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The effect of diet enriched with lipoic acid in the accumulation and metabolization of metals in different organs of *Litopenaeus vannamei*

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Abstract

The bioaccumulation of metals is a problem for shrimp farming due to toxicity. As the water used in the culture tanks comes from environment, the shrimps can be exposed directly accumulating these metals, suffering the toxic effect and being a source of exposure for humans. So, alternatives that can minimize/avoid both accumulation and promote metabolism of metals are welcome. In this study, the *Litopenaeus vannamei* received the antioxidant lipoic acid (LA) through diet for 4 weeks and posteriorly were exposed to cadmium (Cd) and arsenic (As) alone or in combination (nominal concentration of 1 mg/L) during 48 hr. A control group also was run in parallel (without LA and then submitted to both metals exposure). The accumulation of Cd and As and the capacity of As metabolization were analysed in gills, hepatopancreas and muscle of shrimp *Litopenaeus vannamei*. Our results showed that the LA administered through diet decrease the accumulation of both metals in gills and muscle and improved the metabolization of As, favouring the accumulation of non-toxic compounds as arsenobetaine (AsB) in all organs analysed. Therefore, a diet enriched with LA is a good form of chemoprevention in aquaculture that improved the resistance of shrimps against metal contamination.

KEYWORDS
arsenic, arsenic metabolism, cadmium, chemoprevention, lipoic acid, *Litopenaeus vannamei*

1 | INTRODUCTION

The white shrimp *Litopenaeus vannamei* is one of the most cultivated species worldwide (Duan, Zhang, Sun, & Wang, 2018), mainly due to their high resistance to environmental variables such as temperature, salinity and oxygen levels (Zhang, Zhang, Li, & Huang, 2006). Additionally, this species presents good tolerance to environmental contaminants such as toxins and metals (Yu et al., 2016; Zimba, Camus, Allen, & Burkholder, 2006). In fact, metals are ubiquitous in aquatic environments and can cause several adverse effects in organisms including carcinogenicity, mutagenicity and cytotoxicity (Chang et al., 2009).

Generally, shrimps culture tanks use seawater from the environment, and if this water becomes enriched with metals, the farmed animals can incorporate and accumulate these metals, causing several toxic effects (Chuang, Chen, & Ju, 2016). In fact, part of Lagoon Patos, located nearby of Rio Grande city (southern Brazil) has been shown to be contaminated with cadmium and arsenic, due mostly to anthropogenic activities (Mirlean & Roisenberg, 2006). Lobato et al. (2013) showed that *L. vannamei* exposed to cadmium (Cd) and/or arsenic (As), incorporated and accumulated these metals in the hepatopancreas, inducing a pro-oxidant situation. Once aquatic

organisms have incorporated and accumulated metals in their tissues, the final consumer can be put at risk when ingesting these animals because the food is a significant route of exposure to contaminants (Abdel-Tawwab, El-Sayed, & Monier, 2017; Abdel-Tawwab, El-Sayed, & Shady, 2017).

Cadmium is a non-essential metal that exerts toxic effects, both in aquatic organisms and humans (Rehman, Fatima, Waheed, & Akash, 2018). Cd exposure induces oxidative stress, histological changes and molecular damages in aquatic organisms, including *L. vannamei* (Chuang et al., 2016; Yu et al., 2016). Other studies evaluating the effect of Cd in *L. vannamei*, showed that this metal increases glutathione-*S*-transferase (GST) activity and metallothionein-like protein levels, indicating a pro-oxidant condition (Lobato et al., 2013). In humans, Cd exposure is associated with damages in respiratory, neurological and renal systems, as well as with the development of prostate and breast cancers. Additionally, exposure can induce diabetes and affect reproduction capacity (Rehman et al., 2018).

Arsenic is a metalloid that is widely distributed in the environment and is mostly caused by human activity. As this metalloid coexists with phosphate, their presence in the aquatic environment is inevitable (Tseng, 2004). Some studies have shown that As exposure is linked to oxidative stress generation in aquatic organisms (Greani et al., 2017; Kim & Kang, 2015; Ventura Lima, Bogo, & Monserrat, 2011).

The toxicity of As depends on its chemical, and the organic forms are considered less toxic than inorganic ones (Aposhian, Zakharyan, Avram, Sampayo-Reyes, & Wolleenberg, 2004). However, in aquatic environments, the predominant form is inorganic (As^{+V}; oxidized form—arsenate) and once incorporated by organisms this metalloid may be metabolized into organic forms (Ventura-Lima et al., 2011). The metabolization process involves successive steps of oxidation–reduction reactions, methylation, and conjugation with other molecules (e.g., betaine) (Aposhian et al., 2004).

