DOI: 10.5433/1679-0359.2025v46n3p771

Influence of dietary protein content on the biology of *Spodoptera* complex caterpillars *(Lepidoptera: Noctuidae)*

Influência do teor proteico da dieta na biologia de lagartas do complexo *Spodoptera (Lepidoptera: Noctuidae)*

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Highlights

Artificial diets with reduced protein levels increase rearing viability.
Spodoptera eridania and *S. frugiperda* adapt better to changes in protein.
SPF software is a viable alternative to measure caterpillar head capsules.
Lower protein levels did not influence the number of instar larvae.

Abstract

Spodoptera frugiperda, S. eridania, and *S. cosmioides* (Lepidoptera: Noctuidae) are polyphagous pests of great economic relevance due to the damage they cause to crops such as corn, soybean, and cotton. Understanding the biology and nutritional requirements of these insects is essential for the development of control programs. In this context, the objective of this study was to evaluate the biological parameters of *S. frugiperda, S. eridania,* and *S. cosmioides* reared on artificial diets containing different protein levels under laboratory conditions (24 ± 2 °C, RH of 70 ± 10 %, and photophase of 14 h). Four artificial diets were used: the diet described by Greene et al. (1976) (D1), and three modified diets with reductions in the protein components wheat germ, soy protein, brewer's yeast, and powdered milk (casein) by 25% (D2), 50% (D3), and 75% (D4). The following parameters were evaluated: viability of the larval, pupal, and adult stages, as well as egg viability. The data were subjected to the non-parametric Kruskal-Wallis test followed by the Bonferroni *post hoc* test, both at a significance level of 0.05. To evaluate the influence of

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Received: Sept. 22, 2024 Approved: Apr. 22, 2025

diets with different protein contents on the number of instars in each species, caterpillar head capsules were measured daily using a digital microscope and SFP software. A multimodal frequency distribution curve was then plotted, followed by linear regression to confirm the number of instars. For *S. frugiperda* and *S. eridania,* diets D1 and D2 resulted in similar viabilities, whereas for *S. cosmioides,* the most suitable diet was D1. Diets D1, D2, and D3 produced larvae with six instars for all three species. The artificial diet with a 25% reduction in protein content (D2) shows potential for use in laboratory rearing of *S. frugiperda* and *S. eridania.*
Key words: Head capsule. Insect nutrition. Instar. Mass rearing. Proteins.

Resumo

Spodoptera frugiperda, S. eridania e *S. cosmioides* (Lepidoptera: Noctuidae) são pragas polífagas de grande relevância econômica devido aos danos causados em culturas como milho, soja e algodão. Para o desenvolvimento de programas de controle, é fundamental o conhecimento da biologia e das exigências nutricionais dos insetos. Nesse contexto, o objetivo desse estudo foi avaliar parâmetros biológicos de *S. frugiperda, S. eridania* e *S. cosmioides* criadas em dietas artificiais contendo diferentes teores de proteína em condições de laboratório (24 ± 2 °C, UR de 70 ± 10 % e fotofase de 14 h). Foram utilizadas quatro dietas artificiais: dieta descrita por Greene et al. (1976) (D1) e outras três modificadas, com redução dos componentes proteicos gérmen de trigo, proteína de soja, levedura de cerveja e leite em pó (caseína), em 25 % (D2), 50 % (D3) e 75 % (D4). Foram avaliados: viabilidade das fases larval, pupal e adulta e dos ovos. Os dados foram submetidos ao teste não-paramétrico de Kruskal-Wallis seguido pelo teste post hoc de Bonferroni, ambos ao nível de significância de 0,05. Para avaliar a influência das dietas com diferentes teores proteicos sobre o número de ínstares de cada espécie, foi feita a medição diária da cápsula cefálica das lagartas através do uso de microscópio digital e do *software* SFP. Posteriormente, foi plotada uma curva multimodal da distribuição de frequência, seguida de regressão linear para confirmação do número de ínstares. Para *S. frugiperda* e *S. eridania,* as dietas D1 e D2 resultaram em viabilidades semelhantes, enquanto que, para *S. cosmioides*, a dieta mais adequada foi a D1. As dietas D1, D2 e D3 apresentaram fase larval com seis ínstares para as três espécies. A dieta artificial com redução de 25 % no teor proteíco (D2) apresenta potencial de utilização em criações de laboratório para as espécies *S. frugiperda* e *S. eridania.*
Palavras-chave: Criação massal. Cápsula cefálica. Instar. Nutrição de insetos. Proteínas.

Introduction

In the Americas, the species *Spodoptera frugiperda* (J. E. Smith, 1797), *Spodoptera eridania* (Stoll, 1782), and Spodoptera cosmioides (Walker, 1858) are highly relevant pests in several economically important crops. In Brazil, they are known as the "*Spodoptera* complex," which comprises caterpillars with a high potential for defoliation and destruction of reproductive structures (Parra et al., 2022; Bordin et al., 2023), mainly in corn, soybean, and cotton crops (Parra et al., 2022).

Currently, caterpillars of the genus *Spodoptera* are mainly controlled through the intensive use of insecticides and genetically modified plants, which can result in the selection of resistant populations (Palli et al., 2023; Ribas et al., 2022). To improve the efficiency of insect control, it is essential to develop new strategies based on Integrated Pest Management (IPM) (Mamahit & Kolondam, 2023).

