

# Artificial Diets With Different Protein Levels for Rearing *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Subject Editor: Muhammad Chaudhury

Received 16 March 2021; Editorial decision 24 May 2021

## Abstract

*Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is a pest of great economic importance which can feed on more than 300 plant species. As it is polyphagous, its host plants may have variable physical and chemical constitutions. This may influence larval development, as protein and carbohydrate levels are important factors for adequate biological development. The aim of this study was to evaluate insect developmental parameters as well as to compare the food consumption of *S. frugiperda* larvae reared using diets with different protein levels under laboratory conditions. Three artificial diet formulations were used: one typically used for routine laboratory rearing, based on bean, wheat germ and brewer's yeast (D<sub>1</sub>); one containing half the original amount of protein (D<sub>2</sub>), and the other with twice the original amount of protein (D<sub>3</sub>). The relative consumption rate (RCR), relative growth rate (RGR), and efficiency of conversion of ingested food (ECI) for *S. frugiperda* fourth instar larvae varied among diets. The protein present in the diet influenced the duration of larval and pupal periods and pupal weight, but did not affect larval survival, fecundity and longevity of adults. The different protein levels in the diets did not negatively influence population growth, so these three diet variations can be used for mass rearing in the laboratory. However, the influence of these diets on successive generations of the insect remains untested.

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

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is an important maize crop pest which can feed on more than 300 plant species, including some of substantial economic value such as soybean, cotton, sugarcane, and wheat (Barros *et al.* 2010, Silva *et al.* 2017, Montezano *et al.* 2018). This species is widely distributed in the western hemisphere and has been gaining greater importance because of its spread into Africa and India (Clark *et al.* 2007, Goergen *et al.* 2016, Ganiger *et al.* 2018).

Characterizing and quantifying biological parameters, nutritional requirements, and insect behavior are very important for the development of Integrated Pest Management programs (IPM), and these studies are facilitated by the availability of insects from mass rearing under laboratory conditions (Panizzi & Parra 2009). To support development of rearing methodology several studies were carried out aiming to develop a rearing methodology able to supply a large number of *S. frugiperda* individuals with good biological quality using an artificial diet (Silva & Parra 2013).

Polyphagous herbivorous insects feed on nutritionally (chemically) and physically different plants, and this can directly influence larval development (Sarate *et al.* 2012). The most important nutritional factors are the protein and carbohydrate levels, especially their

proportions, as these are fundamental components for growth and development, and are part of the composition of enzymes and hormones (Bae & Sicher 2004, Sarate *et al.* 2012). Studies on lepidopterans such as *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) and *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) have shown that low-protein diets promote changes in developmental period, weight and larval mortality, pupation rate, percentage of adult emergence, and nutritional indices (Borzoui *et al.* 2018, Truzi *et al.* 2019).

For the impact of the proportion of nutrients in the artificial diet of *S. frugiperda* on the development of the larval period has not been well explored. This impact may be positive or negative, and it may alter biological parameters of the adult insect, such as its reproductive capacity (Scriber & Slansky Jr. 1981, Panizzi & Parra 2009, Cohen 2015).

Based on the impact of nutrition on insect population growth, we aimed to evaluate developmental parameters of *S. frugiperda* and compare the consumption and use of food by larvae reared using diets containing different protein levels under laboratory conditions, seeking to better understand the pest behavior exposed to these conditions.

## Materials and Methods

The research was conducted at the Laboratory of Biology and Insect Rearing (LBIR), Department of Agricultural Production Sciences (Plant Protection), São Paulo State University (UNESP), Jaboticabal, São Paulo. The insects were maintained under laboratory-controlled conditions (temperature  $25 \pm 1^\circ\text{C}$ , relative humidity  $70 \pm 10\%$ , and 12:12 L:D).

