Animal (2017), **11:5**, pp 755–761 © The Animal Consortium 2016
doi:10.1017/S175173111600224X

animal

Protective potential of *Lactobacillus* species in lead toxicity model in broiler chickens

M. F. Jahromi[1,2], J. B. Liang[1†], R. Ebrahimi[1], A. F. Soleimani[1], A. Rezaeizadeh[3], N. Abdullah[1] and P. Shokryazdan[1]

[1]*Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia;* [2]*Agricultural Biotechnology Research Institute or Iran (ABRII), East and North-East Branch, P.O.B. 91735844, Mashhad, Iran;* [3]*Department of Endocrinology, Sydney Medical School, University of Sydney, Sydney, NSW, Australia*

(Received 23 December 2015; Accepted 22 September 2016; First published online 2 November 2016)

To alleviate adverse effects of heavy metal toxicity, diverse range of removing methods have been suggested, that is usage of algae, agricultural by-products and microorganisms. Here, we investigated lead (Pb) biosorption efficacy by two lactic acid bacteria species (LABs) in broiler chickens. In an in vitro *study, Pb was added to culture medium of LABs (*Lactobacillus pentosus *ITA23 and* Lactobacillus acidipiscis *ITA44) in the form of lead acetate. Results showed that these LABs were able to absorb more than 90% of Pb from the culture medium. In follow-up* in vivo *study, LABs mixture was added to diet of broiler chickens contained lead acetate (200 mg/kg). Pb exposure significantly increased lipid peroxidation and decreased antioxidant activity in liver. The changes were recovered back to normal level upon LABs supplementation. Moreover, addition of LABs eliminated the liver tissue lesion and the suppressed performance in Pb-exposed chicks. Analysis of liver and serum samples indicated 48% and 28% reduction in Pb accumulation, respectively. In conclusion, results of this study showed that* L. pentosus *ITA23 and* L. acidipiscis *ITA44 effectively biosorb and expel dietary Pb from gastrointestinal tract of chickens.*

Keywords: *Lactobacillus*, lead, heavy metal, broiler chickens

Implications

Diverse range of heavy metal removing methods have been suggested, that is usage of algae, agricultural by-products and microorganisms. Here, we showed that lead (Pb) can be effectively biosorbed by two lactic acid bacteria species (LABs) both under *in vitro* and *in vivo* conditions. Animal feed producer may incorporate these LABs in feed to benefit from both probiotic function and heavy metal removal.

Introduction

Pb is a widespread environmental pollutant causing broad range of physiological and biochemical dysfunctions in animal. It is normally released to environment from number of anthropogenic sources such as agricultural fertilizer, sewage sludge, metal mine and smelting (Scheuhammer, 1987). Animal accumulate Pb in various tissues and subsequently transfer it via food chain to human (Fox, 1987). Mahesar *et al.* (2010) reported that amount of Pb in most of commercial poultry feed samples is higher than the minimum harmful levels. Chickens particularly during the younger age are susceptible to as little as 1.0 mg Pb/kg diet (Bakalli *et al.*, 1995). It has been suggested that the biochemical and molecular mechanisms of Pb toxicity involve the induction of oxidative stress in target cells and generation of reactive oxygen species, followed by DNA damage and apoptosis, partly via (i) direct effect of Pb on cell membranes, (ii) Pb–hemoglobin interactions, (iii) consistent reduction in blood Δ-aminolevulinic acid dehydratase, an erythrocyte enzyme sensitive to Pb and (iv) effect of Pb on the antioxidant defence systems of cells (Ercal *et al.*, 2001). Thus, Pb exposure is manifested with high lipid peroxidation and poor animal growth performance (Erdogan *et al.*, 2004).

Various approaches has been suggested to remove heavy metal pollution such as usage of algae (Davis *et al.*, 2003), agricultural by-products (Demirbas, 2008), garlic (Senapati *et al.*, 2001), yeast (see review by Soares and Soares, 2012), lactic acid bacteria (Teemu *et al.*, 2008) and fungi (Kapoor *et al.*, 1999). Although heavy metal removal ability of microorganisms has been reported previously in wastewater filtration, no report is available on their functionality within *in vivo* biological systems. Animal's digestive system is a dynamic microenvironment that can affect stability of

† E-mail: jbliang@upm.edu.my

reversible surface binding between microorganism and heavy metal. Successful excretion of heavy metal from animal's body require a stable bound throughout the digestive system. Thus, here we aimed to investigate *in vitro* and *in vivo* potential of two *Lactobacillus* isolates (LABs) from mulberry silage in biosorption of Pb and reduction of its preceding toxicity in broiler chickens. Previously, we have reported strong probiotic properties for these LABs (Altaher *et al*., 2015; Jahromi *et al*., 2016).

