

# Food Consumption Utilization, and Life History Parameters of *Helicoverpa armigera* (Lepidoptera: Noctuidae) Reared on Diets of Varying Protein Level

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## Abstract

*Helicoverpa armigera* (Hübner) is an important pest of crops worldwide, and several studies have focused on the development of this species on different artificial diets. However, studies evaluating the insect's food consumption and utilization using nutritionally different diets are scarce. The aim of this study was to evaluate the biology and to compare the consumption and use of food by *H. armigera* larvae on diets with different protein levels provided by several dietary ingredients used in the diets. The nutritional index, the relative consumption rate, the relative metabolic rate, the relative growth rate, and the apparent digestibility were higher in the diet with higher than the optimum level of protein. On the other hand, the conversion efficiency of digested food was lower, resulting in a higher metabolic cost. In terms of biological aspects, larval survival was higher for the diet with optimal protein content and lower for the diet with a higher protein level. The pupal period was longer for the diet with a higher protein content, while pupal survival was lower. Among the evaluated diets, the diet with an optimal protein containing white bean 75 g, wheat germ 60 g, soy bran 30 g, milk powder 30 g, brewer's yeast 37.5 g as the protein sources resulted in a higher net reproductive rate, a shorter time for the population to double in number, and the highest rates of population growth. The results suggest that lower or higher protein contents in the diets of *H. armigera* negatively affect the biological aspects of this species.

**Key words:** insect biology, mass rearing, nutrition

*Helicoverpa armigera* (Hübner) is an important pest of agricultural crops worldwide and stands out with polyphagia, facultative diapause, a high dispersal capacity, adaptation to different environments, and a high reproductive potential (Fitt 1989, CABI 2014). This pest species can feed on vegetative organs and reproductive structures, causing significant losses and control costs of about US\$5 billion worldwide (Lammers and Macleod 2007, Fathipour and Sedaratian 2013).

Although several studies have investigated artificial diets for *H. armigera*, few studies have been carried out evaluating its food consumption and utilization on nutritionally different diets. Larval development is dependent on the ratio of proteins and carbohydrates (Sarate et al. 2012). Protein digestion during the larval period is a complex process and is performed by the proteases present in the insect's gut (Kotkar et al. 2009).

In other lepidopteran species, such as *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), a diet low in proteins and

carbohydrates can influence several biological aspects (Bouayad et al. 2008).

The development of artificial diets suitable for rearing insects has prompted a great advancement in integrated pest management programs. The consumption and use of food are basic conditions for insect growth, development, and reproduction, and the quantity and the proportion of nutrients in the ingredients of the larval diet influence the acceptance of the food, besides affecting the performance of adults (Panizzi and Parra 2009). These aspects may have more or less severe effects on the biology of insects, facilitating or impeding their development (Scriber and Slansky Jr. 1981, Behmer 2009, Panizzi and Parra 2009, Schowalter 2011, Parra 2012, Cohen 2015).

In this context, the aim of this study was to evaluate the biology and to compare the consumption and use of food in *H. armigera* larvae on diets containing several dietary ingredients providing three different levels of total proteins.

## Material and Methods

The rearing of *H. armigera* and the experiments were conducted at the Laboratory of Biology and Insect Rearing (LBIR), Department of Plant Protection, São Paulo State University (UNESP), Jaboticabal, SP. The insects were kept under controlled laboratory conditions at a temperature of  $25 \pm 1^\circ\text{C}$ , a relative humidity of  $70 \pm 10\%$ , and a photoperiod of 12:12 (L:D) h.

### Rearing

*H. armigera* individuals were obtained from soybean crops in Luís Eduardo Magalhães, BA, Brazil ( $12^\circ 5' 58''\text{S}$ ,  $45^\circ 47' 54''\text{W}$ ), and were reared in the laboratory for five generations following the methodology described by Abbasi et al. (2007). The larvae were placed in Petri dishes (6 cm diameter  $\times$  2 cm height) containing an artificial diet described by Greene et al. (1976), with modifications, and monitored until the larvae reached the pupal stage. At the pupal stage, they were separated by sex and transferred to copula and oviposition cages of polyvinyl chloride (20 cm diameter  $\times$  20 cm height), lined with paper towels and supported on a plastic cover (23.5 cm diameter  $\times$  3 cm height) lined with paper towels, with the top closed with voile fabric fastened with elastic. Twenty couples were conditioned per cage and fed with a 10% honey–water mixture on a piece of soaked cotton packed inside a plastic top (3 cm diameter  $\times$  1.5 cm height). Eggs were collected from the paper towel where the females oviposited. The eggs were removed and placed in plastic containers (25  $\times$  15  $\times$  12 cm) until hatching.

