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The influence of the host plants and artificial food on the life cycle of endangerd species of *Troides helena* (Lepidoptera: Papilionidae)

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Abstract

Troides helenea is one of the Lepidoptera species that rare and protected. Based on Convention on International Trade in Endangered Species (CITES), this species categorized in CITES Appendix II. Species that belong to this category are permitted to be utilized on research, educational activities or as the scientific collection in museum. One of the causal factors of population decline of *T. helena* is the least number of host plants. Therefore, this research conducted to discover the alternative feed by comparing the effect of natural feed and synthetic feed giving on the length of *T. helena* life cycle. The natural feed that used was *Aristolochia tagala* (jungle vine) which is the natural host plant of *T. helena* in the nature, meanwhile the synthetic feed that used was the feed that formulated. The life cycle period *T. helena* which fed by using the natural feed and synthetic feed is statistically tested by using T-test. The result of T-test showed that there were life cycle periods difference between the individual which fed by using natural feed and the individual which fed by using synthetic feed. The individual life cycle which fed by using natural feed was shorter than the individual which fed by using the artificial food.

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Introduction

Indonesia has a tropical climate and its connect to high biodiversity. One of important fauna is butterfly which a greatest abundance and diverse. Lepidopterian plays an important role in the balance of the ecosystem, one role is as a pollinator in procces of pollination of flowering plants that can enrich the plant diversity.

Accoding to Peggie (2011). The diversity of plant determine type of butterfly type. The existence of host plants proficiency level will support the survival of the butterfly as a food source, place / media to lay eggs and protector of both the larval stage and in stadia imago (adult butterflies). Given the importance of butterflies in the sustainability of ecological systems it is necessary to research on the life table of butterfly populations in order to predict the potential growth of the butterflies in the future.

In preparing the conservation of butterflies, it is necessary to feed its availability as a primary source for its life. Sorting of hosts in the life support necessary to obtain optimal results in terms of growth, development and pupa time on these processes. This is manifested as an alternative feed preparation for a time when natural food is in its natural habitat is depleted or experienced shortages (Soekardi, 2000).

The consequences is Indonesia is well known for endemic fauna including butterflies. One of important is belong to Troides. Since Indonesia covering 15.677 islands, each Troides developed into different species (Subaaar and Annisa, 2010). Indonesia government set the protection of 12 species of 15 species Troides in Indonesia by Law no. 5 1990 and PP 7 In 1999. One of the protected species and endangered is *Troides helena*.

This species under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) including the in CITES Appendix II. Species included in the CITES Appendix II are permitted its use for the purposes of research, education or scientific collections in museums. *Troides helena* is a butterfly-sized with a wingspan 9.8-15.0 cm and 6.0 to 9.5 cm long front wing.

Habitat segemtation cause in decreasing the diversity of host plants becomes one factor in the decline in the diversity of butterflies. Therefore the researchers and collectors of butterflies began to aware the presence of a butterfly with how to maintain and preserve it in the form of a captive. It is intended as an attempt to conserve or preserve a species of animals and plants in order to prevent the extinction of (Morton, 1979).

The main cause of the decreasing number of *T. helena* is the limitation of food which its larvae are monofagus that only eat one kind of plant *Aristolochia tagala*. *A. tagala* is one of the key factors for the life of *T. helena* (Yushio dan Ishii, 1996). *A. tagala* generally grows in the forest, vines on trees or bushes (Chin, 2014). Deforestations and excessive use of herbicides cause a reduction of population *T. helena* (Peggie, 2011).

There are some efforts to prevent *T. helena* by use synthetic. Synthetic feed is a mixture of various ingredients needed in an effort multiplication of insects which will be used as research material in the laboratory (Wiklund, 1975). Previous research showed success in the use of synthetic feed on the growth cycle of the butterfly *Papilio machoon*, Pyrgus malvae, Pieris brassicae, Lycaena phlaeas, and Aglais urticae (Morton, 1979). On this reseach an investigation to study the effect of natural and synthetic feed on the length of the life cycle of *Troides helena* (Lepidoptera: Papilionidae) were nedeed.