Between As species, inorganic forms, such as arsenite (As^{+III}) and As^{+V} are considered more toxic while mono- and dimethylated forms (monomethylarsonate [MMA] and dimethylarsonate [DMA], respectively) are considered moderately toxic. Also, there are forms considered non-toxic, such as arsenobetaine (AsB), arsenocholine (AsC) and trimethylarsonic acid (TMAO) (Thomas, 2007). All forms can be found in different tissues of organisms exposed to As; however, some forms are predominant, depending on the metabolization capacity of each organism (Ventura-Lima et al., 2011). In general, marine organisms accumulate a major percentage of arsenic as a nontoxic compound, but this is variable, according to both the environmental conditions and metabolization capacity of the organism (Zhang, Guo, Song, Du, & Zhang, 2018).

As contact of farmed shrimps with metals present in their tank water seems to be inevitable, alternatives for reducing metal, as well as those that favour metabolization of arsenic are welcome, once metals can affect the health of shrimps and fish and, consequently, human health. Feed supplements that can improve resistance against metal exposure or facilitate the elimination of metals from the body have been shown to be an appropriate alternative in aquaculture (Lobato et al., 2013).

Once that both Cd and As are identified by induction of a pro-oxidant situation, the use of antioxidants that can chelate metals would be a good alternative. In fact, Drag-Kozak et al. (2018) showed the protect effect of melatonin by decrease in Cd levels in different tissues of Prussian carp (*Carassius gibelio B.*). Another antioxidant that has shown protective effect of against metal toxicity in shrimp is the lipoic acid (LA) (Lobato et al., 2013). Lipoic acid (1,2-dithiolane-3-pentanoic acid; $C_8H_{14}O_2S_2$) is an amphipathic molecule that is an important cofactor of Krebs cycle enzymes, and is crucial to energy metabolism (Kütter, Romano, Ventura-Lima, Tesser, & Monserrat, 2014). Lipoic acid is considered to be an ideal antioxidant, because in addition to neutralization and/or interception of reactive oxygen species (ROS), it can also act as a metal chelator (Flora, 2009).

Some studies have shown that LA can improve the antioxidant capacity of aquatic organisms (Amado, Garcia, Pereira, Yunes, & Monserrat, 2011; Monserrat et al., 2008). In *L. vannamei*, the supplementation with LA improved the resistance of shrimp against Cd and As toxicity (Lobato et al., 2013). However, whether or not this antioxidant can minimize the accumulation of Cd and As, and/or favour the metabolization of arsenic in different organs of *L. vannamei*, is still unknown.

So, the objectives of this study were to evaluate if a diet enriched with LA can minimize the accumulation of Cd and As and increase the metabolization capacity of arsenic in the *L. vannamei*. If so confirmed, then this antioxidant, administered through diet, could be used as chemoprotectant in aquaculture.

2 | MATERIALS AND METHODS

2.1 | Obtaining and maintenance of shrimps under laboratory conditions

The shrimps *L. vannamei* (juvenile, both sexes, with an average weight of 9.0 ± 0.7 g) were obtained from the Marine Aquaculture Station, Oceanography Institute (IO), Federal University of Rio Grande (FURG) and transferred to the Institute of Biological Science (ICB) of the same Institution, where they were maintained under controlled conditions (pH around 8.0; salinity 30, temperature 20 0.5°C, photoperiod 12 hr light/dark cycle, feedings twice a day, constant aeration in order to maintain dissolved oxygen at 7.2 mg O_2 L^{-1}). The animals were acclimated at least 2 weeks prior to the experimental period in the aquatic bioterium of Institute of Biological Science. During acclimatization period, the shrimps were fed with commercial ration (45% crude protein, Purina).

2.2 | Supplementation of lipoic acid in the feed of shrimps

The commercial ration (45% crude protein, Purina) was manually macerated using a grade and pistil. Next, the LA was incorporated in

triturated ration in a proportion of 70 mg/kg of food (Monserrat et al., 2008; Terjensen et al., 2004) and mixed with ultrapure water (MilliQ), and dried at 50°C to obtain pellets that were kept at 4°C during all experiments. A control ration also was prepared as described above except for the incorporation of LA.

2.3 | Administration of ration supplemented with LA to shrimps

Forty eight shrimps were randomly divided into two groups: (a) the experimental group that received LA through of ration (+LA); and (b) the control group that received only commercial ration without LA (−LA). Both groups were fed their respective diets twice daily over 4 weeks, receiving a quantity of ration corresponding to 1% of mean body weight. To adjust the quantity of ration, the animals were weighed weekly, ensuring that the animals received the appropriate amount of food during the experimental period. Each treatment was performed in duplicate. The experiment of supplementation with LA was performed in the bioassay laboratory of Institute of Biological Science of FURG.

2.4 | Exposure to cadmium and/or arsenic

To evaluate exposure to metals, the animals were divided according to their diet (with or without LA as described in the Section 2.3) and both groups were submitted to exposure to Cd ($CdCl_2$ from Merck, PA grade) and/or As (NaAsO2, from VETEC, PA grade). Stock solutions (2 g/L) of each metal was prepared and added in the aquariums according with treatment. The final concentration used in this study was of 1 mg/L of each metal (alone or in combination) during 48 hr. The concentration of each metal was based on Lobato et al. (2013) study. Each treatment was performed in duplicate.

