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Enzyme biomarker response in *Zenaida asiatica* from an agricultural area of Campeche, Mexico

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ABSTRACT

Objective: To evaluate the response of CAT, AChE, and GST enzyme biomarkers in *Zenaida asiatica* from an agricultural area of the municipality of Champotón, Campeche.

Design/methodology/approach: The biomarkers AChE, CAT, and GST were analyzed in tissues of 24 birds from backyards and agricultural areas of Champotón, Campeche. The enzymatic activities in the tissues were compared by means of a t test and comparison of means (Tukey $p \leq 0.05$), with the Statistica v. 7 software.

Results: The highest AChE activity occurred in the brain ($p=0.00001$), CAT activity in liver ($p=0.00001$), and GST activity in liver and brain ($p=0.001$).

Study limitations/implications: To evaluate the effect of pesticides on wild birds, a larger number of individuals is required in different agricultural areas.

Findings/conclusions: In the tissues of *Z. asiatica* from agricultural areas, greater activity of the biomarkers AChE, CAT, and GST was found, reflecting the excessive use of pesticides.

Keywords: *Zenaida asiatica*, Acetylcholinesterase, Catalase, Glutathione S-Transferase.

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INTRODUCTION

The agricultural area cultivated in the Mexican Republic comprised 22,148,245.07 ha in the year 2015 (SIAP, 2016). The state of Campeche has 314,812.03 ha (SIAP, 2016), of which 14.3% are located in the Champotón municipality, which grows as its main crops maize (*Zea mays* L.) and sugar cane (*Saccharum* spp.), with 34.1% and 32.4%, respectively (SIAP, 2016).

The main issues affecting production within these systems include disease and pests, which are mainly controlled with synthetic pesticides (Aktar *et al.*, 2009), such as carbamates, pyrethroids, organochlorines (OCs),

Image by Alejandro Hernández Ortiz at Pixabay

and organophosphates (OFs), among others (CICOPLAFEST, 2013). However, OC pesticides have a high risk of accumulation in the exposed fatty tissues of organisms and of biomagnification in the food web (Chaiyarat *et al.*, 2014); meanwhile, OFs mainly affect the nervous system of vertebrates and invertebrates through the phosphorylation of the acetylcholinesterase enzyme (AChE) in nerve endings (Ghorab and Khalil, 2015).

In these agricultural systems, organisms coexist at different levels of the food chain, as is the case with birds (Padoa-Shioppa *et al.*, 2005), which are highly sensitive to the toxic effects of OFs (Robles *et al.*, 2007). Juveniles (nestlings) have the greatest propensity to intoxication, as a consequence of the low concentration of cholinesterase (AChE) per unit of brain tissue, leading to greater absorption of pesticides through contaminated food (grain and insects), through contact with their parents or because their nests are located within agricultural areas (Burgess *et al.*, 1999).

The effects caused by exposure to toxic residue can be detected through various biomarkers which quantify stress proteins and the activity of the enzymes catalase (CAT), glutathione-S-transferase (GST), and acetylcholinesterase (AChE) (Arago, 2012). These effects have been evaluated in bird species such as *Gallus domesticus*, exposed to 3-phenoxybenzyl (permethrin), causing a 10% reduction of liver activity for GST (Ezeji *et al.*, 2012), and under a dosage of 30 ppm of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide (endosulfan). This caused reductions in CAT activities and GSH content in blood, as well as an increase in AChE activity (Aggarwal *et al.*, 2008). In white stork (*Ciconia ciconia*) juveniles and adults, AChE activity in plasma was found to be inhibited by paraoxon-methyl followed by carbofuran and carbaryl (Oropesa *et al.*, 2013). In the blood of *Passe domesticus* exposed to the use of malathion (Ops) in agricultural areas of Durango, an 11.58% inhibition of butyrylcholinesterase took place (Bujdud *et al.*, 2019). In Palizada, Campeche, Mexico, AChE inhibition in the brains of *Dendrocygna autumnalis* in rice plantations was recorded (Rendón *et al.*, 2005).

