

## Effect of lead on thyroid function in sheep

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### Summary

The objective of this experiment was to determine the effect of long-term low-dose administration of lead (Pb) on thyroid function in sheep. In this experiment 10 healthy Iranian male sheep, aged about one-year-old were randomly allocated into two equal groups of control (n = 5) and experiment (n = 5). Both groups were kept under the same conditions of food and environment. The treatment group received lead acetate (5 mg/kg/day) orally for 8 weeks. Blood sampling of two groups was done on the same days, on days 0 (before receiving lead acetate in treatment group), 14, 28, 42, 56 and 70 after lead administration, every morning (8 to 9 a.m.). The function of the thyroid was evaluated by measuring the levels of serum thyroid hormones T3, T4, FT3, FT4 and TSH. The hepatic function in both groups was evaluated by measuring hepatic enzyme activities including alanin amino transferase (ALT), aspartate aminotransferase (AST),  $\gamma$  glutamyl transferase (GGT), and total bilirubin (TBIL). Serum levels of T3 (on days 14, 56 and 70) T4 (on days 14, 28, 42, 56 and 70), FT3 (on days 56 and 70), FT4 (on days 42, 56 and 70) and TSH (on days 14, 28, 42, 56 and 70) significantly decreased in lead-treated sheep when compared to the control group (P<0.05). Serum enzyme activities of ALT (on days 28 and 42), AST (on days 28, 42, 56 and 70) and TBIL (on days 28 and 70) in Pb treated sheep increased significantly in comparison with the control group (P<0.05). Our findings suggest that Pb exposed sheep can be at risk of low thyroid function.

**Key words:** Sheep, Lead, Thyroid function

### Introduction

Lead (Pb) is considered to be one of the major environmental pollutants and has been incriminated as a cause of accidental poisoning in domestic animals more than any other substance (Casas and Sordo, 2006). Lead that contaminates the environment is largely air-borne but is redeposited by dust into soil and water and is taken up by or exists on the surface of plants which are grazed by livestock (Bolter *et al.*, 1975). Cattle, sheep, and horses are good indicators of pollution on vegetation (Debackere, 1983). One of the primary sources of lead contamination in air, soil and water is combustion of fuels containing lead additives. The main source of excess lead

intake for cattle was that in paint (Clapp, 2001) until the restrictions on the use of lead-base pigments in paints. Other sources of ingestible lead include storage battery plates, radiator repair, secondary smelters, putty, linoleum, asphalt roofing, engine oil, insecticide baits, and contaminated feeds (Buck, 1970; Christian and Tryphonas, 1971; Singh *et al.*, 1994; Yu, 2004; Casas and Sordo, 2006). Lead can cause profound hematological, neurological, gastrointestinal, renal, rheumatological and endocrine manifestations in man even at levels previously considered safe (Cullen *et al.*, 1983; Pagliuca *et al.*, 1990; Lyons and Pahwa, 2005). Lead exposure also causes functional impairment of pituitary-adrenal axis as well as the pituitary-thyroid axis

(Singh and Dhawan, 1999). There are conflicting reports in the literature regarding the effect of occupational lead exposure on the thyroid functions in experimental animals and humans (Singh *et al.*, 2000). The thyroid gland plays an important role in energy usage, synthesis of RNA protein, consumption of oxygen by cells, overall body metabolism, growth processes and neurological development (Vanderpump and Tunbridge, 2008). In adult sheep, poor wool growth, depressed milk yield, reduced weight gain, impaired reproductive performance with loss of libido in rams and late abortions or birth of weak lambs with visibly enlarged thyroid glands as well as an increased susceptibility to infectious agents are the most prominent clinical findings in hypothyroid cases (Sipos *et al.*, 2004). As there was a knowledge gap on the effect of Pb on the thyroid function in sheep, the present study was designed to provide some data on this issue.