Each experimental group was identified as follows: (a) the control group (no metals) + LA; (b) the group exposed to Cd + LA; (c) the group exposed to As + LA; (d) the group exposed to both Cd + As + LA; (e) the control group (no metals) – LA; (f) the group exposed to Cd – LA; (g) the group exposed to As – LA; and (h) the group exposed to Cd + As– LA. After 48 hr, the animals were killed by freezing, and gills, hepatopancreas and muscle were dissected and lyophilized. The experiment of metals exposure was performed in the toxicology laboratory of Institute of Biological Science of FURG.

2.5 | Determination of total Cd and As in organs of *L. vannamei*

The accumulation of Cd and As in gills, hepatopancreas and muscle was determined in the groups fed both diets (with or without LA as described in the Section 3). The employed methodology followed the validated method of Fattorini et al. (2008).

First, the lyophilized (SpeedVAc) samples were digested under pressure with nitric acid and hydrogen peroxide (proportion of 5:1) using a microwave (CEM Xpress, CEM Mars6, CEM Holding Corporation, Matthews, NC, USA), and the elements were analysed by atomic absorption spectrophotometry using an electrofurnace atomization with Zeeman effect (Agilent SpectrAA 240Z, Agilent Technologies, Santa Clara, CA, USA). For all chemical analyses, quality assurance and quality control were previously checked by processing blank and standard reference materials (mussel tissue standard reference materials [SRM] 2977, National Institute of Standards and Technology [NIST]). The concentrations obtained from these SRM were always within the 95% confidence intervals of the certified values. Cd and As tissue concentrations were expressed as μg/g dry weight (dw) (means values ± standard deviation, $n = 5$ for each tissue analysed).

2.6 | Chemical speciation of arsenic

Arsenic chemical speciation was conducted according to previous validated practices (Fattorini et al., 2013). In brief, after dissection, muscle, gills and hepatopancreas were homogenized in methanol (1:10 w/v) (purity > 99%, HPLC grade, Fluka), and arsenic compounds were extracted using a microwave (Mars CEM, CEM Corporation) at 150 W and 55°C for 15 min. Samples were centrifuged (2000 x *g* for 15 min). A SpeedVac (RC1009; Jouan, Nantes, France) was used to concentrate the supernatants. Next, the samples were recovered in 1 ml of a methanol:water (70:30) solution. Separation of arsenic compounds was performed by high-performance liquid chromatography (HPLC). Anionic forms were obtained using a Supelcosil liquid chromatography-SAX1 column (25 cm, 4.6 mm ID, 5 mm, Supelco, Bellefonte, PA, USA) with 15 mM KH_2PO_4 (pH 6.1) as the mobile phase at a flow rate of 1 ml/min. The cationic exchange was realized through a Supelcosil liquid chromatography-SCX column (25 cm, 4.6 mm ID, 5 mm, Supelco, Bellefonte, PA, USA) with 2.5 mM pyridine (pH 2.65) as the mobile phase at a flow rate of 1 ml/min. Forty fractions were collected every 30 s from injection with 0.5 ml of nitric acid (purity > 65%, Fluka), and the arsenic content was determined as previously described. Procedures of extraction and separation were verified and checked using selected SRMs, including DORM-2, DOLT-1, DOLT-2, National Research Council, Canada and BCR627, Institute for reference Materials and Measurements, European Commission, and pure standards of As^V, dimethylarsonate (DMA), tetramethylarsonium (TETRA) and AsB to control for the accuracy, precision and recovery of various arsenic species. Pellets obtained after centrifugation were recovered and washed three times with a saline solution (0.5% NaCl in ultrapure water) before being digested and tested for insoluble and non-extractable arsenic using the previously described procedure. Analyses of arsenic chemical speciation were applied on one representative sample for each organ and experimental condition, obtained by pooling at least three replicates per group. The arsenic compounds were expressed as μg/g dw.

2.7 | Statistical analysis

Statistical differences were analysed through factorial ANOVA, taken into account the following factors: treatment (control, As, Cd and As

+ Cd) and LA enrichment (with or without LA). Assumptions of normality and variance homogeneity were previously checked and mathematical transformation was made when necessary. A Newman–Keuls test was used as post hoc comparison. In all cases, the significance level was fixed at 5% (Zar, 1984).

3 | RESULTS

The comparisons of accumulation capacity for both Cd and As in groups treated with As or Cd alone or in combination were performed comparing with the control group without LA (–LA). To verify if LA could decrease the accumulation of these metals, the comparisons were made of the same treatments but considering the presence or absence LA (+LA and –LA respectively). This comparison criterion also was employed in the analysis of arsenic metabolism.