Material and method

This study was conducted in February - July 2014 at UPI the Botanical garden. Exprimental design were divided into three part: preparation, treatment and data analaysis.

Preparation

Making of artificial food for *T. helena* larvae were based on prescription of [4] which has been modified. *T. Aristolochia* leaves washed and then dried at a temperature of 100°C. After drying the leaves crushed and mixed with other ingredients. An erlenmeyer taht contains of leaf powder is mixed with the material as much as 3 g soy flour, red bean flour, yeast extract, sucrose, chloramphenicol and as much as 93.1ml of distilled water and heated until homogeneous. In the second erlenmeyer in mix order with 150ml of distilled water to the boil and homogeneous. after which the two materials in erlenmeyer mixed, after the material is homogenous and the temperature had dropped to 35°C± add sorbic acid, vitamin D and methyl esters that have been diluted with 1.9ml of alcohol, after all the ingredients mixed and then poured into a petri dish with thickness of 0.5 cm and stored at 4°C.

Treatment

Twenty Eggs that female *T. helena* lying eggs on *A. tagala*. were placed into a jar containing host plants and artificial food for one up second instrar. In the third instar larvae were transferred to the jar size larger and were observe until pupae. Observations was conducted on 9:00 to 10:00 a.m. Observation of parameters that covered on this reseach were life cycle, egg viability, morphology of each stage, and the size of each stage. Data analysis. Data were analyzed using SPSS software release for Windows version 16 to test for normality (Kolmogorov-Smirnov) and homogeneity (Levene Statistic).

Result and discussion

Morphology of T. helena

T.helena larval body has 12 segments with in each segment has a protrusion on its back and besides. After the eggs hatch, the larvae that first appeared or called larval 1st instar has a body color black, orange patterned head and has fine hairs (Fig. 1).

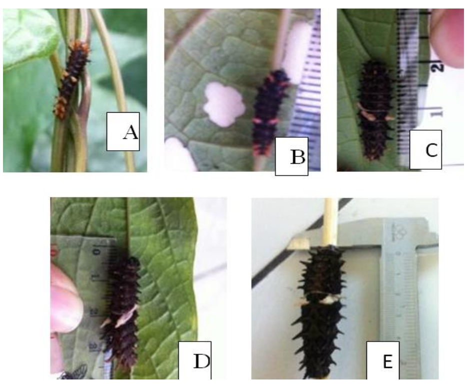

Fig. 1. Morphology of larvae: A. 1th Instar 1: B. 2nd Instar 2: C. 3rd Instar 3: D. 4h Instar: E. 5th.

Instar Body morphology of larvae subsequently, on 2th Instar is almost the same as 1st instars but it has has disappeared and the color head has turned black, in 3rd instar , morphology larvae have other characteristics that there is a line like a saddle pink or white-brown on the abdominal segments to 3-4, while at the end of the protrusions of each segment there are orange. In the fourth instar line like a saddle on the stomach has become increasingly apparent white and orange tip protrusion faded, then the fifth instar, the color line as saddle 1looks older and orange bulge had disappeared. Morphology of all stadia can be seen in Fig. 1.

Before form to pupae, the fifth instar larvae search for a suitable and safe site to turn over become pupae. Afterward it formed a thick silk dot on the tip of her abdomen to hang the tail and thick silk to make two points on the right and left to hang its body. When its reach pupa stage, its changes color into green aferward one week later turn to yellowish green the folowwing week turn to yellow. Its color turned into a bit transparent, shortly before becoming imago (Fig. 2).

Fig. 2. Imago of *T. helena*

Imago of *Troides helena* (Fig. 3) is polymorphic so imago males and females can be distinguished. *T. helena* has wings with a large size with black color on the front wings (fore wings) and yellow on the rear wings (hind wings). Males Imago of *T. helena* have yellow on the rear wings (hind wings) that is thicker or dark stains black dot that is less than the *T. helena* females.