The characterization and quantification of biological parameters, nutritional requirements, and insect behavior are fundamental to the development of IPM programs. These studies are facilitated by the availability of insects from mass rearing under laboratory conditions (Panizzi & Parra, 2013a).

In herbivorous insect diets, the most important nutritional components are protein and carbohydrate levels, particularly their proportions, as these components are essential for insect growth, development, reproduction, and survival, in addition to being part of the composition of enzymes and hormones (Sarate et al., 2012; Bayrak et al., 2023).

The study of artificial diets for the genus *Spodoptera* is of utmost importance, as the quantity and proportion of nutrients, such as proteins, directly influence insect development (Lamb & Loschiavo, 1981). Previous studies on artificial diets for the genus *Spodoptera*, such as that by Kasten et al. (1978), report the possibility of developing an artificial diet with reduced protein content and production cost without compromising the efficiency of *S. frugiperda* caterpillar production.

Thus, considering the relevance of the topic, the objective of this study was to evaluate the biological parameters of *S. frugiperda, S. eridania,* and *S. cosmioides* maintained on artificial diets with different protein contents.

Material and Methods

Characterization of the trial

The study was conducted at the State University of Northern Paraná (UENP), Luiz Meneghel campus, in the Entomology and Nematology Laboratory. The insects used in the experiment were obtained from laboratory colonies of *S. frugiperda, S. eridania,* and *S. cosmioides,* maintained on the diet of Greene et al. (1976) at a temperature of 24 ± 2 °C, relative humidity of 70 ± 10 %, and a photophase of 14 h. Bioassays were performed to assess viability and to determine the number of instars during the larval stage of each species.

Viability assessment

Four artificial diets were used: the diet described by Greene et al. (1976) (D1), and three modified diets containing reduced levels of the protein components wheat germ, soy protein, brewer's yeast, and powdered milk (casein) by 25% (D2), 50% (D3), and 75% (D4) (Table 1). The diets were prepared according to Parra (2001).

Table 1
Composition of artificial diets used in the laboratory rearing of *Spodoptera frugiperda, S. eridania,* and *S. cosmioides*

Component	D1	D2	D3	D4
Wheat germ	100.00 g	75.00 g	50.00 g	25.00 g
Soy protein	50.00 g	37.50 g	25.00 g	12.50 g
Brewer's yeast	62.50 g	46.88 g	31.25 g	15.63 g
Milk powder (casein)	37.50 g	28.13 g	18.75 g	9.38 g
White beans	125.00 g	125.00 g	125.00 g	125.00 g
Ascorbic acid	6.00 g	6.00 g	6.00 g	6.00 g
Sorbic acid	3.00 g	3.00 g	3.00 g	3.00 g
Vanderzant vitamin mixture	10.00 g	10.00 g	10.00 g	10.00 g
Nipagin	5.00 g	5.00 g	5.00 g	5.00 g
Tetracycline	125.00 mg	125.00 g	125.00 g	125.00 g
40% formaldehyde	6.00 mL	6.00 mL	6.00 mL	6.00 mL
Agar	20.00 g	20.00 g	20.00 g	20.00 g
Water	1700.00 mL	1700.00 mL	1700.00 mL	1700.00 mL

D1: diet by Greene et al. (1976); D2: diet with a 25% reduction in protein content; D3: diet with a 50% reduction in protein content; D4: diet with a 75% reduction in protein content.

For each artificial diet, 45 newly hatched caterpillars of each species were individually placed in plastic cups (50 mL) containing diet at approximately 1/3 of the cup's capacity, a quantity sufficient for the insect to complete the larval stage. The cups were then sealed with paraffin-coated cardboard lids, and the caterpillars were kept in the containers until the pupal stage. Larval viability (%) was assessed. Upon reaching the pupal stage, the individuals were sexed (Butt & Cantu, 1962) with the aid of a stereoscopic microscope (Nikon© SM-5). Finally, they were returned to the cups until adult emergence. Pupal viability (%) and adult viability (%) were then determined.

The largest possible number of couples was formed for each treatment, using adults that emerged within a maximum interval of 24 h. The couples were kept in PVC tube cages (20 cm high × 10 cm in diameter). The inner walls of the tubes were lined with sulfite paper (used as an oviposition substrate), the tops were covered with tulle fabric secured with an elastic band, and the bases were placed on Styrofoam plates (15 cm × 15 cm) also lined with sulfite paper. Adults were fed a 10% honey solution provided on small cotton rolls soaked in water and placed in 12 mL glass jars, which were replaced every two days. Eggs laid by the couples were collected daily and stored in 100 mL plastic cups until hatching.

Egg viability (%) was subsequently determined. The experiments were conducted in a climate-controlled rearing room at a temperature of 24 ± 2 °C, relative humidity of 70 ± 10 %, and a photophase of 14 h. The experimental design was completely randomized, with 45 replicates, each caterpillar representing one replicate.

Assessment of number of instars

To evaluate the influence of nutrition on the number of instars in *S. frugiperda, S. eridania,* and *S. cosmioides caterpillars,* 250 newly hatched caterpillars of each species were used for each artificial diet (D1, D2, D3, and D4). Five neonates were placed in each plastic cup (50 mL) containing diet up to half of its capacity, and the cups were closed with paraffin-coated cardboard lids. The caterpillars were maintained in a climate-controlled rearing room at a temperature of 24 ± 2 °C, relative humidity of 70 ± 10 %, and a photophase of 14 h until they reached the pupal stage.