Material and methods

Bacterial isolates

The isolation, identification, characterization and screening procedure of probiotic bacteria used in this study has been elucidated in previous publication from our laboratory (Shokryazdan *et al*., 2015). Briefly, a total of 50 initial isolates from mulberry (*Morusalba*) silage were tested and two isolates of *Lactobacillus pentosus* ITA23 (GenBank accession number: KF297814) and *Lactobacillus acidipiscis* ITA44 (GenBank accession number: KF297816) were selected as highly resistant to bile and *acid, and reduced the pH level of culture medium to 3.2 and 3.7, respectively*. The pH reduction was mainly due to production of lactic acid (534 and 433 mM for *L. pentosus* ITA23 and *L. acidipiscis* ITA44, respectively) and acetic acid (91 and 93 mM for *L. pentosus* ITA23 and *L. acidipiscis* ITA44, respectively).

In vitro *study*

Approximately 3 ml of deMan–Rogosa–Sharpe broth (with or without Pb) was transferred to 5-ml tubes. The concentration of Pb was 155 mg/l by addition of lead acetate (200 mg/l; Nacalai Tesque, Kyoto, Japan). The medium samples were inoculated by 5% of inoculums (overnight culture of each bacterium) and incubated at 37°C for 24 h. At the end of incubation, the culture broth was quantitatively assessed using spectrophotometer at 650 nm. In order to determine the absorption rate of Pb by the cells, 1 ml of each culture was centrifuged at 10 000 r.p.m. for 5 min and the cell-free supernatant was assayed for Pb concentration using microwave digestion and inductively coupled plasma mass spectrometry (ICP-MS) (Ahmed *et al*., 2013).

In vivo *study*

Experimental design, birds and diets. The experiments were conducted in accordance to procedures approved by the Universiti Putra Malaysia Institutional Animal Care and Use Committee. Male broiler chicks aged 108 days (Cobb500) were purchased from a commercial hatchery, weighed and assigned at random to 18 battery cages of six chicks each in an open-sided house under natural tropical environment. Equal number of birds (six cages) were randomly allocated to one of three dietary treatments as follows: (i) basal diet (no lead acetate supplementation; control) (Table 1), (ii) basal diet +200 mg lead acetate/kg (equal to 155 mg Pb/kg) and (iii) basal diet +200 mg lead acetate/kg +1000 mg LABs/kg.

Table 1 *Ingredient composition and nutrient content of the basal diet (g/kg as fed unless otherwise stated)*

Ground yellow corn	538.9
Soybean meal	361.9
Fish meal	30.0
Palm oil	37.4
Choline chloride (60%)	2.5
Trimix[1]	1.0
Common salt	2.0
DL-Methionine	1.8
Limestone	13.0
Dicalcium phosphate	11.5
Calculated nutrient content	
CP	220.0
Crude fat	63.1
Crude fiber	38.0
Calcium	10.2
Phosphorus	4.5
Metabolizable energy (MJ/kg)	13.06

[1]Trimix (per kg Trimix): iron 100 g; manganese 110 g; copper 20 g; zinc 100 g; iodine 2 g; selenite 0.2 g; cobalt 0.6 g; santoquin 0.6 g; folic acid 0.33 g; thiamin 0.83 g; pyridoxine 1.33 g; biotin 2% 0.03 g; riboflavin 2 g; cyanocobalamin 0.03 g; D-calcium pantothenate 3.75 g; niacin 23.3 g; retinol 2000 mg; cholecalciferol 25 mg; α-tocopherol 23 000 mg IU.

Diets were prepared as mash form using raw ingredients according to Table 1. Lead acetate was product of Nacalai Tesque and the probiotic was an equal combination of two species of *Lactobacillus* (*L. pentosus* ITA23 and *L. acidipiscis* ITA44) with final cell viability of 10^9/g. The inclusion level of Pb was selected based on the National Research Council (1994) recommendations for minimum toxic level of Pb in chicken's diet. No feed additives or antimicrobials were used throughout the study. Chicks were maintained on a continuous light schedule and allowed *ad libitum* access to feed and water.

Data collection. Chicks were weighed individually on the 1st day and following 21 days of feeding experimental diets. Feed intake (FI) was recorded and feed conversion ratio (FCR) was calculated. On day 21, two birds from each cage were decapitated and blood, cecal content, jejunum and liver samples were collected.