Life cycle of T. helena

Growth and development of larvae instar larval 1 to 5 takes different for each individual on artificial food and host plant. Length of time first instar of larvae turn into a second instar larvae in host plant ranges from 3-5 days, while for artificial food ranges from 5-11 days. Length of time on the second instar larvae in host plant ranges from 2-6 days moreover for while artifial food sranges from 4-6 days. In the third instar larvae of the time required for the development of fourth instar larvae at natural food ranges from 4-6 days, while the time required to feed the synthetic ranges from 5-8 days. In the fourth instar larvae of time required for development of the fifth instar larvae in natural food ranges from 5-6 days, while the time required to feed the synthetic 3-6 days. In the fifth instar larvae of time required for the development of the next stage, namely stadia prepupa on natural food range 3-4 days while on synthetic feed ranges from2-4 days (Table 1).

Table 1. Life cycle of *T. helena*.

Stadia	Natura Food			Artificial Food		
	N	range (day)	Average (day)	N	Range (day)	Average (day)
Egg	16	7 -8	7,42±0,51	16	7-8	7,33±0,49
1st Instar	12	3-5	3,75±0,62	12	5-11	4,9±0,7
2nd Instar	11	2-6	5,4±0,7	10	4-6	5,8±0,63
3rd Instar	10	4-6	5±0,47	10	5-8	7,5±0,53
4th Instar	9	5-6	5,78±0,4 4	7	3-6	5±0,8
5th Instar	8	3-4	3,75±0,46	4	2-4	3±0,81
Prepupae	8	1	1±0	4	1	1±0
Pupae	7	19-20	19,29±0,49	3	20	20±0
Imago	7	51-53	51,57±0,79	2	53-55	54±1

While the two has more value than the significance level is used so there is no significant difference of feeding natural and synthetic feed on larval instar 2. It can also be seen from the results of the descriptive statistical calculations where the average day second instar larvae natural (host plant) feed with artificial food does not have a distinct difference far.

In the process of development of butterflies, fifth instar larvae grow and develop into prepupa. The time it takes each individual stadia feed prepupa either artificial food and host plant is 1 day. The pupa stage in the natural food has a range of 19-20 days to develop into an adult imago whereas synthetic feed has 20 days umtuk imago develops into an adult. On the Host plant the range of the required number of individuals from egg to become imago is 51-53 days with an average of 51.57±0.79 days, whereas the synthetic has a number of feeding days required range from egg to become imago is 53-55 days with an average of 54±1 days. From calculations using descriptive statistics can be seen that the difference in the average length of a day in the life cycle of *T. helena* between synthetic and natural foodthere is a difference of 3 days.

Differences in the effect of feeding natural and synthetic feed powered by a decrease in the number of individuals living in each stadia. Fig. shows the difference in the number of individuals who survive at every stage.

Number of individuals who survive on natural food more in comparison with synthetic feed. In the natural food only a slight decrease in the number of individuals in each stadianya compared to synthetic feed. Instar larval stage 1 through 3 instars consume the leaves of host plants are provided, while in the 4 to 5 individual instar larvae do not only eat leaves but consume the stems of the host plant. According to Hoskin (2012), the consumption of plant stems will slow the digestive process in insects up will inhibit the metabolism process. Reason instar larval instar 4 and 5 consume the stems of the host plant in order to be able to stop eating and larvae develop into pupae.

Overall the number of individuals who have survived larvae to be prepupa are 4 individuals with the percentage of survival by 33.4%. According to the observations, most likely this is due to the unavailability of a component in synthetic feed suitable for the development of fourth instar larval instar larvae 5. In natural food, 4 to 5 stadia instar larvae eat the leaves of the plant not only *A.tagala* but also eat the stem of the plant, while the weft synthetic feed containing the synthetic feed formula may be less suitable for development instar larval instar 4 to 5. On the original food increasing was expected because of nutritional feed, meeting the criteria. Though the insects feed quality can affect the rate of consumption (Slansky, 1993). Any changes in the quality of food, the larvae leave a response through active rejection of food after knowing that the food contains pathogens Scriber and Slansky, (1993).

It was reported that the larvae migdut there are various i.e protease trypsin, chemotripsin, aminopeptidase and carboxipeptidase. At any given time due to the presence of infection, activity becomes disturbed and not optimum. In such circumstances the body's metabolism becomes permanent normal larvae (Waldbauer and Friedman, 1981) as it includes the most important activity of the enzyme digestiv contained in the butterfly is an enzyme protease (Chapman, 1969).