To measure the head capsule, five randomly selected caterpillars from each treatment were killed daily by freezing until the pre-pupal stage. Dead caterpillars were examined to facilitate measurement (Parra & Haddad, 1989).

Head capsules were measured using photographic images obtained with a digital microscope (Knup® KP-8012), positioned at a 90º angle relative to the caterpillar, with variable height adjusted to the size of the capsule being measured. Measurements were performed using SFP software (software for determining areas), version 1.5.2 (Sachs et al., 2005), by evaluating the maximum width of the head capsule.

Data analysis

The viability data for the larval, pupal, adult, and egg stages were subjected to the non-parametric Kruskal-Wallis test followed by the Bonferroni post hoc test, both at a significance level of 0.05. Statistical analyses were performed using RStudio software (R Core Team [R], 2022).

To determine the number of caterpillar instars, a multimodal curve of the frequency distribution of daily head capsule width was plotted, in which the probable number of instars corresponded to the number of peaks observed in the distribution. The number of instars was then confirmed using linear regression (Parra & Haddad, 1989), with the hypothesis considered valid based on the following parameters: high coefficient of determination (R^2) of the linear regression, absence of overlap in the confidence intervals of head capsule width means between successive instars, and agreement between the estimated growth ratio (K) and the K variation interval proposed by Dyar (1890).

Results and Discussion

Viability of Spodoptera frugiperda, S. eridania, and S. cosmiodes

The viability of the larval, pupal, adult, and egg stages of *Spodoptera frugiperda, S. eridania,* and *S. cosmioides* fed different diets is presented in Table 2.

Table 2
Viability (%) of the larval, pupal, and adult stages and eggs of *Spodoptera frugiperda, S. eridania,* and *S. cosmioides* fed artificial diets with different protein contents *under laboratory conditions* (24 ± 2 ºC, RH of 70 ± 10%, and photophase of 14 h)

Species	Viability	Artificial diet			
		D1	D2	D3	D4
S. frugiperda	Larval stage (%)	78.9a	77.8a	67.8a	45.6b
	Pupal stage (%)	84.5a	68.6ab	55.7bc	31.7c
	Adult stage (%)	98.3a	97.9a	100.0a	100.0a
	Eggs (%)	86.8a	96.1a	43.1b	68.5ab
S. eridania	Larval stage (%)	73.3a	83.3a	65.6ab	54.4b
	Pupal stage (%)	36.4ab	40.0a	44.1a	16.3b
	Adult stage (%)	91.7a	96.7a	96.2a	100.0a
	Eggs (%)	0.0c	-	79.7b	100.0a
S. cosmioides	Larval stage (%)	74.4a	48.9b	47.8b	30.0c
	Pupal stage (%)	73.1a	65.9ab	44.2b	63.0ab
	Adult stage (%)	98.0a	89.7a	94.7a	100.0a
	Eggs (%)	100.0a	91.0a	0.0b	0.0b

*Means followed by the same letter in the row do not differ from each other according to the Kruskal-Wallis test with Bonferroni correction at 5% significance.

Diets D1 (without reduction in protein content), D2 (with a 25% reduction in protein content), and D3 (with a 50% reduction in protein content) did not differ statistically from each other in terms of larval viability for *S. frugiperda* and *S. eridania.* In the pupal stage, D1 and D2 did not differ statistically for *S. frugiperda* and *S. cosmioides,* while for *S. eridania,* D1, D2, and D3 showed no significant differences. No statistical difference was observed among diets for adult viability in any of the three species. Regarding egg viability, D1, D2, and D4 did not differ significantly for *S. frugiperda,* whereas for *S. cosmioides,* D1 and D2 showed no statistical difference.

Although the artificial diet by Greene et al. (1976) (D1) is commonly used for rearing *S. eridania,* this treatment did not yield viable eggs, suggesting that the small number of couples formed (n = 1) for this treatment (small sample size) may have influenced the outcome. Therefore, the generation of infertile adults is attributed to chance. Due to the low viability in the pupal phase and, consequently, the reduced number of adults obtained, it was not possible to assemble couples with emergence intervals of up to 24 h for diet D2.

The 10 essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) cannot be synthesized by insects and must be acquired through the diet. In addition to being precursors in the synthesis of other amino acids, they are essential for egg production in adult insects and for the biosynthesis of proteins and enzymes, through peptide bonds. Thus, considering the value of protein based on the insect's capacity to digest amino acids as well as its content (composition), the essentiality of both aspects throughout development is evident, with high concentrations required at specific stages for satisfactory growth (Panizzi & Parra, 2013a).

Food intake and utilization are fundamental for insect growth, development, and reproduction. The quantity and quality of food consumed during the larval stage affect growth rate, development time, weight, survival, fecundity, and adult longevity. Consequently, insects that feed inadequately as larvae produce low-quality pupae and adults (Panizzi et al., 2013b; Kenis et al., 2018).

