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CONCENTRATION AND ACTIVITY OF CHOLINESTERASE IN COWS PLASMA AS AFFECTED BY ORGANOPHOSPHATE PESTICIDES

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ABSTRACT

Unhealthy environment such as landfills, will result in the exposure of livestock grazing there, and affect the growth and development of these animals. The activity of blood cholinesterase enzymes in animals is used to diagnose and monitor poisoning caused by organophosphate pesticides. Quantitative research with a survey design using a cross sectional design using 35 samples of cows (wild). The study was conducted in January - June 2020. Measurement of blood plasma cholinesterase enzyme activity concentration using the A15 biosystem tool DGKC-Colorimetric Kinetic method. This study aims to evaluate the level of the cholinesterase enzyme as a marker of organophosphate poisoning in cows. Blood samples were taken from a ventrolateral vein of a cows neck, male and female, aged 1-5 years collected from local cows in the landfill. The results showed that cows that were farmed in the land fills (TPA) were exposed to organophosphate pesticides as indicated by a decrese in the concentration and activity of the cholinesterase enzyme in the cows plasma. Cholinestrase enzyme levels in male cows was 0.173 ± 0.081 U/mL and in female cows was 0.188 ± 0.071 U/ml. The highest concentration of cholinesterase enztme activity was at 1.5 years old cows that was 0.22 ± 0.042 U/ml and the lowest was at 3 years old cows that was 0.14 ± 0.078 U/ml.

KEY WORDS: Cholinesterase, Cow, Landfills, Organophosphate

INTRODUCTION

Pesticide pollution in the environment plays an important role in contributing to the fundamental causes of fertility problems. Evidence from the above phenomena includes that female cows that consume food (food resources) from waste from landfills that are suspected of being exposed to pesticides show changes that include poor reproductive behavior, subfecundity, infertility, miscarriages, growth retardation, death intra uterine fetus and ovarian failure. Pesticide residues have an adverse effect on the reproductive system and are known as toxic poisons and disrupt the endocrine system. These toxicants modulate or interfere with reproductive hormones by acting at various sites including the hypothalamus, pituitary and reproductive organs. Pesticide residues can harm the male reproductive system by causing toxicity to sperm plasma membrane (Choudhary *et al.*, 2018)

The use of pesticides can cause resistance to pests as well as side effects in some beneficial and non-target species. Many pesticide residues are found in food and feed originating from environmental pollution. The stability of chemicals in the environment causes contamination of food ingredients especially those that have high fat content such as meat products (Kiranmayi *et al.*,

2016). Pesticides are the main group of residues that cause chemical contamination related to agricultural practices, animal husbandry and production processes that can cause contaminants in meat and meat products because they are contaminated by the environment (Marilena *et al*., 2017).

Organophosphates are a class of insecticides, some of which are highly toxic and all of them can potentially cause acute and subacute toxicity. Organophosphate is used in agriculture, homes, gardens and veterinary practices. All have the same mechanism of inhibition of cholinesterase and can cause similar symptoms, although there are some differences in class. Mechanisms of exposure to the same organophosphate through various routes or multiple organophosphates with various routes can cause serious additive toxicity (Bates and Campbell, 2008). Pesticide residues in livestock are generally accumulated by two ways, namely through direct application to animals or agricultural crops and animal feed. Crops and fodder contaminated with pesticides can accumulate residues in edible tissue. Animals can accumulate pesticides from contaminated food and water (Chawla *et al.*, 2018)

Animals that breathe polluted air or consume forage plants, grasses and feed ingredients that are contaminated with pesticides have high lipid solubility. Pesticides are slowly metabolised in the liver before being released into the circulatory system and finally stored in fatty tissue (Pardío *et al.*, 2012). Pesticide residues in meat are of concern because of possible adverse effects on humans. Pesticide levels are assessed in five edible parts of livestock namely muscle, liver, kidney and tongue tissue to determine human health risks associated with consumption of these tissues (Tongo and Ezemonye, 2015)

