**Egyptian Journal of Animal Health**

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

# Biotherapeutic Role of Bactozyme against Heavy Metals Contamination of Broiler Feed

**Amany M. Salama[*], Seif E. Salem[*] and Ola F.A. Talkhan[**]**

[*] Toxicology Unit, Biochemistry Dept., Animal Health Research Institute, Agriculture Research Center
[**]Chemistry Dept., Animal Health Research Institute-Shiben El- Kom lab., Agriculture Research Center

**Article History**
Received in 4/1/2021
Received in revised from 27/1/2021
Accepted in 2/3/2021

**Keywords:**
Probiotic
Heavy metals
Broiler feed

## ABSTRACT

Contamination of poultry feed by heavy metals represents a risk to human health as they bioconcentrates in the food chain. As probiotics are worldwide distributed in many different products and with proven benefits to both human and animal health. This study is designed to investigate the role of the probiotic; Bactozyme; to overcome contamination of broiler feed by heavy metals. Forty chicks at age of 20 days were divided into 4 groups; $1^{st}$ group served as control, $2^{nd}$ group was administered with 0.5 gm Bactozyme/ liter water; $3^{rd}$ group received contaminated feed with heavy metals, and $4^{th}$ group received both contaminated feed and Bactozyme. All chickens were sacrificed at the day $40^{th}$ of age. Sera were collected from all groups to determine liver and kidney function tests, samples from liver, kidney and muscles were collected and preserved at -20°C for residual detection of heavy metals using Atomic Absorption Spectrometry.

The results showed that feeding broilers on heavy metals contaminated diet led to significant increase of liver enzymes (AST, ALT& ALP), significant decrease of total protein, globulin, and significant increase of creatinine while albumin is insignificantly decreased. Residues of lead, cadmium and arsenic were significantly increased in all examined tissues. The groups received bactozyme with/ without heavy metals contaminated feed showed improvement of all tested parameters that strengthen the hypothesis of efficiency of robotics to tolerate heavy metals toxicity in broilers.

## INTRODUCTION

The increased demand of the Egyptian population for low-cholesterol and high protein meat sources has led to high consumption of the poultry meat due to its affordability. Many heavy metals such as arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg) and others are found in certain animal foods, or poultry feed additives **(Jadhav et al. 2007).** There is no standard definition for heavy metals. Generally, it is referred to a group of metals that are not required or required in trace amounts, they

Corresponding author: Amany M. Salama, Toxicology Unit, Biochemistry Dept., Animal Health Research Institute, Agriculture Research Center
E-mail: amanysultan2025@gmail.com
DOI: 10.21608/EJAH.2021.160805

have high densities, atomic weights, or atomic numbers. Their bioavailability is controlled by chemical and physical factors like temperature, absorption, and physiological characteristics of the exposed species **(Stern 2010)**.Their contribution in industry, medicine and agriculture have resulted in their environmental bioaccumulation that raising concerns about their toxic effects on human health and environment. High exposure to heavy metals can occur through ingestion, inhalation or dermally causing several health problems such as neurological and neurobehavioral disorders, blood abnormalities, cancers, and cardiovascular diseases **(Mehrandish et al. 2019).**Also increasing the frequency of chronic diseases like renal failure, liver cirrhosis, and anemias among Egyptian population **(Salem et al. 2000).**There are several potential sources of toxic metals for poultry. Heavy metals may contaminate soil and water through sewage sludge application, industrial waste disposal, application of pesticides and fertilizers and atmospheric deposition **(Adaikpoh et al. 2005).**

Arsenic compounds have been used for treatment of diseases like syphilis, amoebic diarrhea and trypanosome **(Centeno et al. 2006)**. Arsenicis available in the form of oxides, sulfides or as a salt of iron, calcium, copper, etc. **(Singh et al. 2007).**Phenyl arsenic acids are widely used as poultry feed additive to control coccidiosis, improves production, and imparts attractive color to the chicken flesh **(Ghosh et al. 2012; Mondal 2020).** Although arsenic isessential for synthesis of methionine metabolites in poultry, but it is carcinogenic **(Balos et al. 2019)**,Trivalent arsenic compounds are more toxic than the pentavalent forms. It produces toxicity when the concentration of arsenic compounds is 2-10 times higher than the recommended dose, which is usually 100 mg/ kg complete feed. Most of the toxic effects arise from exposure to inorganic arsenic from commercial uses such as wood preservatives and herbicides. Its toxicity usually attributed to low methylating ability **(Suganya et al. 2016)**. "Arsenic, however, is not a well-documented element when it comes to biota and particularly to birds" **(Sánchez-Virosta et al. 2015).**

Lead is a highly toxic metal naturally present in the earth's crust, it has been disseminated throughout the environments by industries like battery manufacturing, motor vehicle repair, piping, paints, food wrapping, tobacco and as an additive in gasoline **(Alloway and Ayres 1993)**. Lead is a "multimedia" contaminant, with sources include air, water, soil, dust, food, paint. Contamination of food can arise from food processing, food handling, or deposition of atmospheric lead on agricultural crops. Inorganic lead has been only detected in food **(WHO 2011).** Lead poisoning can occur in domestic animals as horses, poultry, and dogs **(Khan et al. 2008). (Suganya et al. 2016)** reported that diet contains as low as 1.0 ppm of lead can cause significant growth suppression in chickens and decline in blood Delta-aminolevulinic acid dehydratase.