## Materials and Methods

This study was performed under regulation of Shiraz University for using animals in scientific procedures. In this experiment 10 healthy male sheep, aged about one-year-old were randomly allocated into two equal groups of control ( $n = 5$ ) and experiment ( $n = 5$ ). The two groups were kept under the same conditions of food and environment. Water and hay were supplied *ad libitum* to all animals. Before commencing the experiment, samples of blood and faeces were submitted for hematological, bio-chemical and parasitological examinations to monitor the health status of the animals. The treatment group received 5 mg/kg/day of lead acetate orally for 8 consecutive weeks. Blood sampling of both groups was done on days 0 (before receiving lead acetate in treatment group), 14, 28, 42, 56 and 70 after lead administration, every morning (8 to 9 a.m.). Blood samples were collected by jugular veinipuncture in sterile silicone-coated vacutainers, allowed to clot, and then centrifuged at 3000 g for 10 min. Serum was separated and stored at  $-20^{\circ}\text{C}$  until use. Serum T3, T4, FT3, FT4 and TSH were

measured by RIA (Bruss, 1997). Serum samples were digested and Pb was measured by atomic absorption spectrophotometry (Shimadzu AA-670; Shimadzu Corp, Kyoto, Japan) at a wavelength of 283.3 nm (Szkoda and Żmudzki, 2005). All glassware was acid washed with 2 M nitric acid. Standards were prepared from a certified Pb standard solution. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured by modified Reitman-Frankel method. Serum GGT concentration was measured by Kinetic colorimetric test according to Szasz/Persijn (Szasz *et al.*, 1974). Total bilirubin was measured using Zist Chimi Kits (Zist Chimi, Iran). Data were checked for normality before performing analysis. All analyses utilized parametric statistical methods. Results were expressed as mean  $\pm$  SE. All data were analysed using SPSS for Windows, Version 15.0. In order to compare the mean control values of different parameters with the mean values of the same parameters measured in the treatment group on days 0, 14, 28, 42, 56 and 70 of Pb administration, Repeated measure ANOVA and Bonferroni post hoc test were used. The level of significance for each test was at  $P < 0.05$ .

## Results

The results are indicated in Table 1 and 2. Serum Pb concentration (on different sampling days following lead administration) and serum enzyme activities of alanin amino transferase (ALT) (on days 28 and 42), aspartate aminotransferase (AST) (on days 28, 42, 56 and 70) and total bilirubin (TBIL) concentration (on days 28 and 70) increased significantly in lead treated sheep when compared with the control values ( $P < 0.05$ ) (Table 1). No significant changes were observed in  $\gamma$  glutamyl transferase (GGT) activity in the treatment group when compared with the control values. Serum T3 (on days 14, 56 and 70), FT3 (on days 56 and 70) (Fig. 1), T4 (on days 14, 28, 42, 56 and 70), FT4 (on days 42, 56 and 70) (Fig. 2) and TSH (on days 14, 28, 42, 56 and 70) (Fig. 3) concentrations decreased significantly in the

**Table 1: Concentrations (mean ± SE) of serum Pb and total bilirubin and serum hepatic enzyme activities before and after lead acetate administration on different days in sheep (n = 10)**

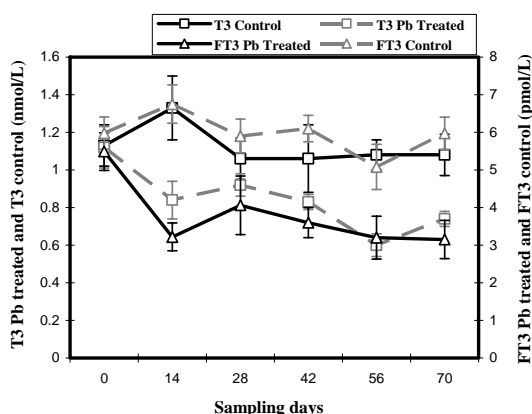
Sampling days	0	14	28	42	56	70
Pb treatment (µmol/L)	0.00 ± 0.00	1.60 ± 0.60*	1.27 ± 0.36*	1.92 ± 0.06*	1.23 ± 0.21*	0.66 ± 0.25*
Pb control (µmol/L)	0.13 ± 0.13	0.18 ± 0.09	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
ALT treatment (IU/L)	39 ± 1.70	40 ± 3.83	61 ± 5.17*	141 ± 7.47*	33 ± 5.00	30 ± 4.37
ALT control (IU/L)	38 ± 2.34	41 ± 3.24	39 ± 1.93	34 ± 2.40	39 ± 4.50	38 ± 2.35
AST treatment (IU/L)	104 ± 1.70	110 ± 14.42	83 ± 6.60*	141 ± 7.50*	127 ± 9.12*	84 ± 7.33*
AST control (IU/L)	103 ± 3.05	95 ± 4.60	109 ± 8.51	117 ± 6.35	93 ± 3.57	103 ± 3.43
GGT treatment (IU/L)	60 ± 2.60	58 ± 7.02	70 ± 4.90	63 ± 7.50	48 ± 3.67	84 ± 4.53
GGT control (IU/L)	61 ± 3.32	52 ± 3.51	78 ± 4.62	59 ± 5.46	57 ± 5.50	61 ± 3.40
TBIL treatment (mg/dl)	0.37 ± 0.02	0.47 ± 0.07	0.64 ± 0.05*	0.37 ± 0.03	0.24 ± 0.05	0.19 ± 0.03*
TBIL control (mg/dl)	0.36 ± 0.02	0.36 ± 0.04	0.34 ± 0.03	0.40 ± 0.02	0.36 ± 0.03	0.37 ± 0.02