In gills, there was a significant Cd accumulation (p 0.05) in the Cd and Cd + As groups when compared to the control group (–LA). However, in the same groups, the pretreatment with LA showed a decrease ($p < 0.05$) of this accumulation (Figure 1a).

In the hepatopancreas, in the groups exposed to cadmium (alone or in combination with arsenic), there was also observed a significant ($p < 0.05$) accumulation of this metal compared to the control group (–LA). In this organ, the pretreatment with LA was shown to increase the Cd levels ($p < 0.05$) with respect to the Cd (–LA) group (Figure 1b).

In muscle, the groups exposed to Cd (alone or in combination with As) presented higher Cd content ($p < 0.05$) compared to the control group (–LA). However, the group Cd + As was shown to accumulate less Cd than the group exposed only to Cd. The pretreatment with LA was shown to decrease ($p < 0.05$) the Cd accumulation in both experimental groups (Figure 1c).

In gills, a significant (p 0.05) accumulation of As was observed in the group exposed only to As, compared to the control group or group Cd + As (–LA). In this organ, all groups treated with LA showed a reduction in As accumulation ($p < 0.05$) (Figure 2a).

In the hepatopancreas, there was a significant accumulation ($p < 0.05$) of arsenic in the groups As (–LA) and As + Cd (–LA) when compared with the control group (–LA). The pretreatment with LA showed an increase ($p < 0.05$) in As accumulation with respect to the group exposed only to As (–LA). However, in the group Cd + As, the LA was shown to decrease the As levels, compared with group Cd + As (–LA) ($p < 0.05$) (Figure 2b).

In muscle, both groups exposed to As (–LA) (alone or in combination with Cd) showed a significant accumulation (p 0.05) of As compared with the control group (–LA). In the LA groups, the Cd + As treatment showed a decrease ($p < 0.05$) in the As levels, compared with the same treatment but without LA (Figure 2c).

Considering the As metabolization in gills, it was observed that the groups that received pretreatment with LA (+LA) decreased the percentage of DMA (considered moderately toxic) in the groups exposed to As and Cd + As (3.2% and 1.3%, respectively) compared with the same treatments without LA (–LA) (12.5% and 8% respectively). In the group exposed to only As, the LA showed to reduce

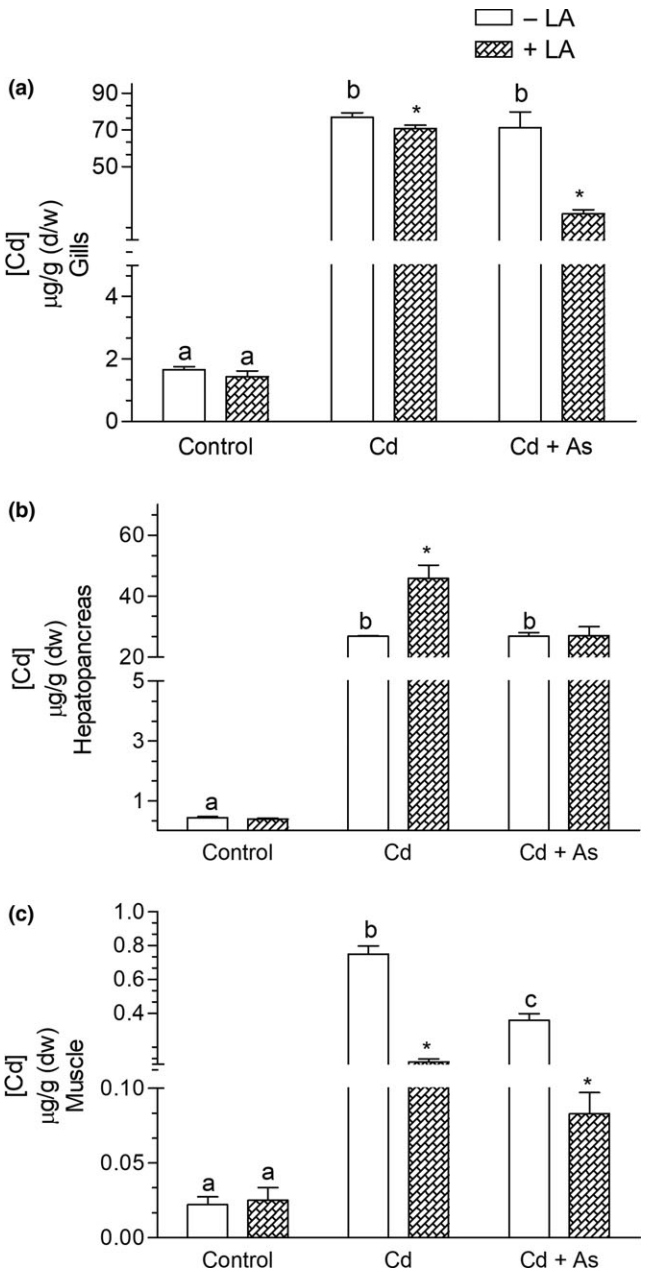

FIGURE 1 Total cadmium concentrations are expressed as µg/g dry weight (dw) in: (a) gills, (b) hepatopancreas and (c) muscle. Results are expressed as the mean ± 1 standard. Different letters indicate the significant difference ($p < 0.05$) between means of treatment compared to control group. Asterisks (*) indicate the significant difference ($p < 0.05$) between the group exposed to Cd (alone or in combination with As) pretreated with LA (+LA) compared to the same treatment and not exposed to LA (–LA). ($n = 5$)

