Effects of Various Concentrations of Dietary Lead on Antioxidant Gene Expressions in Different Tissues of Pigs

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Abstract

Twenty weaned pigs were allocated to four dietary Pb levels for four weeks, such as control, low-level (LL, 10 mg kg^{-1}), maximum tolerable level (MTL, 25mg kg^{-1}) and high toxic level (HTL, 250 mg $kg^{-}1$). The differential expression of twelve antioxidant genes encoding stressor biomarkers was investigated by quantitative reverse transcription-PCR in muscle, liver and kidney of pigs. The expression results indicated that, the antioxidant enzymes such as GPx1, CAT, GR, SOD, G6PD, TXNRD1 and PGC-1α were gradually down regulated ($P<0.05$) in muscle, liver and kidney of LL, MTL and HTL dietary Pb treatment groups. CYP1A1 and MT1A gene expression rates were higher in LL and MTL dietary treatments and lower in HTL, when compared with the control group. The TP53 regulates reactive oxygen species generation, was affected with increasing ($P<0.05$) dietary Pb concentrations. The Pb accumulation rate in tissues are in the order of Kidney> Liver > muscle. As a result, dysfunction occurred in those tissues after dietary Pb supplementation according to the accumulation rate and there was an inhibition of the antioxidant system.

Key words: lead, antioxidant, pig, oxidative stress, mRNA expression, reactive oxygen species

1. Introduction

Heavy metals are considerably toxic, due to their cumulative nature in the various body organs, leading to unwanted effects (Sathawara et al., 2004). Heavy metals are inclined to bioaccumulate in all environment system and biomagnified in food chains (Caggiano, et al., 2004). In which, their levels may reach toxic confines even when they are found at very low concentrations in environmental samples (Caggiano et al., 2004). Toxic elements can implicate the functions of enzymes liable for many diseases, particularly renal, cardiovascular, carcinogenic, mutagenic, nervous and bone disorders (Jarup, 2003; Steenland and Boffetta, 2000; Dolk and Vrijheid, 2003).

Lead, heavy metal is a common cause of poisoning in domestic animals and human life. Lead affects all the organs and system of the human and animal body (Gilman et al., 1991). Lead was considered as a main environmental toxin, therefore animals may be exposed to low concentrations of Pb through contaminated feed, water, and feed supplement (EI et al., 2000). Toxicological brunts of Pb have been widely studied in both animals and human, and it has been suggested that definite physiologic disorders accredited to this heavy metal are directly or indirectly connected to the production of oxidative stress (Reglero et al., 2009; Koivula and Eeva, 2010). The number of studies has already confirmed the effects of Pb exposure on growth and development in humans and animals (Polák and Flaherty, 1995; Martin et al., 1996). The pigs showed a decreased growth rate at 25 mg Pb/kg diet, but still there is no sufficient information to establish MTL in this species (NRC, 2005). Reactive oxygen species (ROS), such as superoxide (O^{2-}), hydroxyl radicals (·OH), and hydrogen peroxide (H_2O_2), are formed during aerobic conditions (Schieber et al. 2014). Usually, the potential of oxidation and reduction maintains a balance in the body (Monaghan et al., 2014).

In case ROS are not removed from the body properly in a timely manner by the antioxidant system, an imbalance between free radical production and the removal would lead to oxidative stress. GPx, along with SOD and CAT, are considered as the main antioxidant enzymes in mammals. According to Evans et al. (1997), mammalian cells may encounter oxidative stress causing the destruction of macromolecules and leading to the abnormal functions in organs. Consecutively, animals may show modifications in physiology and behavior, showing a poor growth performance and suffering from various types of diseases.

Lead resulted in the generation of oxidative stress, such as lipid peroxidation, protein modification, DNA damage, and other effects, all of these are involved in many physiological diseases and disorders. Consequently, Pb associated toxicity may be the reason for oxidative tissue damage (Patrick., 2006; Reglero et al., 2009). In this way, it has been suggested that the Pb-mediated production of oxidative stress might play a key role in disrupting immune function, which might be caused by Pb-mediated alteration of gene expression of immunomodulators (Lynes and Fontenot 2006; Hemdan et al., 2007). In this study, we performed the pig growth rates in control and various dietary Pb level groups and also carried out m-RNA expression analysis of twelve important antioxidant genes in muscle, liver and kidney tissues of dietary Pb supplemented pigs, as stressor biomarkers for Pb metal.

2. Materials and Methods

2. 1. Ethics Statement

The protocol for the animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (No. 2015-147).

