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RESEARCH PAPER

The effect of artificial diet made of soybeans (*Glycine max* L.) on the rearing of *Spodoptera frugiperda* (Lepidoptera:Noctuidae)

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ABSTRACT

Spodoptera frugiperda is a novel invasive pest of maize crops. Therefore, studies on this pest are limited. Research on the use of artificial diets in *S. frugiperda* larvae rearing is essential to facilitate the provision of test larva stocks for lab-scale study purposes. The aim of this research was to figure out the effect of an artificial diet from soybeans as a protein source on *S. frugiperda* rearing. The parameters observed in this research were larval-stage longevity, larval survival rate, pupa size, sex ratio, pupal-stage longevity, pupal survival rate, fecundity, and nutritional index. The results showed that an artificial diet with soybeans had a significant effect on sex ratio, pupal survival rate, relative consumption rate (RCR) 4.89 g/g/day (4th instar); 1.99 g/g/day (5th instar), approximate digestibility (AD) 98.39% (4th instar); 95.45% (5th instar), and fecundity, but it did not have any significant effect on larval-stage longevity, larval survival rate, pupa size, pupal-stage longevity, the efficiency of conversion of ingested food (ECI) 3.68% (4th instar); 7.84% (5th instar), and efficiency of conversion of digested food (ECD) 3.76% (4th instar); 8.27% (5th instar). Artificial diet-based soybean was highly potential to be used in *S. frugiperda* rearing.

Key words: artificial diet, rearing, soybeans, *Spodoptera frugiperda*

INTRODUCTION

Spodoptera frugiperda J. E. Smith or FAW (fall armyworm) is a new invasive pest of maize (*Zea mays* L.) in Indonesia, estimated to be first introduced in March 2019 in Pasaman Barat Regency, West Sumatra (Sartiami et al., 2020). This pest originated from America and spread across various other continents, including Asia. Being a robust flyer and a voracious pest with a high level of adaptability, *S. frugiperda* is capable to spread rapidly (Lubis et al., 2020; Nonci et al., 2019). *S. frugiperda* is polyphagous with a vast range of host plants from a variety of families, mainly Poaceae, which consists of maize, sorghum, and rice, while soybean, cotton, and other monoculture crops are alternative host plants (Montezano et al., 2018).

In Indonesia, studies on *S. frugiperda* are limited as this pest has just been introduced. An in-depth study on this pest is critical to gain knowledge of the pest's behavior and appropriate control techniques. For a lab-scale study, the researcher will definitely require a large quantity of readily available test larva stock. One way to obtain test larva stocks is by rearing that involves artificial diets.

Artificial diets are highly preferable to researchers for rearing as it is convenient to prepare and is readily available regardless of the season. The artificial diet used in this research was made of soybeans as the main ingredient. The soybean (*Glycine max* L.) is one of the alternative host plants of *S. frugiperda* that contains high protein, that is, 36 g/100 g dry matter. Protein is one of the essential macronutrients for insects, having a role in the formation of enzymes and hormones, such as ecdysone. Ecdysone is a hormone that induces insect growth and cell molting activity (Hidayanti & Asri, 2019).

Based on the description above, it is deemed necessary to conduct a study on the role of an artificial diet in *S. frugiperda* rearing in the laboratory.

MATERIALS AND METHODS

Research Site. The research was carried out at the Laboratory of Plant Pests, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Hasanuddin, Makassar, from August 2020 to February 2021.

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Rearing of *S. frugiperda*. The *S. frugiperda* used in this research were collected from the Galesong Selatan District of Takalar Regency and the Barombong District of Gowa Regency in the form of larvae. The *S. frugiperda* larvae were reared in plastic containers on a baby corn diet until reaching the F2 generation before being subjected to a biological observation of insects. Insect rearing was carried out under controlled conditions of temperature and humidity, using a temperature of 26–28 ºC, with a humidity of 70%.

