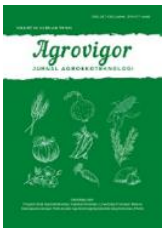
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Article

Evaluation of Artificial Diets Formulations for Laboratory-based Rearing *Spodoptera frugiperda*

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ABSTRACT

Corn is an important food commodity that susceptible to pest attacks such as armyworm *(Spodoptera frugiperda*). This invasive pest attacks the growing points of corn plants and causes decreased productivity. Supporting biological research on this pest, the development of artificial feed is crucial considering the limited natural feed. This study aimed to evaluate the effect of artificial diet formulations on the preferences of *S. frugiperda* larvae. Three formulations with different compositions are studied in this research. Instar 3 larvae were subjected to a preference test to determine the rapid response of the larvae and the weight of the feed consumed (daily consumption). The preference test was carried out in duration 1, 2, 4, 6, and 24 hours. The results showed that Formula 3, which uses natural ingredients such as baby corn and corn sprouts, has the highest initial attractiveness (30% in 1 hour). The physical and chemical characteristics of artificial diet affect the preferences of larvae with formula 3 being the most effective for rapid response, and formulas 1 and 2 being more stable for continuous use. The highest daily consumption of larvae was in formula 3 (0.066 ± 0.027 grams). However, it was not significantly different from formula 1 (0.058 ± 0.029 grams). The use of artificial feeds in the laboratory rearing of *S. frugiperda* offers a viable alternative to natural feeds, with Formula 3 showing a high initial preference, Formula 1 remaining attractive over time, and Formula 2 showing lower consumption, likely due to its efficient nutritional composition, as evidenced by feeding activity through visible bite marks and feed perforations.

INTRODUCTION

Corn is one type of food crop commodity that has the second highest carbohydrate content after rice. Corn is considered a food that is consumed directly or indirectly as a processed product. Corn consumption is considered broad, including as a source of human food, animal feed, industrial raw materials, and plant seeds for cultivation. According to BPS (2018), the production of dry corn kernels in 2018 increased, but compared to the total production in 2017, production decreased by 2.07% from 151,967 tons to 148,815 tons. The government promotes corn surpluses to increase integrated agricultural production, which handles pest and disease infestations in corn cultivation.

The armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is an insect pest that attacks corn during the vegetative phase. This pest targets the growing points that cause failing shoot formation, disturb the plant's growth, and reduce corn productivity in Indonesia (Yani *et al*., 2019). Previous research reported a new variety of corn armyworm, *S. frugiperda* in Tuban, East Java with an incidence of 100% (Megasari *et al*., 2021). This pest originates from tropical and subtropical areas of America which is invasive and threatens the food security of corn commodities. Monitoring the existence and population development of *S. frugiperda* is crucial because it is polyphagous with a wide range of potential hosts, such as food crops from the Graminae group such as corn (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L.), and sugar cane (*Saccharum officinarum* L.). CABI (2019) stated that *S. frugiperda* pest attacks cause yield losses of 55% due to this pest attack in African and European countries ranging from 8.3 to 20.6 million tons per year with economic losses of between US$ 2.5-6.2 billion per year.

S. frugiperda research in the laboratory was carried out by rearing target insects as research materials in large numbers. The required nutrients are obtained from a natural material that is limited because it needs time that coincides with insect growth, so its availability is often insufficient, in addition, a natural diet is influenced by many factors that can affect the results. Nurhajijah *et al*., (2022), the availability of natural feed in large quantities and a fresh state for a long time, the leaves can wilt if stored for too long. An artificial diet is used to simulate natural conditions and meet the nutritional needs of pests so that they can grow and develop well in a controlled environment. The advantage of using an artificial diet is flexibility in adjusting the composition of nutrients, allowing one to observe the specific effects of various nutrients on pest development. Research by Elvira *et al*. (2010), states that artificial diet is a solution in insect rearing because it is easy to prepare and available in various seasons. The use of artificial diets in insect rearing can mitigate the risk of feed contamination, which is affected in insect survival rate. According to Ho *et al*., (2024), contaminated feed can cause insect mortality up to 100 %. Artificial diet also contributes to increasing the fecundity of adult insects, caused by better nutritional content. Insect growth is better when insects are fed artificial diet compared to natural feed (Kumar et al., 2021).