Determination of Pb. Microwave digestion was used for determination of Pb concentrations (Ahmed *et al*., 2013). Briefly, 0.2 g of feed, liver, cecal content or 0.2 ml of serum were added to a solution of 6 ml nitric acid (17 M), 2 ml hydrogen peroxide (8.8 M) and 2 ml hydrogen fluoride (0.02 M) and digested in a microwave oven (Model MARS-5; CEM, Matthews, NC, USA). All standard and blank solutions were prepared as above and were analyzed using ICP-MS system (PE SCIEX ELAN 6000; PerkinElmer, Beaconsfield, Buckinghamshire, UK).

Histopathological study. Liver and jejunum samples were fixed in 10% formalin, dehydrated for 16 h in an automatic

tissue processor (Leica ASP 3000, Leica Microsystems Nussloch GmbH, Heidelberg, Germany) and embedded in paraffin wax using a paraffin embedding system (Leica EG 1160, Leica Microsystems Nussloch GmbH). Each sample was cut to 4-μm thick sections using a rotary microtome (Leica RM 2155, Leica Microsystems Nussloch GmbH). The sections were fixed on a glass slide, heated at 57°C until dried, stained with hematoxylin and eosin and examined under a light microscope (Dialux, LeitzWetzlar, Germany) as described previously (Grenier *et al*., 2012). A lesion score per animal was recorded in a blind way by a pathologist, taking into account the severity type and its intensity extent (scored from 0 to 3).

Antioxidant activity assay. Preparation of the liver tissue for antioxidant assay was according to the method of Bourogaa *et al*. (2013). Briefly, 1 g of liver tissue was homogenized in 2-ml tris buffered saline (TBS) (50 mM Tris, 150 mM NaCl, pH 7.4) in a homogenizer and centrifuged at 10 000 r.p.m. for 10 min. The supernatants were transferred into new 2-ml tube and stored at −80°C for subsequent analysis. The antioxidant activity of liver was determined using two methods of ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC). The TEAC method is also known as 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS) method. Different concentrations (0 to 100 μmol) of Trolox (water-soluble analogue of vitamin E) were used as standard and results expressed as μmol Trolox equivalent (TE)/g liver tissue.

Lipid peroxidation (malondialdehyde (MDA)). The lipid oxidation in liver samples was measured using thiobarbituric acid-reactive substances (TBARS). One gram of liver samples was homogenized in 4 ml 0.15 M KCl and 0.1 mM butylated hydroxytoluene (BHT). Homogenate samples (200 μl) were incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 ml) and 2.8% (w/v) trichloroacetic acid (0.25 ml) in water bath at 95°C for 60 min until pink color development. After cooling, 1 ml of distilled water and 3 ml of n-butyl alcohol were added to extracts and vortexed. The mixtures were centrifuged at 5000 r.p.m. for 10 min. Absorbance of supernatant was read against an appropriate blank at 532 nm using spectrophotometer (Secomam, Domont, France). The TBARS were calculated from a standard curve of 1, 1, 3, 3-tetraethoxypropane and expressed as microgram MDA per gram of liver.

Microbial quantification using quantitative real-time PCR. The populations of selected microbiota in the cecal content were determined using method described by Navidshad *et al*. (2012). Total DNA was extracted using QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, USA) according to manufacturer's instructions. The extracted DNA were then stored at −20°C until use. The standard curves were constructed using number of copies of the 16S ribosomal RNA (rRNA) gene plotted against quantification cycle obtained from 10-fold serial dilutions of PCR products from pure culture of each bacterial group. For preparation of standard curves, DNA was extracted from the pure culture of each target bacteria (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Enterobacteriaceae* and *Escherichia coli*) and conventional PCR with specific primers were used to amplify bacterial DNA (Table 2). PCR products of the target bacteria were run in 1% agarose gel and specific bands were purified using the MEGAquick-spin™ purification kit (iNtRON Biotechnology, Seoul, Gyeonggi-do, Korea). Purity and concentration of 16S rRNA gene in each sample was measured using Nanodrop ND-1000 spectrophotometer (Implen NanoPhotometer™, Munich, Germany). Copy number of 16S rRNA gene/ml of elution buffer was calculated using the following formula:

$$\text{Number of copies} = \frac{\text{Amount of DNA } (\mu\text{g / ml}) \times 6.022 \times 10^{23}}{\text{Length (bp)} \times 10^{9} \times 650}$$

Table 2 *Primers sequences and annealing temperatures for quantified microorganisms*[1]

Target group	Sequence 5′–3′	Annealing temperature (°C)
Lactobacilli	F: CATCCAGTGCAAACCTAAGAG R: GATCCGCTTGCCTTCGCA	58
Bifidobacteria	F: GGG TGGTAATGCCGGATG R: TAAGCCATGGACTTTCACACC	60
Enterococcus genus	F: CCCTTATTGTTAGTTGCCATCATT R: ACTCGTTGTACTTCCCATTGT	60
Enterobacteriaceae family	F: CATTGACGTTACCCGCAGAAGAAGC R: CTCTACGAGACTCAAGCTTGC	60
Escherichia coli	F: GTGTGATATCTACCCGCTTCGC R: AGAACGCTTTGTGGTTAATCAGGA	60

[1]Primers and annealing temperatures were according to Navidshad *et al*. (2012).