Cadmium is a metal chemically similar to zinc and occurs naturally with zinc and lead in sulfide ores. It is a by-product of zinc production. Cadmium is mostly released to environment from industrial processes, waste disposal and cigarette smoke. Contamination with cadmium in the animal feed industry is mostly in conjunction with the use of zinc sulfate. Other sources include mining, discarded cadmium-chloride products, and use of phosphate and sewage sludge fertilizer. Cadmium is considered one of contaminants that are widely distributed in foods, can concentrate along the food chain reaching human body, it has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans **(WHO 2011).** Cadmium exposure can induce oxidative stress, alter the activities of antioxidant enzymes of erythrocytes of the adult poultry birds **(Kant et al. 2011).**

Eliminating of these heavy metals from animal food supply is difficult because these metals are found in the air, water, and soil **(AAFCO 2019)**. Indestructibility with bioaccumulation of heavy metals contribute to the possibility of being as toxicants. The possible approach of their removal is their chelation as metals cannot be catabolized **(Tokar et al. 2015)**.

Removal of toxic metals by microbial biomass is a promising inexpensive conventional method that has been introduced for decontamination of food **(Zoghi et al. 2014)**. Robotics are live nonpathogenic, non-toxicogenic microbe sused as feed supplements that beneficially affect the host animal by improving microbial balance **(Fuller 1989)** according to WHO and FDA probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host **(WHO/FAO 2001)**. Probiotics can exert benefits to both human and animal health as proven in hundreds of scientific research.es, and they are marketed commercially indifferent brands all over the world **(Soccol et al. 2010).** Probiotic species like Lactobacillus, Streptococcus, Bifid bacterium, Enterococcus, Aspergillums and Saccharomyces have a beneficial effect on broiler performance **(Ashayerizadeh et al. 2009)** and on modulation of intestinal micro flora and pathogen inhibition **(Higgins et al. 2007)**.

The populations of microorganism which are found within the gastrointestinal tract of poultry have of two types of microorganisms at their gastrointestinal tract. The first type called autochthonous bacteria which colonize the gut from the environment due to feeding behavior or other activities **(Gusils et al. 1999)**. The second type is allocthonous bacteria which are exogenous and introduced to gastrointestinal tract as a dietary supplement through the feed or drinking water as direct fed microbial (DFM) or probiotics **(Chichlowski et al. 2007).** Probiotics have great adhesive properties that significantly inactivate toxins by surface binding **(Zoghi et al. 2014)**. They have been investigated to have the capacity to bind targets like aluminium, arsenic, lead, and cadmium, and eliminate them out of body (**Abdel-Megeed 2020).** In addition**,** Lactic acid bacteria are reported to remove heavy metals *in vitro* **(Halttunen et al. 2008)** as they can bind to cationic heavy metals, cadmium, and lead **(Zoghi et al. 2014)**. Combinations of specific probiotic strains is thought to be more effective than one depending on the target **(Timmerman et al. 2004).**

Probiotics were previously recorded to its powerful capacity to bind numerous targets and eliminate them with feces. These targets may be aluminum, cadmium, lead, or arsenic Probiotics were previously recorded to its powerful capacity to bind numerous targets and eliminate them with feces. These targets may be aluminum, cadmium, lead, or arsenic powerful capacity to bind targets like aluminium, arsenic, lead and cadmium, and eliminate them out of body (**Abdel-Meg** This study is designed to investigate the biomodulation effect of the probiotic; Bactozyme; to overcome the undesirable effects of feeding broilers with heavy metals contaminated feed.

## MATERIALS& METHODS

### Materials:

**Broiler chicks:** forty chicks at age of one day are raised on healthy feed till the beginning of the experiment at day 20 of age.

**Probiotic:** Bactozyme, obtained from MILES Chemical Solution, LLC, USA, consists of a synergistic blend from direct feed microbial (Lactobacillus acidophillus $40x10^9$ CFU, Aspergillus oryzae fermentation extracts 40GM, Bacillus subtilis fermentation extracts 50GM and Bifidobacterium bifidum $2x10^9$ CFU) in combination with enzymes and butyrate.

**Broiler feed:** Was purchased from local market, it contains protein (21%), fat (4.04%), 2.44% ash and energy 3000 kcal/kg ration.

### Methods:

Chickens were randomly divided into 4equal groups; 1st group received a balanced diet pre-examined to be free of heavy metals (Arsenic, lead& cadmium) using atomic absorption spectrometry; 2nd group received balanced diet and Bactozyme (0.5 gm/ liter water); 3rd group received contaminated diet that pre-examined and confirmed to contain high levels of heavy metals, as described in (Table1) and 4th group received both the contaminated diet and Bactozyme (0.5 gm/ liter water). Birds were maintained at 24-h light schedule. Feed and water were supplied ad libitum throughout the entire experiment. All chickens were sacrificed at the end of 6th week of age, fresh blood samples were immediately collected into sterile centrifuge tubes and centrifuged at 3000rpm for 15 minutesfor sera collection. The clear supernatant serum was aspirated into dry sterile vials and stored at (-20ºC) until used for estimation of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase(ALP), total protein, albumin, globulin, and creatinine.

Both ALT and AST were determined colorimetrically according to **Reitman and Frankel (1975)** and **Tietz et al. (1983)**, respectively, while ALP was estimated according to **Henry et al. (1960)**.

Total protein was determined according to the method of **Henry (1964)** using Biuret Method, and albumin was determined colorimetrically according to **Doumas et al. (1971)**.

Creatinine was determined according to **Teitz (1986)** using colorimetric method.

Samples from liver, kidney and muscles from each group, and their feed, were cut and preserved at -20°C for residual detection of heavy metals using atomic absorption spectrometry model No. A7942 according to **AOAC(2002)**.