An artificial diet is prepared to provide nutrients for armyworms by using natural ingredients mixed with additional components to extend shelf life and improve feed quality. Susrama's research (2017) states that the manufacture of artificial feed derived from the raw materials of insect feed needs to pay attention to the quantity and quality contained, including ascorbic acid, yeast, carbohydrates, protein, plant materials, lipids, sterols, preservatives, sorbic acid, benzoic acid, sodium sulfite, sodium bisulfite, chlorine chloride, antibiotics. Caterpillars fed with high protein content tend to reach the final instar faster and have a greater body weight than those fed natural feed (Hidayanti & Asri, 2019). Feed with high protein content plays a major role in the formation of tissue and ecdyson hormones which are important for the molting process. According to Kumar et al., (2021), artificial diets contain the main nutrients including carbohydrates, proteins, lipids, nucleic acids, vitamins, and ascorbic acid.

Artificial diets have various physical characteristics, including hardness, texture, homogeneity, water content, and other factors. The preparation of the artificial feed procedure also affects the stability and physical form of the final product (Vanderzant, 1969). Artificial feed must still pay attention to the balance of nutritional components, both quality and quantity. An artificial diet is characterized by larval survivability of more than 70%, shorter larval stages, fewer instars, and increased larval and pupal weight (Maldonado *et al*., 2011). This study aimed to assess the suitability of different artificial diets composition for laboratory rearing of *S. frugiperda*. The results are expected to provide information on a suitable artificial diet for the mass rearing of *S. frugiperda* in the laboratory.

MATERIAL AND METHODS

The research was conducted at the Plant Health Laboratory 2, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur in May-August 2024. The tools used in this study were insect maintenance boxes, microscopes (Labomed Luxeo 6Z), tweezers, Petri dishes, cotton, wire, plastic rope, plastic trays, scales, stationery, and documentation tools. The materials used were Madura 3 variety corn microgreen (14 days old), third instar *S. frugiperda* larvae (from the second generation of rearing, different parental pairs and distinct egg batches), distilled water, honey, labels, polybags, soil, manure, and 3 formulas of artificial diet compositions**.**

The artificial diet in this study was modified by Nalim and Parra (1991). The artificial diet was made by boiling 240 grams of green beans with 500 ml of distilled water for 20 minutes and cooling, then blended with corn flour as the first solution. The second solution was made from 20 grams of agar boiled with 500 ml of distilled water until boiling, then cooled to a temperature of 60 °C and blended until smoothed. The two solutions were mixed and added with 72 grams of yeast, 7.3 grams of ascorbic acid, 2.4 grams of sorbic acid, 4.4 grams of nipagin, 10 ml of vitamin solution, and then blended again until smooth. The final solution was then molded in an artificial feed mold. The formulation evaluated was a feed ratio with corn flour substitution. There were 3 artificial diet formulations with several differences in composition (Table 1.).

Third-stage larvae were prepared for preference test (feeding test) in Petri dishes (15.0 cm diameter × 2.0 cm height) containing one cube of each artificial diet (2 × 2 cm) arranged at equal distances. Each petri dish was filled with 10 third-stage larvae of *S. frugiperda,* with subsequent recording of the number of insects attracted to each artificial diet formula at five different evaluation durations (1, 2, 4, 6, and 24 hours). The experiment used a complete randomized design with 3 replications, where each dish was considered one replication. Larvae were recorded in each artificial diet if the test larvae were present on the diet cube at each interval. The observation was counting the larvae on each diet formula. Third-stage larvae were examined to determine the consumed feed weight (daily consumption). There were 3 treatments (formulations 1, 2, and 3) with 7 replications. Feed before and after treatment was weighed every day until all larvae turned into pupae. Early instar larvae are more susceptible to mortality due to stress or dietary variation; therefore, the use of third instar larvae reduces data variability caused by early stage mortality. At this stage, too, nutrient requirements increase significantly, so the effects of differences in artificial diet composition become more evident.