Real-time PCR was performed with BioRad CFX96 Real-time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). Each reaction consisted of 12.5 μl of 2× SYBR Green Master Mix (Thermo Scientific, PA, USA), 1 μl of 10 μM forward and reverse primers, 2 μl DNA and 8.5 μl nuclease-free water. Each sample was assayed in duplicate. No-template control was included to rule out any cross-contamination. Real-time PCR cycling condition comprised initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 20 s, primer annealing (according to Table 2) for 30 s and extension at 72°C for 20 s. Upon completion of amplification, specificity of the amplified product was confirmed using melting curve analysis. The real-time PCR products were incubated by raising the temperature from 65°C to 95°C in 0.5°C increments with a hold of 5 s at each increment.

Statistical analysis

Experimental data were analyzed using one-way ANOVA procedure of SAS program version 9.2 (version 9.3; SAS Institute Inc., Cary, NC, USA) followed by multiple comparison among the means using Duncan's new multiple range test. Differences were considered significant if $P < 0.05$.

Results and discussion

In vitro *study*

Addition of lead acetate had no negative effect on LABs growth (Figure 1a). The concentration of Pb in the control culture medium and after incubation in the presence LABs is presented in Figure 1b. Although the Pb concentration was 155 mg/l in control medium, it was significantly reduced by more than 90% to 14.29 mg/l and 15.49 mg/l in cultures of *L. pentosus* ITA23 and *L. acidipiscis* ITA44, respectively. The mechanism behind such biological Pb absorption could be attributed to negative charge of carboxyl and phosphoryl groups in cell membrane of LABs. These negative-charge molecules are able to bind with any positive-charge molecules such as Pb. The ion exchange mechanism of biosorption of heavy metal by microorganisms previously reported in *Aspergillus niger* (Kapoor and Viraraghavan, 1997), *Rhizopus arrhizus* (Tobin *et al*., 1990), *Lactobacillus fermentum* ME3 and *Bifidobacterium longum* 46 (Teemu *et al*., 2008). In all of these studies, heavy metal biosorption was significantly reduced by chemical intervention that neutralize the negative charge of carboxyl and phosphoryl groups. Teemu *et al*. (2008) confirmed the existence of such biosorption using transmission electron micrographs showing the cell membrane binding to Pb.

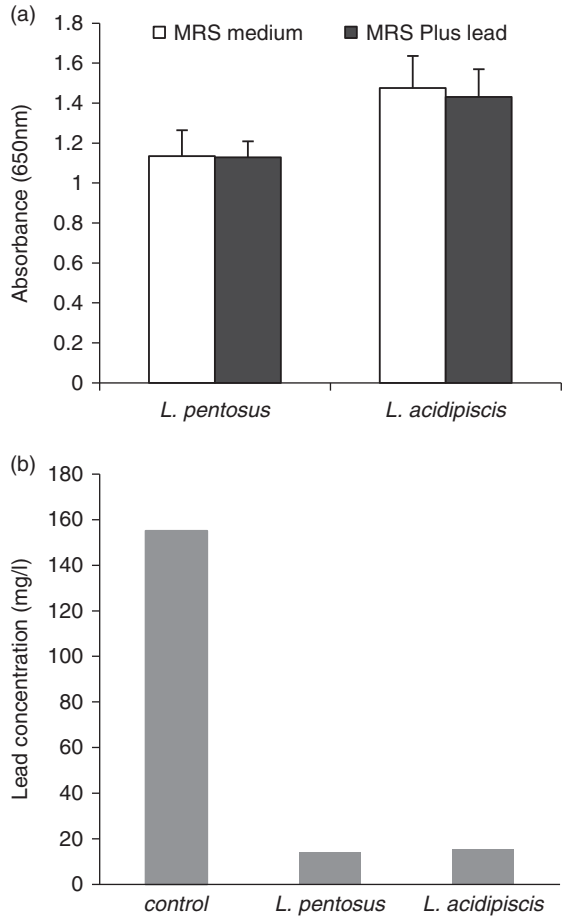

Figure 1 Lead (Pb) reduction potential of two selected *Lactobacillus* species *in vitro*. Growth of either of *Lactobacillus* species in deMan–Rogosa–Sharpe (MRS) medium was not inhibited by Pb (a) and both species largely reduced Pb level in the culture medium (b). The reduction rate was 90.8% and 90.0% for *Lactobacillus pentosus* and *Lactobacillus acidipiscis*, respectively.