Artificial Diet Preparation. The artificial diet was developed based on a modified method by Pinto et al. (2019), by substituting carioca common beans (*Phaseolus vulgaris* L. var carioca) with soybeans (*Glycine max* L.). The use of soybeans powder in this method was under the assumption that the protein content in soybeans is higher than in carioca common beans, that is, 36 g/100 g dry matter.

Biological Observation. There were 100 test insects in the larval form used in this research, placed each in a cup (ϴ 10 cm). In the larval phase, the variabels observed were larva length, larva mass, larval survival rate, and nutritional index, calculated using the Waldbauer formulas (1968) below:

a. RCR (Relative Consumption Rate) (g/g/day)

$$\frac{F}{A \times T}$$

b. RGR (Relative Growth Rate) (g/g/day)

$$\frac{G}{A \times T}$$

c. AD (Approximate Digestibility) (%)

$$\frac{F - E}{F} \times 100\%$$

d. ECI (Efficiency of Conversion of Ingested Food) (%)

$$\frac{G}{F} \times 100\%$$

e. ECD (Efficiency of Conversion of Digested Food) (%)

$$\frac{G}{F - E} \times 100\%$$

f. MC (Metabolic Cost) (%)

$$100 - ECD$$

T = duration of feeding period (days);
F = amount of food consumed (g);
A = average larva weight, calculated as (initial larva weight + final larva weight)/2 (g);
E = feces weight (g);
G = increase in larva weight, calculated as final larva weight-initial larva weight.

In the pupal phase, the variabels observed were pupa length, pupal-stage longevity, and pupal survival rate. Meanwhile, in the imago phase, the parameters observed were sex ratio and fecundity : the number of eggs laid by the female during the life of the imago.

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RESULTS AND DISCUSSION

Biological Characteristics. An investigation into biological characteristics was conducted to gain a picture of the extent to which an artificial diet would affect the biological potential of *S. frugiperda*. The biological characteristics observed in this research are shown in Table 1.

S. frugiperda would typically remain in the larval stage for 14 to 22 days on the host plant (FAO & CABI, 2019). Table 1 revealed that the treatment of a soybean based diet resulted in the longest stage longevity of 32.95 days and the lowest larval survival rate of 60%. The larval-stage longevity and the larval survival rate certainly were influenced by the diet consumed during the phase. The better suited the nutrition contained in the diet the better the larval growth. As stated by da Cunha et al. (2008), the nutritional quality of each diet affected the duration of the larval stage of *S. frugiperda.*

The hormone is another factor affecting the life cycle rate of an insect. There are two hormones playing a role in the molting process, namely ecdysone and juvenile hormones. The two hormones work under the influence of the protein content of the food. Protein is composed of simple monomers called amino acids. Juvenile hormones are produced by the corpora allata, whose initiation is influenced by neuropeptides. According to Nugroho (2016), insect development is controlled by juvenile hormones exclusively produced by the corpora allata (CA), a pair of small glands behind the brain. In insects, CA activity is controlled by the brain mediated by neuropeptides, which are collections of amino acids. Leucine, isoleucine, and threonine are some amino acids in neuropeptides that play a role in the synthesis of juvenile hormones. Soybeans contained a considerable amount of amino acids, including leucine, isoleucine, and threonine at 8.17 g, 5.16 g, and 4.19 g, respectively (Szostak et al., 2019).

In Table 1 on average the pupal stage in the treatment with a soybean-based diet generated the longest longevity and the highest survival rate. The time taken by a pupa to reach the stage of imago was influenced by a number of factors, chief among which was the food. In agreement with this, Azwan (2020) stated that, the amount of food consumed by an insect in the larval stage would affected its pupal stage: the less the food consumed the longer the pupal stage because optimal growth and development were directly proportional to balanced nutrition.

The soybean-based diet was found to be able to result in a high survival rate. This showed that the nutritional composition of the diet was able to support the larvae' survival until they reached the stage of imago. Flasz et al. (2021) stated that, one of the important components of a diet was ascorbic acid or vitamin C. Ascorbic acid is an antioxidant to insects and it is able to improve larval and pupal survival rates. The soybean is known to be among legumes that contain a variety of vitamins, one of which is vitamin C. As stated by Szostak et al. (2019), the soybean contains various dissolved vitamins such as vitamins B1, B2, B3, B6, folic acid, B12, C, and E. The artificial diet in this research was added with ascorbic acid at a dose referring to Pinto et al. (2019).

