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Major Digestive Enzymes of Butterfly of *Eurema blanda* and *Catopsilia pomona*

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Abstract : Butterflies are the largest number of insect orders with a relatively short life cycle and their utilization is limited. The genus Eurema and Catopsilia (Pieridae) are the most abundant species in the Arboretum of Padjadjaran University, West Java, Indonesia. This study was aimed to determine the major digestive enzymes activity of*Eurema blanda* and *Catopsilia pomona* butterfly at three various temperature and pH. *Eurema blanda* and *Catopsilia pomona* have higher amylases activity than proteases and lipases activity at optimum temperature and pH, i.e. 8.141 ± 0.010 and 8.209 ± 0.017 IU/mg, respectively.
Keywords : Amylase, Protease, Lipase, Hydrolytic enzyme, Butterfly.

Introduction

Butterflies are the greatest number of populations compared to other orders in the insecta class. They are scattered from the lowlands to 750 m and found in forests, fields, shrubs, and along the waterways[1,2]. There are estimated 2500 species of butterflies. Butterflies have a short life cycle (2-6 months), breed rapidly, high and continuous availability[2]. The gut is the site for secretion of digestive enzymes, food digestion, and nutrients absorption[3]. Macromolecular in the foods are catalyzed by three major digestive enzymes in the gut, i.e. amylases, proteases, and lipases. Amylases catalyze the hydrolysis of α-D-(1,4)-glucan linkage in carbohydrates, which serve as energy sources[4]. Serine proteinases, such as trypsin and chymotrypsin, hydrolyze peptide bonds inside the protein (endopeptidases) or at the ends of protein (exopeptidases)[5]. Lipases catalyze the hydrolysis of triglycerides to glycerol and free fatty acids. They are soluble in water and hydrolyze insoluble substrates to more polar lipolytic products[6].

The genus Eurema and Catopsilia (Pieridae) are the highest abundance in nature so it is easy to find and often used for scientific studies. The most abundant species of genus Eurema and Catopsilia in the arboretum area of Padjadjaran University, Indonesia, are *Eurema blanda* (common grass yellow) and *Catopsilia pomona* (common emigrant or lemon emigrant).There has been no study on the major digestive enzymes of these two butterflies.This study was aimed to determine the major digestive enzymes (amylases, proteases, and lipases) activity of butterfly of *Eurema blanda* and *Catopsilia pomona*.

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Materials and Method

Materials

Eurema blanda and *Catopsilia pomona* butterflies were taken from December 2014 to February 2015 from the arboretum area of Padjadjaran University, West Java, Indonesia. Butterflies were identified by Laboratory of Animal Taxonomy, Department of Biology, Padjadjaran University. This study was conducted after approved by Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University No 732/UN6.C2.1.2/KEPK/PN/2014.

Chemical Materials

Sodium phosphate, sodium hydrogen phosphate, sodium chloride, soluble amylum, iodine, potassium iodide, hydrochloric acid, brilliant blue coomation, phosphoric acid, sodium hydroxide, acetonitrile, and ethanol were purchased from Merck. Bovine serum albumin, tyrosine, *p*-nitrophenyl palmitate (*p*-NPP), and *p*-nitrophenol (*p*-NP) were purchased from Sigma-Aldrich.

ProteinExtraction

Butterfly wings were cut, then weighed 4 g and extracted for 2 hours at 4 °C with 25 mL of 0.02 M sodium phosphate buffer (pH 6.8) containing 10 mM NaCl. This mixture was centrifuged at 13,000 G for 30 min at 4 °C. The supernatant was collected and stored at -20 °C[7].

Determination of Total Protein Content

Total protein content was determined by Bradford's method[8]using bovine serum albumin as a standard[9].

Amylase Activity Assay

Amylase activity was analyzed by modified Fuwa's method. Extract (250μL) and amylum solution (3 mL) were incubated for 8 min. Then, added hydrochloric acid (0,5 mL) and iodine (0,5 mL). The absorbance was measured at 627 nm[10].

$$\text{U/mg} = \frac{\text{Astd} - \text{Asample}}{\text{Astd}} \times \frac{\text{(mg) amylum}}{\text{(mL)enzyme in extract x total volume x t}}$$

Protease Activity Assay

Extract (250 μL) and casein solution (1 mL) were incubated for 30 min. Then, added ethanol (1 mL) and incubated in a boiling waterbath for 5 min, then cooled to room temperature. This solution was centrifuged at 13,000 G for 10 min. The absorbancewas measured at 275 nm[11].

