Acta Scientiarum

http://periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v47i1.70329

Diets for in vivo inoculation of *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) (Lefavirales: Baculoviridae)

Lorena Contarini Machado*, Hugo José Gonçalves dos Santos Junior, Laura Vaillant Ribeiro Mauri, Julianne Almeida Rodrigues, Nathália Puppim de Oliveira Benachio, Hugo Bolsoni Zago and Carlos Eduardo Costa Paiva

Departamento de Agronomia, Universidade Federal do Espírito Santo, Alto Universitário, s/n, 25000-000, Alegre, Espírito Santo, Brazil. *Author for correspondence. E-mail: contarini.machado@gmail.com

ABSTRACT. The *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) has been studied and applied in controlling the fall armyworm, *Spodoptera frugiperda*. This pest serves as the primary host for in vivo replication of this biological control agent. For viral inoculation, the virus is introduced into an artificial diet, which is also used for large-scale host multiplication. In this study, we tested more cost-effective diets to optimize the viral inoculation stage. Each diet was treated with the virus and monitored daily for host mortality. Viral production parameters were subsequently quantified. Although the evaluated diets did not achieve the same yield levels as those used for large-scale *S. frugiperda* multiplication, the D7 diet showed similar cost-effectiveness to the D2 diet in terms of producing one dose per hectare. Additionally, larvae consuming diets higher in crude protein exhibited reduced viral polyhedra production.

Keywords: artificial diet; fall armyworm; microbial control; *Spodoptera frugiperda*.

Received on November 13, 2023
Accepted on October 29, 2024

Introduction

Baculoviruses are DNA-based viruses that specifically infect arthropods, including many species of significant agricultural importance, making them valuable tools in pest management programs (Lacey et al., 2015; Grzywacz & Moore, 2017; Hussain et al., 2021). The coevolution of baculoviruses with their host species has resulted in high host specificity, allowing for targeted pest control with minimal risk to humans, the environment, or beneficial insects (Costa et al., 2019; García-Banderas et al., 2020; Hussain et al., 2021; Zanella-Saenz et al., 2022).

The *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) has been widely studied and utilized as a biological insecticide for controlling *S. frugiperda* (Lepidoptera: Noctuidae) larvae, commonly known as the fall armyworm (Barrera et al., 2011; Hussain et al., 2021; Stinguel et al., 2022; Velasco et al., 2022). Despite advances in in vitro production techniques, the commercial production of SfMNPV faces challenges due to issues like virus strain instability in cell cultures, production yield limitations, and high costs (Claus et al., 2012; Reid et al., 2014; Lacey et al., 2015). As a result, in vivo production remains the primary method for producing SfMNPV-based bioinsecticides, which necessitates the large-scale rearing of host insects (Valicente et al., 2010; Grzywacz & Moore 2017).

In vivo production of viral polyhedra is influenced by multiple factors, including larval cannibalism, temperature, food availability, larval stage, density, and the timing of infected larva collection (Elvira et al, 2010a, 2010b; Valicente et al., 2013; Zamora-Avilés et al., 2017; Ramírez-Arias et al., 2019; Rios-Velasco et al., 2012; Stinguel et al., 2022; Velasco et al., 2022). These variables offer numerous avenues for optimizing production processes. For instance, since the primary route of larval infection is through virus ingestion, the type of food used during in vivo inoculation of the pathogen plays a critical role. Currently, an artificial diet formulated specifically for mass rearing the hos is typically used for viral inoculation, except when treated with formaldehyde, which inactivates the virus (Machado et al., 2022; Stinguel et al., 2022).

A contamination-free and nutritionally balanced diet is essential for mass rearing of host insects in the laboratory. However, during the virus inoculation stage, when infected hosts do not complete their life cycle, the use of such high-quality artificial diets can be wasteful. After infection, larvae continue to feed for a short

period before gradually reducing intake as the infection progresses (Silva & Parra 2013; Pinto et al., 2019; Truzi et al., 2021).