### Artificial Diets

The artificial diet used was modified from the diet described by Greene et al. (1976) for velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae). Three formulations were used; the modified diet of Greene et al. (1976) (D<sub>1</sub>), and two variations of the diet in the amount of total protein, one with twice the amount of protein (D<sub>2</sub>) and the other with half the amount (D<sub>3</sub>). The compositions of the diets are shown in Table 1. The vitamin mixture used for the diets is presented in Table 2.

### Nutritional Indices

When the larvae reached the fourth instar, as determined when they were about (12 mm in length) (Ali et al. 2009) and dark tubercles in the dorsal region of the first abdominal segment (Matthews 1999),

10 insects were removed from the Petri dishes, weighed, killed by freezing, and oven-dried. Another 10 insects were weighed and kept in the Petri dishes; where when they reached the fifth instar, 20 mm of length (Ali et al. 2009), as confirmed by the presence of ecdysis, and were treated as above. In addition, dietary leftovers and excrements from the insects during the fourth instar and 10 whole artificial diet cubes were weighed and oven-dried. After a drying period of 3 d, the diets and larvae were weighed. Thus, the weight of the dry larvae, the food consumed, and the weight gain of the larvae were obtained for the determination of the indices of food consumption and use.

The methodology proposed by Waldbauer (1968) and modified by Scriber and Slansky Jr. (1981) was used to determine the quantitative nutritional indices of fourth instar of the larvae stage. For the calculation of these indices, the following parameters were used (in dry matter weight):

T = duration of feeding period (d);

Af = weight of food supplied to the insect (g);

Ar = weight of leftover food provided to the insect (g) after T;

F = weight of excretory produced (g) during T;

B = (I - F) - M = weight gain by larvae (g) during T;

$\bar{B}$  = mean weight of larvae (g) during T;

I = weight of food consumed (g) during T;

I - F = food assimilated (g) during T;

M = (I - F) - B = food metabolized during T (g).

The indices of consumption and use for each treatment were determined using the following equations:

$$\text{Relative consumption rate (g/g/d) (RCR)} = \frac{I}{B \times T};$$

$$\text{Relative growth rate (g/g/d) (RGR)} = \frac{B}{B \times T};$$

$$\text{Relative metabolic rate (g/g/d) (RMR)} = \frac{M}{B \times T};$$

$$\text{Approximate digestibility (\%)} (AD) = \frac{I - F}{I} \times 100;$$

$$\text{Efficiency of conversion of ingested food (\%)} (ECI) = \frac{B}{I} \times 100;$$

$$\text{Efficiency of conversion of digested food (\%)} - \text{ECD} = \frac{B}{I - F} \times 100;$$

$$\text{Metabolic cost (\%)} (CM) = 100 - \text{ECD}.$$

### Biological Parameters (Aspects)

For each diet, 60 newly hatched larvae (<24 h) were kept individually in Petri dishes (6 cm diameter  $\times$  2 cm height), where they were monitored until reaching the pupal stage. Artificial diet cubes (2  $\times$  2 cm) were supplied and replaced after approximately 80% had been consumed. We evaluated the following biological parameters:

