

Cholinesterase concentration in three types blood of cattle raised on exposed land by Organophosphates

Ali Harokan¹, Totong Kamaluddin², Daniel Saputra², Nurhayati Damiri^{2,3*}

¹Student of Doctoral Program of Environmental Science, Graduate school, Universitas Sriwijaya

²Lecturer of Doctoral Program of Environmental Science, Graduate School, Universitas Sriwijaya

³Lecturer of Pest and Plant Diseases Department, Faculty of agriculture, Universitas Sriwijaya

*Corresponding Author : nurhayati@fp.unsri.ac.id

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Abstract: Organophosphate pesticides are one of the causes of contamination in animal feed. The cholinesterase enzyme test is used to diagnose toxic exposure due to organophosphate pesticides. Quantitative research with cross sectional survey design used 35 samples of cattle. The study was conducted in March - September 2020. Concentration measurement on cholinesterase enzyme concentrations of blood plasma used the biosystem A15 DGKC-Colorimetric Kinetic method. This study aims to evaluate the concentration of the enzyme cholinesterase as a marker of organophosphate poisoning in cattle. Blood samples were collected from the ventrolateral neck veins of bulls and cows aged 1-7 years which collected from local breeders in the Ogan Ilir area. The results showed that the cattle raised on exposed land by organophosphate pesticides as concentration decreasing indication of cholinesterase enzyme in cows plasma. Cholinestrace enzyme levels in cows were 0.20 ± 0.067 U / ml and bulls were 0.27 ± 0.183 U / ml. The highest concentration of cholinesterase enzyme levels were occurred in cattle aged 2 years, 0.25 ± 0.34 U / ml and the lowest was in cattle aged 7 years, 0.12 ± 0.04 U / ml.

Key words: Cholinesterase, Environment, Cattle, Organophosphate, Plasma

Abstrak. Pestisida organofosfat merupakan salah satu penyebab kontaminasi pada pakan ternak. Pemeriksaan enzim cholinesterase digunakan untuk mendiagnosis pemaparan keracunan akibat pestisida organofosfat. Penelitian kuantitatif dengan desain survei menggunakan desain *cross sectional* dengan menggunakan 35 sampel sapi. Penelitian dilakukan pada bulan Maret - September 2020. Pengukuran Kadar konsentrasi enzim cholinesterase plasma darah menggunakan alat biosystem A15 metode DGKC-Colorimetric Kinetic. Penelitian ini bertujuan untuk mengevaluasi kadar konsentrasi enzim kolinesterase sebagai penanda keracunan organofosfat pada sapi. Sampel darah diambil dari vena ventrolateral leher sapi jantan dan betina berumur 1-7 tahun dikumpulkan dari peternak lokal di wilayah Oganllir. Hasil penelitian menunjukkan bahwa sapi yang dibudidayakan di peternak terpapar pestisida organofosfat yang ditunjukkan dengan penurunan konsentrasi dan kadar enzim kolinesterase dalam plasma sapi betina. Kadar enzim kolinestrace pada sapi betina $0,20 \pm 0,067$ U / ml dan sapi jantan $0,27 \pm 0,183$ U / ml. Konsentrasi kadar enzim kolinesterase tertinggi berdasarkan usia sapi jantan dan betina terjadi pada sapi umur 2 tahun yaitu $0,25 \pm 0,34$ U / ml dan terendah pada sapi umur 7 tahun yaitu $0,12 \pm 0,04$ U / ml.

Kata kunci: Cholinesterase, Lingkungan, Sapi, Organofosfat, Plasma

1. Introduction

Pesticides are strong acid esters or phenols which can cause skin irritation, redness and dermatitis on the skin. Its use can cause nervous

system disorders. Which occurred in the peripheral nerves and central nervous system[1]. Contaminated animal feed is the main source of pesticide entry into the livestock[2].

Pesticides have lipophilic properties that

could be stored in the fatty tissue of the animal's body. Pesticide residues present in feed which eaten by livestock will transfer to meat through the food supply chain as a result, then animal products such as meat containing pesticide residues that endanger public health[3].