Therefore, investigation of diets that are both cost-effective and capable of producing high yields of viral polyhedra could provide an alternative to the artificial diet currently used for host rearing during the virus inoculation phase (Truzi et al., 2021). Such a reduction in production costs would increase the market competitiveness of SfMNPV-based organic products (Grzywacz & Moore, 2017). While there is a substantial literature on artificial diets for large-scale host rearing (Silva & Parra, 2013; Pinto et al., 2019; Truzi et al., 2021), there is a notable gap in studies focusing on more economical diets specifically designed for SfMNPV inoculation that still support strong viral polyhedra production. This research aims to investigate artificial diets for the in vivo viral inoculation phase of SfMNPV to identify viable, cost-effective options.

Material and methods

Trials were conducted at Laboratory of Microbial Insect Control within the Entomology Sector of *Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas e Doenças* (NUDEMAFI). This facility is located in the Center for Agricultural Sciences and Engineering at *Universidade Federal do Espírito Santo* (CCAE/UFES) in the city of Alegre, Espírito Santo, Brazil.

Mass multiplication and maintenance of *Spodoptera frugiperda*

The multiplication and maintenance of *Spodoptera frugiperda* were performed in a climate-controlled BOD incubator set at 25 ± 2°C with a relative humidity of 60% and a 12-hour photoperiod. Adults were housed in PVC cages and fed with absorbent cotton soaked in a 10% sucrose solution. For oviposition, white paper sheets lined the inside of the cages, which were replaced every two days to collect the eggs. Eggs were transferred to transparent plastic jars and kept there until hatching. Neonate larvae were then moved, using a soft-bristled brush, to 100 mL plastic containers with an adapted artificial diet made from beans, wheat germ, brewer's yeast, and carrageenan, as described by Nalim (1991 apud Souza et al., 2001). Larvae were kept on this diet for five days. Approximately 40 larvae were then transferred to a larger Gerbox® acrylic container (11 × 11 × 3 cm). After 10 days, the larvae were individualized into smaller Gerbox® containers (3 cm in diameter) until they pupated. Upon emergence as adults, they were returned to the rearing cages to continue the cycle.

Obtaining and producing the SfMNPV

For this study, we used Isolate 6 of the SfMNPV, sourced from the Entomopathogen Bank of the Laboratory of Biological Control at *Embrapa Milho e Sorgo*. This isolate was chosen because it does not cause immediate tegument liquefaction after insect death, thereby reducing the loss of viral polyhedra (Vieira et al., 2012).

Virus multiplication began with an initial suspension at 1×10^8 occlusion bodies (OB) mL^{-1}. Pieces of artificial diet (without formaldehyde) were dipped in this suspension and provided to *S. frugiperda* larvae. Larvae were kept in individual 50 mL plastic containers, with fresh artificial diet added as needed. After infection and subsequent death, cadavers were collected and frozen for later maceration and purification.

The purification process followed a modified version of the method described by Hashimoto et al. (2000). Infected larvae were macerated in autoclaved distilled water containing 1% sodium dodecyl sulfate (SDS) and filtered through voile cloth to separate fatty tissues and coarse particles from the liquid. Filtrate was then centrifuged at 6000 rpm for 20 minutes, a step repeated three times. After the final centrifugation, the resulting precipitate was resuspended in sterile distilled water and stored at 4°C for use in subsequent experiments.

Selection of inoculation diet for SfMNPV

The artificial diet typically used for mass rearing of *S. frugiperda*, referred to as Diet D1, was used as a control. Diet D2, which is Diet D1 without added formaldehyde, served as a baseline comparison against the new diets proposed in this study for viral inoculation (Table 1). Selection was conducted in three stages. In the first stage, diets D3 through D7 were tested. In the second stage, diets D8 through D13 were evaluated. Finally, in the third stage, D7 (selected from stage one), D11 (selected from stage two), D14, and D15 were compared (Tables 1, 2, and 3 for details on each diet composition and selection criteria).

After preparing the diets, a 10 mL aliquot was poured into 50 mL plastic cups, forming the experimental units. Treatment D1 served as the control, while the other treatments received a 100 µL aliquot of the SfMNPV

viral suspension (Isolate 6) pipetted beneath the diet surface at a concentration of 1×10^8 OB mL^{-1}. This concentration was determined by counting the OBs in a Neubauer chamber under an optical microscope at 400 × magnification.

Table 1. Components of the artificial *Spodoptera frugiperda* multiplication diet (D1 and D2) and the proposed diets for SfMNPV viral inoculation (D3, D4, D5, D6 and D7) of the first stage.