**Table 1.** Composition of the artificial diets for *Helicoverpa armigera*

Constituent	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
White bean	75.0 g	150.0 g	37.5 g
Wheat germ	60.0 g	120.0 g	30.0 g
Soy bran	30.0 g	60.0 g	15.0 g
Milk powder	30.0 g	60.0 g	15.0 g
Brewer's yeast	37.5 g	75.0 g	18.75 g
Ascorbic acid	3.6 g	3.6 g	3.6 g
Sorbic acid	1.8 g	1.8 g	1.8 g
Methylparahydroxybenzoate (Nipagin)	3.0 g	3.0 g	3.0 g
Vitamin solution	9.0 ml	9.0 ml	9.0 ml
Tetracycline	0.12 g	0.12 g	0.12 g
Formaldehyde (40%)	3.6 ml	3.6 ml	3.6 ml
Agar	23.0 g	23.0 g	23.0 g
Distilled water	1,400 ml	1,400 ml	1,400 ml

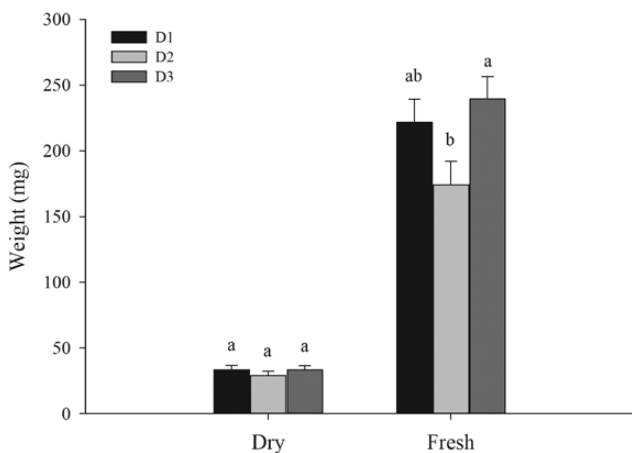
D<sub>1</sub> – Artificial diet modified from Greene et al. (1976), used at rearing. D<sub>2</sub> – Artificial diet modified from Greene et al. (1976), with double protein. D<sub>3</sub> – Artificial diet modified from Greene et al. (1976), with half protein.

larval period, larval survival, pupal weight at 24 h, sex ratio, pupal development duration, and pupal survival.

Cylindrical PCV cages (10 cm diameter × 20 cm height) were constructed and the top closed with voile fabric fastened with elastic, supported on a plastic cover (15 cm diameter × 2 cm height) and lined with paper towels. Two couples of *H. armigera* that had emerged the same day were released in the each cage. During the adult phase, we observed 10 replicates per treatment and each cage was considered a replicate. Adults were fed with a 10% honey–water mixture on a piece of soaked cotton packed inside a plastic top (3 cm diameter × 1.5 cm height). Following daily observation, female fecundity (eggs/female) and longevity of male and female adults were recorded.

**Table 2.** Composition of the vitamin solution used for artificial diets

Component	Amount
Niacinamide	4.0 mg
Calcium pantothenate	4.0 mg
Thiamine HCl	1.0 mg
Riboflavin	2.0 mg
Pyridoxine HCl	1.0 mg
Folic acid	1.0 mg
Biotin	0.08 mg
Vitamin B12	0.008 mg
Distilled water	400 ml



**Fig. 1.** Dry and fresh weight of *Helicoverpa armigera* larvae on artificial diets.

**Table 3.** Nutritional indices of *Helicoverpa armigera* on artificial diets

Index	Diet		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
RCR (g/g/d)	2.3 ± 0.40B <sup>a</sup>	5.5 ± 6.48A	1.9 ± 0.08B
RGR (g/g/d)	0.7 ± 0.00B	1.6 ± 0.41A	0.5 ± 0.01B
RMR (g/g/d)	0.7 ± 0.18B	2.5 ± 0.46A	0.3 ± 0.02B
AD (%)	58.4 ± 2.42B	73.2 ± 2.17A	45.8 ± 10.22C
ECl (%)	30.8 ± 1.90A	30.3 ± 1.21A	29.1 ± 2.21A
ECD (%)	54.4 ± 4.55A	41.9 ± 2.50B	63.8 ± 1.93A
CM (%)	45.6 ± 4.55B	58.1 ± 2.50A	36.2 ± 1.93B

<sup>a</sup>Means ± SE followed by the same letter on the line do not differ by the Tukey test ( $P > 0.05$ ).