In vivo *study*

Concentration of Pb in cecal content, liver and serum is presented in Table 3. Although lead acetate was not added to control diet, our analysis showed some scarce level of Pb in the feed (0.155 mg /kg) and negligible amount in the cecal content, serum and liver samples. Low concentrations of Pb in feedstuff such as fish meal, soybean meal and corn has been frequently reported before (Nicholson *et al*., 1999; Zhang *et al*., 2012). The concentration of Pb in cecal content of chickens supplemented with LABs was 1.7 times higher than the Pb-exposed chicks ($P < 0.05$). This observation together with the *in vitro* results indicated that LABs biosorption of Pb maintained throughout the gastrointestinal tract (GIT) and resulted in by-pass transition of Pb from GIT absorption sites. This is also evident by the significant Pb reduction observed in liver and serum of chicks fed Pb + probiotic diet ($P < 0.05$). Although probiotic was not able to reduce Pb accumulation in liver and serum to the same levels as in the control chicks, the reduction was sufficient to protect the chicks against the Pb adverse effects, as documented by other parameters such as liver lesion and performance. It is noteworthy to mention that the existence of interaction between microenvironment of GIT (i.e. pH) and stability of binding between Pb and LABs is still an open question to be answered.

Oxidative parameters and tissue lesion. The effects of Pb and LABs supplementation on MDA and antioxidant activity (FRAP and ABTS) are presented in Table 4. The MDA level was increased ($P < 0.05$) in liver of Pb received birds by nearly twofolds compared with those of control group (248.06 *v.* 130.66 μg MDA/g). MDA elevation represents a significant increase in lipid peroxidation. As measured by markers of oxidative (MDA) and antioxidative status (FRAP and ABTS), our results attribute the negative effects of Pb exposure to free radical generation. Ercal *et al*. (2001), Erdogan *et al*. (2004) and Prvulovic *et al*. (2015) reported the same finding. Therefore, the elevation of MDA or FRAP and ABTS could be explained by the massive production of free

Table 3 *Recovery of lead (Pb) in the cecal content and its accumulation in liver and serum of broiler chickens in different treatment groups*

	Control	Pb[1]	Pb + probiotic[2]	SEM	*P*-values
Cecal content (mg/kg)	1.88[c]	17.77[b]	30.81[a]	2.07	***
Liver (mg/kg)	0.05[c]	0.64[a]	0.33[b]	0.06	***
Serum (mg/l)	0.01[c]	0.64[a]	0.46[b]	0.03	***

$n = 12$.
[a,b,c]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Pb was added in the form of lead acetate in the concentration of 200 mg/kg feed.
[2]Probiotic was added in the concentration of 100 mg/kg feed (~10^9 cells/kg).
***$P < 0.001$.

Table 4 *Effect of lead (Pb) and probiotic supplementation on oxidative parameters in liver of broiler chickens*

Parameters	Control	Pb[1]	Pb + probiotic[2]	SEM	*P*-values
Lipid peroxidation (μg malondialdehyde/g liver)	130.66[b]	248.06[a]	147.40[b]	17.74	**
Ferric reducing antioxidant power (TE/g liver)	10.64[a]	8.16[b]	11.36[a]	0.80	*
ABTS (TE/g liver)	10.40[a]	8.88[b]	10.49[a]	0.79	*

TE = μmol Trolox equivalent; ABTS = 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation.
$n = 6$.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Pb was added in the form of lead acetate in the concentration of 200 mg/kg feed.
[2]Probiotic was added in the concentration of 100 mg/kg feed (~10^9 cells/kg).
*$P < 0.05$; **$P < 0.01$.

Table 5 *Effect of lead (Pb) and probiotic supplementation on lesion score of liver and jejunum in broiler chickens*

Lesion score[1]	Control	Pb[2]	Pb + probiotic[3]	SEM	*P*-values
Liver	0.98[b]	2.17[a]	1.08[b]	0.09	**
Jejunum	1.12[b]	2.13[a]	1.80[a]	0.18	**

$n = 6$
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]The score for each lesion was obtained by multiplying the severity factor by the extent of the lesion. The organ score was then obtained by calculating the sum of each lesion score. Severity factor (or degree of severity), 1 = mild lesions, 2 = moderate lesions; the extent of each lesion (intensity or observed frequency) was evaluated and scored as 0 = no lesion, 1 = low extent, 2 = intermediate extent, 3 = large extent.
[2]Pb was added in the form of lead acetate in the concentration of 200 mg/kg feed.
[3]Probiotic was added in the concentration of 100 mg/kg feed (~10^9 cells/kg).
**$P < 0.01$.