the levels of iAs when compared with group exposed to As (–LA) (1.2% and n.d [bellow the detection limit] respectively). On the other hand, in the group exposed to Cd + As, the LA increased the iAs levels when compared with the same treatment without LA (0.6% and 3.2% respectively). The LA also showed decrease the TMAO levels in the groups exposed to As and Cd + As (4.6% and 9.0%, respectively) compared with the same treatments without LA (21.5%

and 33.0% respectively). Interestingly, this reduction in TMAO levels induced by LA also was observed in the control groups with and without LA (27.9 and 14.9 respectively).

Concomitant to these results, the LA showed to increase the percentage of non-toxic compounds as AsB in the As and Cd + As groups (71.2% and 67.7%, respectively) when compared with the same groups that did not received LA (39.1% and 50.8% respectively) (Table 1).

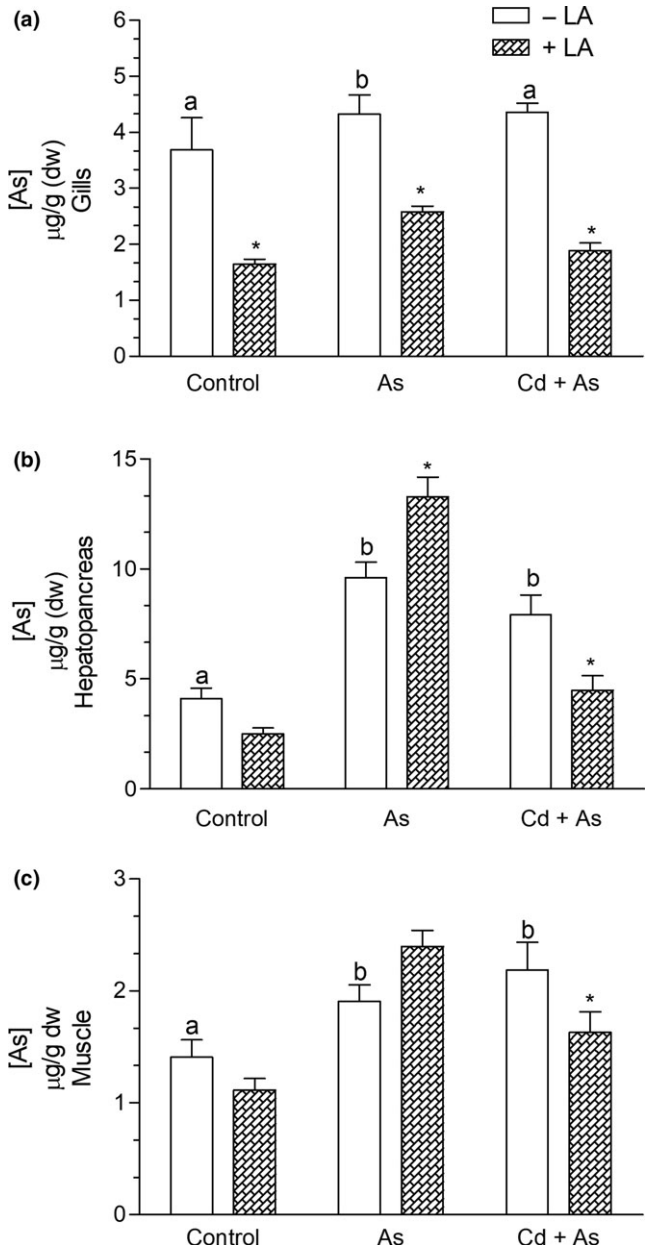

FIGURE 2 Total arsenic concentrations are expressed as µg/g dry weight (dw) in: (a) gills, (b) hepatopancreas and (c) muscle. Results are expressed as the mean ± 1 standard. Different letters indicate the significant difference ($p < 0.05$) between means of treatment compared to the control group. Asterisks (*) indicate the significant difference ($p < 0.05$) between the group exposed to As (alone or in combination with Cd) pretreated with LA (+LA) compared to the same treatment and not exposed to LA (–LA). ($n = 5$)

In the hepatopancreas, the pretreatment with lipoic acid (+LA) showed to reduce the levels of inorganic arsenic (iAs) (highly toxic) in both groups exposed to As (alone or in combination with Cd) (0.5% and 0.3%, respectively) when compared with As and As + Cd groups that were fed with diets without LA (30.1% and 6.3% respectively). However, the LA increased the DMA levels in the group exposed only As compared with group exposed to As without LA (0.2% and 2.1% respectively). The LA also showed to increase the TETRA levels in both groups exposed to As (alone or in combination with Cd) (2.2% and 2.3%, respectively) compared with the same groups that did not received LA (0.7% and 0.4% respectively). Also, there was a considerable increase of AsB (non-toxic compound) levels in the As and As + Cd groups that received LA (88.8% and 87.1%, respectively) compared with the same experimental groups without LA (50.8% and 71.8% respectively) (Table 2).