With the biological parameters obtained, the parameters for the construction of fertility life tables were estimated, according to Birch (1948), Silveira Neto et al. (1976), Southwood (1978), and Price (1984):  $x$  = age of parental females, age is considered starting in the egg phase;  $l_x$  = specific survival rate to age  $x$ , expressed as a fraction per female and male (total adults);  $m_x$  = specific fertility or number of offspring produced per female at age  $x$ ;  $l_x.m_x$  = age-specific maternity. The growth parameters resulting from life tables were  $R_0$  = net rate of population growth,  $T$  = mean generation time,  $r_m$  = intrinsic rate of increase,  $\lambda$  = finite rate of increase. In addition to these parameters, we also determined  $Dt$ , which is the time required for the population to double in number, according to Krebs (1994).

The growth parameters ( $R_0$ ,  $T$ ,  $r_m$ ,  $\lambda$  e  $Dt$ ) were calculated using the following equations:

$$R_0 = \sum (l_x.m_x)$$

$$T = \sum (x.l_x.m_x) / \sum (l_x.m_x)$$

$$r_m = \ln R_0 / T$$

$$\lambda = e^{r_m}$$

$$Dt = \ln(2) / r_m$$

**Statistical Analysis**

Data from nutritional indices and biological characteristics of adult *H. armigera* specimens on different diets were subjected to Kolmogorov and Bartlett tests to determine normality and homogeneity of variance. Data from the RCR, RGR, RMR, ECD, weight of the fresh and dry larvae, and pupal period were transformed by the root of  $x + 0.5$  to meet the requirements of the analysis of variance (ANOVA) and then analyzed using PROC ANOVA. Means were compared by Tukey’s test ( $P < 0.05$ ). Data for larval period, larval survival, weight of pupae, pupal period, pupal survival, fecundity of females, and longevity of males and females were compared using the Student–Newman–Keuls test. All analyses were conducted using the software package SAS (SAS Institute 2015).

Population parameters of fertility life tables were estimated according to the Jackknife methods for estimating the confidence intervals of the parameters and for allowing comparisons between treatments, as described by Maia et al. (2000). Estimates were conducted using SAS software (SAS Institute 2015).

## Results

### Nutritional Indices

The highest fresh weight of *H. armigera* in the fifth instar was obtained in D<sub>3</sub>, whereas in D<sub>2</sub> the larvae had the lowest weight, with a variation greater than 60 mg ( $F_{2,27} = 3.74$ ,  $P = 0.0369$ ). Regarding dry weight ( $F_{2,27} = 0.72$ ,  $P = 0.4978$ ), all treatments were similar (Fig. 1).

RCR ( $F_{2,27} = 17.04$ ,  $P < 0.0001$ ), RMR ( $F_{2,27} = 16.82$ ,  $P < 0.0001$ ), and apparent digestibility (AD) ( $F_{2,27} = 48.74$ ,  $P < 0.0001$ ), which represents the percentage of the feed that was effectively assimilated, were higher in D<sub>2</sub>. For D<sub>1</sub> and D<sub>3</sub>, these indices were smaller and similar to each other, differing in AD, which was lower for D<sub>3</sub> (Table 3).

Regarding the RGR, the lowest values were for D<sub>1</sub> and D<sub>3</sub> ( $F_{2,27} = 23.58$ ,  $P < 0.0001$ ), while for the efficiency of conversion of ingested food (ECI) the values were similar for the three diets, varying from 29.1 to 30.8% ( $F_{2,27} = 0.48$ ,  $P = 0.6261$ ). On the other hand, the ECD was lower in D<sub>2</sub> ( $F_{2,27} = 11.81$ ,  $P = 0.0002$ ), which led to a higher CM for this diet ( $F_{2,27} = 12.54$ ,  $P = 0.0003$ ); while ECD was higher for D<sub>1</sub> and D<sub>3</sub> and CM was lower (Table 3).

### Biological Characteristics

For the larval period, the insects fed with different diets did not present significant differences ( $F_{2,27} = 2.14$ ,  $P = 0.1609$ ), with larval periods varying from 18.2 to 21.0 d. Survival in this period was higher for D<sub>1</sub> and lower for D<sub>2</sub>, with a variation of 38.1%, while D<sub>3</sub> presented intermediate survival ( $F_{2,27} = 8.54$ ,  $P = 0.0049$ ) (Table 4).