The main important source of pesticide residues is feeding livestock with contaminated crops. Pesticide poisoning in animals is generally associated with errors in humans. Errors include improper doses, improper use of compounds or formulations, use of treated seeds as feed, improper spraying and improper storage and disposal of pesticide containers (Choudhary *et al.*, 2018). The bad effects of pesticides on livestock are kept in cages and have a bad effect on different health. Animals can be exposed to pesticides from contaminated feed and drinking water. The lipophilic nature of this pesticide accumulates in animal body fat. The cumulative amount of pesticide residues in meat makes great concern for ensuring food safety and human health (Choudhary *et al.*, 2018). Cholinesterase activity in blood in animals is used to diagnose and monitor organophosphate pesticide poisoning (Mohammad *et al*., 2007).

The acetylcholinesterase enzyme is an enzyme whose role is to neutralize/restore acetylcholine bonds as a neurotransmitter with muscarinic receptors which play a major role in the creation of nerve impulse transmission in the central and peripheral nervous system. When the inhibition of the activity of the cholinesterase enzyme occurs due to this enzyme is inhibited by organophosphate substances (toxins) it will cause excessive activation of the brain and muscle tissue. This situation is caused by the enzyme cholinesterase which is inadequate hydrolyzing at nerve synapses and neuromuscular junction (motor end-plate) (Kolesárová *et al.*, 2013)

In mammals, inhibition of cholinesterase by organophosphates leads to high concentrations in animals. The inhibition of the cholinesterase (Ach^{ase}) enzyme in cholinergic synapses of the central and peripheral nervous system causing clinical signs of organophosphate poisoning is a consequence of excessive stimulation of muscarinic receptors (Larsen *et al.*, 2019). For this reason, it is necessary to study the concentration and activity of cholinesterase in cows plasma as affected organophosphate pesticides.

MATERIALS AND METHODS

This research was located in the Sukawinatan, Palembang, South Sumatera. The study was carried out in January-June 2020. The tools and materials used in this study are: Blood tube (red non-heparin 3 ml), cooler box, needle BD vacu 21 G, holder vacutainer, centrifugation at 10000 rpm (Centurion, UK) and biosystem A15. This study is a quantitative study with a survey design using a cross sectional design using 35 samples of cows that are commonly farmed in landfills (TPA).

Blood samples were taken from the neck ventrolateral vein from cows aged 1-5 years. DGKC-Colorimetric Kinetic Method with A15 biosystem tool. Principle of Butyrylthiocholine + $H_2O \rightarrow$ CHE Thiocholine + Butyric acid. Cows blood is centrifuged at 10000 rpm for 10 minutes for plasma collection. Then A-15 biosystem tool was used. Carefully open the lid of the serum control bottle and add 5 mL of Aquabidest. Close the bottle again

and mix slowly for 30 minutes and avoid forming foam. Put the control serum into the sample cup. Do control with the A-15 biosystem tool. Parameters observed in the study included the concentration of cholinesterase activity in cows plasma, differences in sex and mean age of cows. The data collected was then analyzed both by tabulation and SPSS version 16. The concentration of the cholinesterase enzyme activity of the DGKC-Colorimetric Kinetic method with the A15 biosystem tool.

RESULTS AND DISCUSSION

The final disposal site (TPA) environment is the most widely applied method in several cities in Indonesia, including in the city of Palembang. With the existence of landfill, it will have a negative impact on the environment. The negative impact is the resulting pile of rubbish that is difficult to decompose besides it is produced leachate which has the potential to damage the environment. TPA receives all types of waste originating from households, markets, commercial locations, public facilities. In addition to inorganic waste, TPA also has organic waste such as leaf waste, food scraps, fruit scraps and vegetable scraps. This is what makes one of the sources of feed that is used to feed cows in the landfill. Limited land and the high price of feed is one of the reasons for farmers raising cows to a final landfill where cows are released from their pens from 14.00 to 15.00 western Indonesia time. Cows grazing in the TPA have the potential to contain dangerous elements. Hazardous elements such as pesticides that can accumulate in the blood and organs of the cows body tissue.