### Rearing of *S. frugiperda*

In adulthood, 20 couples were transferred to copula and oviposition PVC cages (20.0 cm diameter  $\times$  20.0 cm height). Each cage was internally lined with a paper sheet (oviposition substrate), closed at the top with voile fabric fastened with elastic, and supported on a plastic dish (23.5 cm diameter  $\times$  3.0 cm height) lined with paper towels. The adults were fed with a solution of honey (10.0%) soaked in a piece of cotton packed inside a plastic top (3.0 cm diameter  $\times$  1.5 cm height). Eggs were collected from the paper sheet and placed in plastic containers (25.0 cm length  $\times$  15.0 cm width  $\times$  12.0 cm height) until the larvae hatched. At this stage, the larvae were kept individually in plastic Petri dishes (6.0 cm diameter  $\times$  2.0 cm height) containing an artificial diet described by [Kasten Jr. et al. \(1978\)](#), until they reached the pupal stage, when they were separated by sex.

### Artificial Diets

Three artificial diet formulations were used following [Kasten Jr. et al. \(1978\)](#). One was the diet recommended by these authors ( $D_1$ ), and the other two were modified diets, containing half ( $D_2$ ) and twice ( $D_3$ ) the original protein amount. The composition of each diet and the respective amounts of ingredients are shown in [Table 1](#).

### Nutritional Indices

After larvae reached the fourth instar, determined by the exuviae, 10 insects were weighed, killed by freezing, and oven-dried to a constant weight. Another 10 insects were weighed and kept in Petri dishes until they reached the fifth instar, also determined by the exuviae, after which the aforementioned procedure was performed, and the weight of the leftover diet and the excrement from the insects was obtained. In addition, 10 whole cubes of each diet treatment, similar

to those offered to the larvae, were weighed and oven-dried to a constant weight. After 3 d of drying, the dry weights of larvae, diets, and excrement were obtained. These parameters (by weight of dry matter) were obtained to determine the indices of food consumption and use (RCR, RGR, RMR, AD, ECI, ECD, MC) during the fourth larval instar, according to [Waldbauer \(1968\)](#) and [Scriber & Slansky Jr. \(1981\)](#), using the following equations:

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

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

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

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

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

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

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

Where, T = duration of feeding period (days); 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).

### Biological Parameters

For each diet treatment, 50 newly hatched larvae (< 24 h) obtained from stock rearing were placed individually in Petri dishes (6.0 cm diameter  $\times$  2.0 cm height) containing artificial diet cubes (2.0 cm  $\times$  2.0 cm) which were replaced when approximately 80.0% of the diet cube had been consumed. These larvae were divided into 10 replicates per treatment and observed daily throughout the larval and pupal periods. The biological parameters obtained were larval period, larval survival, pupal weight at 24 h, sex ratio, pupal period, and pupal survival.

After the emergence of the adults, two newly emerged couples were released into cylindrical PVC cages (10.0 cm diameter  $\times$  20.0 cm height), made in a manner similar to that described in the section on rearing *S. frugiperda*, containing a solution of honey (10.0%) for feeding. Four replicates (cages) per treatment were observed daily, and fecundity (eggs/female) and longevity of male and female adults were determined.

Groups of approximately 100 eggs were collected and placed in Petri dishes (15.0 cm diameter  $\times$  2.0 cm height) to determine the viability of the eggs and the time required for the larvae hatching.

The parameters for the construction of fertility life tables were estimated according to [Birch \(1948\)](#), [Silveira Neto et al. \(1976\)](#),

**Table 1.** Composition of the artificial diets used for *Spodoptera frugiperda* rearing

Component	$D_1$	$D_2$	$D_3$
White bean	240.0 g	120.0 g	480.0 g
Wheat germ	120.0 g	60.0 g	240.0 g
Brewer's yeast	72.0 g	36.0 g	144.0 g
Ascorbic acid	7.3 g	7.3 g	7.3 g
Sorbic acid	2.4 g	2.4 g	2.4 g
Methylparahydroxybenzoate (Nipagin)	4.4 g	4.4 g	4.4 g
Vitamin solution <sup>a</sup>	10.0 ml	10.0 ml	10.0 ml
Tetracycline	0.12 g	0.12 g	0.12 g
Formaldehyde (10%)	6.0 ml	6.0 ml	6.0 ml
Agar	20.0 g	20.0 g	20.0 g
Distilled water	1,000 ml	1,000 ml	1,000 ml

<sup>a</sup>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.