2.2 Animals and experimental design

Twenty weaned castrated male piglets with an average body weight about 30 kg and ~60 days of age, were randomly allocated to the following 4 dietary treatments: control (0 mg kg^{-1}), low-level (LL, 10 mg kg^{-1}), maximum tolerable level (MTL, 25 mg kg^{-1}) and high toxic level (HTL, 250 mg kg^{-1}) Pb fed along with feeding for one month. The metal was added as lead sulfate. For different concentrations of Pb, appropriate quantities were weighed and filled into the capsule and labeled. For convenient feeding, appropriate Pb concentrations were separated into two parts and filled in a separate capsule and supplied along with the feed. Each dietary treatment group consisted of 5 replicate, each pig was kept in separate pen (Fig. 1). The corn-soybean meal basal diet was formulated to meet the nutrient requirements according to NRC (2012) standards, shown in table 1. Pigs were fed with corn-soybean diet twice daily. 1 kg day of feed was supplied for the first 2 weeks and next 2 weeks 2 kg day was provided and dietary Pb supplement was increased based on the feed quantity as well..

Fig. 1. Feeding and dietary Pb treatment. A. Swine form, B. Pb contained capsule treatment along with feed, C. Pig feeding capsule along with the feed

Figure 1. Expression of antioxidant genes quantified by qRT-PCR in pig kidney. Relative mRNA expression of antioxidant genes in various concentrations of dietary Pb pigs represent the comparison vs. control group and reported as a fold change from the value of the control group. Values are mean with standard errors represented by vertical bars. a, b, c, d Mean values with unlike letters were significantly different ($P<0.05$).

Ingredients	%	Nutrient content	%
Corn	58.56	Crude protein	18.00
Soybean meal (46% CP)	14.00	Crude fat	6.00
Soybean oil	1.60	Crude fiber	2.80
Extruded-soybean	12.00	Ashes	4.80
Fish meal	3.45	Calcium	0.75
Whey powder (12% CP)	7.00	Phosphorus	0.45
Calcium hydrophosphate	1.08	Lysine	1.10
Limestone	0.60	Digestible crude protein	15.00
Sodium chloride	0.32	Digestible energy (Mcal/lb)	3.55
L-Lysine hydrochloride	0.43	Se (mg/kg)	0.06
Threonine	0.12		
DL-Methionine (99%)	0.14		
Choline chloride (50%)	0.20		
Vitamin-mineral premix[1]	0.50		
Total	100.00		

Table 1. Ingredients and nutrient levels of the basal piglet diet (% as-fed basis)

1Provided per/kg of diet: vitamin A 11,000 IU, vitamin D3 1,500 IU, vitamin E 44.1 IU, vitamin K3 4.0 mg, vitamin B1 1.4 mg, vitamin B2 5.22 mg, vitamin B5 20.0 mg, vitamin B12 0.01 mg, Niacin 26.0 mg, Pantothenic acid 14 mg, Folic acid 0.8 mg, Biotin 44 µg, Fe 100.0 mg, Cu 16.50 mg, Zn 90.0 mg, Mn 35.0 mg, I 0.30 mg

2.3.Weight measurement

Throughout the experimental period, body weight (BW) was taken every week in the morning before feeding.

2.4. Sampling and processing

After 4 weeks, all control and Pb treated pigs were killed by an anesthetic overdose with the combination of barbiturates and pentobarbital. The samples of liver, kidney and muscle tissues were collected immediately and rapidly frozen in liquid nitrogen, and stored at –80 ^{o}C for further analysis.

2.5. RNA isolation, Reverse Transcription and Quantitative RT-PCR

RNA's were isolated from control and various concentrations of Pb fed pig muscle, liver and kidney samples by using TRIzol® Reagent (Ambion, USA) manufacturer's instructions. The 2 µg of total RNA was used in a final volume of 20 µl to synthesize cDNA using GoTaq® 2-step RT-qPCR system (Promega, USA) kit. Quantitative RT-PCR was used to measure the mRNA expression levels of twelve antioxidant marker genes. Primers for gene of interest were designed based on the pig (*Sus scrofa*) sequences by using Integrated DNA Technologies (IDT) Oligo Analyzer Tool. The primer sequences and amplified product lengths were shown in Table 2. The qRT-PCR was performed using a GoTaq® qPCR master mix (Promega, USA) on an Applied Biosystems 7500 Fast Real-Time PCR System (USA). Amplification was carried out for 1 cycle at 95^{o}C for 5 min; 40 cycles at 95^{o}C for 15 s, 56^{o}C for 30 s, 72^{o}C for 30 s, followed by a melting curve. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin genes were used as internal genes for normalization to make the results more reliable. The relative differences between control and experimental groups were calculated and expressed as relative changes. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative mRNA abundance (Livak and Schmittgen, 2001).