Table 6 *Effect of lead (Pb) and probiotic supplementation on feed intake (FI), BW and feed conversion ratio (FCR) of broiler chickens*

	Control	Pb[1]	Pb + probiotic[2]	SEM	*P*-values
Initial weight (g)	52	53	50	2	Ns
Final weight (g)	676[a]	610[b]	682[a]	21	*
FI (g)	883[a]	843[b]	885[a]	20	*
FCR	1.44[b]	1.55[a]	1.42[b]	0.06	*

$n = 6$.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Pb was added in the form of lead acetate in the concentration of 200 mg/kg feed.
[2]Probiotic was added in the concentration of 100 mg/kg feed (~10^9 cells/kg).
*$P < 0.05$.

radicals and preceding exhaustion of antioxidant enzymatic activity (Sharma and Barber, 2012; Prvulovic *et al*., 2015). Interestingly in our study, LABs supplementation completely restored oxidative and antioxidative status of the birds to its normal state (Table 4). This observation was despite the presence of Pb in liver and serum of Pb + probiotic birds. It seems that the accumulated Pb in tissues of these birds was below the toxicity level. This notion is supported by histopathological results obtained in liver samples (Table 5). Lesion score analysis revealed that Pb presence had detrimental effect on liver tissue ($P < 0.05$), but LABs supplementation eliminated such tissue damage. The beneficial effect of LABs in reducing liver lesion score could be attributed to its role in lowering Pb levels to non-toxic amounts. However, we did not observe the same beneficial effect in jejunum tissue. It is possible that some proportions of Pb were still present in active form in the jejunum and the biosorption process was not fully completed.

Performance parameters. Effect of Pb exposure and LABs supplementation on BW gain, FI and FCR are shown in Table 6. Presence of Pb in the feed significantly reduced FI and weight gain and increased FCR. This is in agreement with previous reports by Damron *et al*. (1969), Seven *et al*. (2012) and Yuan *et al*. (2013). Previous study in our laboratory suggested that downregulation of major nutrient transporter genes in small intestine is one of the possible underlying mechanism for such observation (Ebrahimi *et al*., 2015). The FI reduction and preceding weight gain suppression of Pb-exposed birds could also be attributed to anorexia and anxiety caused by reduction of brain 5-hydroxytryptamine (serotonin) (Haider *et al*., 2013). This is also in part explain the elevation observed in FCR. Furthermore, the negative effect of Pb exposure on FCR and weight gain maybe also associated with Pb effects on metabolic and endocrine responses (Scheuhammer, 1987; Yuan *et al*., 2013). Exposure to Pb elevate plasma corticosterone (Cory-Slechta *et al*., 2004; Haider *et al*., 2013), and consequently a diverse range of glucocorticoids effects take place from immune suppression to reduction of intestinal absorptive surface area (Hu *et al*., 2010). Interestingly, LABs supplementation significantly eliminated the adverse effect of Pb on all performance parameters. The reason for such effect might come from the Pb biosorption by LABs, removing the Pb from intestinal absorption sites, and therefore reducing Pb accumulation in body (Table 6). Lowering Pb accumulation reduce the toxic effects of Pb exposure in birds, as documented here with lower liver lesion and lipid peroxidation, and higher antioxidant capacity. It is noteworthy to mention that the observed performance in our study was lower than the expectations from this strain of broiler chicks. This is probably due to the rearing environment of the chicks. Previous studies also reported such inferior performance in broiler chicks exposed to hot humid tropical condition (Yalcin *et al*., 1997; Yodseranee and Bunchasak, 2012).

Table 7 *Effect of lead (Pb) and probiotic supplementation on population of microorganisms in the cecum of broiler chickens (log10 copy number/g cecal content sample)*

Microorganisms	Control	Pb[1]	Pb + probiotic[2]	SEM	*P*-values
Enterobacter	6.83[a]	6.77[a]	5.57[b]	0.23	*
Escherichia coli	5.67[a]	5.73[a]	5.27[b]	0.24	*
Lactobacillus	7.23[b]	7.11[b]	7.77[a]	0.22	*
Bifidobacteria	6.53[ab]	6.41[b]	6.77[a]	0.15	*
Enterococcus	5.98	6.12	6.46	0.33	Ns

$n = 12$.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Pb was added in the form of lead acetate in the concentration of 200 mg/kg feed.
[2]Probiotic was added in the concentration of 100 mg/kg feed (~10^9 cells/kg).
*$P < 0.05$.