$D_1$ : Artificial diet from [Kasten Jr. et al. \(1978\)](#) used for rearing.

$D_2$ : Artificial diet from [Kasten Jr. et al. \(1978\)](#) with half the protein level.

$D_3$ : Artificial diet from [Kasten Jr. et al. \(1978\)](#) with double the protein level.

Southwood (1978), and Price (1984):  $x$  = age of parental females, with age considered since egg phase;  $lx$  = specific survival rate to age  $x$ , expressed as a fraction per female and male (total adults);  $mx$  = specific fertility or number of offspring per female produced at age  $x$ ;  $lx.mx$  = age-specific maternity. The life table resulted in the following growth parameters:  $R_0$  = net rate of population increased;  $T$  = average generation time;  $r_m$  = innate ability to increase in number; and  $\lambda$  = finite rate of increase. The parameter  $Dt$  (time required for the population to double in number) was also determined, according to Krebs (1994).

The following equations were used to calculate the growth parameters ( $R_0$ ,  $T$ ,  $r_m$ ,  $\lambda$ , and  $Dt$ ):

$$R_0 = \sum (lx.mx)$$

$$T = \frac{\sum (x.lx.mx)}{\sum (lx.mx)}$$

$$r_m = \frac{\ln R_0}{T}$$

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

$$Dt = \frac{\ln(2)}{r_m}$$

### Statistical Analysis

The results obtained for the biological parameters and nutritional indices of *S. frugiperda* on the three diet formulations were subjected to Kolmogorov and Bartlett tests to determine the normality and homogeneity of variance needed for analysis of variance – ANOVA. Data on female fecundity, male longevity, and nutritional indices met these requirements, while data on female longevity were transformed using  $\log(x + 1)$  and then analyzed using PROC ANOVA procedure (SAS Institute 2015). Means were compared by Tukey's test ( $P < 0.05$ ). The larval period, larval survival, pupal weight, sex ratio, pupal period, and pupal survival did not meet the requirements, and were compared using the Student Newman Keuls test ( $P < 0.05$ ) (SAS Institute 2015).

The Jackknife method was used to estimate the population parameters of the fertility life table and the confidence intervals, as

described by Maia *et al.* (2000). The comparison among treatments was performed using the Student *t*-test (SAS Institute 2015).

## Results

### Nutritional Indices

The dry weight of fifth instar larvae of *S. frugiperda* was similar among treatments, ranging from 21.9 to 32.7 mg ( $F_{2,23} = 2.42$ ,  $P = 0.1129$ ). Regarding fresh weight, the highest value was obtained in  $D_1$ , while  $D_2$  had intermediate weight, and the larvae in  $D_3$  had the lowest weight ( $F_{2,23} = 3.06$ ,  $P = 0.0680$ ), with a variation greater than 90.0 mg (Figure 1).

The relative consumption rate (RCR) ( $F_{2,23} = 4.33$ ,  $P = 0.0266$ ) and relative growth rate (RGR) ( $F_{2,23} = 6.82$ ,  $P = 0.0052$ ), were higher in  $D_3$  (4.69 g/g/day and 1.44 g/g/day, respectively) than in the other treatments. Diet  $D_1$  had the lowest RCR (2.04 g/g/day), while  $D_3$  had the highest RGR (1.44 g/g/day) (Table 2).

Regarding the relative metabolic rate (RMR) ( $F_{2,23} = 1.89$ ,  $P = 0.1756$ ), approximate digestibility (AD) ( $F_{2,23} = 0.72$ ,  $P = 0.4967$ ), efficiency of conversion of digested food (ECD) ( $F_{2,23} = 0.83$ ,  $P = 0.4486$ ), and metabolic cost (MC) ( $F_{2,23} = 0.83$ ,  $P = 0.4486$ ), no differences were observed among the evaluated diets. Though for the efficiency of conversion of ingested food (ECI) there was a difference between diet types and was higher for  $D_1$  (32.22%) than for the other treatments ( $F_{2,23} = 4.44$ ,  $P = 0.0247$ ), indicating that a higher amount of food ingested was transformed into biomass and was lowest for  $D_2$  (26.17%) (Table 2).