Table 2. The list of real-time quantitative PCR primers for oxidative stress-responsive genes and their accession numbers and product sizes

S. No	Gene name	Accession Number[1]	Primer sequence	Product size
1	TP53	NM_213824	F: GGCCATCTACAAGAAGTCAGAG R: CAAGTACTCGGCCCGTAAAT	142
2	PGC-1α	AB106108	F: TCCGTATCACCACCCAAATC R: GACCTTGATCTTGACCTGGAATA	122
3	SOD1	NM_001190422	F: TGCAGGTCCTCACTTCAATC R: GAGGGCGATCACAGAATCTT	142
4	TXNRD1	NM_214154	F: GGAGGAACGTGTGTGAATGTAG R: CCCAGTCGTGTTTAATCGTCTC	130
5	GPX1	NM_214201	F: TTCGAGAAGTGTGAGGTGAATG R: CACTGGAGACCAGGTGATAAAC	132
6	HMOX1	NM_001004027	F: CGAATGAACACTCTGGAGATGA R: GAGGGTCTCTGGTCCTTAGT	140
7	CAT	NM_214301	F: CTCACAGCGAATACCCTCTTATC R: GTGAGTGTCAGGATAGGCAAATA	176
8	G6PD	GBZA01000237	F: TGCTTTCCATCAGTCGGATAC R: GCCCACGATGTATGTGTCTT	136
9	CYP1A1	NM_214412	F: CTGGTGTCAGTAGCCAATGT R: ACGGAGGATAGGGATGAAGT	153
10	GR	XM_003483635	F: GCCGACTGAACACCATCTATC R: GTGAGGAGCTGTGTACTTCTTC	131
11	MT1A	NM_001001266	F: CGTGCAAAGCCTGCAGAT R: ACAGCAGCTGCACTTGTC	122
12	PRDX6	NM_214408	F: GTGACAGCTCGTGTGGTATTT R: GTCTCCATTCTTCCAGTCAACC	171
13	β-actin	DQ452569	F: GGACCTGACCGACTACCTCAT R: GGGCAGCTCGTAGCTCTTCT	181
14	GAPDH	AF017079	F: GTCTGGAGAAACCTGCCAAATA R: CCCAGCATCAAAGGTAGAAGAG	152

[1]TP53=tumor protein p53; PGC-1α = PPARγ coactivator 1α; SOD1= Superoxide dismutase1; TXNRD1= Thioredoxin reductase 1; GPx1= Glutathione peroxidase 1; HMOX1= Heme oxygenase 1; CAT= Catalase; G6PD= Glucose 6 phasphate; CYP1A1= Cytochrome P450 1A1; GR= Glutathione reductase; MT1A= Metallothionein 1A; PRDX6= Peroxiredoxin

2.6. Statistical analysis

All sample data are presented as mean ± standard deviation (S.D.). Different group means were compared using one-way ANOVA then followed by Duncan's multiple range test, when suitable, to identify differences among groups. The two-group comparisons of control and dietary treatment groups were carried out using the Student's t-test. Statistical significance was assigned at $p<0.05$.