In muscle, the group Cd + As with LA pretreatment showed a reduction in the levels of iAs when compared with the Cd + As (–LA) groups (52% to 23% respectively). A different result was observed in the group exposed only to As, where LA was shown to increase the iAs levels when comparing the same treatment but without LA (–LA) (20.5% and 5.9% respectively). Also, the LA pretreatment seems to have induced a decrease in AsB levels in the As group when compared with As (–LA) group, although LA exposure was shown to increase TETRA (other non-toxic compounds) levels in As group. On the other hand, in the group exposed to Cd + As (+LA), an increase in AsB levels (63.5%) was observed when compared with the same treatment without LA (40.5%) (Table 3).

TABLE 1 Percentage of distribution of different compounds of As in gills of *Litopenaeus vannamei* exposed to 1 mg of As/L (alone or in combination with 1 mg of Cd/L). These percentages are referring to sum of total As

% of As	Ctl (–LA)	As (–LA)	As +Cd (–LA)	Ctl (+LA)	As (+LA)	As +Cd (+LA)
iAs	n.d	1.2%	0.6%	n.d	n.d	3.2%
MMA	n.d	n.d	n.d	n.d	n.d	4.8%
DMA	11.6%	12.5%	8.0%	8.2%	3.2%	1.3%
AsB	55.2%	39.1%	50.8%	72.0%	71.2%	67.7%
TMAO	27.9%	21.5%	33.0%	14.9%	4.6%	9.0%
AsC	n.d	n.d	n.d	n.d	6.8%	n.d
TETRA	n.d	n.d	n.d	n.d	11.8%	n.d
ins-As	5.2%	25.7%	7.6%	4.9%	2.4%	13.9%

Note. iAs: inorganic arsenic as sum of As^{+3} and As^{+5}; MMA: monomethylarsonate; DMA: dimethylarsonate; TMAO: trimethylarsine oxide; TETRA: tetramethylarsonium; AsB: arsenobetaine; AsC: arsenocholine; ins-As: not extractable arsenic: usually inorganic arsenic associated to insoluble concretions such as proteins and lipids. n.d: not detectable. (–LA) groups that were not received the pretreatment with lipoic acid; (+LA) animals that received the pretreatment with lipoic acid through of food during 4 weeks and after this period were exposed to As (alone or in combination with Cd).

4 | DISCUSSION

Farmed marine organisms are known by to accumulate metals in their organs, which are a potential source of metals for the final consumer (Kaya & Turkoglu, 2017). Alternatives to minimize the accumulation or to maximize metal elimination from the animal body are of great value for aquaculture. In this study, we evaluated lipoic acid as a feed supplement to verify if this antioxidant molecule could decrease the accumulation of Cd and As, as well as to increase the metabolization capacity of arsenic in different organs of *L. vannamei*.

A significant accumulation of Cd and As in gills, hepatopancreas and muscle, in both groups exposed to Cd or As (alone or in combination) compared to the control group (–LA) was verified (Figures 1 and 2). Although in shrimps, data about Cd and As accumulation after exposure to these metals are scarce, several confirmations exist for fish species. A conspicuous accumulation of Cd in muscle, liver and gills of the fish *Aconthopagrus schegeli* exposed to Cd was verified by Kim, Park, Yeo, Kim, and Han (2017). A similar result also was observed in the same tissues of the fish *Sebastes schlegelii*, exposed to As (Kim & Kang, 2015).

The accumulation of metals in metabolically active organs such as gills, hepatopancreas or liver can cause a loss of energetic efficiency, affecting other physiological processes such as reproduction, growth, energy metabolism and osmoregulation. Wu and Chen (2005) observed a significant delay in the growth of *L. vannamei* after Cd exposure. Also, the exposure to high Cd concentration was shown to reduce the survival and reproduction capacity of shrimp *Palaemontes pugio* (Manyin & Rowe, 2008). In addition, Cd exposure induced DNA damage in *Litopenaeus vannamei* showing a genotoxic effect (Chang et al., 2009). Arsenic exposure also affected reproduction in zebrafish (Boyle et al., 2008) and osmoregulation capacity in tilapia (Hwang & Tsai, 1993) as well as oxidative stress (Sarkar, Mukherjee, Chattopadhyay, & Bhattacharya, 2014). Although information about the combined effect of Cd and As in aquatic organisms was limited in muscle and hepatopancreas of *L. vannamei*, a mixture of these metals did not alter the accumulation capacity of these tissues when compared with groups exposed singly to one kind of metal (Lobato et al., 2013). Also, Doganlar, Doganlar, Muranli, and Guner (2016) observed in zebrafish that the exposure to a high concentration mixture of Cd and As (50 and 100 ppb, respectively) caused a carcinogenic effect.