\* Shows significant difference between treatment and control groups (P<0.05)

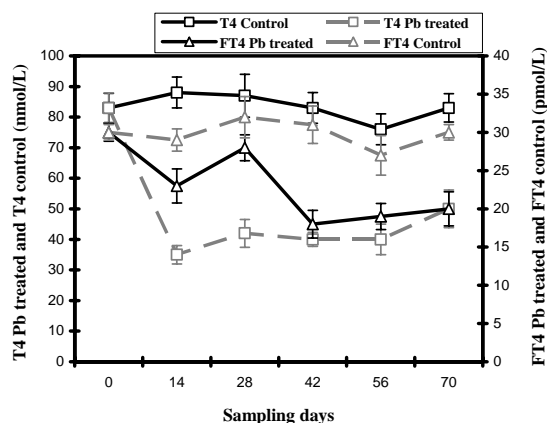
**Table 2: Concentrations (mean ± SE) of thyroid hormones before and after lead acetate administration on different days in sheep (n = 10)**

Sampling days	0	14	28	42	56	70
T3 treatment (nmol/L)	1.12 ± 0.11	0.84 ± 0.10*	0.92 ± 0.06	0.83 ± 0.04	0.60 ± 0.06*	0.74 ± 0.04*
T3 control (nmol/L)	1.13 ± 0.11	1.33 ± 0.17	1.06 ± 0.11	1.06 ± 0.18	1.08 ± 0.08	1.13 ± 0.11
T4 treatment (nmol/L)	83 ± 4.90	35 ± 3.00*	42 ± 4.55*	40 ± 2.92*	40 ± 5.00*	50 ± 6.14*
T4 control (nmol/L)	83 ± 4.81	88 ± 5.05	87 ± 7.03	83 ± 5.00	76 ± 5.05	83 ± 4.61
FT3 treatment (pmol/L)	5.94 ± 0.50	3.22 ± 0.37	4.06 ± 0.78	3.60 ± 0.40	3.20 ± 0.57*	3.15 ± 0.51*
FT3 control (pmol/L)	5.98 ± 0.43	6.75 ± 0.51	5.90 ± 0.45	6.10 ± 0.35	5.08 ± 0.60	5.97 ± 0.43
FT4 treatment (pmol/L)	30 ± 1.15	23 ± 2.24	28 ± 1.70	18 ± 1.80*	19 ± 1.70*	20 ± 2.33*
FT4 control (pmol/L)	30 ± 1.03	29 ± 1.45	32 ± 2.70	31 ± 2.45	27 ± 2.58	30 ± 1.02
TSH treatment (mIU/L)	0.13 ± 0.01	0.03 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.03 ± 0.00*
TSH control (mIU/L)	0.13 ± 0.01	0.11 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.13 ± 0.01

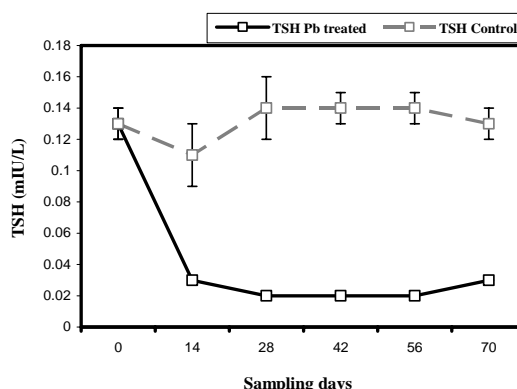
\* Shows significant difference between treatment and control groups (P<0.05)



**Fig. 1: Serum T3 and FT3 concentrations of control and Pb treated sheep**



**Fig. 2: Serum T4 and FT4 concentrations of control and Pb treated sheep**



**Fig. 3: Serum TSH concentration of control and Pb treated sheep**

Pb treated group when compared with the control values (P<0.05) (Table 2).