**Feed pre-examination for residual detection of heavy metals:**

Dried feed sample weighed 2.0g was placed in crucibles with Conc. nitric acid (1cm$^3$) as ashing aid then pre-ashed by placing on a heater until the content charred, then transferred into a muffle furnace at a temperature of 480$^o$C for 2-3hrs. The cooled samples were dissolved by 5cm$^3$ of 30% HCl and then filtered using What man filter papers. The filtrate was individually poured into 50cm$^3$ volumetric flask and made up to the mark with deionized water. The sample solution was analyzed by atomic absorption spectrometry **(Okoye et al. 2011).**

**Tissue preparation for residual detection of heavy metals:**

one gram of each tissue sample was digested with10 ml of nitric / sulfuric / perchloric acids (8:1:1). Initial digestion was made for 4 hours at room temperature followed by heating at 40-45°C for one hour in water bath then temperature was raised to 75°C until the end of digestion. After cooling at room temperature, the cold digest was diluted to 20 ml deionized water and filtered through 0.45 µl Whatman filter paper. The clear filtrate of each sample was kept in refrigerator till analysis using atomic absorption spectrometry **(Okoye et al. 2011)**.

**Statistical analysis**

Data analysis was carried out using analyses of variance (F-test) followed by Duncan's multiple range test. A probability at a level of 0.05 or less was considered significant.

**RESULTS**

This study examined the tissues (liver, kidney& muscles) of broiler chickens after feeding on contaminated diet by heavy metals with/ without the probiotic; Bactozyme. The obtained results detected high levels of arsenic, lead and cadmium in broiler feed as shown in table (1). Regarding the effect on hepatic enzymes, AST, ALT and ALP are significantly increased (76.50 ± 3.33, 12.83 ± 0.49, 184.67 ± 3.28) in the group received contaminated diet in comparison to control group (54.00 ± 7.77, 9.00 ± 0.53, 119.67 ± 7.36)respectively, as recorded in table (2). These parameters are within control levels in groups received contaminated diet with Bactozyme or Bactozyme only. Total protein is high significantly decreased. Creatinine is significantly increased (0.65±0.1)in the group received contaminated dietin comparison to control group. Other groups showed normal levels of albumin, globulin and creatinine except total protein which is significantly increased(6.90 ± 0.24) in the group fed on Bactozyme only and significantly decreased in group fed on contaminated diet with Bactozyme (4.98 ± 0.22) in compare to control group (5.58 ± 0.10) as shown in table (2).

Table (1) Preliminary pre-examination of broiler feed (contaminated feed) for heavy metal residues (ppm) & their maximum tolerance limit.

| Broiler feed / Residue (ppm) | Starter broiler feed | | Grower broiler feed | | Finisher broiler feed | | Maximum tolerance limit |
|---|---|---|---|---|---|---|---|
| | Control | Contaminated | Control | Contaminated | Control | Contaminated | |
| **Cadmium** | 0.034 ± 0.006 | 1.23 ± 0.243 | 0.098 ± 0.04 | 0.79 ± 0.29 | 0.064 ± 0.03 | 1.014 ± 0.214 | 1 mg/kg **FAO/ WHO(1992)** |
| **Lead** | 0 | 0.527 ± 0.108 | 0.03 ± 0.015 | 0.61 ± 0.15 | 0.082 ± 0.02 | 0.52 ± 0.163 | 5 mg/kg **FAO/ WHO (1992)** |
| **Arsenic** | 2.239 ± 1.157 | 9.350 ± 1.623 | 1.457 ± 0.07 | 6.311 ± 1.47 | 1.463 ± 0.79 | 4.426 ± 0.522 | 2000 ug/kg **FAO/WHO (1992)** |

Table (2) Changes in parameters of kidney & liver function tests after feeding broilers with heavy metals contaminated diet with/ without Bactozyme in comparison to control:

| Groups / Parameters | Control group | Bactozyme group | Heavy metal contaminated feed group | Contaminated feed + Bactozyme group |
|---|---|---|---|---|
| **Total protein** | 5.58±0.10 [b] | 6.90±0.24 [a] | 4.17±0.06 [d] | 4.98±0.22 [c] |
| **Albumin (g/dl)** | 2.52±0.31 [a] | 3.41±0.33 [a] | 1.52±0.22 [a] | 3.28±1.06 [a] |
| **Globulin (g/dl)** | 3.29±0.13 [a] | 2.93±0.12 [ab] | 2.32±0.19 [b] | 2.82±026 [ab] |
| **Creatinine (mg/dl)** | 0.32±0.03 [b] | 0.31±0.03 [b] | 0.65±0.1 [a] | 0.41±0.05 [b] |
| **AST(IU/ml)** | 54.00±7.77 [b] | 44.33±3.48 [b] | 76.50±3.33 [a] | 60.00±3.61 [b] |
| **ALT(IU/ml)** | 9.00±0.53 [a] | 9.33±0.67 [a] | 12.83±0.49 [b] | 12.3±0.67 [b] |
| **ALP(IU/ml)** | 119.67±7.36 [c] | 98.00±3.61 [c] | 184.67±3.28 [a] | 143.33±11.26 [b] |

Each value represents means ± S.E. Values with different letters in the same row represent significant difference at P ≤ 0.05 by Duncan multiple range test.