In an attempt to obtain an artificial diet suitable for rearing two biotypes of *S. frugiperda* in the laboratory, Busato et al. (2006) found that the diet with the highest protein content evaluated was the most suitable for insect development, promoting faster development, greater caterpillar mass at maximum growth, heavier pupae, and increased fecundity. *Evaluating Helicoverpa* armigera (Lepidoptera: Noctuidae) caterpillars fed on various host plants, Sarate et al. (2012) observed that diets rich in proteins and/or carbohydrates resulted in higher caterpillar weight and shorter larval periods.

Much of the mortality observed with diet D4 (with a 75% reduction in protein content), as evidenced by reduced viability in the larval stage (%), occurred during the transition from the larval to the pupal stage. This resulted in malformed and non-viable pupae, indicating an effect of insufficient protein in the diet.

When the nutritional needs of insects are not met by the food provided, their performance is impaired, leading to longer development time, reduced fecundity and fertility, and smaller adult size, which may affect their ability to mate and disperse, among other factors (Chapman, 2013). However, high protein levels may also negatively affect insect biology, as observed by Truzi et al. (2019) in *H. armigera,* confirming the importance of maintaining adequate nutritional levels.

In studies by Truzi et al. (2021) on the influence of protein levels on the development of *S. frugiperda,* the different protein concentrations resulted in similar insect development, allowing the use of artificial diets with lower protein levels for rearing. Reducing the amount of ingredients lowers the cost of diet preparation and, consequently, the overall cost of insect production. This is highly advantageous for research and the mass production of biological control agents such as *Telenomus remus* (Hymenoptera: Scelionidae) and *Baculovirus spodoptera,* since *S. frugiperda* is used as a host species in laboratory rearing (Valicente et al., 2010; Vieira et al., 2017; Truzi et al., 2021).

The amount of protein and amino acids in each protein component of the artificial diet is directly related to the insect's biological performance (Cohen, 2004). Thus, in the present study, the variation in survival among *S. frugiperda, S. eridania,* and *S. cosmioides* maintained on different diets was possibly due to the protein concentrations and, mainly, to differences in the protein:carbohydrate ratio in the artificial diets.

The diet proposed by Greene et al. (1976) does not contain specific carbohydrate sources, such as sucrose, glucose, and fructose (Parra, 2013). However, this diet includes protein-rich components such as white beans (19 g of protein/100 g), wheat germ (23 g of protein/100 g), powdered milk (23 g of protein/100 g), brewer's yeast (43 g of protein/100 g), and soybean meal (35 g of protein/100 g) (United States Department of Agriculture [USDA], 2023). Diet D2, with a 25% reduction in protein content compared to D1, showed similar viabilities to D1 for *S. frugiperda* and *S. eridania,* indicating that an adequate protein:carbohydrate ratio was maintained for both species.

According to Panizzi and Parra (2013a), an ideal artificial diet for the mass rearing of insects should provide high larval viability, result in adults with high reproductive capacity, be suitable for more than one species, include low-cost components that are readily available on the market, ensure total viability above 75%, and maintain insect quality over generations.

Although none of the diets evaluated achieved total viability above 75% for the three species studied, diet D2 showed similar viability to D1 (the standard diet used for rearing the *Spodoptera* genus) for *S. frugiperda* and *S. eridania,* demonstrating potential for use in laboratory rearing of these two species.

Nonetheless, for *S. cosmioides,* all diets except D1 resulted in low larval viability, indicating that this species requires a higher protein intake compared to the other two species. It is important to note that the present study was conducted over only one generation, making it necessary to investigate the influence of artificial diets with varying protein levels across successive generations of the *Spodoptera* genus.

Number of instars

This study was the first to use SFP software version 1.5.2 (Sachs et al., 2005) for measuring caterpillar head capsules. The measurement results were satisfactory, suggesting that the software is a viable alternative to the use of micrometric ocular lenses attached to a stereoscopic microscope, which remains the most commonly used method for measuring head capsules (Parra & Haddad, 1989).

Figures 1, 2, and 3 illustrate the multimodal distribution curves of head capsule widths (mm) of *S. frugiperda, S. eridania,* and *S. cosmioides* caterpillars fed different artificial diets, respectively. It was not possible to determine the number of instars in caterpillars fed diet D4 using the methodology applied, due to low larval viability and inadequate insect development. For all three species, six peaks indicated by arrows were observed in diets D1, D2, and D3, suggesting the occurrence of six instars during the larval phase.