## Discussion

Serum levels of thyroid hormones, including T3, T4 and TSH, are commonly used as reliable indicators of the thyroid function in humans and experimental animals (Paier *et al.*, 1993; Chaurasia *et al.*, 1996; Kelly, 2000). All reactions necessary for the formation of T3 and T4 are influenced and controlled by pituitary

thyroid-stimulating hormone (TSH) which stimulates follicular cells in the thyroid gland (Beers and Berkow, 1999; Kelly, 2000). Pituitary TSH secretion is controlled by a negative feedback mechanism modulated by the circulating level of free T<sub>4</sub> and free T<sub>3</sub> and by conversion of T<sub>4</sub> to T<sub>3</sub> in the pituitary thyrotropic cells. T<sub>3</sub> is the metabolically active iodothyronine. TSH secretion is also influenced by thyrotropin-releasing hormone (TRH), a 3-amino acid peptide synthesized in the hypothalamus which stimulates the pituitary to release TSH (Beers and Berkow, 1999; Kelly, 2000; Higgins, 2007). In our study mean serum concentrations of T<sub>3</sub> (on days 14, 56 and 70), T<sub>4</sub> (on days 14, 28, 42, 56 and 70), FT<sub>3</sub> (on days 56 and 70), FT<sub>4</sub> (on days 42, 56 and 70) and TSH (on days 14, 28, 42, 56 and 70) decreased significantly in lead-treated sheep. Hypothyroidism occurring in human subjects with occupational exposure to lead is suggestive of a negative effect of the element on thyroid function (Lasisz *et al.*, 1992). Also Singh and Dhawan (1999) showed that lead treatments on rats at a dose rate of 50 mg/kg body weight for a period ranging from 1-4 months caused a significant depression in T<sub>3</sub> and T<sub>4</sub> levels. On the other hand, a dose related depression of thyroid functions had been observed in humans during occupational exposure to inorganic lead (Gustafson *et al.*, 1989). It has been proposed that Pb can accumulate in the organism and its actions include damage to cell membranes and disorders of the oxido-reductive processes in the cells (Lasisz *et al.*, 1992). Therefore thyroid dysfunction in our study might be related to structural damage of thyroid follicular cells due to accumulation of Pb in the thyroid gland. As the thyroid gland is the only organ involved in T<sub>4</sub> synthesis (Kelly, 2000), the decrease in the serum level of this hormone in the Pb treated sheep may suggest that Pb-induced thyroid dysfunction is mediated by influencing the production and/or secretion of T<sub>4</sub> by the follicular cell of the thyroid gland. Although on different days following lead administration free thyroid hormones reduced in our Pb exposed sheep, TSH didn't respond to low thyroid hormones and conversely significantly decreased. The significant decrease of serum TSH on

different days following lead administration in Pb treated sheep may also be the result of Pb effect on regulatory enzymes associated with hypothalamic pituitary thyroid (HPT) axis. On the other hand, thyroid hormones are metabolized in peripheral tissue by deiodination, conjugation, deamination, and decarboxylation enzyme reactions. Therefore, alterations in these metabolic pathways may significantly affect thyroid hormone metabolites and influence thyroid function at the cellular level (Paier *et al.*, 1993; Chaurasia *et al.*, 1996; Kelly, 2000). Most of the circulating T<sub>3</sub> originates from the extra-thyroidal tissues. The peripheral deiodination of T<sub>4</sub> to T<sub>3</sub>, taking place mainly in the liver, is dependent on 5'-monodeiodinase (5'-D) activity (Paier *et al.*, 1993; Chaurasia *et al.*, 1996; Gibney *et al.*, 2003). A common feature of thyroid hormone dysfunction at cellular levels is a low level of circulating T<sub>3</sub>, with generally normal to slightly elevated blood T<sub>4</sub> levels and either normal or slightly suppressed TSH levels (Kelly, 2000). Significant increase in serum enzyme activities of ALT, AST and TBIL in Pb treated sheep in our study could be an indication of hepatic cells damage (Latimer *et al.*, 2003). Hepatic pathology influence serum thyroid hormone concentrations, secondary to the effects on peripheral enzyme pathways (Kelly, 2000). Therefore, partial decrease of serum T<sub>3</sub> concentration in Pb treated sheep might be related to hepatic dysfunction. In conclusion, our study shows that long-term low-dose exposure to lead in sheep affects thyroid function due to hypothyroidism.

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