### Biological Parameters

The larval period of insects fed with different diets ranged from 17.9 to 18.9 d ( $F_{2,130} = 5.39$ ,  $P = 0.0057$ ), and it was longest for the diet with double protein level ( $D_3$ ). The percentage of larval survival ( $F_{2,149} = 0.42$ ,  $P = 0.6606$ ) was not significantly different among the three protein level conditions, and it was always higher than 84.0% (Table 3).

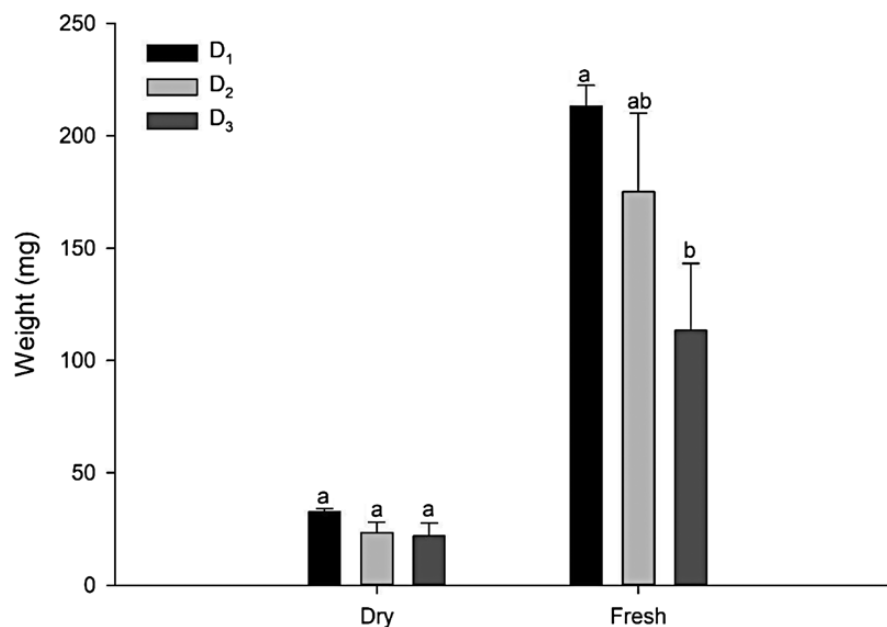


Fig. 1. Dry and fresh weight of *Spodoptera frugiperda* larvae fed with artificial diets containing different protein levels.  $D_1$ : Artificial diet used for rearing;  $D_2$ : Artificial diet modified with half the protein level;  $D_3$ : Artificial diet modified with double the protein level.

**Table 2.** Nutritional indices of *Spodoptera frugiperda* larvae fed with artificial diets containing different protein levels

Index	Diets		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
RCR (g/g/day)	2.04 ± 0.09b <sup>a</sup>	3.55 ± 0.77ab	4.69 ± 0.96a
RGR (g/g/day)	0.65 ± 0.02b	0.90 ± 0.15b	1.44 ± 0.27a
RMR (g/g/day)	0.46 ± 0.08a	1.14 ± 0.47a	1.39 ± 0.41a
AD (%)	55.09 ± 4.08a	54.44 ± 4.64a	62.93 ± 6.29a
ECI (%)	32.22 ± 1.34a	26.17 ± 1.41b	30.32 ± 2.37ab
ECD (%)	61.42 ± 5.44a	52.28 ± 6.59a	50.98 ± 7.24a
MC (%)	38.57 ± 5.44a	47.71 ± 6.59a	49.01 ± 7.24a

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

D<sub>1</sub>: Artificial diet used for rearing, D<sub>2</sub>: Artificial diet modified with half the protein level, D<sub>3</sub>: Artificial diet modified with double the protein level. RCR: Relative consumption rate, RGR: Relative growth rate, RMR: Relative metabolic rate, AD: Approximate digestibility, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food, MC: Metabolic cost.

