles, and serum are given in Table 1. The overall mean concentration of Pb was in the order: kidney > liver > serum > muscle, and ranged 1.51±0.054-2.37±0.241, 0.79±0.030-1.06±0.171, 0.44±0.063-0.90±0.140, and 0.63±0.116-1.04±0.132 mg/kg in kidney, liver, muscles, and serum, respectively. The kidney, liver, and serum samples showed non-significantly higher Pb concentrations than

control. The highest Pb concentrations in kidney, liver, muscle, and serum were observed during the first week of sampling from SH.

On an individual sample basis, the 50.00-80.00% kidney, 26.67-40.00% liver, and 10.00-26.67% muscle sample from the slaughterhouse during a period of 6 weeks showed Pb concentrations above the permissible limits of 1 mg/kg [16]. When the Pb concentration in each sample was compared with maximum permissible limits of 0.5 mg/kg for edible offal and 0.1 mg/kg for lean meat [17-18], the 66.67-96.67% kidney, 60.00-73.33% liver, and 66.67-90.00% muscle samples showed higher Pb concentrations than the international permissible levels (Table 2).

The edible offal and lean muscle (37.78, 65.00, and 82.78%, respectively) showed Pb concentrations below the permissible limit of 1 mg/kg for food items [16]. The lean meat showed the highest ratio of samples with safe level of Pb residues (below the permissible limit) and hence provided evidence of safety for consumers (Table 3).

Pb has known toxic effects on many systems of the body, including kidney, liver, and muscles of food animals. The presence of Pb residues in edible organs of a food animal is an important aspect of public health

Table 2. The percentage of samples containing Pb level (week) above the internationally recommended permissible limits.

Week	Kidney (%)		Liver (%)		Muscle (%)	
	>1mg/kg*	>0.5 mg/kg**	>1mg/kg*	>0.5 mg/kg**	>1mg/kg*	>0.1 mg/kg***
Week 1	73.33	96.67	40.00	66.67	26.67	80.00
Week 2	80.00	90.00	26.67	66.67	13.33	73.33
Week 3	53.33	66.67	40.00	60.00	16.67	66.67
Week 4	50.00	70.00	33.33	63.33	13.33	73.33
Week 5	60.00	70.00	30.00	70.00	20.00	90.00
week 6	60.00	73.33	40.00	73.33	10.00	80.00

*Maximum permissible Pb level recommended by ANZFA, 2001 for food items

**Maximum permissible Pb level recommended by WHO/FAO for edible viscera

***Maximum permissible Pb level recommended by WHO/FAO for lean muscle

Table 3. Kidney, liver, and muscle samples (360) showing variable Pb concentrations.

Pb residues	Kidney (%)	Liver (%)	Muscle (%)
Below detection level (ND)	10.56	22.22	22.78***
0.10-0.50 mg/kg	11.67	11.67	30.56
	10.56+11.67 = 22.23**	22.22+11.67 = 33.89**	
0.60-1.00 mg/kg	15.55	31.11	29.44
	10.56+11.67+15.55 = 37.78*	22.22+11.67+31.11 = 65.00*	22.78+30.56+29.44 = 82.78*
>1.00 mg/kg	62.22	35.00	17.22

*Samples (%) possessing Pb level below the maximum permissible limit (1mg/kg) by ANZFA for food items

** Samples (%) possessing Pb level below the maximum permissible limit (0.5mg/kg) by WHO, FAO for offal

*** Samples (%) possessing Pb level below the maximum permissible limit (0.1mg/kg) by WHO, FAO for lean muscle

Table 4. Values (Mean±SE) of hematological parameters of Lohi sheep.