A potential relationship between these effects on enzyme biomarkers and pesticides used in the field has been documented. González-Gómez *et al.* (2020) reported polychlorinated biphenyls (PCB), organochlorine pesticides (OCs), and polybrominated biphenyls concentrations on 90% of the feathers of 71 individuals of *Columna livia domestica* collected in Asturias and Galicia. However, there is no information about the enzymatic activities of birds present in agricultural areas of the state of Campeche. Therefore, the objective of this study was to evaluate the response of enzyme biomarkers CAT, AChE, and GST in the species *Zenaida asiatica* from an agricultural area in the municipality of Champotón, Campeche, Mexico.

MATERIALS AND METHODS

The study was conducted during the March-August 2017 period in the locality of Santo Domingo Kesté, Champotón, Campeche (19° 30' 54" N, 90° 26' 41" W). The weather is warm and sub-humid with an average annual temperature of 26 °C and the town is located 24 meters above sea level. The soil is argillaceous and used mainly for agriculture (INEGI, 2014). The population is largely dedicated to relay agriculture, growing maize (*Zea mays*), cushaw pumpkin (*Cucurbita argyrosperma* Huber), beans (*Phaseolus* spp.), hibiscus (*Hibiscus*

sabdariffa), and peanuts (*Arachis hypogaea*), which they use for sale and self-consumption (INEGI, 2010). In maize crops, 61.78% of farmers apply pesticides prior to germination and 55.20% apply some type of insecticide, mainly to stave off armyworms after 30 days of germination (Uzcanga *et al.*, 2015). Insecticides, pesticides, and granular fertilizers are applied, in order to grow cushaw pumpkins (Ireta-Paredes *et al.*, 2017).

Collecting samples of white-winged doves (*Zenaida asiatica*)

Twenty-four white-winged doves were divided into two groups, with group 1 (G1) consisting of 12 birds from the backyards of the Santo Domingo Kesté locality (fed with fruit and vegetable residue) and group 2 (G2) of 12 birds donated by farmers (caught within farming areas for self-consumption).

In order to obtain tissue from birds, the recommendations issued by Official Mexican Standard 033-SAG/ZOO-2014 were taken into consideration, following the bird disinfection protocol. Afterwards, each bird was dissected, with a longitudinal incision in order to extract the liver, heart, muscle, and brain, which were placed into 1.5-ml eppendorf tubes, duly labeled with the name of tissue, bird number, and location.

Preparation and determination of acetylcholinesterase (AChE) activity

In order to analyze the activity of biomarker AChE in the tissues of *Zenaida asiatica*, the methodology proposed by Ellman *et al.* (1961) was employed, whereas to determine the quantity of proteins, the Bradford (1976) method was used.

Preparation and determination of glutathione S-transferase (GST)

The activity of biomarker GST in dove tissue was evaluated through the methodology proposed by Habig and Jakoby (1974). Protein quantity was determined through the Bradford (1976) method.

Preparation and determination of catalase (CAT) activity

The analysis of biomarker CAT in the tissues of the *Z. asiatica* dove was measured through the methodology proposed by Aebi (1984), as modified by Regoli (1998). All readings were carried out using a Thermo Scientific® microplate spectrophotometer.

Data analysis

The response of biomarkers AChE, GST, and CAT on tissue (heart, liver, brain, and muscle) were compared through a t test, in order to determine significant differences between both groups (G1 and G2). Tukey's test was used as a multiple comparison test; in both cases, the confidence level was $p \leq 0.05$. Data was analyzed using Statistica v. 7 software.

RESULTS AND DISCUSSION

Biomarker response

Acetylcholinesterase (AChE) Activity: The greater AChE activity was found in brain tissue ($F=17.71$, $P=0.00001$), with 105.32 ± 34.55 nmol/min/mg protein, followed by heart, liver,

and muscle, with 50.75±17.90, 40.21±19.82, and 33.06±25.08 nmol/min/mg protein, respectively. *Agelaioides badius* had a similar response under exposure to imidacloprid, exhibiting greater AChE values in the brain (Poliserpi *et al.*, 2021).

When these groups were compared, a greater inhibition (T=130.81, P=0.0321) of AChE activity was observed in the brains of doves in G2 than in G1, with 83.24±22.92 and 127.33±30.71 nmol/min/mg protein, respectively (Figure 1).