$$\text{U/mg} = \frac{\text{μmol tirosin which produce}}{\text{mg enzyme in extract x t}}$$

Lipase Activity Assay

Substrate solution was prepared by phosphate buffer: ethanol: *p*-NPP solution (95:4:1). Extract (250 μL) and substrate solution (1 mL) were incubated for 15 min. Then, added ethanol (1 mL) and measured theabsorbanceat405 nm[12].

$$\text{U/mg} = \frac{\text{μmol p−NP which produce}}{\text{mg enzyme in extract x t}}$$

StatisticalAnalysis

Significant differences between butterfly enzymes were determined using single factor ANOVA with replication in conjunction with Duncan Multiple Range Test (DMRT). Data was considered to be significantly different if p value < 0.05[9].

Results and Discussion

The butterflies were collected during December 2014 to February 2015, because Jatinangor, Indonesia, experienced the highest rainfall that could increase butterfly populations related to the level of plant fertility as the butterfly host plant (Davies and Butler, 2008). There were 142 *E. blanda* butterflies (4.695 g) and 66*C. pomona* butterflies (10.411 g).

Determination of Total Protein Content

The maximum wavelength of the CBGG-protein complex was 573 nm. The total protein content of *E. blanda* (76.54 ± 0.16 ppm) was higher than *C. pomona* (71.98 ± 0.18 ppm) which significantly different (p = 5.34 x 10^{-6}). These values were higher than total protein content of *Helicoverpa armigera* (5.82 ± 0.02 ppm)[7].

Amylase Activity Assay

Amylase activity was determined by Fuwa method based on the formation of the remaining amylum with iodine. The formed amylum-iodine complex depends on the amylum type, due to the difference of the glucose number[10]. The reason for selecting the Fuwa method was the amylase activity in the sample measured with the iodine assay (mg of amylum equivalents consumed/min) was five times higher than the activity units (mg of glucose equivalents produced/min) measured with the dinitrosalysilic acid method[13].

Table 1.Amylase activity in various temperature and pH

Parameter		Amilase activity (U/mg)		*p* value
		E. blanda	***C.pomona***	
Temperature (° C)	27	5.60 ± 0.02	5.85 ± 0.01	9.37 x 10^{-6}
	37	8.14 ± 0.01	8.21 ± 0.01	4.11 x 10^{-3}
	47	7.07 ± 0.01	7.74 ± 0.11	4.40 x 10^{-4}
pH	5.8	2.29 ± 0.02	2.67 ± 0.01	8.90 x 10^{-6}
	6.8	5.60 ± 0.02	5.85 ± 0.01	9.37 x 10^{-6}
	7.8	1.08 ± 0.02	1.24 ± 0.05	4.56 x 10^{-3}

Amylase activity of both butterflies was significantly different($p < 0.05$, Table 1). The amylase activity of*E. blanda* and *C. pomona* was smaller than *Andrallus spinidens* beetle, i.e. 13.46 U/mg[14], but higher than *Aspergillus niger*, i.e. 5.7 U/mg[15].

Amylasesof *E. blanda*and *C. pomona*were worked optimum at 37°C and pH 6.8. At optimum temperature and pH, amylasesactivity of *C. pomona*was better than *E. blanda*. The optimum amylase temperature of these two butterflies is in the optimal temperature range of stomach amylase of *Eurygasterintegriceps* (Hemipteran bugs), i.e. 25-40 °C[16]. Most butterflies are active at 16-42 °C and 28-30 °C arerequire for flight. Butterflies are often in the open place to get sunlight and increase body temperature[17]. The cause of the decreased amylase activity after the optimum temperature is the denaturation of the hydrophobic residues, such as tryptophan and phenylalanine, on the enzyme surface which important for stabilize the amylase structure[18]. At the optimum pH, the charge of amino acid residuesare in an appropriate state, so the enzyme activity is efficient. The optimum pH of *E. blanda*and *C. pomona*were almost equal to the optimum pH of amylase in hemipteran insects, i.e. 6.5[19].

Protease Activity Assay

The protease activity in casein hydrolysis was determined based on the amount of produced tyrosine and measured at 277 nm, i.e. the maximum wavelength of the aromatic amino acids[5].