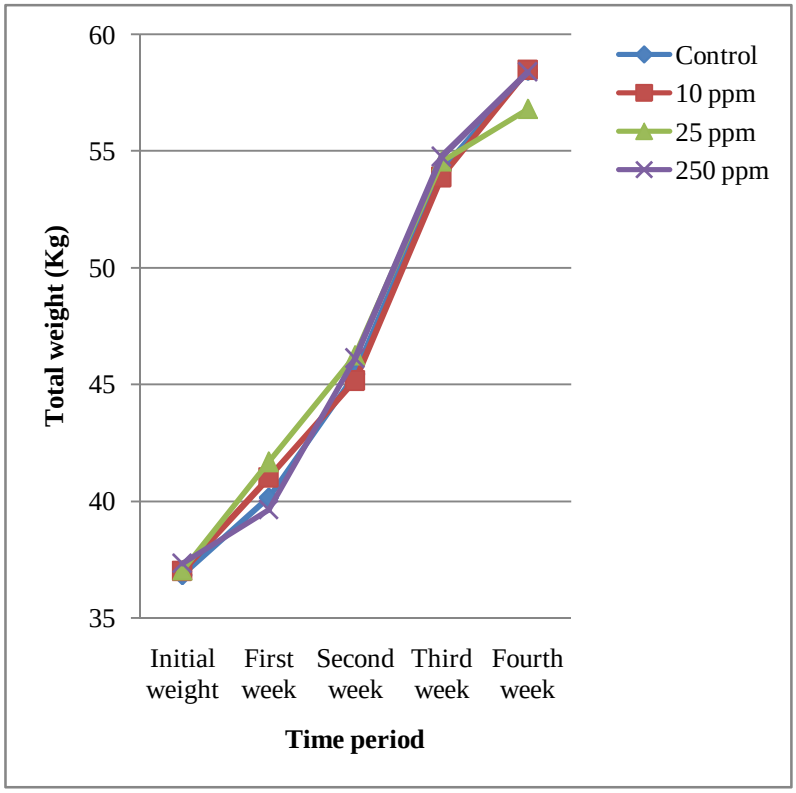
3. Results

3.1. Growth performance and daily weight gain of pigs supplemented with different concentrations of Pb

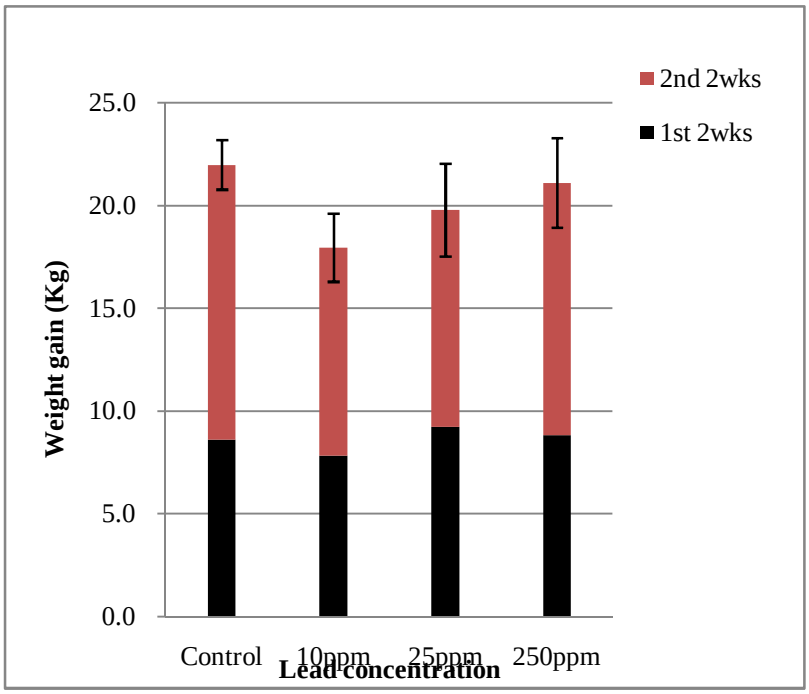
During the experiment, pigs consumed Pb along with the feed based on their appropriate dietary concentrations. BW was measured every week during the experimental time. Four week BW of the LL (10 ppm), MTL (25 ppm) and HTL (250 ppm) treatment groups showed slight variation, when compared with the control group (Fig. 2A). For first two weeks, daily weight gain (DWG) of the pigs in control, LL, MTL and HTL treatment groups were almost similar. The DWG of the dietary Pb treatment groups during last two week showed slightly lower than the control group (Fig. 2B).

Fig. 2. Pigs growth rate and weight gain. A. Control, 10 ppm, 25ppm, and 250 ppm dietary Pb pigs body weight for four weeks during treatment period; B. Control, 10 ppm, 25ppm, and 250 ppm dietary Pb pigs weight gain for 1st two weeks and 2nd two weeks.

(A)



(B)



3.2. Antioxidant gene expressions in muscle supplemented with different concentrations of Pb

Real-time quantitative PCR was used to investigate the mRNA levels for different antioxidant genes in muscle tissues of control, LL, MTL and HTL lead treated pig groups (Fig. 3). The mRNA expression of GPx1, CAT, GR, SOD1 and PGC-1α were significantly decreased (<0.05) in muscle tissue with increasing dietary Pb concentrations, when compared with the control group.