Microbial populations. Table 7 showed the population of various microbiota in the cecum. There was no significant difference in microbial population between the control and Pb-exposed birds. However, supplementation of LABs significantly increased beneficial microbe population at the cost of pathogens. It has been reported that a modes of action of probiotics is their pathogen interference and antagonistic activity, whereby probiotic strains inhibit the growth and colonization of other microorganisms, such as *E. coli* (Mountzouris *et al*., 2007; Meimandipour *et al*., 2010). This is a competitive exclusion where microbes are competing for nutrient and attachment sites on intestinal epithelial wall (Fuller, 2001). It is therefore reasonable to attribute some portion of the performance recovery in Pb + probiotics birds to effect of LABs supplementation in favoring microbes such as *Lactobacillus* and *Bifidobacteria*.

Conclusions

Results of *in vitro* and *in vivo* studies suggested that supplementation of *L. pentosus* ITA23 and *L. acidipiscis* ITA44 besides the classical effect of favoring beneficial microbes at the cost of pathogens; it may also biosorb Pb and reduce its absorption by broiler chickens GIT. Therefore, LABs supplementation may protect the birds against the adverse effects of Pb exposure on growth performance, lipid peroxidation, antioxidant activity and tissue damage.

Acknowledgments

This study was sponsored by the Long-Term Research Grant Scheme (LRGS 2/2012) provided by the Ministry of Education Malaysia.

References

Ahmed AY, Abdullah MP, Wood AK, Hamza MS and Othman MR 2013. Determination of some trace elements in marine sediment using ICP-MS and XRF (a comparative study). Oriental Journal of Chemistry 29, 645–653.

Altaher YW, Jahromi MF, Ebrahim R, Zulkifli I and Liang JB 2015. *Lactobacillus pentosus* ITA23 and *Lactobacillus acidipiscis* ITA44 enhance feed conversion efficiency and beneficial gut microbiota in broiler chickens. Revista Brasileira de Ciência Avícola 17, 159–164.

Bakalli RI, Pesti GM and Ragland WL 1995. The magnitude of lead toxicity in broiler chickens. Veterinary and Human Toxicology 37, 15–19.

Bourogaa E, Nciri R, Mezghani-Jarraya R, Racaud-Sultan C, Damak M and El-Feki A 2013. Antioxidant activity and hepatoprotective potential of Hammada scoparia against ethanol-induced liver injury in rats. Journal of Physiology and Biochemistry 69, 227–237.

Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD and Bauter MR 2004. Maternal stress modulates effects of developmental lead exposure. Environmental Health Perspectives 112, 717–730.

Damron BL, Simpson CF and Harms RH 1969. The effect of feeding various levels of lead on the performance of broilers. Poultry Science 48, 1507–1509.

Davis TA, Volesky B and Mucci A 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Research 37, 4311–4330.

Demirbas A 2008. Heavy metal adsorption onto agro-based waste materials: a review. Journal of Hazardous Materials 157, 220–229.

Ebrahimi R, Jahromi MF, Liang JB, Soleimani AF, Shokryazdan P and Zulkifli I 2015. Effect of dietary lead on intestinal nutrient transporters mRNA expression in broiler chickens. Biomed Research International 28, 149745.

Ercal N, Guerer-orhan H and Aykin-burns N 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal induced oxidative damage. Current Topics in Medicinal Chemistry 1, 529–539.

Erdogan Z, Erdogan S, Aksu T and Baytok E 2004. The effects of dietary lead exposure and ascorbic acid on performance, lipid peroxidation status and biochemical parameters of broilers. Turkish Journal of Veterinary Animal Sciences 29, 1053–1059.

Fox MR 1987. Assessment of cadmium, lead and vanadium status of large animals as related to the human food chain. Journal of Animal Science 65, 1744–1752.

Fuller R 2001. The chicken gut microflora and probiotic supplements. The Journal of Poultry Science 38, 189–196.

Grenier B, Bracarense AP, Schwartz HE, Trumel C, Cossalter AM, Schatzmayr G, Kolf-Clauw M, Moll WD and Oswald IP 2012. The low intestinal and hepatic toxicity of hydrolyzed fumonisin B1 correlates with its inability to alter the metabolism of sphingolipids. Biochemical Pharmacology 83, 1465–1473.

Haider S, Saleem S, Tabassum S, Khaliq S, Shamim S, Batool Z, Parveen T, Inam QU and Haleem DJ 2013. Alteration in plasma corticosterone levels following long term oral administration of lead produces depression like symptoms in rats. Metabolic Brain Disease 28, 85–92.

Hu XF, Guo YM, Huang BY, Bun S, Zhang LB, Li JH, Liu D, Long FY, Yang X and Jiao P 2010. The effect of glucagon-like peptide 2 injection on performance, small intestinal morphology, and nutrient transporter expression of stressed broiler chickens. Poultry Science 89, 1967–1974.