Table (3) The concentration of heavy metals; Cadmium; Lead and Arsenic in µg/gm tissue (ppm) in broilers fed on heavy metals contaminated diet with/ without Bactozyme in comparison to control & their maximum permissible limits according to E.O.S.Q.C and FAO/WHO:

| Heavy metal residue (ppm) | Groups / Tissues | Control group | Bactozyme group | Heavy metal contaminated feed group | Contaminated feed+ Bactozyme group | Heavy metal maximumpermissiblelimit (ppm) |
|---|---|---|---|---|---|---|
| Cadmium | Liver | 0.065±0.032$^{b}$ | 0.057±0.0198$^{b}$ | 0.178± 0.037$^{a}$ | 0.04±0.015$^{b}$ | E.O.S.Q.C(2005): 0.5 FAO/WHO(1992):0.05 |
| | Kidney | 0.046±0.025$^{b}$ | 0.028±0.11$^{b}$ | 0.192±0.053$^{a}$ | 0.05±0.02$^{b}$ | E.O.S.Q.C(2005): 0.1 FAO/WHO(1992):0.05 |
| | Muscles | 0.02±0.01$^{b}$ | 0.063±0.014$^{b}$ | 0.126±0.028$^{a}$ | 0.032±0.005$^{b}$ | E.O.S.Q.C(2005): 0.1 FAO/ WHO(1992):0.05 |
| Lead | Liver | 0±0$^{b}$ | 0.01±0.009$^{b}$ | 0.89±0.3$^{a}$ | 0.007±0.007$^{b}$ | E.O.S.Q.C(2005): 0.1 FAO/ WHO(1992):0.05 |
| | Kidney | 0±0$^{b}$ | 0.076±0.076$^{b}$ | 0.74±0.46$^{a}$ | 0.022±0.02$^{b}$ | E.O.S.Q.C(2005): 0.1 FAO/ WHO(1992):0.05 |
| | Muscles | 0.027±0.027$^{b}$ | 0.013±0.01$^{b}$ | 0.096±0.05$^{a}$ | 0.005±0.005$^{b}$ | E.O.S.Q.C(2005): 0.1 FAO/ WHO(1992):0.05 |
| Arsenic | Liver | 1.19±0.24$^{b}$ | 0.868±0.27$^{b}$ | 6.82±3.16$^{a}$ | 1.37±0.784$^{b}$ | 2000µg/kg **US FDA (FDA Regulations, 1992).** |
| | Kidney | 1.27±0.27$^{b}$ | 1.04±0.2$^{b}$ | 3.69±1.08$^{a}$ | 0.696±0.184$^{b}$ | 2000µg/kg **US FDA (FDA Regulations, 1992).** |
| | Muscles | 0.653±2.13$^{a}$ | 0.832±0.273$^{a}$ | 4.05±1.01$^{a}$ | 0.94±0.12$^{b}$ | 500 µg/kg **(FDA Regulations, 1992)**. |

Each value represents means ± S.E.

Values with different letters in the same row represent significant difference at P ≤ 0.05 by Duncan multiple range test.

In concern to residue concentrations of heavy metals in different tissues, cadmium, lead and arsenic are significantly detected in all examined tissues (liver, kidney & muscles) of the group fed on contaminated diet. While the groups received Bactozyme with/ without heavy metals contaminated feed, the residue concentrations are within control levels as shown in table (3).

## DISCUSSSION

Increases in economic activities and industrialization have led to excessive environmental pollution especially with heavy metals. Heavy metals toxicity is particularly dangerous to birds, due to their high metabolic rate and greater threat of accumulating toxic metals in different body organs (**Felsmann, 1998).** The results of the current study revealed significant increase of AST, ALT & ALP in the group fed on heavy metals contaminated diet that indicate alterations in hepatic tissue that supposed to be due to toxicity of heavy metals. These results agree with **(Irfan et al. 2013)** who reported that cadmium adversely affect the enzymatic systems of cells. (**Djurasevic et al. 2017)** observed high levels of AST& ALT in male rats received 1.25 mg of $CdCl_2$/100 g body mass/day over 5weeks**.** Also, high levels of AST, ALT & ALP were recorded in arsenic-intoxicated broilers **(Khan et al. 2013)**; cattle **(Rana et al. 2008)** and birds (**Halder et al. 2009). Castagnetto et al. (2002)** described that Cadmium forms cystein-metallothionein complex in the liver that causes hepatotoxicity. Lead alter the haematological system by inhibiting the activities of enzymes essential for haem biosynthesis, mainly**γ-**aminolaevulinic acid dehydratase (ALAD) **(WHO 2011).**The ionic mechanism of lead toxicity occurs by replacement of lead metal ions to bivalent cations as $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$ and monovalent cations like Na+, so disturbs cell metabolism and causes changes in biological processes such as cellular signaling, protein folding, ionic transportation, enzyme regulation, and release of neurotransmitters **(Flora et al. 2012).**

The results showed that total protein and globulin are significantly decreased, while albumin is insignificantly decreased, creatinine is significantly increased in group received contaminated diet that may indicate toxic effect of heavy metals, while total protein is significantly increased, albumin, globulin and creatinine are within control limits in group fed on Bactozyme with/ without heavy metals contaminated diet which may attribute to the ameliorative effect of the probiotic. These results agree with the findings of **Siadati et al. (2017)** and **Yazhini et al. (2018)** who observed that plasma protein level was increased with probiotic supplementation in Japanese Quail and broilers, respectively. **Mikulec et al. (1999)** as well, described that lactic acid bacteria competitively exclude the pathogenic bacteria which reduce the breakdown of proteins to nitrogen and reduce the efficiency of dietary protein, so amino acids and proteins are efficiently utilized. In addition, **Yazhini et al. (2018)** reported that encapsulated probiotic increase villi height that could increase the protein absorption in broilers. Increasing protein level with probiotic supplementation may be due to increase of feed intake as observed by **Samanta and Biswas (1995)** and **Jin et al. (1996)**. As well, **(Chiang and Hsiegh 1995)** reported probiotic improvement in weight gain: Feed ratio with reduction in ammonia production in broiler chickens fed diets mixed with six probiotics up to six weeks of age. In addition, **(Naik et al. 2000)** and**(Safalaoh et al. 2001)** reported the probiotic(Lactobacillus acidophilus, Saccharomycs cerevisiae and their combination) involvement in improvement of feed efficiency in broilers. However, the findings of **(Li et al. 2014)** and **(Abdel-Hafeez et al.2017)** disagrees with our results as they observed significantly low serum total protein concentration in birds supplemented with probiotic.