The content of cholinesterase in cow blood plasma.

Test analysis of the concentration of cholinesterase enzyme activity in cows by sex is presented in Table 1.

Table 1 shows that the concentration of cholinesterase enzyme activity in the plasma of male cows shows a mean value of 0.173 U/ml, lower compared to cholinesterase activity in the blood plasma of female cows of 0.188 U/ml. The results of the examination of the concentration of cholinesterase obtained an average of 0.19 ± 0.076 U/ml. However, the value of cholinesterase activity in plasma of male and female cows has decreased which indicates the exposure by organophosphate pesticides.

Determination of cholinesterase activity in plasma and erythrocytes was performed by the Ellman method, the initial mean value for acetylcholinesterase was 624.65 ± 39.32 IU/L in male and female cows. Administration of topical inhibiting effects of chlorpyrifos on peak concentrations of acetylcholinesterase activity (males: 10.920 ± 4.18 µg/L; females: 12.12 ± 3.88 µg/L (Picco *et al.*, 2008). Cholinesterase activity in the control group in blood plasma 0.220 ± 0.083 IU/mL (Pardío *et al.*, 2001)

The cholinesterase activity of all bulls is lower than that of female cattle cholinesterase. These findings differ from those reported in humans having high plasma cholinesterase activity (Mohammad *et al.*, 2007). However, like the results of this study, sex differences in blood cholinesterase activities should not be excluded. Differences in blood cholinesterase activity between animal species and also between sexes can be considered normal physiological differences and they can form the basis of differential variations and blood fractions that depend on sensitivity to organophosphate pesticides (Mohammad *et al.*, 2007).

Cows that are bred in landfills (landfills) have been exposed to organophosphate pesticides. This is

Table 1. Concentrations cholinesterase in plasma in male and female cows

Parameter	Sex	
	Male	Female
Mean Average cholinesterase enzyme activity (U/ml)	0.173	0.188
Standard Deviation	0.081	0.071
Standar Error	0.026	0.014
95% Confidence Interval for mean		
Lower bound	0.11	0.16
Upper bound	0.23	0.22
Min	0.03	0.07

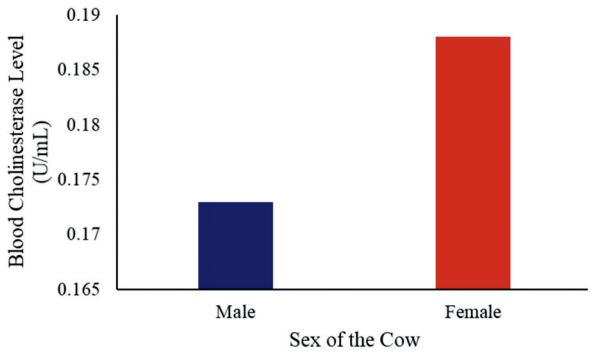

Fig. 1. Average levels of the cholinesterase enzyme in cows blood by sex

indicated by the level of cholinesterase activity in blood plasma. Cholinesterase activity in male cows is of 0.173 U/ml and female cows of 0.188 U/ml.

The content of the cholinesterase enzyme in cow blood plasma.

Test analysis of cholinesterase concentrations in cows by sex is presented in Table 2. The concentration of cholinesterase activity in blood plasma of 1 year old cows was 0.20 ± 0.046 U/ml, while at 1.5 years old cows was 0.22 ± 0.042 U/ml, 2 years old 0.19 ± 0.090 U/ml , 3 years old 0.14 ± 0.078 U/ml and age 5 years 0.18 ± 0.072 U/ml. Cholinesterase activity in cows blood plasma is highest at 1.5 years of age and the lowest is 3 years. However, the value of cholinesterase activity in cows blood plasma by age decreases the concentration of cholinesterase activity in cows age 1 year, 1.5 years and age 3 years old. This overall result is a decrease in cholinesterase indicating the exposure of cows by organophosphate pesticides.