The weight of pupae at 24 h of age was highest for D<sub>1</sub> (288.2 mg) and reduced for both diets with changes in protein levels, for D<sub>3</sub> 275.0 mg and for D<sub>2</sub> 246.1 mg ( $F_{2,130} = 26.21, P < 0.0001$ ). The sex ratio did not differ among treatments ( $F_{2,29} = 0.10, P = 0.9074$ ), with value of 0.5 in the three diets (Table 3).

Diets influenced the pupal period of *S. frugiperda*, which was longest for D<sub>1</sub> (10.6 d), intermediate for D<sub>3</sub> (10.3 d), and shortest for D<sub>2</sub> (10.2 d) ( $F_{2,122} = 3.19, P = 0.0447$ ). The percentage of survival until the end of the pupal phase did not significantly differ among the evaluated diets ( $F_{2,149} = 0.94, P = 0.3924$ ), with values above 78.0% in the three conditions (Table 3).

Females of *S. frugiperda* have longer longevity than males; however, no significant difference was found in the longevity of males ( $F_{2,23} = 1.75, P = 0.1987$ ) and females ( $F_{2,23} = 0.22, P = 0.8010$ ) among diets. Feeding during the larval phase also did not influence female fecundity ( $F_{2,11} = 0.15, P = 0.8587$ ), which varied between 592.9 and 667.5 eggs/female (Table 4).

The reproductive period of *S. frugiperda* fed on an artificial diet started one day after emergence of the females. The average duration of the reproductive period was 6.0, 10.0, and 12.0 d for D<sub>3</sub>, D<sub>2</sub>, and D<sub>1</sub>, respectively. The total number of female offspring and survival period of the insects were proportional, and they were highest for D<sub>1</sub>, intermediate for D<sub>2</sub>, and lowest for D<sub>3</sub>, with the values of 677.2, 551.4, and 531.9 female offspring and 45.0, 42.0 and 40.0 d, respectively (Figure 2).

The results of the fertility life table for *S. frugiperda* showed no differences among diets. The net reproduction rate ( $R_0$ ) was between 231.22 and 255.86 females/female per generation. Numerically, the highest intrinsic increase rate ( $r_m$ ) observed was 0.199 females/female/day, while the maximum finite increase rate ( $\lambda$ ) was 1.220. The average generation time (T) varied from 27.8 to 29.4 d, and it took at least 3.5 d for the population to double in size (Dt) (Table 5).

## Discussion

When comparing the nutritional indices of *S. frugiperda* larvae in diets with different protein levels, some differences were observed. The dry weight of the larvae was similar in the three diets; however, there was a difference in fresh weight of fifth instar larvae, with the highest weight observed in D<sub>1</sub>, whereas the diet with half protein

**Table 3.** Biological parameters of the larval and pupal stages of *Spodoptera frugiperda*, with larvae fed with artificial diets containing different protein levels

Characteristic	Diets		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
Larval period (days)	18.3 ± 0.22b <sup>a</sup>	17.9 ± 0.22b	18.9 ± 0.19a
Larval survival (%)	88.0 ± 4.64a	90.0 ± 4.28a	84.0 ± 5.24a
Pupae weight (mg)	288.2 ± 4.06a	246.1 ± 4.55c	275.0 ± 4.09b
Sex ratio	0.5 ± 0.06a	0.5 ± 0.06a	0.5 ± 0.08a
Pupal period (days)	10.6 ± 0.11a	10.2 ± 0.11b	10.3 ± 0.12ab
Pupal survival (%)	80.0 ± 5.71a	88.0 ± 4.64a	78.0 ± 5.92a

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

D<sub>1</sub>: Artificial diet used for rearing, D<sub>2</sub>: Artificial diet modified with half the protein level, D<sub>3</sub>: Artificial diet modified with double the protein level.