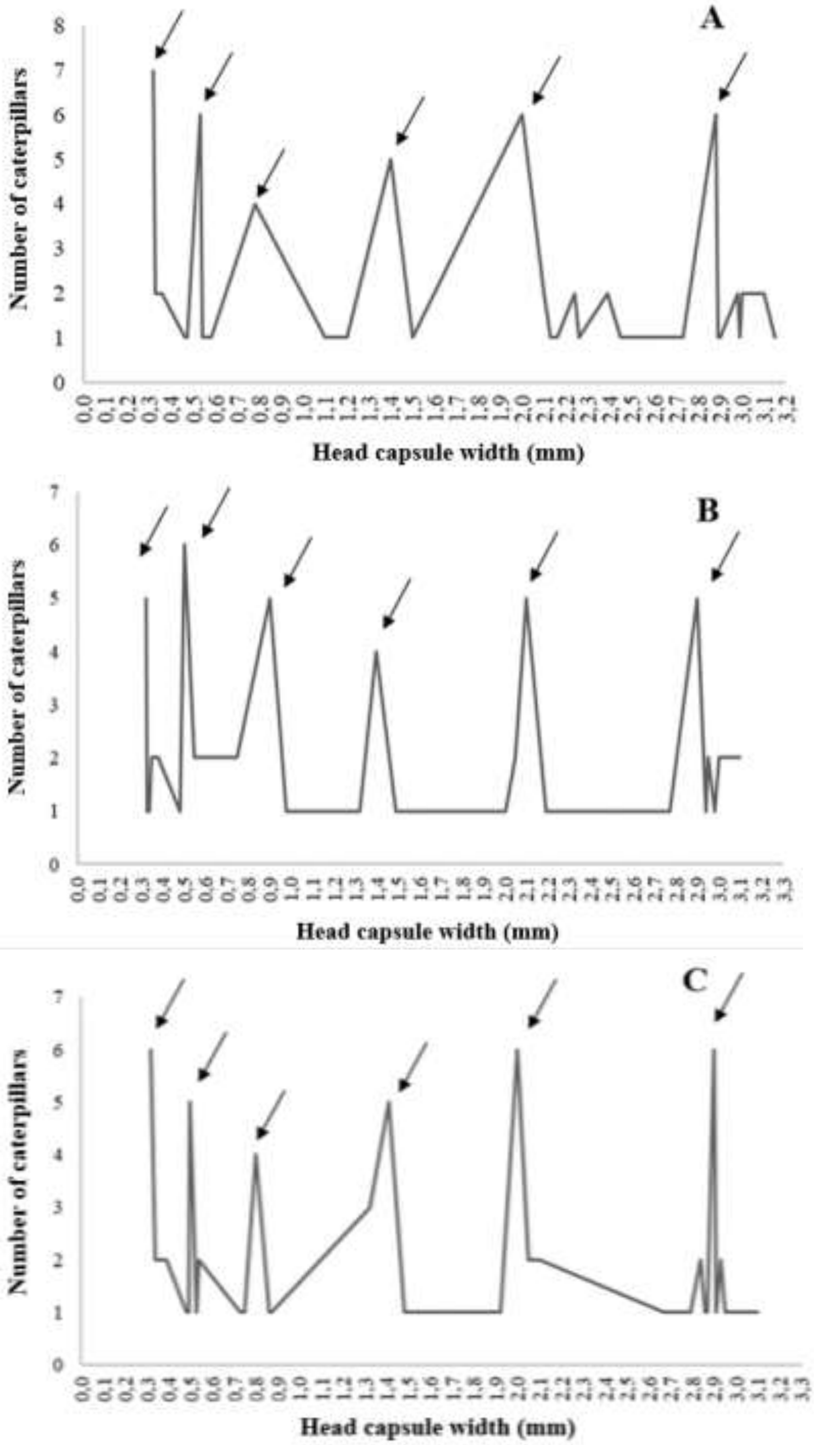

Figure 1. Distribution curve of head capsule width (mm) in *Spodoptera frugiperda* caterpillars fed diets with different protein contents and kept under laboratory conditions (24 ± 2 ºC, RH of 70 ± 10%, and photophase of 14 h).
A: artificial diet by Greene et al. (1976) (n=70). B: artificial diet with a 25% reduction in protein content (n=70). C: artificial diet with a 50% reduction in protein content (n=75). Arrows indicate the probable instars.