Jahromi MF, Altaher YW, Shokryazdan P, Ebrahimi R, Ebrahimi M, Zulkifli I, Goh YM, Tufarelli V and Liang JB 2016. Dietary supplementation of a mixture of Lactobacillus strains enhances performance of broiler chickens raised under heat stress conditions. *International Journal of Biometeorology* 60, 1099–1110.

Kapoor A and Viraraghavan T 1997. Heavy metal biosorption sites in *Aspergillus niger*. Bioresource Technology 61, 221–227.

Kapoor A, Viraraghavan T and Cullimore DR 1999. Removal of heavy metals using the fungus *Aspergillus niger*. Bioresource Technology 70, 95–104.

Mahesar SA, Sherazi STH, Niaz A, Bhanger MI and Abdul Rauf S 2010. Simultaneous assessment of zinc, cadmium, lead and copper in poultry feeds by differential pulse anodic stripping voltammetry. Food Chemical and Toxicology 48, 2357–2360.

Meimandipour A, Shuhaimi M, Soleimani AF, Azhar K, Hair-Bejo M, Kabeir BM, Javanmard A, Muhammad Anas O and Yazid AM 2010. Selected microbial groups and short-chain fatty acids profile in a simulated chicken cecum supplemented with two strains of Lactobacillus. Poultry Science 89, 470–476.

Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G and Fegeros K 2007. Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poultry Science 86, 309–317.

National Research Council 1994. Nutrient requirements of poultry, 9th revised edition. The National Academy Press, Washington, DC, USA.

Navidshad B, Liang JB and Jahromi MF 2012. Correlation coefficients between different methods of expressing bacterial quantification using real time PCR. International Journal of Molecular Sciences 13, 2119–2132.

Nicholson FA, Chambers BJ, Williams JR and Unwin RJ 1999. Heavy metal contents of livestock feeds and animal manures in England and Wales. Bioresource Technology 70, 23–31.

Prvulovic D, Kojic D, Popovic M and Grubor-Lajsic G 2015. Inhibitory effects of aluminosilicates on lead acetate toxicity in selected organs of broilers. The Thai Veterinary Medicine 45, 255–261.

Scheuhammer AM 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review. Environmental Pollution 46, 263–295.

Senapati SK, Dey S, Dwivedi SK and Swarup D 2001. Effect of garlic (Allium sativum L.) extract on tissue lead level in rats. Journal of Ethnopharmacology 76, 229–232.

Seven I, Aksu T and Tatli-Seven P 2012. The effects of propolis and vitamin C supplemented feed on performance, nutrient utilization and carcass characteristics in broilers exposed to lead. Livestock Science 148, 10–15.

Sharma R and Barber I 2012. Histopathological alterations in developing duodenum of Swiss mice, exposed to lead acetate. Journal of Chemical Biological and Physical Sciences 2, 1312–1318.

Shokryazdan P, Liang JB, Jahromi MF and Abdullah N 2015. Probiotic potential of lactic acid bacteria isolated from Mulberry silage. Journal of Pure and Applied Microbiology 9 (Spl. Edn. 2), 443–452.

Soares EV and Soares HM 2012. Bioremediation of industrial effluents containing heavy metals using brewing cells of Saccharomyces cerevisiae as a green technology: a review. Environmental Science and Pollution Research 19, 1066–1083.

Teemu H, Seppo S, Jussi M, Raija T and Kalle L 2008. Reversible surface binding of cadmium and lead by lactic acid and bifidobacteria. International Journal of Food Microbiology 125, 170–175.

Tobin J, Cooper D and Neufeld RJ 1990. Investigation of the mechanism of metal uptake by denatured *Rhizopus arrhizus* biomass. Enzyme and Microbial Technology 12, 591–595.

Yalcin S, Settar P, Ozkan S and Cahaner A 1997. Comparative evaluation of three commercial broiler stocks in hot versus temperate climates. Poultry Science 76, 921–929.

Yodseranee R and Bunchasak C 2012. Effects of dietary methionine source on productive performance, blood chemical, and hematological profiles in broiler chickens under tropical conditions. Tropical Animal Health and Production 44, 1957–1963.

Yuan C, Song HH, Jiang YJ, Azzam MMM, Zhu S and Zou XT 2013. Effects of lead contamination in feed on laying performance, lead retention of organs and eggs, protein metabolism, and hormone levels of laying hens. Journal of Applied Poultry Research 22, 878–884.

Zhang F, Li Y, Yang M and Li W 2012. Content of heavy metals in animal feeds and manures from farms of different scales in northeast China. International Journal of Environmental Research and Public Health 9, 2658–2668.