The weight of pupae at 24 hours of age ( $F_{2,27} = 2.01$ ,  $P = 0.1821$ ) and the sex ratio ( $F_{2,27} = 0.18$ ,  $P = 0.8341$ ) did not differ significantly between the diets evaluated, with the weight varied between 310.6 and 337.6 mg and the sex ratio between 0.3 and 0.4. The different diets influenced the pupal period of *H. armigera* ( $F_{2,27} = 13.21$ ,  $P = 0.0012$ ), where the duration was higher for D<sub>2</sub> and similar between D<sub>1</sub> and D<sub>3</sub> (Table 4).

Survival to the end of the pupal phase was lower for D<sub>2</sub>, while D<sub>1</sub> and D<sub>3</sub> showed similar survival rates ( $F_{2,27} = 9.50$ ,  $P = 0.0034$ ) (Table 4).

In adulthood, there was no significant difference in terms of longevity between males ( $F_{2,27} = 0.34$ ,  $P = 0.7143$ ) and females ( $F_{2,27} = 0.42$ ,  $P = 0.6539$ ). However, females presented a higher longevity when compared to males in more than 3 d (Table 5).

The different diets had no impact on female fecundity ( $F_{2,27} = 0.25$ ,  $P = 0.7853$ ), which varied between 206.5 and 268.7 eggs/female (Table 5).

The reproductive period of *H. armigera* started 1d after the emergence of the females when fed artificial diets. The average duration of the reproductive cycle was 12, 10 and 11 d when the larvae fed D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>, respectively (Fig. 2). The females produced 8.9

female offsprings per day when fed D<sub>1</sub>, 25.5 females per day when fed D<sub>2</sub> and 8.0 females per day when fed D<sub>3</sub>. We observed that on total, 142.6, 332.2, and 120.3 female offsprings were obtained per female when the *H. armigera* fed D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>, respectively (Fig. 2). The D<sub>2</sub> was the one that provided the highest production of offspring, but it was the one that provided less survival, only 10% of the individuals reached adulthood.

The Fertility Life Table, based on the results obtained for the biological parameters of *H. armigera*, showed differences between the evaluated diets. The net reproduction rate ( $R_0$ ), although higher for D<sub>1</sub>, was similar for all treatments, ranging from 22.7 to 61.3 females/females per generation. Regarding the average generation time (T), the lowest value was found for D<sub>1</sub>, while D<sub>3</sub> presented the highest value. The intrinsic increase rate ( $r_m$ ) was higher for D<sub>1</sub> and lower for D<sub>3</sub>, from 0.052 females/female/d. The finite increase rate ( $\lambda$ ) was also higher for D<sub>1</sub>, presenting similar values between D<sub>2</sub> and D<sub>3</sub>. The time for the population to double in size (Dt) was significantly lower for D<sub>1</sub>; while for D<sub>3</sub>, it took 3 more days for the population to double in the number of individuals when the larvae were fed on these diets (Table 6).

Among the diets evaluated, D<sub>1</sub> had the highest net reproduction rate, the shortest time for the population to double in number, and the highest rates of population growth (Table 6).

## Discussion

By comparing the consumption, feed use, and biology of *H. armigera* larvae in diets with different protein contents, significant differences were identified.

The highest fresh weight of fourth instar larvae was related to the protein content, since the lower the protein content of the diet, the greater the weight of the larvae. These results differ from those found

**Table 5.** Biological characteristics of *Helicoverpa armigera* adults on artificial diets

Characteristic	Diet		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
Male longevity (d)	6.7 ± 1.96A <sup>a</sup>	5.5 ± 1.55A	5.0 ± 1.12A
Female longevity (d)	10.1 ± 1.84A	9.2 ± 1.31A	9.1 ± 1.33A
Fecundity (eggs/female)	229.7 ± 77.60A	268.7 ± 65.10A	206.5 ± 28.02A

<sup>a</sup>Means ± SE followed by the same letter on the line do not differ by the Student–Newman–Keuls ( $P > 0.05$ ).