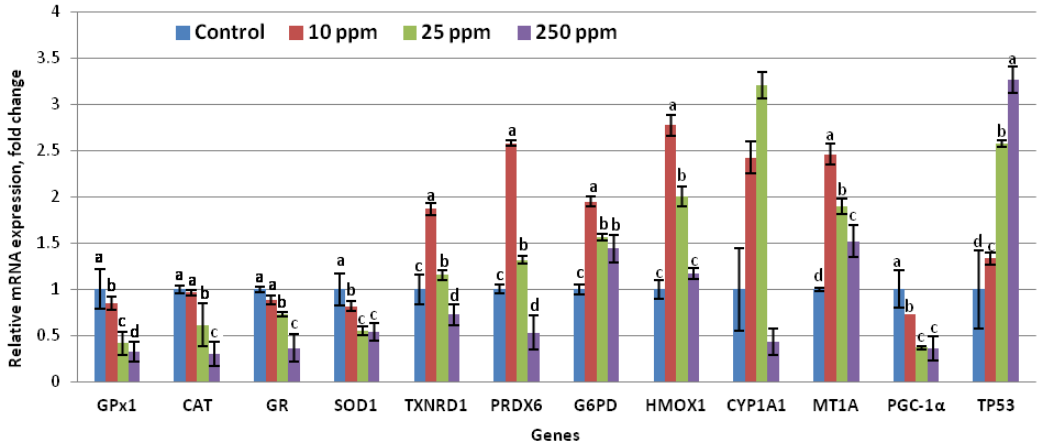


Figure 3. Expression of antioxidant genes quantified by qRT-PCR in pig muscle. Relative mRNA expression of antioxidant genes in various concentrations of dietary Pb pigs represent the comparison vs. control group and reported as a fold change from the value of the control group. Values are mean with standard errors represented by vertical bars. a, b, c, d Mean values with unlike letters were significantly different (P<0.05).

The mRNA levels of G6PD, HMOX1 and MT1A transcripts are significantly up regulated in LL, MTL and HTL treatments than the control group, but the expression levels are decreasing with increasing dietary Pb treatments. Compared with the control group, TXNRD1, PRDX6 and CYP1A1 genes were up- regulated in LL and MTL and down-regulated in the HTL dietary group. The mRNA expression of TP53 gene fold changes were increased with increasing Pb fed, the expressions were 1.3, 2.5 and 3.26 fold changes at LL, MTL and HTL dietary groups, respectively.

3.3. Antioxidant gene expressions in liver supplemented with different concentrations of Pb

Transcriptional changes in liver tissue-derived antioxidant genes were investigated at various concentrations of dietary Pb groups (Fig. 4).

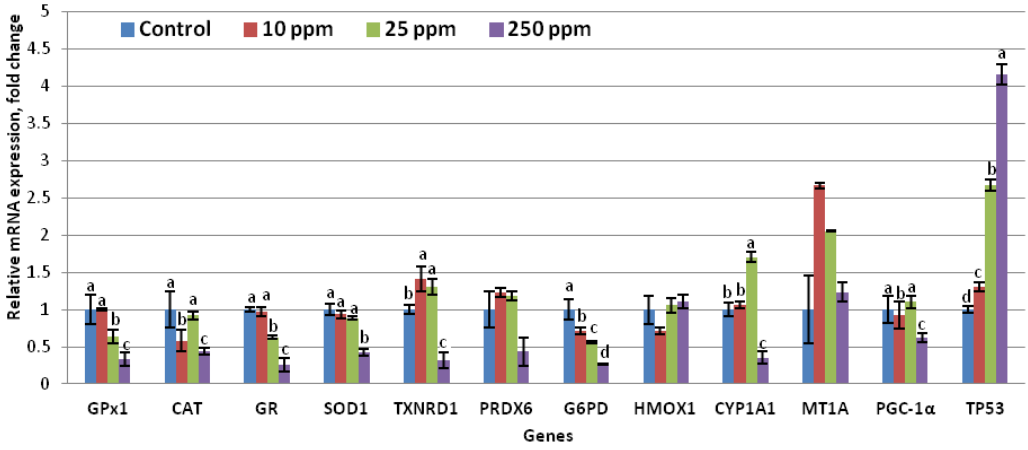


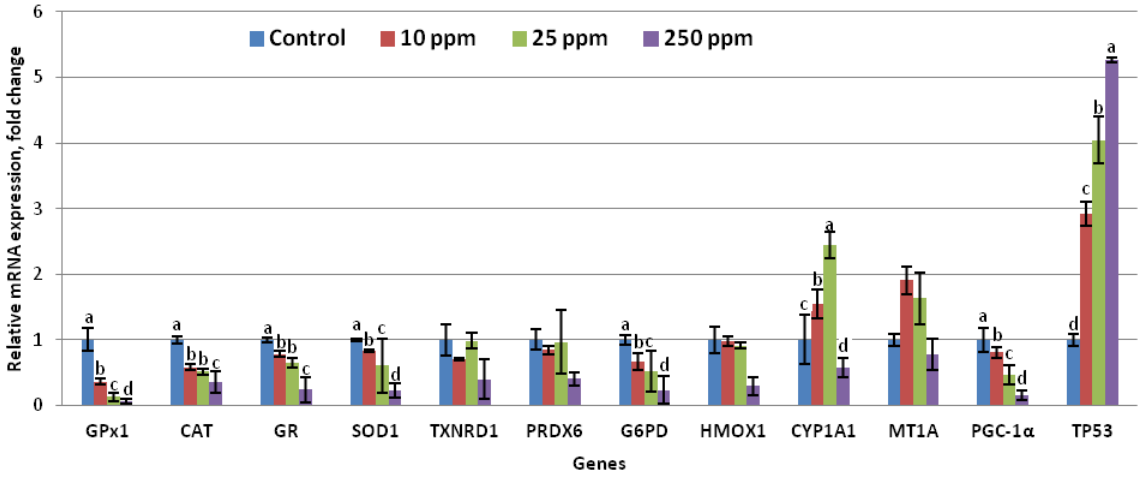
Figure 4. Expression of antioxidant genes quantified by qRT-PCR in pig liver. Relative mRNA expression of antioxidant genes in various concentrations of dietary Pb pigs represent the comparison vs. control group and reported as a fold change from the value of the control group. Values are mean with standard errors represented by vertical bars. a, b, c, d Mean values with unlike letters were significantly different (P<0.05).