Heavy metals can cause cell malfunction and toxicity by binding with protein sites and displacing original metals from their natural binding sites. Also, heavy metals bind to macromolecules as DNA and nuclear proteins resulting in oxidative distress **(Flora et al. 2008)**. Their ions bind to albumin, the most abundant protein in plasma. They bind to the free sulfhydryl group of terminal cysteine residues and to histidine residues **(Ferguson et al. 2001),** that causing malfunctioning of cell respiration, cell enzymes and mitosis especially by Arsenic **(Gordon and Quastel 1948).** Moreover, cadmium stimulates the formation of beta 2-microglobulin in urine that results in renal tubular dysfunction **(Suganya et al. 2016)**. As well, lead toxicity may be due to its affinity for thiol groups (–SH) and other organic ligands in proteins **(WHO 2011).**

Examination of broiler tissues (livers, kidney, and muscles) after feeding on heavy metals contaminated diet with/without the probiotic; Bactozyme; showed significant levels of cadmium, lead and arsenic residues in all tissues after feeding on contaminated diets shown in table(3). Pb liver level (0.89 ± 0.3) is by far the uppermost, followed by kidney (0.74 ± 0.46) and the lower Pb concentration is detected in muscles (0.096 ± 0.05). Cadmium concentration is the uppermost in kidney (0.19 ± 0.05) followed by liver (0.178 ± 0.037) and muscles (0.126 ± 0.028). Arsenic is highly increased in liver (6.82 ± 3.16) followed bymuscles (4.05 ± 1.01), and kidney (3.69 ± 1.08) respectively. According to European Union Regulation (EC) No (1881/2006), the maximum tolerance levels for lead and cadmium in liver, including poultry liver, are 0.5 ppm (mg/kg) for both **(Ghimpeteanu et al. 2012).** The maximum permissible limit for cadmium, lead and arsenic in different broiler tissues according to **E.O.S.Q.C (2005)**, **FAO (1992)** and **FDA (1992)** are mentioned in table (3**). Suganya et al. (2016)** reported that chickens can tolerate up to 500 mg lead /kg, without affecting the rate of weight gain. Levels of accumulated lead in chickens are the uppermost in bone, followed by kidney and liver then skeletal muscle. Most of the studies showed that residues of arsenic, cadmium, mercury and lead are the most detected heavy metals in poultry liver. Lead is the most toxic elements, because it binds and inactivates essential enzymes **(Baykov et al. 1996).** Cadmium binds to cystein-rich protein as metallothionein. It can replace zinc present in metallothionein as they have the same oxidation states, so inhibit it from acting as a cell scavenger of free radical, causing hepatotoxicity in liver and circulates to the kidney, accumulates in the renal tissue causing nephrotoxicity **(Castagnetto et al.**

**2002).** Cadmium mainly accumulates in the proximal tubular cells and cause bone mineralization through bone damage or by renal dysfunction **(Jaishankar et al. 2014).** Arsenic is biotransformed in liver and kidneys **(Ford, 2002)**, and the methylated metabolites are distributed throughout the body **(Dopp et al. 2004).** The amount of accumulated element in the organs, depends on the interval of exposure, the quantity of ingested element, as well as animal age and breed **(Massányi et al. 2000).** So, more deleterious changes to tissues are expected on long run exposure to heavy metals through contaminated feed as these effects are time-dose dependent. Maximum tolerable Cd level for poultry is 0.50ppm. The maximum permissible hygiene limits for Cd in meat (0.1mg/kg) and liver (0.5 mg/kg) according to the Codex Alimentorum. High Cadmium (0.12-1mg) in the poultry feeds may have bad environmental consequences if poultry manure is used to fertilize plants or to treat soil **(Suganya et al. 2016)**.

In this study, all parameters that showed alterations in broilers fed on heavy metals contaminated diet were obviously improved in the group received either Bactozyme only or with the contaminated diet. That may indicate the role of the probiotic in modulation of heavy metal toxicity in broilers. These results are concomitant with the findings of **Djurasevic et al. (2017)** who speculated obvious elimination of cadmium from intoxicated rats by probiotic administration. Many studies support our findings as **Dierck (1989)** mentioned that probiotics act in poultry by improving feed intake. Probiotics canalter metabolism by increasing digestive enzyme activity; decreasing bacterial enzyme activity and ammonia production **(Yoon et al. 2004);** maintaining normal intestinal microflora by competitive exclusion and antagonism **(Kizerwetter-Swida and Binek, 2009)** and stimulating the immune system **(Kabir et al. 2004).**In case of heavy metals toxicity, probiotic can detoxify them by binding of metallic ions to cell wall of bacteria **(Liu et al. 2009)**. **Zoghi et al. (2014)** discussed that toxins removal by bacteria is not attributed to bacterial metabolism but to binding of protein and carbohydrate components in cell wall of either yeasts or bacteria to the toxic compounds. **Beveridge and Murray (1980)** illustrated that Bacillus subtilis can bind to lead that start with a stoichiometric reaction between metallic cations and surface binding sites, then inorganic deposition of more metal. **Halttunen (2007)** discussed that lactic acid bacteriahave cationic binding sites for the removal of anionic As (V), and chemical modifications can increase As (V) removal.