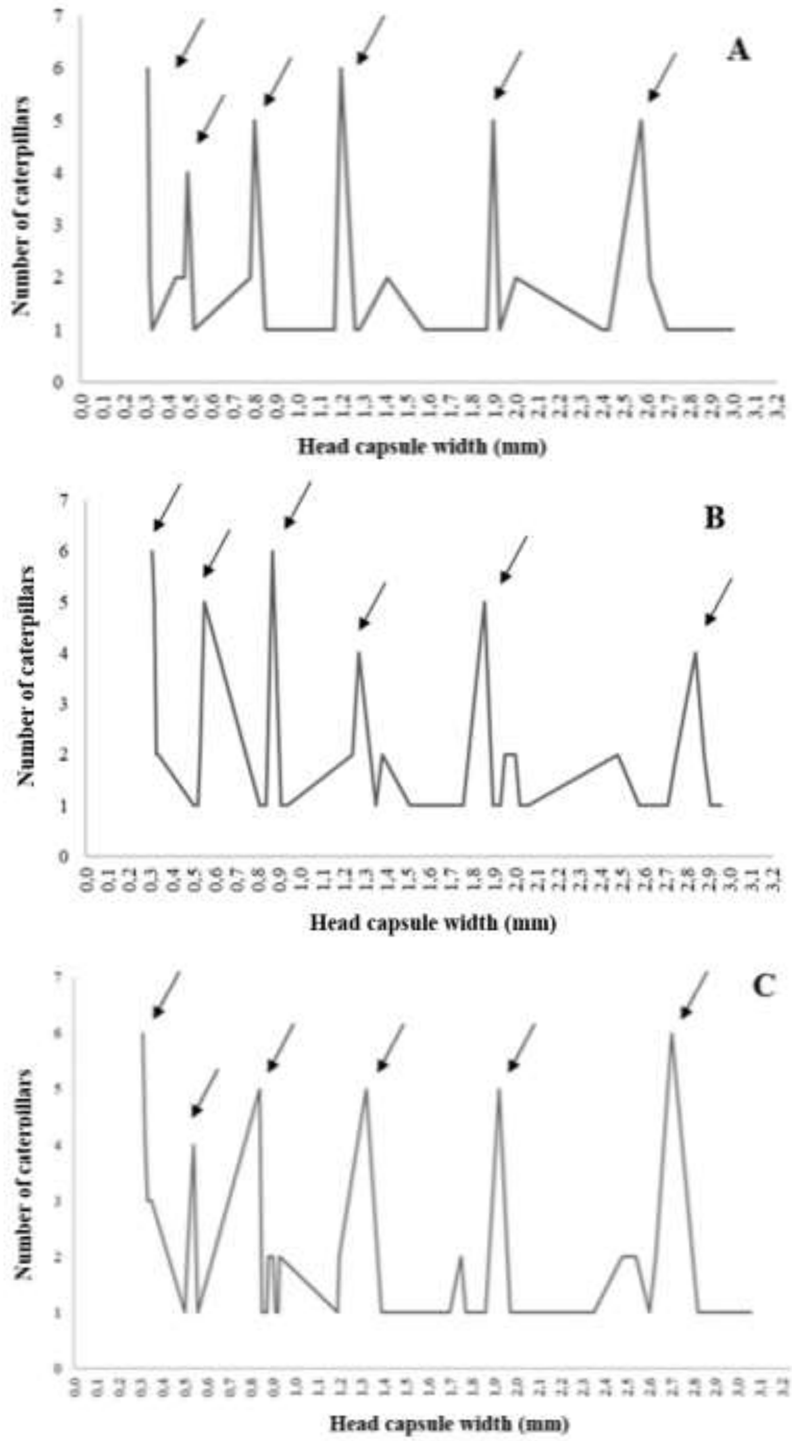

Figure 2. Multimodal distribution curve of head capsule width (mm) in *Spodoptera eridania* caterpillars fed diets with different protein contents and kept under laboratory conditions (24 ± 2 ºC, RH of 70 ± 10%, and photophase of 14 h).
A: artificial diet by Greene et al. (1976) (n=75). B: artificial diet with a 25% reduction in protein content (n=80). C: artificial diet with a 50% reduction in protein content (n=90). Arrows indicate the probable instars.