Compared with the control group, GPx1, CAT, GR, SOD1, G6PD and CYP1A1 genes were significantly down-regulated in the order of LL, MTL and HTL groups. The mRNA expression of TXNRD1 and PRDX6 genes were slightly expressed in LL (1.4 and 1.2 fold, respectively) and MTL (1.3 fold and 1.17 fold, respectively) groups and down-regulated in TXNRD1 (~3 fold) and PRDX6 (~2.5 fold) genes at MTL, when compared with the control group. The MT1A mRNA abundance was 2.6, 2.0 and 1.23 fold higher in LL, MTL and HTL groups in the order, while compared with the control group.

The HMOX1 gene showed down regulated in LL treatment, when compared with the control, but there is no significant differences in MTL and HTL groups. The transcript of PGC-1α was down-regulated in HTL when compared with control and no significant difference was observed in LL and MTL. The TP53 gene is up-regulated with increasing levels of Pb. The TP53 gene was highly expressed in HTL (4.1 fold), and also increased to 2.6 and 1.3 fold in MTL and LL respectively, when compared with the control group.

3.4. Antioxidant gene expressions in different concentrations of Pb fed pig Kidney tissues

Quantitative RT-PCR was used to investigate transcriptions of various antioxidant genes in kidney after exposure to different levels of Pb (Fig. 5).



The transcripts of GPx1, CAT, GR, SOD1, TXNRD1, G6PD, PRDX6, HMOX1 and PGC-1α were gradually (<0.05) decreased from lower to a higher concentrations, while compared with the control group. At higher toxic level (250 ppm), mRNA expression of all the above genes was higher than LL and MTL treatment groups. The mRNA expression of CYP1A1 gene was significantly up-regulated in LL (1.54 fold) and MTL (2.44 fold) groups, when compared with the control group. Transcript of MT1A gene mRNA expression showed 1.9 fold increased in the LL dietary group, but there were no significant differences at MTL and HTL, when compared with the control group. The TP53 gene expression was increased with increasing Pb concentrations, and this gene showed highest expression in HTL (5.2 fold), following MTL (4.04 fold) and LL (2.9 fold) groups, when compared with the control.

4. Discussion

Overall, within four weeks there were no significant differences in DWGs between the control and different Pb treated groups. According to Phillips et al. (2003), 137 days feeding 25 mg Pb/kg diet as lead acetate treatment resulted in decreased weight in growing pigs. However, Short period, providing dietary Pb treatment for 30 days only in our study may not affect the growth of early pigs.

The transcriptional profiles of twelve genes encoding stressor biomarkers (GPx, CAT, GR, SOD1, TXNRD1, PRDX6, G6PD, HMOX1, CYP1A1, MT1A, PGC-1α and TP53) were examined in muscel, liver and kidney tissues extracted from pigs after exposure to various concentrations, such as 0 ppm (control), 10 ppm (LL), 25 ppm (MTL) and 250 ppm (HTL) of toxic metal, Pb for four weeks. Few studies have analyzed transcriptional changes in antioxidant and stress-related genes in the pig, although such modifications represent first responses of living organism to environmental change. In the present study, we considered a total set of genes encoding antioxidant enzymes and stress-responsive proteins. Here we discussed the transcriptional changes and the biological functions of these genes. Several studies suggested in the literature that oxidative stress is involved in Pb toxicity, even at very low exposure levels (Stohs and Bagchi, 1995). The oxidative stress may be caused for the direct or indirect generation of ROS, but, also to modifications caused in the antioxidant defense systems of cells (Mateo et al., 2003; Patrick, 2006; Nemsadze et al., 2009).

Many researchers believed that intracellular antioxidant enzymes, GPx, SOD, GR and CAT are important parts of these systems and play an important role in protecting tissues from ROS reactions (Mishra and Delivoria-Papadopoulos, 1988; Sohal et al., 1990).