RESULT AND DISCUSSION

Tested Larvae Preferences

The preference of the studied larvae for an artificial diet is an essential indicator for determining the success of feed formulation in providing nutritional requirements or enticing larvae. The observation result graph showed three diet formulas (Formula 1, Formula 2, and Formula 3) were tested on larvae and observed during certain durations, at 1, 2, 4, 6, and 24 hours after feeding (haf) (Figure 2).

Table 1. Artificial Diet Composition.

Materials	Formula 1	Formula 2	Formula 3
Mung bean flour	240 g	240 g	-
Baby corn	-	-	60 g
Corn microgreen	-	120 g	120 g
Corn flour	240 g	-	-
Nutritional yeast	72 g	72 g	72 g
Ascorbic acid	7,3 g	7,3 g	7,3 g
Sorbic acid	2,4 g	2,4 g	2,4 g
Methyl parahydroxybenzoate (nipagin)	4.4 g	4.4 g	4.4 g
Vitamin solution	10.0 ml	10.0 ml	10.0 ml
Agar	20 g	20 g	20 g
Destilled water	1000 ml	1000 ml	1000 ml

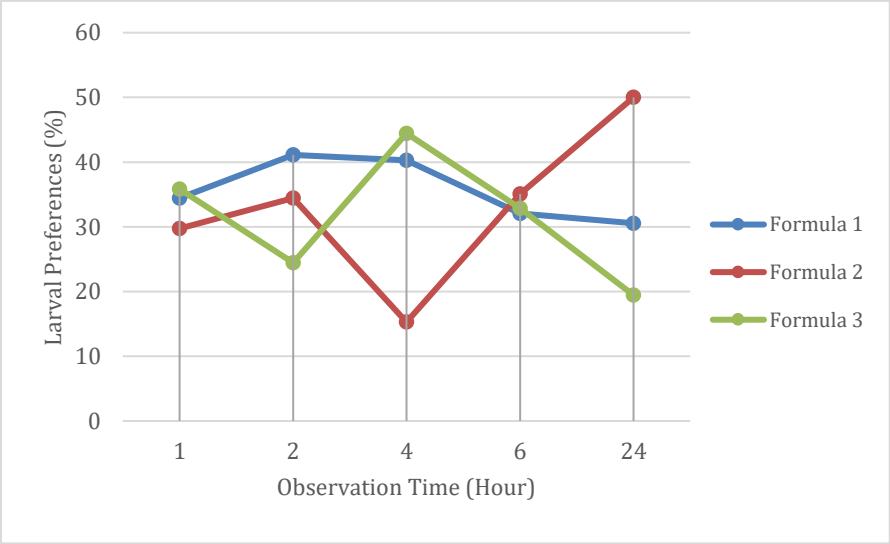


Figure 2. Result of *S. frugiperda* larvae preference to artificial diet formula.

Each diet formula has a specific compound that is effective in attracting larvae, which is measured in the percentage of larval preference in each observation period. The observations at 1 haf showed that formula 3 experienced the highest preference among the three formulas at 35.8%. Formula 3 has a stronger initial attraction to larvae than Formula 1 and Formula 2. The physical or chemical properties of Formula 3 immediately attracted larvae. The artificial diet formula 3 composition contains both, pure and processed carbohydrates, while the pure carbohydrates come from corn sprouts and young corn. According to Isnawati (2009), carbohydrates are the main energy source in metabolism.