## Conclusion

There are increasing evidence of the beneficial effects of probiotics which induced by various mechanisms. This study examined the effect of a blend of probiotics; Bactozyme; on heavy metals intoxicated broilers. We conclude that probiotics potentially play an effective role in biomodulation of lead, cadmium, and arsenic toxicities in broilers, as one of the main sources of nutrition for humans. So, the study recommends incorporation of probiotics into broiler feeding to minimize any deleterious effects caused by incorporation of these heavy metals in to broiler feed.

## REFERENCES

Abdel-Hafeez HM, Saleh ESE, Tawfeek SS, Youssef IMI, Abdel-Daim ASA. 2017. Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. Asian Aust. J. Anim. Sci., 30(5): 672-682.

Abdel-Megeed R. 2020. Probiotics: A Promising Generation of Heavy Metal Detoxification. Biological Trace Elements Research. Available from: https://doi.org/10.1007/s12011-020-02350-1.

Adaikpoh EO, Nwajei GE, Iogala JE. 2005. Heavy metals concentrations in coal and sediments from River Ekulu in Enugu, Coal City of Nigeria. J. Appl. Sci. Environ. Mgt. 9 (3): 5– 8.

Alloway BJ, Ayres DC.1993. Chemical Principles of Environmental Pollution, 1st ed. Blackie Academic and Professional, London.

AOAC. 2002. AOAC Official method 999.10 Lead, Cadmium, Zinc, Copper and Iron in foods. Atomic Absorption Spectrophotometry after microwave digestion. First Action 1999- NMLK- AOAC Method.

Ashayerizadeh A, Dabiri N, Ashayerizadeh O, Mirzadeh KH, Roshanfekr H., Mamooee M. 2009. Effect of dietary antibiotic, probiotic and prebiotic as growth promoters, on growth performance, carcass characteristics and hematological indices of broiler chickens. Pakis. J. Biol. Sci. (12): 52-57.

Association of American Feed Control Officials (AAFCO).2019.AAFCO Official Publication. Champaign, IL: FASS Inc. Available from:http://www.aafco.org/Publications.

Balos MZ, Jaksic S, Pelic DL. 2019. The role, importance, and toxicity of arsenic in poultry nutrition. World's Poultry Science Journal, 75(3): 375-386.

Baykov BD, Stoyanov MP, Gugova ML. 1996. Cadmium and lead bioaccumulation in male chickens for high food concentrations. Toxicol. Environ. Chem. (54): 155-159.

Beveridge TJ, Murray RGE. 1980. Site of metal deposition in the cell wall of Bacillus subtilis. J. Bacteriol. 141 (2): 876-887.

Castagnetto JM, Hennessy SW, Roberts VA, Getzoff ED, Tainer JA, Pique ME. 2002. MDB: the metalloprotein database and browser at the Scripps Research Institute. Nucleic Acids Res. 30(1): 379–382.

Centeno JA, Tchounwou PB, Patlolla AK, Mullick FG, Murakata L, Meza E.2006. Managing Arsenic in the Environment: From Soil to Human Health. Australia: CSIRO Publishing;311-8.

Chiang SH, Hsiegh WM. 1995. Effect of direct fed microorganisms on broiler growth performance and litter ammonia level. Asian Australian journal of Animal Science, 8 (2):159-162.

Chichlowski M, Croom J, McBride BW, Havenstein GB, Koci MD. 2007. Metabolic and Physiological Impact of Probiotics or Direct-Fed-Microbials on Poultry: A Brief Review of Current Knowledge. International Journal of Poultry Science, 6(10): 694-704.

Dierck NA. 1989. Biotechnology aids to improve feed and feed digestion: Enzymes and fermentation. Arch. Anim. Nutr. Berl. (39): 241-261.

Djurasevic S, Jama A, Jasnic N, Vujovic P, Jovanovic M, Mitic-Culafic D, Knezevic-Vukcevic J, Cakic-Milosevic M, Ilijevic K, Djordjevic J. 2017. The Protective Effects of Probiotic Bacteria on Cadmium Toxicity in Rats. J Med Food 20 (2): 189–196.

doi: 10.1089/jmf. 2016.0090

Dopp E, Hartmann LM, Florea AM, Recklinghausen U, Pieper R, Shokouhi B, Rettenmeier AW, Hirner AV, Obe G. 2004. Uptake of inorganic and organic derivatives of arsenic associated with induced cytotoxic and genotoxic effects in Chinese hamster ovary (CHO) cells. Toxicol. Appl. Pharmacol., (201): 156–165.

Doumas B, Watson WA, HG, Biggs HG. 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chem. Acta.31(1):87-96. doi: 10.1016/0009-8981(71): 90365-2.

EOSQC [Egyptian organization for standrization and quality control]. 2005. Egyptian standers for requirements of frozen meat and edible meat offal, No.1473 -1522.

FAO 1992. Manual of food quality control Part 4 food and Agriculture organization of United Nation, Rome.

FDA Regulation. Code of Federal Regulations, 21 CFR 556.60. 1992. The Office of the Federal Register National Archives and Records. Food and Drug Administration, Washington, DC.

Felsmann MZ. 1998. The level of chosen xenobiotics and morphological changes of internal organs in mallards (Anas platyrhynchos L.) and coots (Fulicaatra L.). Doctor's thesis, ART. Olsztyn, 10

Ferguson CJ, Wareing M, Ward DT, Green R, Smith CP, Riccardi D. 2001.Cellular localization of divalent metal transporter DMT-1 in rat kidney. Am J Physiol Renal Physiol. (280): F803–F814.