The brain was the organ with the highest sensitivity for determining AChE activity, which matches the findings of Lari *et al.* (1994) and Fossi *et al.* (1996), who observed a direct correlation between plasma cholinesterase and brain cholinesterase in the Japanese quail. Consequently, a 50% inhibition in plasma cholinesterase is equivalent to a 20-50% inhibition in brain cholinesterase, indicating that the bird is in an exposure zone or reversible effect zone (toxicity). This activity has been recorded in *Dendrocygna autumnalis* in rice plantation areas exposed to 0,0-diethyl 0-(3,5,6-trichloro-2-pyridine) phosphorothioate, N-(Phosphonomethyl) glycine, and 2,3-Dihydro-2,2-dimethyl-7-benzofuranol methyl carbamate: 34% AChE inhibition in brain tissue was reported by Rendón, Soares, and Guilhermino (2005). In *Columba livia gaddi*, *Streptopelia decaocto*, and *Coturnix coturnix*, exposed to the 2,2-dichlorovinyl-dimethyl phosphate pesticide, there are records of a cholinesterase activity inhibition of 21-98% in the brain and 9-100% in plasma cholinesterase (Alias *et al.*, 2011). Likewise, Burkepile *et al.* (2002) examined the effects of O,O-Diethyl-O-(4-nitrophenyl) phosphorothioate on AChE activity and the productive behavior of *Zenaida asiatica* in southern Texas, USA, finding that ≥4,5 ppm levels inhibit 25% of AChE activity and have negative effects on egg laying and incubation.

Catalase (CAT) activity: In analyzing CAT activity among bird tissue, no significant differences were observed between groups (p>0.05) (Figure 2). The greatest CAT activity was observed in the liver (F=22.65, p=0.00001), followed by the heart, brain, and muscle, with 21.30±8.23, 7.35±4.18, 7.23±3.41, and 3.85±3.82 nmol/min/mg of protein, respectively. In this light, Poliserpi *et al.* (2021) mention that CAT activity was only detected

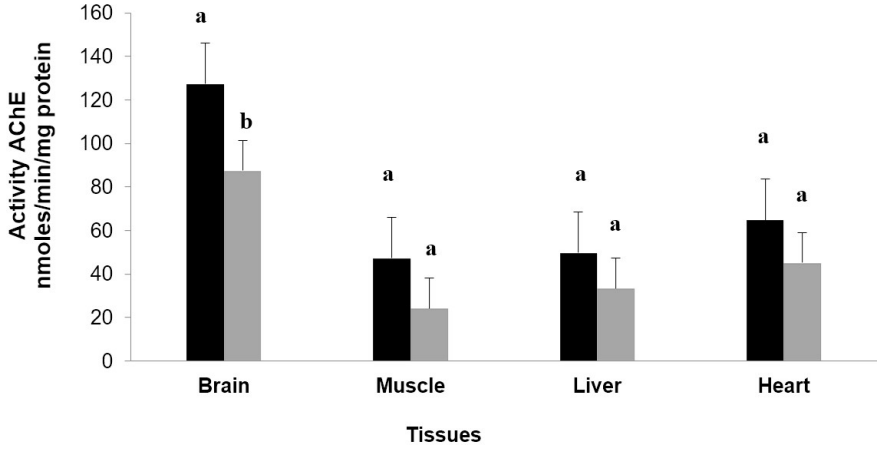

Figure 1. Acetylcholinesterase (AChE) activity in four tissues of Dove *Zenaida asiatica*. Dark column (■) G1, ligth column (■) G2. The date are expressed as mean ± standard deviation (N=24). The literals indicate significant difference between groups by tissues of bird p≤0.05.