The sex ratio obtained in this research showed that the female imagoes was outnumbered their male counterparts at a ratio of 70% or 1.43: 1. A higher number of female than male adults was desirable because it was hoped that the number of eggs produced (fecundity) by a female adult and the number of progenies will also be high. This is as stated by Kong et al. (2021); Fitz-Earle & Barclay (1989) that if a maximum number of eggs per rearing facility per female is to be obtained, then the optimum sex ratio that must be reached is 1: 3 (male: female).

Nutritional Index. The nutritional index is a parameter that depicts the approximate digestibility, consumption and growth rates, energy used for metabolism, and efficiency of conversion of ingested and digested food into the biomass of a larva. The calculation of the nutritional index was referred to Waldbauer's gravimetric method (1968). The results of the calculation were presented in Table 2.

Table 2 showed that in the treatment of diet from soybeans as a protein source, the RCR from 4^{th} to 6^{th} instar decreased. This decrease in rate marked that the larval digestibility declined with every shift all the way to the pupal stage. On this Hidayanti & Asri (2019) stated that *S. frugiperda* larvae that have reached the final instar stage will lose their weight, and for this reason, the 6^{th} instar stage prior to pupal formation is called the critical weight stage. RCR is strongly influenced by diet quality and quantity. Diet texture that grows coarser and drier will unquestionably inhibit the feeding process of the larva, especially when the larva is still in the early instar stage. As stated by Panizzi (2012); Pinto et al. (2019), physical characteristics such as texture, surface velocity, and form or condition of the diet are key indicators in figuring out the diet quality, which undoubtedly will influence the insect's capacity to consume and digest the diet.

AD, ECI, and ECD were interrelated. The low ECI and ECD found in this research illustrate that not all of the food consumed and assimilated was completely converted into biomass. This was also reflected in the high MC, as can be seen in Table 2. According to Truzi et al. (2021) and Ambarningrum et al. (2009) low ECD reflects the high larval metabolic cost. Moreover, high MC also marks that the diet consumed by the larva contains toxic allelochemicals, requiring the larva to spend much energy on detoxifying them in its body to prevent them from disturbing its physiology (Lucchese-Cheung et al., 2021).

The low ECD in this research was compensated by increased AD. On this Waldbauer (1968) stated that every increase in ECD was compensated by increased AD, and vice versa. High AD is explained by the larva's digestive system being exposed to enzymes

Table 1. The biological characteristics of *S. frugiperda* in the larval and pupae stage

Characteristics	Soybean	Carioca common beans in artificial diet*	Baby corn**
Larval-stage longevity (days)	32.95	15.6	19.9
Larval survival rate (%)	60	92.0	89.91
Pupa size (mm)	14.79	-	16
Pupal-stage longevity (days)	12.70	11.6	10
Pupal survival rate (%)	96	73.4	63.21
Sex ratio (Sex ratio) (%)	70	56	68

According to *Pinto et al. (2019) and **Nurfauziyah et al. (2019).

for a longer period of time. Besides, the urgent need for energy for growth, development, and detoxification requires that the larva must increase the amount of food digested in order for its growth not to be disturbed. The increase in AD could be seen in the number of feces produced by the test larvae. Not only is it assumed to come from unabsorbed digestive remains, the feces is also assumed to result from the peritrophic membrane that was destroyed due to the allelochemicals that were present in the diet (Lucchese-Cheung et al., 2021; van Huis, 2013).

Da Silva et al. (2017) also added that, herbivorous insects have a number of detoxification enzymes, such as mixed-function oxidases (MFOs) and general esterases, in their digestive system, to be exact in the mid-gut, to digest plant allelochemicals. Otherwise put, the allelochemicals of the Family Poaceae (DIMBOA and MBOA) induce MFO activity in *S. frugiperda* more efficiently than the allelochemicals in soybeans (*Phytoalexin glyceollin*).