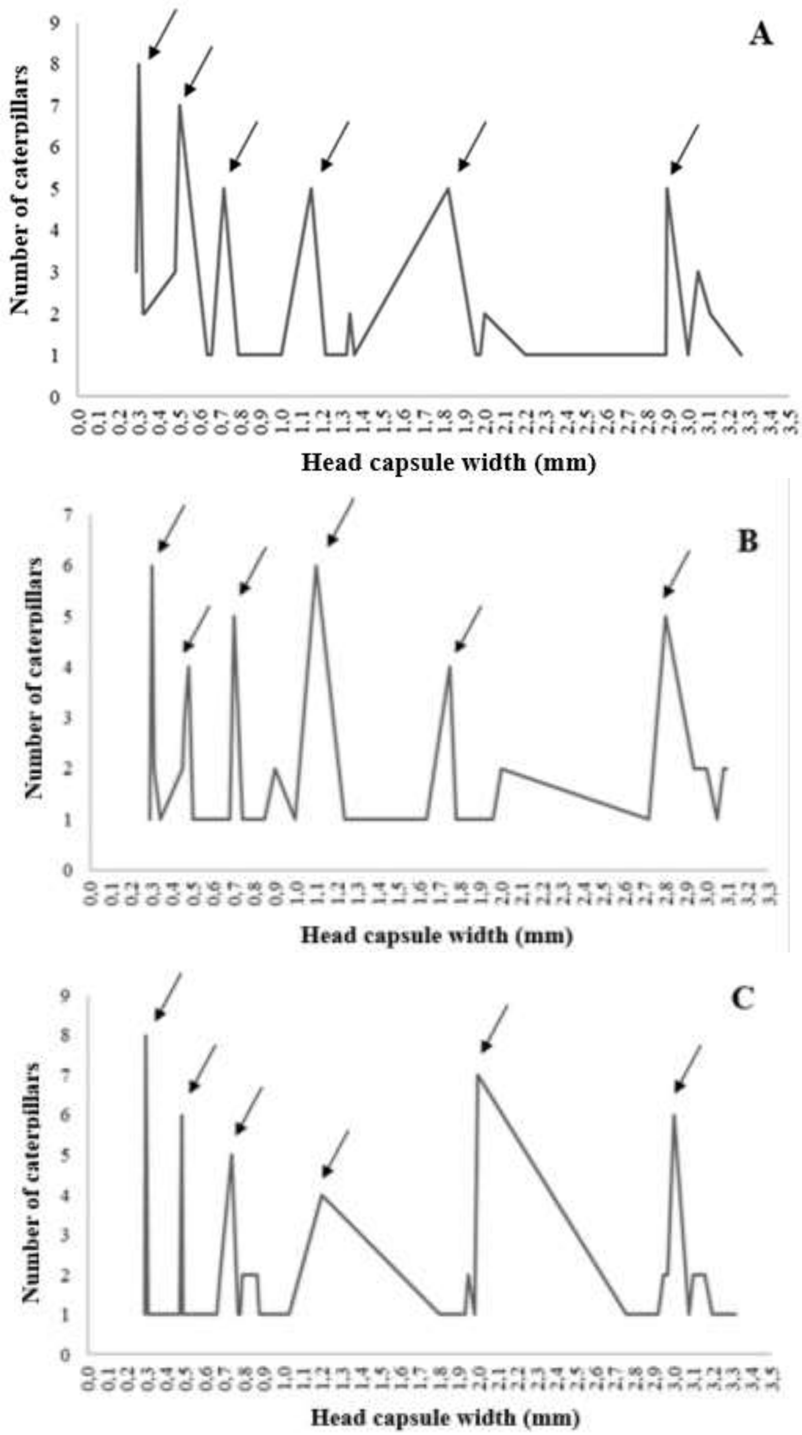

Figure 3. Multimodal distribution curve of head capsule width (mm) in *Spodoptera cosmioides* caterpillars fed diets with different protein contents and kept under laboratory conditions (24 ± 2 ºC, RH of 70 ± 10%, and photophase of 14 h).
A: artificial diet by Greene et al. (1976) (n=80). B: artificial diet with a 25% reduction in protein content (n=75). C: artificial diet with a 50% reduction in protein content (n=85). Arrows indicate the probable instars.

Based on the multimodal distribution curves, Tables 3, 4, and 5 were generated, containing the number of instars, head capsule width and mean width (mm), confidence interval (CI), instar duration (days), and growth ratio (K) for *S. frugiperda, S. eridania,* and *S. cosmioides* caterpillars.

Table 3
Number of instars, head capsule width range and mean, confidence interval (CI), instar duration, and growth ratio (K) of *Spodoptera frugiperda* caterpillars fed artificial diets containing different protein levels under laboratory conditions (24 ± 2 ºC, RH of 70 ± 10%, and photophase of 14 h)

Artificial diet	Instars	Range (mm)	Mean (mm)	CI (P<0.05)	Duration (days)	K
D1	I	0.32-0.36	0.33	0.33-0.34	3.00	-
	II	0.46-0.58	0.52	0.52-0.53	1.33	1.57
	III	0.78-1.10	0.84	0.80-0.87	1.67	1.60
	IV	1.18-1.50	1.36	1.33-1.39	2.22	1.63
	V	2.00-2.67	2.20	2.17-2.24	2.78	1.62
	VI	2.72-3.10	2.93	2.92-2.96	3.00	1.33
	Mean K	1.55				
	R^2	0.992				
D2	I	0.32-0.39	0.35	0.34-0.35	3.00	-
	II	0.48-0.55	0.51	0.50-0.51	1.78	1.47
	III	0.86-1.02	0.89	0.87-0.91	2.05	1.75
	IV	1.32-1.74	1.49	1.47-1.52	2.15	1.68
	V	2.00-2.77	2.26	2.24-2.29	2.33	1.52
	VI	2.90-3.10	2.96	2.94-2.98	2.69	1.31
	Mean K	1.54				
	R^2	0.989				
D3	I	0.32-0.39	0.35	0.34-0.36	3.00	-
	II	0.48-0.54	0.51	0.50-0.52	2.00	1.44
	III	0.73-0.87	0.80	0.78-0.82	2.00	1.58
	IV	1.32-1.49	1.43	1.40-1.45	2.53	1.78
	V	1.92-2.79	2.34	2.25-2.42	2.36	1.64
	VI	2.84-3.10	2.95	2.94-2.97	3.11	1.26
	Mean K	1.54				
	R^2	0.989				

D1: artificial diet by Greene et al. (1976). D2: artificial diet with a 25% reduction in protein content. D3: artificial diet with a 50% reduction in protein content.
K: growth ratio.
R^2: coefficient of determination.

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Table 4
Number of instars, head capsule width range and mean, confidence interval (CI), instar duration, and growth ratio (K) of *Spodoptera eridania* caterpillars fed artificial diets containing different protein levels under laboratory conditions (24 ± 2 ºC, RH of 70 ± 10%, and photophase of 14 h)

Artificial diet	Instars	Range (mm)	Mean (mm)	CI (P<0.05)	Duration (days)	K
D1	I	0.31-0.48	0.34	0.33-0.35	2.44	
	II	0.49-0.78	0.50	0.49-0.50	2.06	1.47
	III	0.80-1.17	0.82	0.81-0.83	2.63	1.66
	IV	1.20-1.87	1.30	1.28-1.33	2.50	1.59
	V	1.90-2.43	1.90	1.89-1.91	2.30	1.46
	VI	2.58-3.00	2.65	2.62-2.68	3.07	1.40
	Mean K	1.55				
	R^2	0.992				
D2	I	0.31-0.34	0.32	0.32-0.33	3.00	
	II	0.50-0.55	0.53	0.53-0.54	2.00	1.67
	III	0.81-0.94	0.88	0.88-0.89	2.35	1.65
	IV	1.24-1.55	1.36	1.34-1.38	3.02	1.53
	V	1.76-2.68	2.09	2.04-2.14	2.25	1.54
	VI	2.70-2.96	2.83	2.82-2.85	3.38	1.36
	Mean K	1.55				
	R^2	0.993				
D3	I	0.31-0.35	0.33	0.32-0.33	3.88	
	II	0.50-0.56	0.53	0.52-0.54	2.00	1.62
	III	0.84-0.93	0.88	0.87-0.88	3.13	1.66
	IV	1.19-1.77	1.43	1.40-1.47	3.08	1.63
	V	1.84-2.56	2.16	2.11-2.21	2.37	1.51
	VI	2.60-3.06	2.75	2.73-2.78	3.54	1.27
	Mean K	1.54				
	R^2	0.985				

D1: artificial diet by Greene et al. (1976). D2: artificial diet with a 25% reduction in protein content. D3: artificial diet with a 50% reduction in protein content.
K: growth ratio.
R^2: coefficient of determination.