A relevant result for human health was the accumulation of Cd and As in muscle (Figure 1c and Figure 2c, respectively) when compared to control group (–LA). The muscle is the edible part of shrimp, and even though accumulation in muscle is lower than other organs, its consumption can induce harmful effects in the final consumer (Shivakumar, Thippeswamy, Tejaswikumar, & Prashanthakumara, 2014). However, the animals that received LA through diet showed a reduction in the accumulation of Cd in gills and muscle and a reduction in As levels when animals were exposed to Cd + As (Figure 1a,c and Figure 2a,c respectively). This decrease in accumulation represents a great benefit to aquatic organisms,because it can avoid or decrease damage in the gills preserving their physiological role in gas exchange and osmoregulation. While in muscle, the decrease in metal accumulation can represent a benefit to human health because seafood is a source of some compounds such as omega-3 polyunsaturated fatty acid, high-quality proteins and minerals (Kaya & Turkoglu, 2017).

TABLE 2 Percentage of distribution of different compounds of As in the hepatopancreas of *Litopenaeus vannamei* exposed to 1 mg of As/L (alone or in combination with 1 mg of Cd/L). These percentages are referring to sum of total As

% of As	Ctl (–LA)	As (–LA)	As +Cd (–LA)	Ctl (+LA)	As (+LA)	As +Cd (+LA)
iAs	n.d	30.1%	6.3%	n.d	0.5%	0.3%
MMA	n.d	0.8%	0.4%	n.d	0.1%	0.5%
DMA	n.d	0.2%	0.9%	n.d	2.1%	0.9%
AsB	72.9%	50.8%	71.8%	77.2%	88.8%	87.1%
TMAO	13.0%	9.4%	10.7%	3.4%	4.3%	5.1%
AsC	7.5%	1.8%	2.5%	8.9%	1.1%	2.5%
TETRA	1.7%	0.7%	0.4%	4.6%	2.2%	2.3%
ins-As	4.9%	6.2%	7.0%	5.9%	1.0%	1.2%

Note. iAs: inorganic arsenic as sum of As^{+3} and As^{+5}; MMA: monomethylarsonate; DMA: dimethylarsonate; TMAO: trimethylarsine oxide; TETRA: tetramethylarsonium; AsB: arsenobetaine; AsC: arsenocholine; ins-As: not extractable arsenic: usually inorganic arsenic associated to insoluble concretions such as proteins and lipids. n.d: not detectable. (–LA) groups that were not received the pretreatment with lipoic acid; (+LA) animals that received the pretreatment with lipoic acid through of food during four weeks and after this period were exposed to As (alone or in combination with Cd).

TABLE 3 Percentage of distribution of different compounds of As in muscle of *Litopenaeus vannamei* exposed to 1 mg of As/L (alone or in combination with 1 mg of Cd/L). These percentages are referring to sum of total As

% of As	Ctl (–LA)	As (–LA)	As +Cd (–LA)	Ctl (+LA)	As (+LA)	As +Cd (+LA)
iAs	n.d	5.9%	52%	n.d	20.5%	23%
MMA	n.d	n.d	n.d	n.d	n.d	n.d
DMA	n.d	n.d	n.d	n.d	n.d	n.d
AsB	84.6%	78.2%	40.5%	80.8%	63%	63.5%
TMAO	n.d	n.d	n.d	n.d	n.d	n.d
AsC	n.d	n.d	n.d	n.d	n.d	n.d
TETRA	8.2%	5.7%	2.4%	9.7%	8.0%	4.5%
ins-As	7.2%	10.3%	4.3%	9.5%	8.6%	9.0%

Note. iAs: inorganic arsenic as sum of As^{+3} and As^{+5}; MMA: monomethylarsonate; DMA: dimethylarsonate; TMAO: trimethylarsine oxide; TETRA: tetramethylarsonium; AsB: arsenobetaine; AsC: arsenocholine; ins-As: not extractable arsenic: usually inorganic arsenic associated to insoluble concretions such as proteins and lipids. n.d: not detectable. (–LA) groups that were not received the pretreatment with lipoic acid; (+LA) animals that received the pretreatment with lipoic acid through of food during 4 weeks and after this period were exposed to As (alone or in combination with Cd).