The preference observation at 2 hours after feeding (haf) showed a fluctuative trend. Most larvae prefer Formula 3 at 1 haf but decreased slightly in the next hours. Meanwhile, the number of larvae that prefer Formula 1 was increased to equilibrate Formula 3. The active ingredient of Formula 1 is an increase in attracting the larvae over time. The slower release of the compound increases attractiveness over time. The decrease in preference for formula 3 was not significant. However, it indicates that the larvae are familiar with the composition of the formula or the initial attraction was not persistent. Larva preferred Formula 2 was decreased but still showed a stable trend. The observation at 4 haf on formula 3 reached its peak preference (44.4%) which made it the formula with the highest attractiveness in this period. Preference for Formula 2 decreased to 15.2%. Formula 3 contained ingredients that work optimally to attract larvae. The preference decreased for formula 1 and formula 2 indicates that the initial attraction of the formula is not strong enough compared to formula 3. This decrease is caused by the active ingredient components that are not long-lasting or the aroma attraction that is reduced gradually after a few hours. According to Susrama (2017), a mixture of chemical and natural components in the diet for *S. frugiperda* consists of corn sprouts and young corn. Kartini (2015) stated that diet modification by maintaining the nutritional content.

Observation of larva preference at 6 haf showed trend changing. Formula 1 showed a significant increase surpassing Formula 3 which showed a sharp decline while Formula 2 remained stable at the preference level. The significant increase in preference for formula 1 indicates that the active ingredients in the formula tend to be more stable and attractive in the longer term. Conversely, the decrease in preference for formula 3 indicated that its appeal is temporary. Formula 2 showed stability but could not compete with the appeal of Formula 1. According to Elvira et al. (2010), the balance of nutrient intake is crucial in supporting insect growth and reproductive cycle, thus allowing maintenance sustainability for several generations.

The 24-hour observation time of all formulas showed an overall decrease in preference. Formula 2 had the highest percentage at 50%, while Formula 3 showed the lowest preference (19.4%). The significant decrease in Formula 3 indicates that this formula is

effective for a short time, while other formulas have a more persistent although they experienced decreases at 24 hours. The decrease is caused by several factors, such as reduced effectiveness of the active ingredients in the formula, larva adaptation to the formula that leads to decreases of attraction, and the changing of formula physical properties, such as aroma or moisture. This is supported by Singh's statement (1977), that 4 principles must be considered in making an artificial diet. The 4 principles include physical factors (including texture, hardness, water content, and size of artificial feed), chemical factors (including nutrients and organic matter), nutritional balance, and microbial contamination.

S. frugiperda larvae finished the feed in formula 3 until there was nothing left, the data showed that formula 3 had the highest preference. Formula 3 has natural carbohydrate ingredients derived from baby corn and corn sprouts, while formulas 1 and 2 use processed carbohydrates, green bean flour, and corn flour. According to Susrama (2017), carbohydrate content is important in the artificial diet because insects need it as an energy source. Sugars such as sucrose are required to stimulate appetite. Some insects have absolute carbohydrate needs, while others do not. In theory, insects require carbohydrates considered not essential because they can synthesized from amino acids or lipids.

Daily Consumption of Tested Larvae

The third instar larvae were observed to determine the feed consumed amount each day (Table 3). The larvae were placed in different containers and fed with each formula. The daily consumption of *S. frugiperda* larvae of artificial diet formulations is greatly influenced by the nutritional composition of the feed. A diet with high protein content such as soybeans and corn leaves, not only increases consumption weight but also supports optimal growth of the larvae. According to Hidayati & Asri (2019), one of the requirements for insect propagation or rearing is the provision of feed or nutrition.

The daily consumption observations showed that formula 3 had the highest daily consumption (0.066 ± 0.027 grams), was not significantly different from formula 1 (0.058 ± 0.029 grams). Montezano et al. (2018) stated that *S. frugiperda* larvae fed an artificial diet with balanced protein and carbohydrate content showed faster growth than those with a natural diet (e.g., corn leaves). An artificial diet can provide more optimal nutrition than natural food sources. Formula 3 has a natural carbohydrate content with a softer feed texture, while formulas 1 and 2 are from processed carbohydrates. According to Putra & Khotimah (2021), *S. frugiperda* utilizes carbohydrates to increase body weight.

Table 3. Average Daily Consumption of *Spodoptera frugiperda* Larvae on Artificial Diet Formulations Until Pupal Stage .