**Table 4.** Biological parameters of *Spodoptera frugiperda* adults with larvae fed with artificial diets containing different protein levels

Characteristics	Diets		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
Male longevity (days)	8.1 ± 1.02a <sup>a</sup>	8.8 ± 0.44a	6.5 ± 1.13a
Female longevity (days)	11.5 ± 1.36a	10.4 ± 0.73a	10.3 ± 0.49a
Fecundity (eggs/female)	667.5 ± 126.93a	618.6 ± 77.38a	592.9 ± 75.62a

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

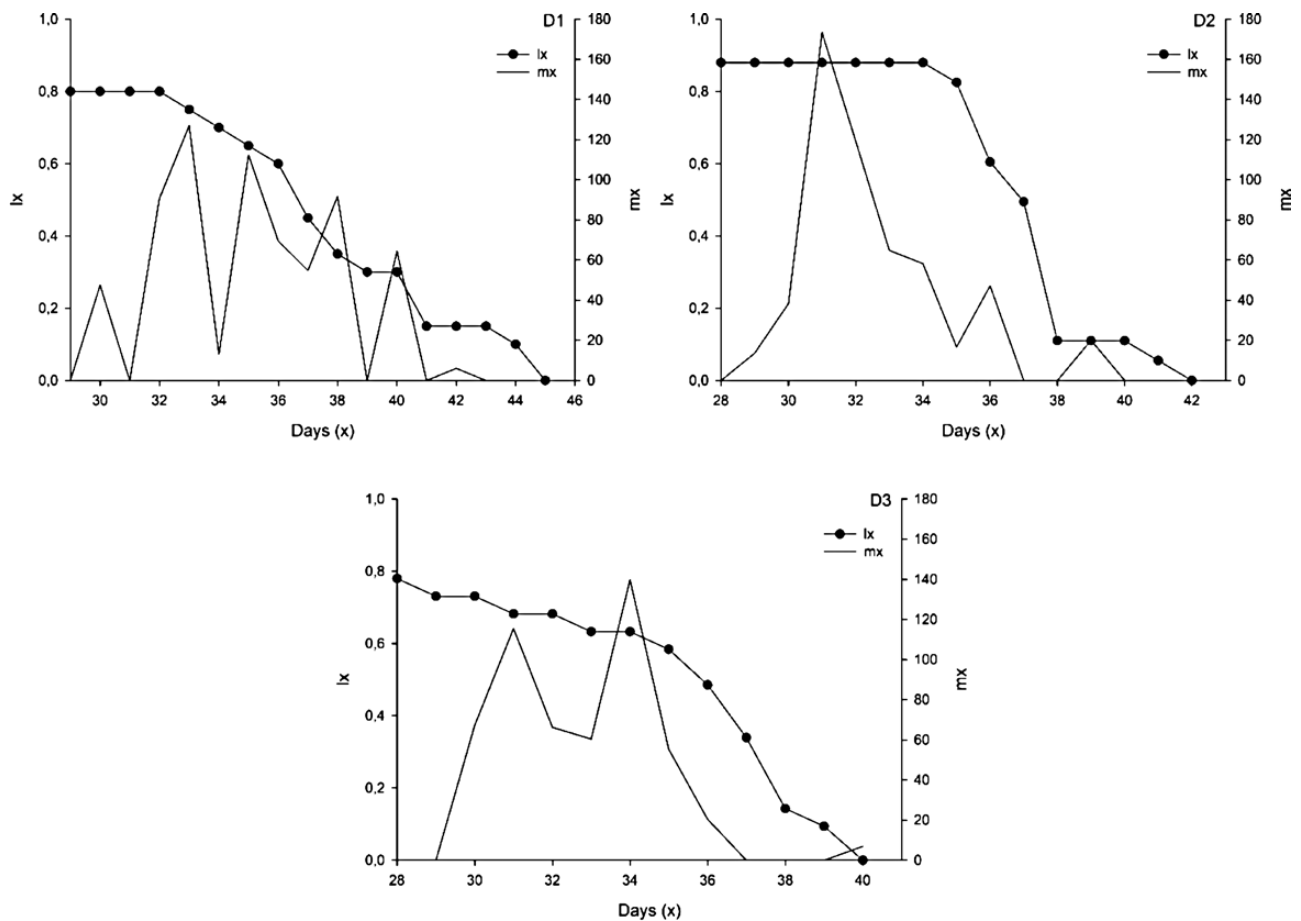
D<sub>1</sub>: Artificial diet used for rearing, D<sub>2</sub>: Artificial diet modified with half the protein level, D<sub>3</sub>: Artificial diet modified with double the protein level.

level provided intermediate weight and the diet with double protein level had the lowest weight. Pinto *et al.* (2019) obtained similar results when evaluating artificial diets with the addition of green corn or corn flour in the composition, differing only from D<sub>3</sub> herein used, which showed a high reduction in the fresh weight of insects.