Organophosphate pesticides are able to inhibit the activity of the cholinesterase enzyme through nerve impulse mechanism by binding cholinesterase, so that acetylcholine hydrolysis does not occur. The inhibition of cholinesterase activity may last from a few hours to several weeks depending on the type of anticholinesterase. Acetylcholine is a neurotransmitter located between the nerve endings and muscles which has functions continuing stimulation to nerves[4].

Acetylcholinesterase activity plays a role during the acetylcholine-assisting inflammatory response. Acetylcholinesterase enzymes biologically has function to help the neurotransmitter acetylcholine as an anti-inflammatory mediator. Choline ester acetylcholinesterase is produced by many cells, including lymphocytes in the blood and central nervous system [5]. This study aims to evaluate the levels of the cholinesterase enzyme concentration as a marker of organophosphate poisoning in cattle.

2. Materials and Methods

This research was carried out in farm in Oganllir Regency. The research was conducted in March-September 2020. The equipment and materials used in this study were: Blood tube (3 ml non heparin red color), cooler box, Eppendorf, needle BD vacu 21 G, holder vacutainer, centrifugation at 10000 rpm (Centurion, UK) and biosystem A15. The tool used to analyze the cholinesterase enzyme was biosystem A15. These research was a quantitative study with a survey *cross sectional* design. In this study, sample selection used simple random sampling technique. The number of samples used the formula below:

$$n = \frac{Z\alpha^2 \times P \times Q}{L^2}$$

Information:

- n = Minimum sample size required
- Z α =The standard value of the distribution corresponds to a = 5% = 1.96

- P = Prevalence of outcomes 30 %
- Q = 1 – P
- L = Level of Accuracy 10
- n = $\frac{(1.96)^2 \times 0.3 \times 0.3}{(0.1)^2} = 34,57$ (35 sample)

Blood samples were taken in the ventrolateral neck vein of cattle aged 1-7 years. DGKC-Colorimetric Kinetic method us biosystem A15. The working principle of DGKC – Colorimetric Kinetic is Butyrylthiocholine + H2O CHE Thiocholine + Butyric acid. In this study, there were 35 cattle consisting of 3 bulls and 32 cows. Cattle blood was centrifuged at 10000 rpm for 10 minutes to collect blood plasma. Then the blood plasma was analyzed using the biosystem A-15. The control serum is carefully opened from the bottle and add 5 ml of aquabides, The control serum container is closed again then stir slowly for 30 minutes by avoiding the formation of foam, then enter the control serum into the sample cup. Carrying out control is carried out by using biosystem A-15.

Parameters observed in the study were included cholinesterase concentration in cattle plasma, differences in mean sex and mean age of cattle using pearson’s statistical test. The data collected were analyzed both tabulated and statistical SPSS version 16. Concentrations of cholinesterase enzyme levels with the DGKC-Colorimetric Kinetic method used the biosystem A15.

3. Results and Discussion

Characteristics of cattle

The characteristics based on the type of cattle are listed in Table 1: Types of cattle available to breeders

Type of cattle	Percentages
PO (Ongole breeding)	74.3
Brahman	5.7
Local Cattle	8.6
Brangus	2.9
Bali	8.6

Table 1, shows the types of cattle available in the farm are Ongole breeding (PO) (74.3%), Brahman (5.7%), local cattle (8.6%), Brangus (2.9%) and Bali. (8.6%).



The content of cholinesterase in cattle blood plasma

Cholinesterase enzyme concentration

cattle according to sex are presented in Table 2

Table 2. Cholinesterase enzyme levels in blood plasma of male and female cattle.

Sex	Mean cholinesterase enzyme levels (U / ml)	Standard Deviation	Standard Error	95% Confidence Interval for mean		Min	Max
				Lower	Upper		
Male	0.27	0.183	0.105	0.17	0.73	0.12	0.48
Female	0.20	0.067	0.011	0.17	0.22	0.06	0.30

Table 2, presented the cholinesterase enzyme concentration in male an average value of 0.27 U / ml, higher than the cholinesterase enzyme levels in female of 0.20 U / ml. However, the value of cholinesterase enzyme levels in the plasma of cattle has been decrease as indicatore organophosphate pesticides exposure.