The main cellular antioxidant is the reduced glutathione; it can be regenerated most efficiently by GR and reduced NADP. The SOD provides the efficient dismutation of O^{2-}; leading to the formation of H_2O_2, and it can remove by GPx and CAT (Rodriguezet al., 2004).

In the absence of suitable compensatory reply from the endogenous antioxidant network, GPx, CAT, and SOD concentrations are reduced as a result oxidative stress developed. In our present study, we examined the mRNA expression of genes related to the antioxidant enzymes such as GPx, SOD1, GR and CAT enzymes in pig muscle, liver and kidney tissues. The antioxidanats of GPx, SOD1, GR and CAT are significantly down-regulated in all tissues with increasing Pb concentrations. According to our opinion, especially at higher toxic level (250 ppm) the free radical generation is higher and it suppresses the activity of antioxidants. For this reason the down regulation of antioxidant genes is increased from lower to higher dietary Pb concentration. According to Zhu et al. (2012), GPx and CAT gene expressions were down-regualted in weaning pigs when compared with the control group. The down-regulations of GPx, SOD1, GR and CAT gene expression were increased in the order of muscle, liver and kidney tissues. Similarly, rat exposed to 50 mg Pb/L water as lead acetate for 90 days accumulated Pb in tissues in the order of Kidney> Liver > muscle (ATSDR, 1999). In our study, the gene expression down-regulated rate is higher in the most sensitive tissues, kidney and liver, but muscle is not a vulnerable site of Pb, so the down-regulation of antioxidant genes were lower than kidney and liver. Similarly, according to Xu et al. (2007), the gene expression of antioxidants mRNA and activity showed various profiles in different tissues of pigs, and the regulation of antioxidants is not strongly coordinated in either tissue.

The CYP1 is responsible for the metabolism of many xenobiotic compounds, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and pesticides. Stimulation of CYP1A mRNA by organic pollutants has been extensively reported (Williams et al., 2003; Fisher et al., 2006). Although, oxidative stress is known to suppress CYP1A1 and CYP1A2 expression in hepatocytes (Barker et al., 1994). In contrast, our present study CYP1A1 transcript showed up-regulation at all tissues of LL and MTL dietary treatments and down regulation in HTL. Especially muscle CYP1A1 showed higher expression in LL and MTL and down regulation in HTL (Fig. 3). There are no extensive studies on CYP1A1 mRNA expression in pig. Probably muscle didn't show any effect at 10 (LL) and 25 (MTL) ppm Pb concentration, but it was suppressed in the transcript at high lead toxicity level (250 ppm). Compared with the muscle tissue; liver and kidney tissues are sensitive to Pb exposure, so they were slightly up-regulated at LL and MTL and down-regulated at HTL. Thus, this gene may serve as a useful biomarker for Pb.

The G6PD assists in the detoxification of ROS and supplies reducing power by increasing the production of NADPH (Pandey et al., 2003). In the present study, muscle G6PD transcript was induced 1.9 fold, 1.5 fold and 1.4 fold in LL, MTL and HTL treatments respectively, when compared with control. This gene is down-regulated in in all dietary Pb treatments of liver and kidney. Usually, Pb accumulation in muscle is lower than the liver and kidney, for this reason G6PD showed up-regulation in muscle and down regulation in liver and kidney tissues with increasing Pb concentration order (Fig 4 and 5). TXNRD1 is a key selenoprotein antioxidant enzyme and also able to reduce thioredoxin and other compounds, therefore detoxifying cells from oxidative injuries (Arner, 2009; Turanov et al., 2010). PRDX6 is also a member of an antioxidant protein, playing an important role in oxidative stress, catabolism of lipids and phospholipid liposomes (Fujii and Ikeda, 2002; Hofmann et al, 2002).

In this study, compared with the control, TXNRD1 and PRDX6 transcripts showed higher expression rate in LL, MTL of muscle and liver, but no significant differences in LL, MTL of kidney, but both genes were down-regulated at HTL of all the tissues (Fig. 3, 4, 5). Based on our results, TXNRD1 and PRDX6 transcripts didn't show any effects at LL and MTL dietary Pb treatments, but at HTL the free radicals can be produced higher and then suppress the activity of TXNRD1 and PRDX6 genes. Kidney and liver tissues are more sensitive than muscle on Pb treatment. For this reason muscle tissue expression levels were higher in LL and MTL than liver and kidney. The HMOX1 gene gives a unique model for studying the mechanism by which modifications in cellular redox possibly result in gene expression. The transcriptional up-regulation of the HMOX1 gene followed cellular exposure to agents, like as H_2O_2 (Jang et al., 2009). The HMOX1 via wide variety of stimuli can induce HO-1 expression, including heme, heavy metals, H_2O_2, lipoprotein, nitric oxide and nitric oxide donors and growth factors (Ryter and Tyrrell, 2000). In this study, HMOX1 gene was up-regulated in LL and MTL groups of muscle and down-regulated at LL of liver and HTL of kidney tissues, when compared with the control group. Due to lower metal accumulation rate in muscle, LL and MTL group showed higher expression, when compared with the liver and kidney.