According to Abdelsalam and Ford (1985), Normal cholinesterase activity ((µmol / ml / min) measured in cattle's blood and plasma is obtained in cattle aged 3-6 months 0.39 ± 0.11, ages 1-2 years 0.19 ± 0.03, ages 2 years and above 0.22 ± 0.04. The level of acetylcholinesterase activity in complete blood is 5510 ± 600 IU/L in complete blood and 190 ± 30 IU/L in blood plasma for bulls aged one to two years (Picco *et al.*, 2008). The value of cholinesterase activity in the Michel method (ΔpH/30 min) obtained an average of 0.315 ± 0.0499 Micro mol/min/g.

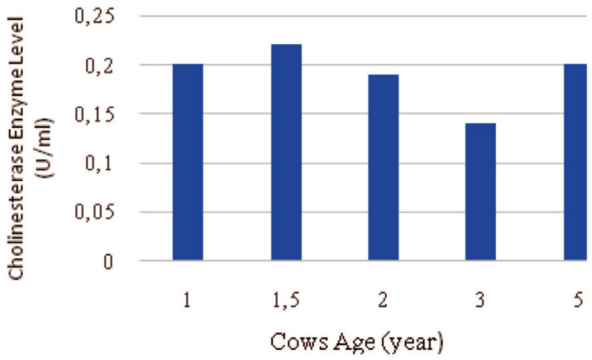

Fig. 2. Average levels of cholinetrase enzyme in cows blood at various ages

The value of cholinesterase activity by the Ellaman method (" Absorbance / min) obtained an average of 0.644 ± 0.135 Micro mol/min/g (Khalil and Abass, 2017). Normal cholinesterase enzyme activity in the reporting range is 2.06 ± 30 µmol /g $^{-1}$ /min^{-1} (Salaramoli, 2008)

Seventeen different pesticides were detected in fish tissue with 81% of captured animals containing at least one molecule of pesticides. The phenvaleric pyrethroid insecticide and bifenthrin were most commonly detected, respectively in 41.8% and 36.4% of the samples tested. Dichlorvos and very poisonous pirimiphos-methyl are dropped. This finding proves that the pesticides used today can accumulate and have an impact on health (Brodeur *et al.*, 2017). Acetylcholinesterase (AChE) activity in sheep exposed to chlorophyllphos (CPF) and pyrethroid cypermethrin (CPM) groups. chlorpyrifos (CPF) showed blood plasma AChE 172 ± 2.00 nmol min^{-1} mg^{-1}, for pyrethroid cypermethrin (CPM) > 2,000 (Larsen *et al.*, 2019)

Cholinesterase activity in humans, there was a significant difference in $p < 0.05$ of cholinesterase activity in 3 groups compared to the control group. Also the relationship of cholinesterase activity regarding the age of the control group subjects was 0.93 ± 0.04, the seller group 38.0 ± 13.22, the agricultural group 47.3 ± 08.62 and the veterinary group 42.1 ± 12.40. Where the normal activity of cholinesterase in the control group is 0.93 ± 0.04, the seller group is 0.74 ± 0.07, the agricultural group is 0.83 ± 0.07 and the veterinary group is 0.84 ± 0.07 (Al-Haseni and Yahya, 2012)

Cows that are farmed in landfills (TPA) have been exposed to organophosphate pesticides. This is

Table 2. Concentrations of cholinetrase enzyme in cows plasma based on age

Age	Mean cholinesterase enzyme activity (U/ml)	Standard Deviation	Std.Error	95% Confidence Interval for mean		Min	Max
				Lower bound	Upper bound		
1 year	0.20	0.046	0.027	0.09	0.31	0.162	0.251
1.5 year	0.22	0.042	0.019	0.17	0.27	0.156	0.27
2 year	0.19	0.090	0.024	0.14	0.24	0.03	0.336
3 year	0.14	0.078	0.032	0.06	0.22	0.072	0.264
5 year	0.18	0.072	0.029	0.11	0.26	0.09	0.258

indicated by the level of cholinesterase activity in cows blood plasma. The level of cholinesterase activity in 1 year old cow was 0.20 ± 0.046 U/ml, 1.5 years old 0.22 ± 0.042 U/mL, 2 years old 0.19 ± 0.090 U/ml, 3 years old 0.078 ± 0.032 U/ml and 5 years old 0.18 ± 0.072 U/ml.