The results regarding larval weights can be explained by the nutritional indices as the RCR, which represents the amount of food ingested per unit weight of insect per day, and the RGR, which shows the insects' biomass gain in relation to weight per day, were higher in the diet with double protein (D<sub>3</sub>). This indicates that the larvae required a larger amount of food and protein to meet their nutritional needs, which led to greater weight gain. The highest RGR probably occurred due to the shorter time interval for instar change, which is confirmed by the lower fresh weight of larvae in this diet.

For the ECI, which represents the percentage of food ingested that was transformed into biomass, the highest value was in D<sub>1</sub>, whereas the lowest value was in D<sub>2</sub>. This indicates D1 was better used by the insect than the other diets. Regarding the other indices, the three artificial diets were similar, indicating that the protein level did not interfere with the metabolism of *S. frugiperda*.

The amount of food consumed as well as its nutritional quality can result in changes in the duration of the larval phase of *S. frugiperda*, as mentioned by Pinto *et al.* (2019). This was also observed in the present study, as the amount of protein in the diet influenced the larval period, prolonging this phase when present at high levels, but



**Fig. 2.** Average number of offspring per female (mx) and survival rate (lx) of *Spodoptera frugiperda* on artificial diets. D<sub>1</sub>: Artificial diet used for rearing; D<sub>2</sub>: Artificial diet modified with half the protein level; D<sub>3</sub>: Artificial diet modified with double the protein level.

**Table 5.** Parameters of the fertility life table of *Spodoptera frugiperda* with larvae fed with artificial diets containing different protein levels

Characteristics	Diets		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>
R <sub>0</sub>	248.77a <sup>a</sup> (158.94–338.60)	255.86a (205.60–306.11)	231.22a (184.52–277.91)
r <sub>m</sub>	0.187a (0.1635–0.2118)	0.199a (0.1931–0.2059)	0.191a (0.1785–0.2049)
λ	1.206a (1.1773–1.2354)	1.220a (1.2130–1.2287)	1.211a (1.1953–1.2272)
T	29.4a (26.94–31.86)	27.8a (27.17–28.42)	28.4a (27.15–29.64)
Dt	3.7a (3.19–4.17)	3.5a (3.36–3.58)	3.6a (3.36–3.86)

<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).

D<sub>1</sub>: Artificial diet used for rearing, D<sub>2</sub>: Artificial diet modified with half the protein level, D<sub>3</sub>: Artificial diet modified with double the protein level.

without impairing the survival of insects. When evaluating different plants as food for *S. frugiperda* under laboratory conditions similar to that of this study, with the exception of the photoperiod (14:10 LD), *Silva et al. (2017)* found a larval period of 12.87 and 19.93 d

in corn and cotton, respectively, whereas the artificial diet of *Greene et al. (1976)* resulted in a larval period of 11.99 d. These results are similar but lower than those obtained in the present study.

The diet with double protein level resulted in a reduction in pupal weight and duration of pupal phase, whereas intermediate levels generated heavier pupae with longer pupal period, with a difference of 13.2 mg and 0.3 d, respectively. Regarding this biological parameter, *Giongo et al. (2015)*, using an artificial diet from *Greene et al. (1976)*, obtained pupae weighing 279.51 mg, similar to that obtained in the present study.