Artificial Diet	Daily Consumption (gr)
Formula 1	0,058 ± 0,029[b]
Formula 2	0,029 ± 0,010[a]
Formula 3	0,066 ± 0,027[b]

Note: Different letter after the number of daily consumption showed significantly different based on DMRT 95%



Figure 3. Shape of Artificial Diet after treatment

Nutrient content variation from various plant types causes a significant impact on the ecdysone hormone that plays a role in the physiological process of *S. frugiperda* larvae, especially their growth and metamorphosis process. One of the main functions of the ecdysone hormone is to control skin changes or molting, which is crucial in larval development (Cholifak et al., 2012). The amount of food consumed will affect the larvae development. The larvae bite an artificial diet until perforated and creates crumbs to occur. Formula 3 has the least remaining feed, some larvae even finish all the feed. Formulas 1 and 2, which tend to be hard textures, are not very popular with larvae. The hardness level and carbohydrate content in the diet affect daily consumption. However, research by Pinto et al., (2019) shows that *S. frugiperda* larvae will consume more food when their nutritional requirements has not been met. Accordingly, the results presented in Table 3 suggest that Formula 2 likely provides sufficient nutritional content to meet the daily requirements of the larvae, as evidenced by their lower daily consumption rate.

The tested artificial diet showed changes in shape. Holes and bite marks indicated that *S. frugiperda* larvae consumed and broke the feed during the maintenance process (Figure 3). The holes are evidence of the larvae's feeding activity. The bite marks look like irregular white patterns. The holes and bite marks on the feed indicate that this feed can attract caterpillars and support consumption, making it an effective medium for laboratory testing. According to Pinto et al. (2019), artificial diet feeding in insect maintenance can provide in-depth insight into biology, behavior, and nutritional needs. This information is crucial in designing a more effective integrated pest management (IPM) program. Research by Gou et al. (2020) states that artificial diets or artificial feeding to reared insects is an innovation that has been widely used in various entomological studies and practical applications in agriculture, forestry, and biotechnology. Artificial diet is specifically designed to meet the nutritional needs of insects, thus allowing for regular, consistent, and controlled insect growth. This feed is an ideal choice for raising insects under laboratory or mass production conditions, where the availability of natural food sources may be limited or inconsistent.

An artificial diet is a food that is specifically designed by humans for insect mass rearing, by considering the nutritional content that supports the insect growth, development, locomotion, immunity, and reproduction. Researchers prefer an artificial diet because it is easier to produce, always available, and adjustable according to the needs of nutritional content (Elvira et al., 2010). The composition of chemicals and natural ingredients in an artificial diet affects insect nutrition. According to Wibisono (1999), an artificial diet is produced using natural ingredients and chemicals. Plant materials including grains, leaves, and fruits are used to meet natural elements, while yeast extract, ascorbic acid, and other compounds can meet chemical elements. Singh's statement (1982) shows that the characteristics of an artificial diet are durable in storage without reducing its nutritional content, and its practical preparation. The modification of an artificial diet must refer to the larvae preferences to contain determined nutrition and minimize microbial and fungal contamination.

CONCLUSION

The use of artificial diet in laboratory maintenance of *S. frugiperda* can be an alternative solution to replace natural diet, which is always available, measurable, and has adjustable nutritional content. Among the three formulas, the third formula showed a fairly high preference in the initial test. However, over time, there was a decrease in attractiveness. In contrast, formula 1 showed a tendency to maintain attractiveness, while formula 2 showed an increase in preference at the end of the test. Formulas 1 and 3 had similarly high consumption levels, but formula 2 showed a lower consumption level. The low consumption level in formula 2 is likely due to its sufficient nutritional content. The hollow and bite marks indicate that *S. frugiperda* was consumed in the diet during the rearing.

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AUTHORS CONTRIBUTION

DM and SK were planning and coordinating the research. DA and AN were formulating the artificial diet. AAM, AN, and DRD were conducting insect

rearing and testing. AAM is responsible for data collection and analysis. DM and DRD were working on the analysis and manuscript draft.

CONFLICT OF INTEREST

The authors declare there were no conflicts of interest.

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