Week	RBC 10 ⁶ /μL	TLC 10 ³ /μL	Hb g/dL	PCV %	ESR mm/hr
Week 1	8.69±0.304 ^a	0.96±0.089 ^a	9.61±0.349 ^a	20.93±0.703 ^a	1.20±0.026 ^a
Week 2	8.67±0.388 ^a	1.69±0.151 ^b	9.53±0.447 ^a	20.32±0.890 ^a	1.18±0.022 ^a
Week 3	8.96±0.162 ^{ab}	2.57±0.253 ^{bc}	9.92±0.196 ^b	20.99±0.574 ^a	1.20±0.026 ^a
Week 4	8.60±0.182 ^{ab}	2.63±0.256 ^{bcd}	9.33±0.139 ^{ab}	19.47±0.723 ^a	1.23±0.018 ^a
Week 5	7.90±0.172 ^{ac}	1.76±0.177 ^{bcd}	8.67±0.318 ^{ac}	18.48±0.707 ^a	1.26±0.018 ^a
Week 6	8.82±0.346 ^{abc}	0.89±0.102 ^{af}	9.04±0.296 ^{abc}	21.39±0.849 ^a	1.22±0.027 ^a
Control	10.11±0.962 ^d	5.68±0.615 ^g	11.35±0.585 ^d	35.93±1.769 ^b	1.30±0.073 ^a

Values with different letters (a-g) within a column differ significantly ($P<0.05$)

[19]. The consumption of Pb-contaminated meat usually produces cumulative effects on consumers [12].

The edible offal and muscle Pb concentration was higher than the permissible limits of 0.1 mg/kg for muscle meat and 0.5 mg/Kg for offal as set by WHO, FAO, and E.O.S [20-21] in the present study. The similar findings of higher Pb concentrations were also observed by other researchers [22-24] in edible offal and muscles of sheep and other food animals.

In the present study, kidney showed the highest concentration of Pb residues, followed by liver and muscle showing the least tendency for Pb accumulation, which was similar to the Pb residues in edible tissues of sheep [10] from KPK, Pakistan. The 82.78% muscles samples exhibited Pb concentration below the permissible limit of 1 mg/kg [16], hence representing the evidence of more safety for the consumers in this trial. The same results were also reported in a mining area [25] where the

Table 5. Values (Mean±SE) of differential leukocyte count (DLC) of Lohi sheep.

Week	Neutrophil %	Eosinophil %	Monocyte %	Lymphocyte %
Week 1	21.64±0.527 ^a	1.41±0.023 ^a	3.02±0.114 ^a	73.92±0.541 ^a
Week 2	18.59±0.286 ^b	1.68±0.087 ^{ab}	2.87±0.112 ^{ab}	76.94±0.337 ^b
Week 3	19.89±1.447 ^{bc}	1.63±0.103 ^{abc}	3.44±0.091 ^{ac}	75.01±1.471 ^{abc}
Week 4	20.90±1.296 ^{abcd}	1.45±0.104 ^{abcd}	3.19±0.087 ^{abc}	74.45±1.378 ^{abcd}
Week 5	17.01±0.638 ^{bcd}	1.21±0.030 ^{de}	3.06±0.074 ^{abce}	78.70±0.660 ^{abcde}
Week 6	21.75±0.374 ^{adf}	1.08±0.017 ^{ef}	3.37±0.108 ^{abcef}	73.78±0.421 ^{adf}
Control	23.08±1.381 ^{afg}	5.55±0.632 ^g	4.20±0.259 ^g	67.16±1.587 ^g

Values with different letters (a-g) within a column differ significantly ($P<0.05$)

Table 6. Values (Mean \pm SE) of ALT, AST, ALP, urea, and creatinine in serum of Lohi sheep.