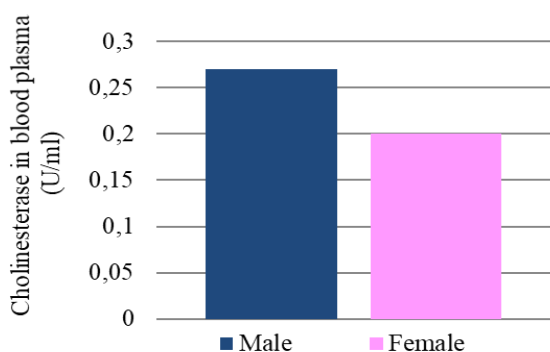


Figure 1. Average levels of the cholinesterase enzyme in the blood of cattle according to sex

Cholinesterase enzyme using the Ellman method, the initial mean value of acetylcholinesterase was 624.65 ± 39.32 IU / L. Cholinesterase enzyme in the control group in blood plasma was 0.220 ± 0.083 IU / ml[6]. Cattle raised in farm have been exposed to organophosphate pesticides. It was indicated by the levels of the cholinesterase enzyme in blood plasma. Cholinesterase enzyme levels in cattle were 0.20 U / ml.

Cholinesterase enzyme levels in cattle blood plasma.

Analysis of the cholinesterase enzyme level test in cattle by age was presented in Table 3

Table 3. Cholinesterase enzyme levels in cattle plasma by age.

Age	Mean cholinesterase enzyme activity(U/ml)	Standard Deviation	Standard Error	95% Confidence Interval for mean		Min	Max
				Lower bound	Upper bound		
2	0.25	0.34	0.19	0.17	0.34	0.24	0.30
3	0.17	0.05	0.02	0.10	0.25	0.12	0.24
4	0.23	0.04	0.01	0.19	0.26	0.18	0.30
5	0.21	0.05	0.01	0.17	0.26	0.12	0.30
6	0.16	0.07	0.03	0.08	0.25	0.06	0.30
7	0.12	0.04	0.02	0.04	0.19	0.06	0.18

Table 3, cholinesterase levels in blood plasma in cattle aged 2 years 0.25 ± 0.34 , 3 years old 0.17 ± 0.05 , 4 years old 0.23 ± 0.04 , 5 years old 0.21 ± 0.05 , 6 years old 0.16 ± 0.07 and 7 years 0.12 ± 0.04 . The highest and lowest cholinesterase

levels in cattle blood plasma were 2 years old and 7 years old respectively. However, the value of cholinesterase levels in cattle blood plasma based

on age decreased on cattle aged 3 years, 5 years, 6 years and 7 years. The result is an overall reduction



in cholinesterase, which indicated the cattle were exposed by organophosphate pesticide.

Table 4. Cholinesterase enzyme levels in plasma by type of cow

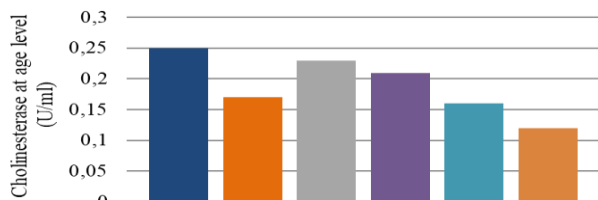


Figure 2. Average levels of the cholinesterase enzyme in the blood of cattle at various ages Cholinesterase enzyme levels in the blood plasma of bulls and cows. The test analysis of the cholinesterase enzyme levels in cattle based on the type of cow were summarized in Table 4.

Type of cow	Cholinesterase enzyme levels mean (U / ml)	Standard Deviation	Standard Error	95% Confidence Interval for mean		Min	Max
				Lower bound	Upper bound		
PO (Ongole breeding)	0.19	0.072	0.0142	0.164	0.223	0.06	0.30
Local Cattle	0.20	0.042	0.029	0.171	0.591	0.18	0.24
Bali	0.23	-	-	-	-	0.24	0.24

Table 4, shows the cholinesterase enzyme levels in cow plasma has a mean value of 0.19 U / ml in PO (ongole breeding), 0.20 U / ml in local cattle and 0.23 UI / ml in Bali cattle. The average cholinesterase levels in the blood of cattle by type of cow are listed in Figure 3.