**Table 4.** Biological characteristics of the larval and pupal stages of *Helicoverpa armigera* on artificial diets

Characteristic	Diet		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
Larval period (d)	18.2 ± 0.53A <sup>a</sup>	20.9 ± 1.54A	21.0 ± 4.97A
Larval survival (%)	60.0 ± 7.29A	21.9 ± 4.63B	40.0 ± 7.29AB
Pupae weight (mg)	335.2 ± 10.23A	337.6 ± 12.29A	310.6 ± 9.08A
Sex ratio	0.4 ± 0.05A	0.4 ± 0.25A	0.3 ± 0.09A
Pupal period (d)	14.9 ± 0.36B	18.5 ± 1.06A	14.6 ± 0.21B
Pupal survival (%)	42.5 ± 5.00A	10.0 ± 4.68B	32.5 ± 6.37A

<sup>a</sup>Means ± SE followed by the same letter on the line do not differ by the Student–Newman–Keuls ( $P > 0.05$ ).

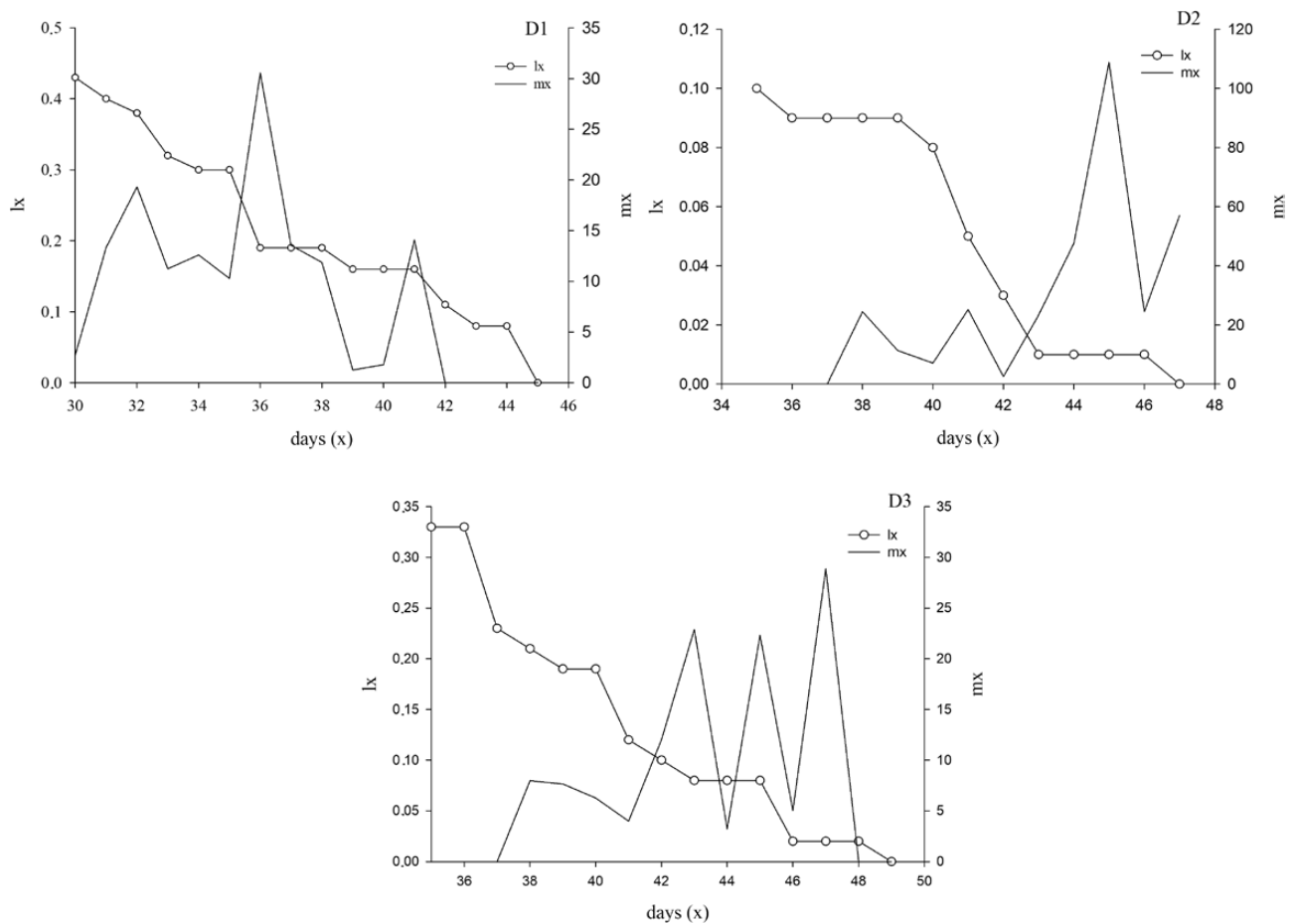


Fig. 2. Average number of offsprings per female (mx) and survival rate lx of *Helicoverpa armigera* on artificial diets.

Table 6. Parameters of the fertility life table of *Helicoverpa armigera* on artificial diets

Characteristicsw	Diets		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
R <sub>0</sub> (females/female)	61.3 (-20.37-143.00)A <sup>a</sup>	23.7 (-108.66-156.05)A	22.7 (-4.65-50.11)A
r <sub>m</sub> (females/female*d)	0.139 (0.1044-0.1756)A	0.091 (-0.0791-0.2605)AB	0.087 (0.0557-0.1180)B
λ (females/female/d)	1.146 (1.1045-1.1866)A	1.096 (0.9123-1.2816)B	1.089 (1.0554-1.1233)B
T (d)	30.1 (25.01-35.31)C	36.0 (34.29-37.81)B	36.8 (35.89-37.70)A
Dt (d)	4.9 (3.62-6.21)B	7.4 (-9.45-24.28)AB	7.9 (4.87-10.86)A

R<sub>0</sub> = net rate of population growth, T = mean generation time, r<sub>m</sub> = intrinsic rate of wincrease, λ = finite rate of increase, Dt = time required for the population to double in number. The values of sex ratio used were 0.42, 0.57 and 0.38 for Diets 1, 2 and 3.

<sup>a</sup>Means (confidence interval) followed by the same letter on the line do not differ by the Student *t*-test (*P* > 0.05).

by Sarate et al. (2012), who fed larvae with several host plants and observed that diets rich in proteins and/or carbohydrates resulted in higher larvae weight and a shorter larvae period. However, the dry larvae mass was similar between the different diets.