Flora G, Gupta D, Tiwari A. 2012. Toxicity of lead: A review with recent updates. InterdiscipToxicol. 5(2): 47–58. doi: 10.2478/v10102-012-0009-2. Available from:www.intertox.sav.sk

Flora SJS, Mittal M, Mehta A. 2008. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian J Med Res (128): 501–523.

Ford M. 2002. Arsenic. In: Goldfrank's Toxicologic Emergencies, 7 th edition, pp. 1183-1195. McGraw-Hill, New York, USA.

Fuller R.1989. Probiotics in man and animals. J Applied bacteriology, 66(5): 378–365.

Ghimpeteanu O, Das K, Militaru M. 2012. Assessment of Heavy Metals and Mineral Nutrients in Poultry Liver using Inductively Coupled Plasma- Mass Spectrometer (ICP-MS) and Direct Mercury Analyzer (DMA). Bulletin UASMV, Veterinary Medicine, 69 (1-2). Print ISSN 1843-5262; Electronic ISSN 1843-5378.

Ghosh A, Awal MA, Majumder S, Sikder MH, Rao DR. 2012. Arsenic residues in broiler meat and excreta at arsenic prone areas of Bangladesh. Bangladesh J Pharmacol, (7): 178-185.

Gordon JJ, Quastel GH. 1948. Effect of organic arsenicals on enzyme system. Biochem J (42): 337-350.

Gusils CS, Gonzalez, Oliver G. 1999. Some probiotic properties of chicken Lactobacilli. Canedian Journal of Microbiology, (45): 981-987.

Halder G, Roy B, Samanta G. 2009. Haematologic aspects of arsenic intoxication with and without supplemental methionine and betaine in layer chicken. Ind. J. Poultry Sci., 44.Available from: http://www. indianjournals.com/ijor.aspx?target=ijor:ijps&volume 44 & issue2 & article027 Accessed on January 30, 2010.

Halttunen T. 2007. Removal of cadmium, lead and arsenic from water by lactic acid bacteria. Functional Foods Forum, Department of biochemistry and food chemistry, University of Turku.

Halttunen T, Collado MC, El-Nezami H, Meriluoto J, Salminen, S. 2008. Mixing of lactic acid bacteria strains may reduce their toxin removal efficiency from aqueous solution. Lett. Appl. Microbiol., 46(2): 160-165.

Henry RJ, Chiamori N, Golub OJ, Berkman S. 1960. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and lactic acid dehydrogenase. Amer. J. clin. Path., (34): 381-398.

Henry RJ. 1964. Clinical chemistry, Harper & Row Publishers. New York p. 181.

Higgins JP, Higgins SE, Vicente JL, Wolfenden AD, Tellez G, Hargis BM. 2007. Temporal effects of lactic acid bacteria probiotic culture on Salmonella in neonatal broilers. Poult. Sci. (86): 1662-1666.

Irfan M, Hayat S, Ahmad A, Alyemeni MN 2013. Soil cadmium enrichment: Allocation and plant physiological manifestations. Saudi J Biol Sci 20(1): 1-10.

Jadhav SH, Sarkar SN, Patil RD, Tripathi H.C. 2007. Effects of subchronic exposure via drinking water to a mixture of eight water contaminating metals: a biochemical and histopathological study in male rats. Arch Environ Contam Toxicol, (53): 667-677.

Jaishankar M, Tenziten T, Anbalagan N, Mathew BB, Beeregowda 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol. 7(2): 60–72. doi: 10.2478/intox-2014-0009.

Jin LZ, Ho YW, Abdullha N, Jalaludin S. 1996. Influence of dried Bacillus subtilis and Lactobacilli cultures on intestinal microflora and performance in broilers. Asian Journal of Animal Sciences, 9(4): 397- 403.

Kabir SML, Rahman MM, Rahman MB, Rahman MM, Ahmed SU. 2004. The dynamics of probiotics on growth performance and immune response in broilers. Int. J. Poult. Sci. (3): 361-364.

Kant, Mehta M, Varshneya C,Chauhan S. 2011. Induction of Oxidative Stress by Subacute Oral Exposure of Cadmium Sulphate in Adult Poultry. Braz J Vet Pathol, 4(2): 117-121.

Khan MSH, Mostofa M, Jahan MS, Sayed MA, Hossain MA. 2008. Effect of garlic and vitamin-B complex in lead acetate induced toxicities in mice. Bangladesh Journal of Veterinary Medicine, 6(2): 203-210.

Khan A, Sharaf R, Khan MZ, Saleemi MK,

Mahmood F. 2013. Arsenic Toxicity in Broiler Chicks and its Alleviation with Ascorbic Acid: A Toxico-patho-biochemical Study. Int. J. Agric. Biol., ISSN Print: 1560–8530; ISSN Online: 1814–9596 13–924/2013/15–6–1105–1111.

Kizerwetter-Swida M, Binek M. 2009. Protective effect of potentially probiotic Lactobacillus strain on infection with pathogenic bacteria in chickens. Pol. J. Vet. Sci.(12): 15 -20.

LiY, Xu, Q, Yang C, Yang X, Lv L, Yin C, Liu X, Yan H. 2014. Effects of probiotics on the growth performance and intestinal microflora of broiler chickens. Pak. J. Pharm. Sci., (27): 713-717.

Liu J, Qu W, Kadiiska MB. 2009. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol (238): 209–214.