Table 5
Number of instars, head capsule width range and mean, confidence interval (CI), instar duration, and growth rate (K) of *Spodoptera cosmioides* caterpillars fed artificial diets containing different protein levels under laboratory conditions (24 ± 2 ºC, RH of 70 ± 10%, and photophase of 14 h)

Artificial diet	Instars	Range (mm)	Mean (mm)	CI (P<0.05)	Duration (days)	K
D1	I	0.28-0.33	0.30	0.30-0.31	3.50	
	II	0.48-0.50	0.49	0.49-0.50	2.00	1.62
	III	0.64-0.82	0.73	0.72-0.74	2.33	1.48
	IV	1.00-1.36	1.22	1.20-1.24	1.97	1.66
	V	1.82-2.20	1.92	1.90-1.95	2.87	1.58
	VI	2.81-3.26	3.00	2.98-3.03	3.33	1.56
	Mean K	1.58				
	R^2	0.997				
D2	I	0.29-0.34	0.31	0.30-0.31	2.50	
	II	0.45-0.50	0.47	0.47-0.48	2.23	1.54
	III	0.66-0.85	0.73	0.72-0.74	2.00	1.55
	IV	0.90-1.33	1.09	1.06-1.11	2.46	1.50
	V	1.64-2.00	1.82	1.80-1.85	2.48	1.68
	VI	2.72-3.10	2.94	2.92-2.96	3.33	1.61
	Mean K	1.57				
	R^2	0.998				
D3	I	0.29-0.31	0.30	0.29-0.30	3.21	
	II	0.45-0.68	0.53	0.52-0.55	2.32	1.78
	III	0.71-0.88	0.78	0.77-0.79	2.32	1.46
	IV	1.03-1.87	1.36	1.27-1.44	1.79	1.74
	V	1.93-2.89	2.17	2.10-2.23	3.45	1.60
	VI	2.92-3.32	3.05	3.04-3.06	3.91	1.41
	Mean K	1.60				
	R^2	0.984				

D1: artificial diet by Greene et al. (1976). D2: artificial diet with a 25% reduction in protein content. D3: artificial diet with a 50% reduction in protein content.
K: growth ratio.
R^2: coefficient of determination.

For *S. frugiperda,* diets D1, D2, and D3 showed coefficients of determination (R^2) of 0.992, 0.989, and 0.989, respectively, indicating good model fit across all diets. The mean K per instar was 1.55, 1.54, and 1.54 for diets D1, D2, and D3, respectively within the range established by Dyar (1890), whose rule states that the head capsule width increases geometrically with each molt, with an average ratio of 1.4, varying between 1.1 and 1.9 (Parra & Haddad, 1989).

In the case of *S. eridania,* R^2 was 0.992, 0.993, and 0.985, while K averaged 1.51, 1.55, and 1.54 for diets D1, D2, and D3, respectively again within the expected range based on Dyar's rule.

For *S. cosmioides,* R^2 values were 0.997, 0.998, and 0.984, and the K means were 1.58, 1.57, and 1.60 for diets D1, D2, and D3, respectively, consistent with the range described by Dyar (1890).

The number of instars is not fixed and typically varies between four and eight in most insect species (Parra, 2001). Several factors beyond species-specific traits can influence the number of instars, including temperature, food quality and quantity, rearing density, and sex, among others (Parra & Haddad, 1989).

Under conditions similar to those in the present study, Montezano et al. (2014) and Specht and Roque-Specht (2016) reported a predominance of six instars in *S. eridania* and *S. cosmioides,* with 3.44% and 4.80% of females having seven instars, respectively.

Using the artificial diet developed by Greene et al. (1976) under similar conditions, Montezano et al. (2019) observed six larval instars in *S. frugiperda.* According to a survey conducted by the same authors, larval development in this species is highly variable, with five to ten instars reported in the literature. This variation is attributed to the biological plasticity of *S. frugiperda,* which enhances its ability to develop and survive under adverse conditions (Esperk et al., 2007).

Although there is no direct correlation between the duration of the cycle and number of instars (Parra, 2001), an increased number of instars may indicate nutritional inadequacy (Parra & Haddad, 1989), since insects may add an instar to compensate for reduced performance when fed a lower-quality diet (Mattana & Foerster, 1988). In the present study, diets D1, D2, and D3 resulted in six instars during the larval period for all three species under the evaluated conditions, indicating that the reduction in protein content did not alter this parameter.

Conclusion

The artificial diet with a 25% reduction in protein content supported development similar to that provided by the diet of Greene et al. (1976) for *Spodoptera frugiperda* and *S. eridania,* indicating its potential for use in the mass rearing of these species in the laboratory, with the advantage of reduced production costs. *Spodoptera cosmioides,* however, did not exhibit satisfactory development on diets with reduced protein content.

Acknowledgments

The authors would like to thank CAPES (Coordination for the Improvement of Higher Education Personnel) for providing fellowships and financial support.

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