Week	ALT U/L	AST U/L	ALP U/L	Urea mg/dL	Creatinine mg/dL
Week 1	43.9 \pm 1.447 ^a	35.9 \pm 1.156 ^a	277.6 \pm 12.037 ^a	32.0 \pm 1.542 ^a	0.81 \pm 0.027 ^a
Week 2	36.1 \pm 1.534 ^b	25.6 \pm 0.488 ^b	218.0 \pm 14.179 ^b	29.7 \pm 1.121 ^{ab}	0.74 \pm 0.017 ^a
Week 3	31.8 \pm 1.411 ^{bc}	25.4 \pm 0.757 ^{bc}	220.9 \pm 13.961 ^{bc}	35.7 \pm 1.285 ^{ac}	0.81 \pm 0.030 ^a
Week 4	43.5 \pm 1.436 ^{abd}	33.3 \pm 2.723 ^{abcd}	224.0 \pm 15.786 ^{bcd}	35.6 \pm 1.069 ^{acd}	0.79 \pm 0.023 ^a
Week 5	36.8 \pm 3.896 ^{abcde}	33.6 \pm 1.012 ^{ade}	238.9 \pm 8.600 ^{abcde}	34.2 \pm 0.559 ^{abc}	0.79 \pm 0.022 ^a
Week 6	40.4 \pm 3.263 ^{abcdf}	34.4 \pm 2.415 ^{ade}	277.9 \pm 12.936 ^{acf}	33.6 \pm 1.871 ^{abc}	0.82 \pm 0.027 ^a
Control	36.2 \pm 1.301 ^{bceef}	28.3 \pm 2.290 ^{abbcde}	235.5 \pm 6.994 ^{bcdeg}	33.2 \pm 0.654 ^{abc}	0.80 \pm 0.068 ^a

Values with different letters (a-g) within a column differ significantly ($P < 0.05$)

muscles showed Pb concentration below the permissible limits of international standards.

The serum Pb concentration was found to be higher than the reference range of 0.05 to 0.25 mg/L [26]. The findings of the present study are in agreement with the findings of other researchers [19, 23] who also reported higher Pb concentrations in blood of different animals.

The values of hematological analysis are given in Table 4. The values of RBC, TLC, Hb, and PCV were significantly ($P > 0.05$) lower than the control, whereas ESR showed a non-significant difference. Neutrophil, eosinophil, and monocyte were significantly ($P > 0.05$) lower, but lymphocyte count was significantly ($P < 0.05$) higher than control (Table 5). The sheep during week 5 showed the lowest values of RBC, Hb, and PCV and neutrophil count.

In the present study, RBC, TLC, PCV, and Hb were decreased, but ESR showed a normal range that was similar to previous findings [27]. A possible reason for lower hematological tests in our study might be due to the uptake of Pb by sheep in a natural environment. In agreement with the present study, sheep showed lower hematological values [28-29] due to Pb intoxication. However, in contrast to our findings, higher TLC [28, 30] has been observed in rat and sheep by different workers. The higher lymphocyte and lower eosinophil counts in our study were in line with the findings of two different researchers [30-31], but disagreed with the findings of the same researchers as they found higher neutrophil and monocyte counts in contrast to our findings.

The values of biochemical parameters – including ALT, AST, ALP, urea, and creatinine – are given in Table 6. The values of all biochemical parameters were observed within the normal range set by different international laboratories. The ALT and AST values were highest and urea was lowest during week one of sampling from SH. The samples of week 6 showed the highest values of ALP and creatinine in this study.

The ALT, AST, and ALP concentrations in the present study were comparable with the normal range, which disagreed with the findings of previous studies

that observed higher enzyme levels in rat [32-33]. This variation might be due to breed difference as Lohi sheep seemed to be more resistant in the natural environment at observed Pb concentrations in the present study.

The serum urea concentration exhibited the normal range in the present study, which varied from higher serum urea level in Pb toxicity in albino rats [28]. This difference might be due to species variation or variable pollution status of the environment. It may also reflect the level of contamination in a slaughterhouse or grazing behavior of sheep. The higher serum creatinine values in the present work were in line with the already reported values [34] where the higher serum creatinine level in Pb toxicity in animals was observed.

Conclusion

It can be summarized that sheep during week one showed the highest levels of liver Pb residues, ALT, and AST, whereas the lowest values were seen during week 3 from the slaughterhouse. Similarly, there was higher kidney Pb and serum urea concentration during week 6 of the trial. So, it can be concluded that Pb may be transferred from a contaminated environment to animals in low doses and accumulate in tissues. Adult Lohi sheep were apparently healthy on antemortem and postmortem inspection; hence it possessed the tendency for accumulation of Pb above the permissible limits in edible tissues without showing the clinical manifestations. Its products like milk and meat may be a source of Pb residues for consumers and hence demand the necessary measures to safeguard public health.

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