According to *Bernardi et al. (2014)*, heavier pupae should result in females with higher fecundity, even when the larval phase of insects is exposed to adverse factors. This relationship was not observed in the present study, as the diet offered during the larval period did not affect female fecundity and adult longevity; this may have occurred because the three protein levels used resulted in similar pupae weight, with a variation of only 42.1 mg. However, the values found in this study are much lower than those of other studies that reported values between 1061.0 and 1850.0 eggs/female, but with longevity of females over 17.0 d (*Bernardi et al. 2014*, *Pinto et al. 2019*). As the duration of the adult phase was reduced by more than 5.0 d, even using a diet similar to those of previous studies, this must have influenced the reduction in the number of eggs per female.

The results of the fertility life table showed no influence of diets on *S. frugiperda* population growth. The net rate of population growth (R<sub>0</sub>) was greater than one, while the intrinsic rate of increase



( $r_m$ ) and the finite rate of increase ( $\lambda$ ) were positive, indicating population growth. The mean generation time (T) was between 27.8 and 29.4 d, indicating that the protein level in the diet, within the tested values, did not interfere with the total insect cycle. The estimated parameters indicate population growth of *S. frugiperda*, and the time required for the population to double in number (Dt) was also similar, indicating that the insect population will reproduce and grow similarly under the three diets, even the one with the lowest protein level.

When evaluating the development of *S. frugiperda* fed with corn plant, Omoto *et al.* (2016) obtained similar values for the fertility life table parameters, except for the generation time, which was longer than that found in this study (36.6 d). This reinforces the statement that the artificial diets evaluated are suitable for the rearing of *S. frugiperda* under laboratory conditions, as there was also a certain acceleration in insect development when compared to its natural host.

Some studies have suggested that herbivorous insects may have behavioral and biochemical mechanisms which, when exposed to nutritionally unideal foods, enable them to control the nutrient intake to maintain nutritional balance (Behmer *et al.* 2002, Bede *et al.* 2007). In this sense, it is known that the larval phase of *Spodoptera* has flexible responses after unbalanced nutrient intake and, to compensate for the influence of proteins in the diet, is able to metabolize and use the carbohydrate 'skeleton' of the amino acids, eliminating excess as nitrogenous waste (Lee *et al.* 2003, Lwalaba *et al.* 2010, Zhang *et al.* 2011). This would justify the alteration of some indices of food consumption and utilization without interfering in the development and population growth of *S. frugiperda*, indicating that the modifications made in artificial diets may not have significantly influenced the rearing of this species.

In addition, the reduction in the amount of ingredients used for mass rearing is interesting, as it reduces the cost of preparing the diet and consequently, the final cost of producing insects. The average price of 1.0 kg of white beans is US\$2.55, of 1.0 kg of wheat germ is US\$3.21, and 1.0 kg of brewer's yeast is US\$4.90 (US\$1.00 = R\$5.36), for example; this would result in a cost of US\$1.35 of these ingredients for 1,000 ml of water in the artificial diet used for rearing ( $D_1$ ) and US\$0.68 in the artificial diet with half protein ( $D_2$ ). The reduction in the cost of rearing *S. frugiperda* is interesting for the development of research especially on control agents such as *Telenomus remus* Nixon, 1937 (Hymenoptera: Platygasteridae) and baculovirus (Haase *et al.* 2015, Vieira *et al.* 2017), which would also have a reduced cost when *S. frugiperda* is used in the rearing/production process.

Future studies should evaluate the concentrations of macro and micronutrients aiming at the elaboration of more adequate diets for rearing. The interference of different protein levels in successive generations should also be studied, as some of the modified components in diets can provide micronutrients that are essential to insects, such as brewer's yeast containing sterols, and the deficiency of these nutrients can be detected only after a few successive generations (Cortes Ortiz *et al.* 2016).

The different protein levels present in the artificial diets herein used promoted similar development of *S. frugiperda*. Therefore, the three artificial diets evaluated can be used for mass rearing in the laboratory, with a reduction in production cost for the one with the lowest level.

## Acknowledgments

We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the scholarship granted (process number 2018/00045-8).

## Author Contributions

CCT planned and designed the work, conducted experiments and statistical analyses, and wrote the manuscript. NFV and JMZ conducted experiments. SAB revised the manuscript.

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