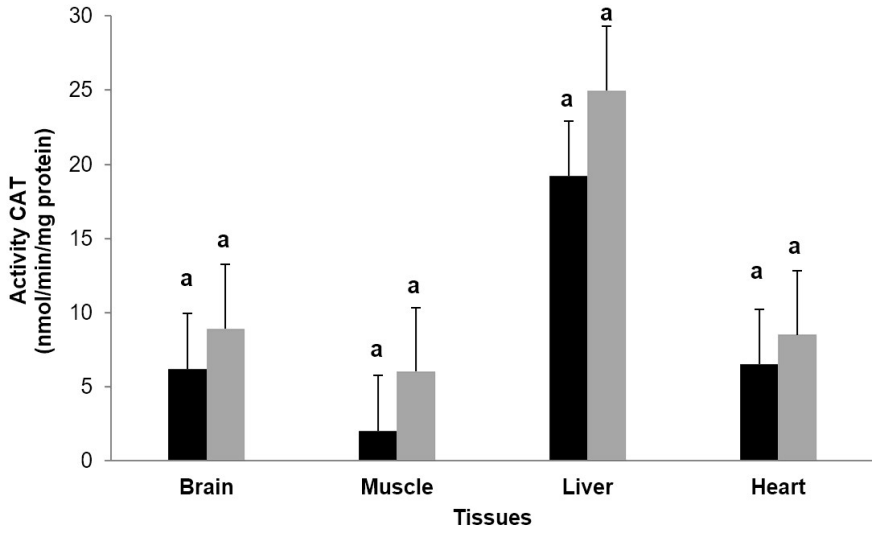

Figure 2. Catalase (CAT) activity in four tissues of Dove *Zenaida asiatica*. Dark column (■) G1, ligth column (■) G2. The date are expressed as mean ± standard deviation (N=24). The literals indicate significant difference between groups by tissues of bird p≤0.05.

in the liver of *Agelaioides badius*, whereas no activity was detected in plasma, brain, muscle, or red blood cells.

Glutathione-S-transferase (GST) activity: GST activity (T=139.22, p=0.04) was significantly greater in the brains of G2 birds than in G1 (Figure 3). Significant differences in GST activity were found among tissues (F=11.91, p=0.001), with the liver and the brain having the highest response (114.57±19.49 and 106.19±50.29 nmol/min/mg of protein), followed by the heart and muscle (75.41±17.93 and 34.79±19.85 nmol/min/mg of protein, respectively). Likewise, Ezeji *et al.* (2012) recorded an increase in GST activity in the liver and serum of *Gallus domesticus* exposed to 3-phenoxybenzyl, given that GST action

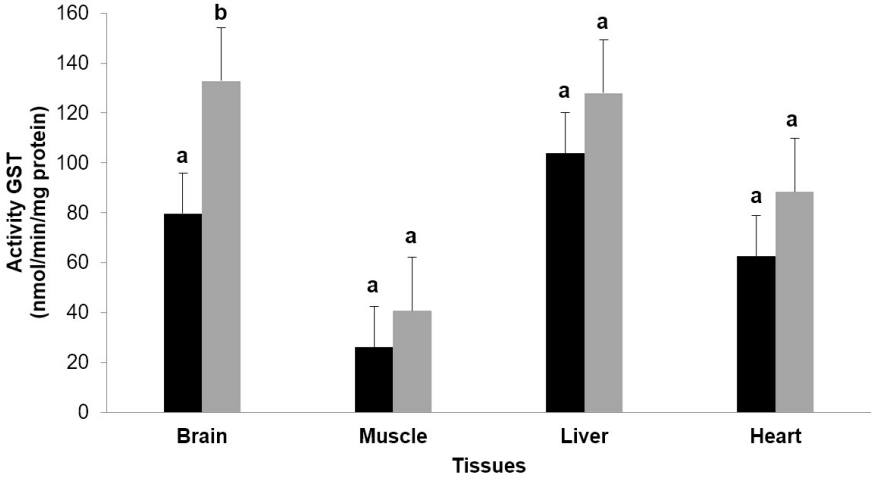

Figure 3. Glutation S-transferasa (GST) activity in four tissues of Dove *Zenaida asiatica*. Dark column (■) G1, ligth column (■) G2. The date are expressed as mean ± standard deviation (N=24). The literals indicate significant difference between groups by tissues of bird p≤0.05.

on OF pesticides can lead to activation or detoxification (Miyamoto and Mikawa, 2005); meanwhile, Poliserpi *et al.* (2021) recorded the highest GST response in plasma, brain, liver, and muscle in the species *Agelaioides badius* exposed to imidacloprid.

CONCLUSIONS

The activity of enzyme biomarkers evaluated in the tissues of *Z. asiatica* have shown a response to pesticides: the brain and the liver are the tissues with the most activity recorded by the biomarkers AChE, CAT, and GST, mainly in birds which come directly from agricultural areas, and less in birds fed in backyards. This shows the impact of pesticides in the study area. Therefore, biomarker evaluation is efficient in indicating possible affectation in avian wildlife species.

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