***S. frugiperda* Fecundity.** The ability of a female imago to lay eggs is called fecundity. Whether or not a diet has potential can be seen from numerous indicators, chief among which is female imago fecundity. The fecundity of *S. frugiperda* fed with the artificial diet could be seen in Table 3.

The average fecundity of female *S. frugiperda* in Table 3. Soybean-based artificial diet feeding yielded an average of 409.57 eggs per female, with a total of 23 females observed. The value obtained was higher than that obtained from using baby corn (Nurfauziyah et al., 2019) but lower than that obtained from using carioca common beans in an artificial diet (Pinto et al., 2019).

Insect fecundity is influenced by many factors, including the protein content of the diet. Soybean contains various amino acids, such as leucine, arginine, lysine, phenylalanine, isoleucine, threonine, valine, histidine, tryptophan, and methionine, at various percentages, which can support *S. frugiperda's* nutritional needs to produce eggs. According to Favetti et al. (2015); Pinto et al. (2019) that proteins or amino acids are required at high concentrations for insect development, including for egg production (fecundity). There are 10 essential amino acids needed to support insect growth and development, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

The average fecundity of *S. frugiperda* fed with the soybean-based artificial diet was 409.57 eggs per female (Table 3), lower than the average fecundity of *S. frugiperda* fed with carioca common beans (Pinto et al., 2019), that was 1,850 eggs/female. The difference in value between the two diets was influenced by the amino acid content. The soybean and the carioca common bean have similar amino acid contents, but they are present at differing percentages. The amino acid content of the carioca common bean is higher than that of the soybean. In the carioca common bean, the lysine, phenylalanine, and alanine contents are higher than those in the soybean (72.0 mg/g vs. 56.90 mg/g, 52.2 mg/g vs. 49.40 mg/g, and 52.2 mg/g vs. 40.2 mg/g,

Table 2. Nutritional index in 4th instar, 5th instar, and 6th instar of *S. frugiperda*

Index	Soybean			Carioca common bean in artificial diet*
	4th instar	5th instar	6th instar	4th instar
Relative consumption rate (RCR) (g/g/day)	4.84	1.99	0.95	2.3
Relative growth rate (RGR) (g/g/day)	0.15	0.14	0.06	0.67
Approximate digestibility (AD) (%)	98.39	95.45	93.24	57.3
Efficiency of conversion of ingested food (ECI) (%)	3.68	7.84	6.64	30.8
Efficiency of conversion of digested food (ECD) (%)	3.76	8.27	7.20	55.3
Metabolic cost (MC) (%)	96.24	91.73	92.80	44.7

*According to Pinto et al. (2019).

Table 3. Average fecundity of *S. frugiperda*

Characteristic	Soybean	Carioca common bean in artificial diet*	Baby corn in regular diet**
Fecundity (eggs/female)	409.57 (n = 23)	1,850 (n = 10)	133.25 (n = 1)

*According to Pinto et al. (2019); **According to Nurfauziyah et al. (2019); n: number of female imagoes.

respectively) (Sońta & Rekiel, 2020; Dukariya et al., 2020; Firdaus, 2019). The number of eggs produced by a female image is also determined by longevity. Longer longevity will produce more eggs than the shorter one (Hasbi et al., 2016).

CONCLUSION

An artificial diet made of soybeans (*Glycine max* L.) was highly potential to be used in *S. frugiperda* rearing. Biological characteristics of the pest fed with the soybean based diet had a significant effect on sex ratio, pupal survival rate, relative consumption rate (RCR), approximate digestibility (AD), and fecundity.

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AUTHORS' CONTRIBUTIONS

ST completed the entire fieldwork, biological data analyzed, and prepare the figures. However, she and NZ had equal input into writing the manuscript. SS contributed to different parts of the nutrition index analysis, SS provided critical insight into writing the manuscript. All authors reviewed the manuscript.

COMPETING INTEREST

The authors have declared that no competing interest exists.

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