Cholinesterases are special carboxylic ester hydrolases that break down choline esters. Pesticides are achyl cholinesterase AChE acetylcholine hydrolase, and butyrylcholinesterase BuChE acylcholine acylhydrolase, also known as nonspecific cholinesterase or pseudocholinesterase. AChE is acetylcholine (ACh). Non-specific cholinesterases such as butyrylcholine and propionylcholine, depending on the type (Krieger, 2010). Cholinesterase activity can be measured in complete blood, erythrocytes, or plasma. The use of whole blood has been recommended as a guideline for international standardization of ChE measurements. It is recommended that complete blood CE monitoring should be adequate for livestock species such as cattle, horses and sheep with 90% of total cholinesterase activity present in red blood cells (Pardío *et al.*, 2001)

Cholinesterase activity was measured in complete blood, plasma and tissue by the Ellman method (1961) using acetylthiocholine iodide as a substrate and 5.5-dithiobis- (2-nitrobenzoic acid) as a chromogenic indicator. Blood diluted 1 in 40 in 0.1 M cold phosphate buffer (pH 8) (Abdelsalam dan Ford, 1985). The activity of the cholinesterase enzyme in 25 crosses from cattle obtained the mean value in blood plasma 203.1 ± 42.06 IU/L^{-1} (Ferré *et al.*, 2018). Cholinesterase is a key enzyme in a range of important areas such as neurobiology, toxicology and pharmacology. Of these, two main groups, acetylcholinesterase and butyrylcholinesterase play important roles in the function and health of humans and animals.

Cholinesterase is present mainly in the central nervous system. It is bound to the excision tissue cell membrane and is involved in the nerve transmission process. Its main biological function is to catalyze the hydrolysis of the neurotransmitter acetylcholine into choline, which is a reaction needed to allow cholinergic neurons to return to rest after activation (Miao, He and Zhu, 2010)

Cholinesterase is an enzyme that is involved in helping the nervous system function properly. There are two separate cholinesterase enzymes in the body. Acetylcholinesterase is found in red blood cells as well as in the lungs, spleen, nerve endings and gray matter of the brain and pseudocholinesterase (butyrylcholenesterase) is found in serum and liver, muscle, pancreas, heart and brain white matter. Acetylcholinesterase is involved in the transmission of nerve impulses by breaking down acetylcholine, a chemical that helps transmit signals across nerve endings. Decreased acetylcholinesterase enzyme activity causes excessive neurotransmitter acetylcholine to nerve endings. This can cause excessive stimulation of nerves in the tissues and organs of the body (Cholinesterase Tests, 2020) from the results of the examination of the concentration of the cholinesterase enzyme in cows blood plasma, the results obtained below the standard value determined which indicates a decrease in the concentration of cholinesterase activity in cows blood plasma.

CONCLUSION

From the study it can be concluded that the cows that were farmed in the landsfills (TPA) were exposed by organophosphate pesticides as indicated by a decrease in the concentration and activity of the cholinetrase enzyme in the cows plasma. Cholinetrase enzyme levels in male cows was 0.173 ± 0.081 U/mL and in female cows was 0.188 ± 0.071 U/mL. The highest concentration of cholinetrase enzyme activity was at 1.5 years old cows that was 0.22 ± 0.042 U/mL and the the lowest was at 3 years old cows that was 0.14 ± 0.078 U/ ml.

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