Consumption rate may affect the use of food after digested by Growth rate. As an illustration, if food is provided with enough nutrients, it will generate good growth. With the persistence of epithelial cell metabolism and membrane peritopik keep functioning. As a result of developing epithelial cells, the larvae become healthy and ultimately appetite rises.

In this study, formula is fixed or the addition of feed components so texture remains the same synthetic feed. This causes the 4th and 5th instar larvae that consume synthetic feed can not stop eating because of the texture that remains soft and continues to discharge so did not experience the development process prepupa into the next stage.

In addition to the effect of the growth and development of feed Troides helena influenced by abiotic factors. Abiotic factors measured in this study such as temperature and humidity. Temperatures in this research ranges between 24-26ºC while the humidity ranges from 84-92%.

Food can influence an effect on the length of the life cycle also affect the morphology of the *Troides helena*. According (Dahelmi *et al.*, 1928) the quality of feed affect the larval stage in the life cycle of the butterfly. For growth, the larvae need water and proteins derived from host plants were eaten, a species will experience a short period of larval stage and then into adult individuals who are small or deformed morphology influenced example is the length of the larvae as shown in Table 2.

Table 2. Average length of larvae Troides helena on natural feed and feed synthetic.

Stadia	Average (cm)	
	Natural food	Artificial food
Instar 1	0,72±0,2	0,57±0,17
Instar 2	1,6±0,55	1,12±0,22
Instar 3	2,68±0,45	2,05±0,42
Instar 4	4,26±0,55	3,34±0,41
Instar 5	5,5±0,33	4,17±0,58

It can be seen that each instar on Natural food have more growth Instead of artificial food. On average instar 1 length to larval feeding on natural was 0.72±0.2 cm, while the artificial food of 0.57±0.17 cm. On the second instar larvae average length on the natural feeding was 1.6±0.55 cm whereas for synthetic feed of 1.12±0.22 cm. On the third instar larvae average length on natural feed was 2.68±0.45 cm, while the synthetic feed of 2.05±0.42 cm. In the fourth instar larvae average length on natural feed was 4.26±0.55 cm, while the synthetic feed and 3.34±0.41 cm on average 5 instar larvae on natural feed length is 5.5±0,33 cm whereas the synthetic feed of 4.17±0.58 cm. At each stage, the length of larvae feeding on natural and synthetic have differences. As described in Table 2 that at each instar, the morphology of the larvae feed length is shorter than the length of synthetic natural feed larvae.

One of External factors affecting life cycle of *T. helena* is the condition of one of them is temperature. Larvae on *A. tagala* is treated a second group which took place in September-October, at the time of the occurrence of increasing average temperatures than in August. High temperatures will accelerate the development process, as expressed Jumar [4] that the insects that live at higher temperatures have more faster and quick than an insect that lives at lower temperatures.

The Nutrient of host plant is also cennected with herbivore compatibility including morphology attributes, chemicals and nutritio composition (Mattson & Scriber, 1987). The good composition of host plant reflect the component of food which amounts of amino acids, proteins, lipids, carbohydrates, fatty acids, water, minerals and vitamins, in which positively or negatively affect the performance of herbivorous insects (Sharma *et al.*, 1982). All these data gave the information that nitrogen content of host plants is an important limiting factor for herbivores (Zhong-Xian *et al.*, 2007). Previous discussion on the comparison of feed can be focused on the content of soursop leaves. Protein content in the A. tagala 3.1%. Protein is very closely related to the formation of a chemical compound that plays a role in metabolism. At protein composed of a substance that is more modest amino acid that plays a role in the synthesis of proteins such as the formation of enzymes and hormones. The availability of high protein allows the readiness of the larvae on the plants to growth on the next development.

Conclusion

Treatment between natural and synthetic food were give a significantly differences on the length of the life cycle of *T. helena*. The results showed that individuals which consume natural food has a long life cycle is shorter than the synthetic feed.

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