Although few studies have considered the role of antioxidants as a strategy to decrease the accumulation of metals, as well as to improve the resistance against metal pollution in the body of aquatic animals, Abdel-Tawwab, El-Sayed, Monier, et al. (2017), Abdel-Tawwab, El-Sayed, and Shady (2017) showed that active charcoal and EDTA administered through diet could minimize the accumulation of metals, such as cadmium and copper in *Oreochromis niloticus*. Also, it was observed in *L. vannamei* that the supplementation with the antioxidant curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) through diet, induced a drop in the accumulation of Cd (Yu et al., 2016). In mammals, LA was shown to decrease the iron levels in brain tissue, minimizing the deleterious effect induced by this metal (Suh, Moreau, Heath, & Hagen, 2005). In addition, LA has been shown to improve the resistance against metal pollution, and increase the antioxidant capacity of *L. vannamei* (Lobato et al., 2013).

On the other hand, in the hepatopancreas, the pretreatment with LA increased the metal levels when the shrimps were exposed to Cd or As singly (Figure 1b and Figure 2b; respectively). One hypothesis to this result is that as LA accumulates, mostly in the liver and hepatopancreas, a higher intracellular concentration of this antioxidant would be expected in these organs. Because it is chelating metals, it would also explain the increase of Cd and As in this organ. In fact, Bhatt and Flora (2009) showed in rat a beneficial role of LA in terms of As chelation. Other hypothesis is that LA can stimulate the increase in GSH levels in living organisms (Flora, 2009) and this tripeptide possess sulfhydryl group in their structure and both Cd and As exhibit affinity for these groups; this hypothesis can also explain the increase both Cd and As in the hepatopancreas in shrimps supplemented with LA. Besides, the LA can stimulate an increase in metallothionein-like levels (Lobato et al., 2013), levels which in turn cause Cd to be unavailable to exert toxic effects in these organisms, it also can be postulated that this organ would not be affected.

In the case of As, it is more important that the quantity of metalloid accumulated is the prevalent chemical form. In fact, inorganic arsenic (iAs such as arsenite and arsenate; As^{3+} and As^{5+}, respectively) are considered highly toxic while methylated forms (MMA and DMA) are moderately toxic and there are forms considered non-toxic at all, such as AsB, AsC, TMAO and TETRA (Ventura-Lima et al., 2011). LA was shown to improve the metabolization capacity of hepatopancreas once that a decrease in iAs levels and increase in AsB content in both groups exposed to As alone or in combination with Cd was registered, when compared with the same treatments without LA (Table 2).

In gills, LA favoured As metabolization by decreasing iAs (except in the group co-exposed to Cd + As) and DMA levels (considered moderately toxic). However, also observed that LA induced a decrease in TMAO (non-toxic compound) levels in the groups exposed to As (alone or in combination with Cd), this decrease also was observed in the control group that received LA, indicating that this antioxidant stimulate the As metabolization because the TMAO is an intermediate among DMA and AsB (Aposhian, 2004). In fact, AsB levels were increased in the groups exposed to As alone or in combination with Cd (Table 1). However, in muscle in the group exposed to As alone, LA did not decrease iAs content, but the decrease of AsB levels was accompanied by an increase in TETRA content, a non-toxic compound (Table 3).

Actually, several studies have considered the As metabolization capacity in aquatic organisms after exposure to this metalloid (Cordeiro et al., 2016; Nunes et al., 2017; Ventura-Lima et al., 2007). However, until now, few studies evaluated the influence of antioxidants in the metabolization process of arsenic. Jin et al. (2010) observed in mice that administration of reduced glutathione (GSH) and methionine increased the levels of methylated compounds when the animals were exposed to iAs, indicating an improvement in the metabolization capacity of As. In fact, the GSH is involved in the metabolism of As, being an electron donor during reduction reactions that precede methylation (Aposhian et al., 2004; Ventura-Lima et al., 2011). The methylated compounds are more easily excreted than inorganic compounds; therefore, the improvement in the capacity of metabolization can decrease the risk to the health of organisms, including humans (Kitchin & Wallace, 2008). López-Carrillo et al. (2016) observed that the uptake of micronutrients (such as vitamin B12 and C, folate, choline, Se and Zn) through diet, increased the levels of methylated compounds, indicating the improvement of arsenic metabolization capacity in humans. It is known that LA induces an increase in GSH levels in some aquatic organisms, including *L. vannamei* (Lobato et al., 2013). Thus, this effect of LA on GSH levels could explain the increase of metabolization capacity of As in the different organs of *L. vannamei* observed in this study.

5 | CONCLUSIONS

Alternatives that minimize or avoid accumulation and favour the metabolization are welcome in aquaculture. In this study, we showed that LA administered through diet decreased the accumulation of Cd and As, alone or in combination, in muscle and gills of shrimps. Moreover, LA improved the metabolization capacity of As in different organs of *L. vannamei*. Beyond the results observed in this study, LA is a potent antioxidant that can modulate the redox system of organisms. So, the supplementation of LA through the diet seems is a good alternative practice in aquaculture, one that increases the resistance against metal contamination.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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