Table 2.Protease activity in various temperature and pH

Parameter		Protease activity (U/mg)		*p* value
		E. blanda	C.pomona	
Temperature (° C)	27	1.89 ± 0.02	0.59 ± 0.01	9.21×10^{-8}
	37	0.80 ± 0.01	0.45 ± 0.01	1.27×10^{-7}
	47	0.44 ± 0.01	0.49 ± 0.01	1.85×10^{-6}
pH	5.8	0.44 ± 0.01	0.64 ± 0.01	1.56×10^{-5}
	6.8	1.89 ± 0.02	0.59 ± 0.01	9.21×10^{-8}
	7.8	1.92 ± 0.01	0.60 ± 0.01	1.64×10^{-10}

Protease activity of both butterflies was significantly different ($p< 0.05$, Table 2).Protease activity of *E. blanda*was higher than *C. pomona*. These values were higher than *Helicoverpa armigera* worm, i.e. 0.10 U/mg[7]and *Bacillus sp*., i.e. 0.25 ± 0.01 U/mg[20].

Proteases of *E. blanda*and *C. pomona*had optimum temperatures at 27°C, while the optimum pH was 7.8 for *E. blanda*and 5.8 for *C. pomona*. The optimal temperature of the stomach proteasesof*Ectomyeloisceratoniae*was 30 °C with casein as the substrate[21].The optimum pH range of protease is 7-9, due to serine proteases in the extract[22]. The optimum pH of *C. pomona*'s protease was out of that range, suggested of conformational changes on the catalytic side, but further studies are required to prove it.

Lipase Activity Assay

The principle of lipase activity assay is oil as a substrate hydrolyzed at ester bonds by lipase to glycerol and its fatty acids[23].

Table 3.Lipase activity in various temperature and pH

Parameter		Lipase activity (U/mg)		*p* value
		E. blanda	*C.pomona*	
Temperature (° C)	27	$5.81 \pm 0.04 \times 10^{-5}$	$10.75 \pm 0.17 \times 10^{-5}$	1.69×10^{-3}
	37	$11.98 \pm 0.06 \times 10^{-5}$	$11.74 \pm 0.04 \times 10^{-5}$	1.31×10^{-10}
	47	$5.99 \pm 0.01 \times 10^{-5}$	$12.38 \pm 0.02 \times 10^{-5}$	5.91×10^{-11}
pH	5.8	$6.17 \pm 0.07 \times 10^{-5}$	$9.33 \pm 0.01 \times 10^{-5}$	1.95×10^{-7}
	6.8	$5.81 \pm 0.04 \times 10^{-5}$	$10.75 \pm 0.17 \times 10^{-5}$	9.36×10^{-7}
	7.8	$8.70 \pm 0.03 \times 10^{-5}$	$0.69 \pm 0.01 \times 10^{-5}$	1.70×10^{-10}

Lipase activity of both butterflies was significantly different ($p< 0.05$, Table 3).Lipase activity of *C. pomona* was higher than *E. blanda*. These values were smaller than *Helicoverpa armigera* caterpillar, i.e. 1.12×10^{-4} U/mg[7] and *Azospirillum sp*., i.e. 5.61 U/mg[24].

The lipaseoptimum temperature was 37°C for *E. blanda*and 47°C for *C. pomona*. The optimum temperature of *E. blanda*lipase was similar to *Chilosuppressalis* caterpillar, i.e. 37-40 °C[25]. The optimum temperature *C. pomona*was higher than *E. balnda*. It is because the increased temperature can increase the catalysis ratedue to increased kinetic energy and the frequency of molecular collisions[26]. The lipaseoptimum pH was 7.8 for *E. blanda*and 6.8 for *C. pomona*. The optimum pH of stomach lipase of*Rhodniusprolixus* (Hemiptera, Reduviidae) was 7-7.5[27]. The pH of the insect lumen was in the range of 6.0 to 7.5[28]. Triacylglycerol hydrolysis into glycerol and fatty acid occurs in neutral and alkaline pH in *Manducasexta* caterpillars(Lepidoptera). The maximum lipase activity was at pH 8.2 and decreased at pH 8.8. At pH below 6.5, lipase almost inactivated[23].

*Euremablanda*and *Catopsiliapomona*butterflieswere potential as a hydrolyticenzyme resource, especially amylases. It is because *E.blanda*and *C.pomona*butterflies have higher amylases, proteases, and lipases activity than other insects, but were lower than bacteria. Butterfly cultivation must be done to maintain the butterfliesavailability, so further studies can be done. Limitations in this study was limited obtained enzyme, so further characterization can not be done.

Conclusion

Eurema blanda and *Catopsilia pomona* have higher amylases activity than proteases and lipases activity at optimum temperature and pH.

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