The RGR, representing the amount of food ingested per unit weight of the insect per day, and the RMR, representing the amount of food spent in metabolism per unit weight, were higher for the diet with a higher protein content, demonstrating that larvae need a greater amount of food to meet their nutritional needs due to the high amount of protein required for their development.

AD, which represents the percentage of ingested food that is effectively assimilated by the insect, was also higher for the diet with a higher protein content and lower for the diet with a lower protein content,

suggesting that the amount of food assimilated by the insect was associated with the protein level; therefore, in diets rich in protein, a higher food intake is necessary to satisfy the nutritional needs of the insect.

In terms of the RGR, which indicates the biomass gain by the insect in relation to its weight, the lowest values were found for the diets with the lowest amounts of protein; however, regarding the ECI, which represents the percentage of food ingested that is transformed into biomass, there was no difference between the three diets. The ECD showed that for diets with high protein contents, a low conversion of the diet to biomass occurred. Due to this low efficiency, this diet presented a high CM.

The protein present in the diet did not influence the length of the larval period (18.2 to 21.0 d). Survival during this period was low



in the high-protein diet, demonstrating that high levels of this nutrient in the diet may impair *H. armigera* population growth. Hamed and Nadeem (2008), who evaluated different artificial diets to rear *H. armigera* in the laboratory, found a larval period varying between 15.6 and 42.8 d, and the results of this study are within this range. Truzi et al. (2017), using an artificial diet similar to D<sub>1</sub>, found a larval period of 14.6 d, a higher value than observed in this study.