Naturally containing metal binding peptides of metallothioneins (MTs) and polychelatins are the main metal sequestering molecules used by cells to immobilise metal ions, presenting selective, high affinity binding sites (Winklemann and Winge, 1994). Over-expression of metal-binding proteins like as MT1A in animal, bacterial and yeast cells resulted in improved metal accumulation. (Strouhal et al., 2003).

In this study, compared with the control, MT1A is significantly up-regulated in all dietary treatments of muscle and liver with decreasing Pb concentrations, but in liver up-regulated only at LL dietary group ($p< 0.05$). Similarly, in pigs supplemented with dietary Zn increases the MT expression in a dose-depended manner, and the expression and metal-binding affinity differs between the different MT isoforms (Alscher et al., 2005). According to Dai et al (2013), MT mRNA and protein levels in livers and kidneys at low dose, intermediate dose and high dose exposed of Pb rats were higher than those in the controls. The present study has revealed that the MT1A production was induced in the presence of Pb and hence it could be recommended as a biomarker in exposure to Pb.The PGC-1α is a wide and powerful regulator of free-radical metabolism and it plays a significant defensive role against stress-induced oxidative stress by regulating the expression of mitochondrial antioxidants, they provide a probable target for the therapeutic manipulation of the essential endogenous toxins (Lu et al., 2010). According to Valle et al. (2005), higher expression of PGC-1α increased mitochondrial antioxidant enzyme expression and decreased oxidative stress and cell death. In the current study, the PGC-1α transcript is significantly down-regulated ($p<0.05$) in all dietary treatments of muscle, liver and. We are suspecting that, with the increasing concentration order of Pb stress stimulated the free-radical production and inhibited antioxidant capacity. Similarly, the PGC-1α transcript is downregulated in weaning pigs (Zhu et al (2012).

The TP 53 gene is a redox-active transcription factor and it organizes and directs cellular responses in the tolerate of a diversity of stresses that lead to genomic instability Stimulation of TP53 can induce a variety of responses, as well as cell cycle arrest, DNA repair, apoptosis, and senescence (Liu and Chen, 2006). In this study, TP53 protein expression rate is higher ($p<0.05$) with increasing Pb concentrations in all dietary treatments of muscle, liver and kidney, when compared with the control group. In the weaning pigs, the TP53 transcript showed higher expression compared to the control group (Zhu et al (2012). Under normal or low cellular stress, reduced concentrations of TP53 encourage the expression of antioxidant genes. At severe cellular stress, high concentrations of TP53 encourage the expression of genes that supply free radical configuration and TP53 mediated apoptosis (Sablina et al., 2005). Therefore, under normal or low stress conditions, TP53 looks to have an antioxidant role and it can protects cells from oxidative damage. Even though this consequence might depend on the concentration of TP53. In this study, the higher content of TP53 in different dietary Pb pigs indicated that TP53 may play the proapoptosis role in the muscle, liver and kidney after Pb dietary treatment.

Conclusion

In this study, we determined the acute effect of heavy metal Pb on twelve genes that encode antioxidant enzymes and stress responsive proteins. Our results recommend that changes of the gene expressions can be considered as effective biomarkers for environmental stress, particularly Pb exposure. Among the twelve antioxidant genes, GPx1, CAT, GR, SOD1 G6PD, TXNRD1 and PGC-1α genes showed distinctly high sensitivity to the increasing Pb concentration. Especially, metal accumulation rate in kidney and liver is higher than in muscle, so for this reason the down-regulation of above genes is higher than muscle.The expression rate of CYP1A1 and MT1A transcripts is higher in LL and MTL treatments and lower in HTL, when compared with the control group. The TP53 protein is highly regulated from lower to higher Pb concentrations of muscle, liver and Kidney samples, particularly higher expression in kidney and liver than the muscle. In this regard, our results are quite consistent with those of previous studies. In conclusion, various concentrations of Pb stress is the major cause for the failure of kidney, liver and muscle functions in dietary Pb fed pigs via promotion of free radical generation, limitation of the antioxidant effects, and decrease in specific organ enzyme activities. Further studies are needed to identify the complete mechanism regulating free radical generation from dietary Pb treatment.

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