Massányi P, TrandŽÍk J, Lukáč N, Strapák P, Kováčik J, Toman R. 2000. The contamination of bovine semen with Cd, Pb, Cu and Zn and its relation to the quality of spermatozoa used for insemination. Folia Veterinaria. 44(3): 150-153.

Mehrandish R, Rahimian A, Shahriary A. 2019. Heavy metals detoxification: A review of herbal compounds for chelation therapy in heavy metals toxicity. J Herbmed Pharmacol. 8(2): 69-77.

Mikulec Z, Serman V, Mas N, Lukac Z. 1999. Effect of probiotic on production results of fattened chickens fed different quantities of protein. Vet. Arhiv., (69): 199-209.

Mondal NK. 2020. Prevalence of Arsenic in chicken feed and its contamination pattern in different parts of chicken flesh: a market basket study Environ Monit Assess Aug 20;192(9): 590. doi: 10.1007/s10661-020-08558-x

Naik DG, Javedmulla A, Shivakumar MC. 2000. Performance of broilers supplemented with probiotics. Karnataka Journal of Agricultural Sciences, 13(4): 957-960.

Okoye COB, Ibeto CN, Ihedioha, JN. 2011. Assessment of heavy metals in chicken feeds sold in south eastern, Nigeria. Advance in Applied science Research, 2(3):63-68.

Rana T, Sarkar S, Mandal T, Batabyal S. 2008. Haematobiochemical profiles of affected cattle at arsenic prone zone in Haringhata block of Nadia District of West Bengal in India. Internet J. Hematol., 4: 2

Reitman S, Frankel S. 1975. Am. J. Clin. Path., 28; 65.

Safalaoh ACL, Smith A, Senanayake YDA, Sangakkara UR. 2001. Effective microorganisms as an alternative to antibiotics in broiler diets: effect on broiler growth performance, feed utilization and serum cholesterol. Sixth International Conference on Kyusei farming. Proceedings of the conference on greater productivity and a cleaner environment through Kyusei nature farming, University of Pretoria, Pretoria, S. Africa. 28- 31 October, 1999. PP: 150-155.

Salem HM, Eweida EA, Farag A. 2000. Heavy metals in drinking water and their environmental impact on human health. ICEHM2000, Cairo University, Egypt, September, PP: 542- 556.

Samanta M, Biswasl P. 1995. Effect of feeding probiotic and lactic acid on the performance of broiler. Journal of Indian Poultry Science, (30):145-147.

Sánchez-Virosta P, Espín S, García-Fernández AJ, EevaT. 2015. A review on exposure and effects of arsenic in passerine birds. Science of The Total Environment, 512-513, 506–525.Available from:http://doi.org/10.1016/j.scitotenv.2015.01.069.

Siadati SA, Ebrahimnezhad Y, Gh SJ, Shayegh J. 2017. Evaluation of probiotic potential of some native Lactobacillus strains on the growth performance and serum biochemical parameters of Japanese quails (Coturnix Coturnix japonica) during rearing period. Bras. J. Poult. Sci., 19: 399-408.

Singh N, Kumar D, Sahu A. 2007. Arsenic in the environment: effects on human health and possible prevention. J Environ Biol 28 (2 Suppl): 359–365.

Soccol CR, Vandenberghe LP, Spier MR, Medeiros ABP, Yamaguishi CT, Lindner JD, Pandey A, Thomaz-Soccol V.2010. The Potential of Probiotics: A Review. Food

Technol. Biotechnol. 48 (4): 413–434.

Stern BR. 2010. Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. J Toxicol Environ Health A.73(2): 114-27.

Suganya T, Senthilkumar S, Deepa K, Muralidharan J, Sasikumar P, Muthusamy N. 2016. Metal toxicosis in poultry- A review. International Journal of Science, Environment and Technology, 5(2): 515 – 524.

Tietz NW.1986. Textbook of clinical chemistry. WB saunders, philadlphia, pp 1271-1281.

Tietz NW, Rinker AD, Shaw LM. 1983. IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase. J Clin Chem Clin Biochem. (21):731- 748.

Timmerman HM, Koning CJ, Mulder L, Rombouts FM, Beynen AC. 2004. Mono-strain, multi-strain and multi-species probiotics-A comparison of functionality and efficacy. Int. J. Food Microbiol. (96):219–233

Tokar EJ, Boyd WA, JH, Waalkes MP. 2015. Toxic Effects of Metals. Casarett & Doull's Essentials of Toxicology. 3rd ed. Chapter (23): 347- 359.

WHO/FAO. 2001. Report on joint WHO/ FAO Expert Consultation on evaluation of health and nutritional properties of Probiotics in food including powder milk with live lactic acid bacteria.

World Health Organization, Geneva. 2011. Safety evaluation of certain food additives and contaminants. Seventy-third meeting of the Joint FAO/ WHO Expert Committee on Food Additives (JECFA). (WHO food additives series; 64). ISSN 0300-0923.

Yazhini P, Visha P, Selvaraj P, Vasanthakumar P, Chandran V. 2018. Dietary encapsulated probiotic effect on broiler serum biochemical parameters. Veterinary World, PP.1344-1348.

Yoon C., Na CS, Park JH, Han SK, Nam YM, Kwon JT. 2004. Effect of feeding multiple probiotics on performance and fecal noxious gas emission in broiler chicks. Kor. J. Poult. Sci. (3): 229-235.

Zoghi A, Khosravi-Darani K, Sohrabvandi S. 2014. Surface Binding of Toxins and Heavy Metals by Probiotics. Mini-Reviews in Med Chem, (14): 84-98.