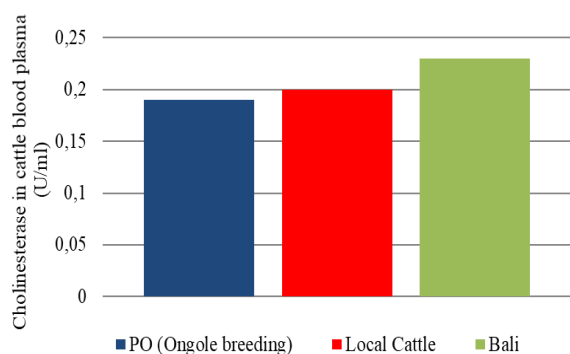


Figure 3. Average levels of the cholinesterase enzyme in the blood of cattle from various types of cattle

Pearson Correlation Analysis to the concentration of the cholinesterase enzyme

The analysis of the Pearson correlation test between age and cholinesterase enzyme levels is summarized in Table 5.

Table 5. Correlation analysis of Pearson age with cholinesterase enzyme levels

	Cholinesterase Enzyme Level Score
Age score	r = - 0.524 p < 0.001 n= 35

From, Table 5, the p value was 0.001 indicating significant correlation between age score and the cholinesterase enzyme level score. Pearson correlation value r = -0.524 indicates strong relationship, and the direction of relationship is inverse (negatively correlated)

The normal cholinesterase enzyme (($\mu\text{mol} / \text{ml} / \text{minute}$) was measured in cattle blood and plasma aged 3-6 months 0.39 ± 0.11 , 1-2 years 0.19 ± 0.03 , 2 years old and above. 0.22 ± 0.04 [7]. Acetylcholinesterase concentrations in blood $5510 \pm$

600 IU / L and 190 ± 30 IU / L in blood plasma for cows aged 1 to 2 years [8].

Cholinesterases were a group of esterases that hydrolyze choline esters at a higher rate than other esters at the hydrolysis rate at optimum and controlled conditions. Cholinesterase was primarily responsible for the rapid removal of acetylcholinesterase within one millisecond after release at the cholinergic synapses, thus allowing proper temporal control of muscle contraction[9].

The in vivo test of sheep, goats and cattle showed cholinesterase inhibition by organophosphate malathion and diazinone insecticides. This insecticide had relatively low toxicity for pets. This pesticide can affect the animal after exposure causing cholinesterase inhibition at nerve endings then generate parasympathetic overstimulation[10].

Cholinesterase enzyme activity is related to genetic relationships, as well as a number of conditions that can decrease cholinesterase enzyme activity, such as liver disease, malnutrition, organophosphate poisoning, burns, renal failure, plasmapheresis and drugs. Several conditions were associated with increased plasma cholinesterase activity, thyroid disease, obesity, nephrotic syndrome and cognitive impairment[11].

Acetylcholinesterase was found in tissues conduction such as nerves and muscles, central and peripheral tissues, motor, sensory fibers, cholinergic and noncholinergic fibers. Acetylcholinesterase activity is higher in motor neurons than sensory neurons. Acetylcholinesterase is also found in the membranes of red blood cells as blood group antigens. In mammals the majority of acetylcholinesterase occurs as a tetrameric with a much smaller number of monomers[12].

In addition organophosphate pesticides, had the several potential metals to suppress activity, , such as Hg^{2+} , Cd^{2+} , Cu^{2+} and Pb^{2+} . The potential effect of lead on erythrocyte acetylcholinesterase activity during metal exposure was used as a biomarker of lead-induced neurotoxicity in exposed subjects. Polycyclic aromatic hydrocarbons directly inhibit acetylcholinesterase. Polycyclic aromatic hydrocarbons were able to inhibit acetylcholinesterase activity in an addictive manner

along with organophosphates to become a noncompetitive acetylcholinesterase inhibitor [13].

4. Conclusion

From the research results, it concluded that cultivated cattle has exposed by organophosphate pesticides indicated by concentration decreasing in the cholinesterase enzyme in cattle plasma. Cholinesterase enzyme levels in cattle were 0.20 ± 0.067 u/ml. The highest levels of cholinesterase enzymes occurred in cattle aged 2 year, 0.25 ± 0.34 u/ml and the lowest in cattle aged 7 years, 0.12 ± 0.04 u/ml. Cholinesterase enzyme in the control group blood plasma was 0.220 ± 0.083 IU /ml. [6].

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