The variation in protein content did not influence pupal weight and sex ratio. However, the pupal period was influenced in that the high-protein diet prolonged this phase of the insect's life cycle. For the diet based on cotton seed and water chestnut, similar values have been found previously (Hamed and Nadeem 2008). However, for artificial diets, this period was shorter (Truzi et al. 2017).

The survival of insects in the larval and pupal phase were higher for the diet with a high protein content, in which was also the case for the larval period, indicating, in this case, the positive influence of high protein levels on the biology of this pest species. However, the number of females produced per female or net rate of population growth ( $R_0$ ) was similar among the diets tested, which showed that the higher the number of females reaching the reproductive period in the D1.

The diet in the larval period did not interfere with the longevity of adults. However, females presented greater longevity than males, which has also been observed in other studies using different diets (Ali et al. 2009, Jha et al. 2014, Truzi et al. 2017).

Female fecundity was not influenced by the diet during the larval period. However, the values found were low when compared to other studies, which reported values between 440 and more than 2,500 eggs/female on different diets during the larval phase (Razmjou et al. 2013, Nasiri et al. 2014, Truzi et al. 2017).

In relation to the fertility life table, for the net reproduction rate ( $R_0$ ), Truzi et al. (2017) found a higher value for the artificial diet, with 255 females per female in each generation. For soybean cultivars, the rate ranged from 16.0 to 270.0 females per female (Soleimannejad et al. 2010), while for tomato cultivars, it was between 7.8 and 186.9 females per females (Safuraie-Parizi et al. 2014). The average generation time (T) was influenced by the protein content, and at lower protein levels, the insects took longer to complete the cycle. The intrinsic rate of increase ( $r_m$ ) was also affected by the low protein level, with a smaller number of females per female being produced per day. When *H. armigera* larvae were reared on soybean cultivars, the values were similar, ranging between 0.084 and 0.114 females per female each day (Soleimannejad et al. 2010), but for artificial diets and for different host plants, larger values have been observed in previous studies (Razmjou et al. 2013, Truzi et al. 2017). For the finite rate of increase ( $\lambda$ ), low and high protein levels resulted in a lower number of females per female each day. *Helicoverpa armigera* presented the shortest time for the population to double in size (Dt) at D<sub>1</sub> with 4.9 d, but this period was higher than that found by Truzi et al. (2017), which was 3.6 d.

The actual protein contents of the ingredients and the information on amino acids in each protein which may be related to the insect performance. The white bean has 19 g protein/100 g, wheat germ 23 g protein/100 g, whole milk powder 23 g protein/100 g, Brewer's yeast 43 g protein/100 g, soy bran 35 g protein/100 g and yeast 40.44 g protein/100 g (Cohen 2015; USDA 2018).

Many insects digest proteins from their food in order to get amino acids. Insects need a dietary source of arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In the absence of one of these essential amino acids, growth and development may impair of some species (Chen 1966). Sometimes, nonessential amino acids motivate growth, because of

the optimization of the nutrient balance and the good organization of the biochemical pathways concerned in the synthesis of the non-essential amino acids. For example, alanine and glycine or serine is necessary for optimal growth in *Bombyx mori* (Nation 2001).

These previous studies may explain some results obtained in this research, where the diet with the highest amount of protein provided the best results for *H. armigera*. The sources of proteins used in the diet, such as wheat germ and soy bran have provided the insects the 10 essential amino acids leucine, isoleucine, valine, threonine, arginine, lysine, methionine, histidine, phenylalanine, tryptophan, and seven nonessential amino acids alanine, glycine, cysteine, glutamic acid, aspartic acid, tyrosine, and serine (Cohen 2015).

The influence of different protein levels on *H. armigera* development, population growth, consumption indices, and feed use was studied, and the information obtained in this study can be used to adapt diets or to develop new diets for mass rearing of this insect species. In addition, future studies should evaluate the influence of different protein levels on successive generations of *H. armigera*.

## Conclusion

The most suitable artificial diet for the mass rearing of *H. armigera* in the laboratory was D<sub>1</sub> (protein content: white bean 75 g, wheat germ 60 g, soy bran 30 g, milk powder 30 g, brewer's yeast 37.5 g), with higher level of protein improving the biological aspects of this pest species.

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