Ingredients	Artificial diet		Diet for viral inoculation				
	D1	D2	D3	D4	D5	D6	D7
	($g\ L^{-1}$)		($g\ L^{-1}$)				
Water (mL)	1000	1000	1000	1000	1000	1000	1000
Beans (g)	138	138	-	-	-	-	-
Wheatgerm (g)	66.28	66.28	-	-	-	-	66.28
Brewer's yeast (g)	42.26	42.26	-	42.26	-	-	-
Ascorbic acid (g)	4.27	4.27	4.27	4.27	4.27	4.27	4.27
Sorbic acid (g)	1.38	1.38	-	-	-	-	-
Nipagin (g)	2.64	2.64	2.64	2.64	2.64	2.64	2.64
Formaldehyde (mL)	10.46	-	-	-	-	-	-
Tetracycline (g)	0.209	0.209	-	-	-	-	-
Carrageenan (g)	13.63	13.63	30	30	30	30	30
Maize flour (g)	-	-	30	30	-	-	-
Soy protein (g)	-	-	-	-	32.5	-	-
Soybean meal (g)	-	-	-	-		21.43	-
Diet cost (BRL L^{-1})	13.91	13.61	7.49	9.45	8.09	7.76	8.98

Table 2. Components of the artificial *Spodoptera frugiperda* multiplication diet (D1 and D2) and the proposed diets for SfMNPV viral inoculation (D8, D9, D10, D11, D12 and D13) of the second stage.

Ingredients	Artificial diet		Diet for viral inoculation					
	D1	D2	D8	D9	D10	D11	D12	D13
	($g\ L^{-1}$)		($g\ L^{-1}$)					
Water (mL)	1000	1000	1000	1000	1000	1000	1000	1000
Beans (g)	138	138	-	-	-	-	-	-
Wheatgerm (g)	66.28	66.28	-	-	-	-	-	-
Brewer's yeast (g)	42.26	42.26	-	-	-	-	-	-
Ascorbic acid (g)	4.27	4.27	-	-	-	-	-	-
Sorbic acid (g)	1.38	1.38	-	-	-	-	-	-
Nipagin (g)	2.64	2.64	2.64	2.64	2.64	2.64	2.64	2.64
Formaldehyde (mL)	10.46	-	-	-	-	-	-	-
Tetracycline (g)	0.209	0.209	-	-	-	-	-	-
Carrageenan (g)	13.63	13.63	25	25	25	25	25	25
Casein (g)	-	-	30	30	-	-	-	-
Milk powder (g)	-	-	-	-	30	30	-	-
Albumin (g)	-	-	-	-	-	-	30	30
Vitamin solution (mL)			-	9	-	9	-	9
Diet cost (BRL L^{-1})	13.91	13.61	35.91	36.04	7.27	7.41	9.07	9.21

Table 3. Components of the artificial *Spodoptera frugiperda* multiplication diet (D1 and D2) and the proposed diets for SfMNPV viral inoculation (D7, D11, D14 and D15) of the third stage.

Ingredients	Artificial diet		Diet for viral inoculation			
	D1	D2	D7	D11	D14	D15
	($g\ L^{-1}$)		($g\ L^{-1}$)			
Water (mL)	1000	1000	1000	1000	1000	1000
Beans (g)	138	138	-	-	138	69.04
Wheatgerm (g)	66.28	66.28	66.28	-	66.28	33.14
Brewer's yeast (g)	42.26	42.26	-	-	42.26	21.13
Ascorbic acid (g)	4.27	4.27	4.27	-	4.27	2.13
Sorbic acid (g)	1.38	1.38	-	-	4.00	4.00
Nipagin (g)	2.64	2.64	2.64	2.64	-	-
Formaldehyde (mL)	10.46	-	-	-	-	-
Tetracycline (g)	0.209	0.209	-	-	-	-
Carrageenan (g)	13.63	13.63	30	25	15	15
Milk powder (g)	-	-	-	30	-	-
Vitamin solution (mL)			-	9	-	-
Diet cost (BRL L^{-1})	13.91	13.61	8.98	7.41	10.34	7.71

Each sample unit was inoculated with one *Spodoptera frugiperda* larvae. The assay was conducted separately with 8-day-old larvae reared at 25 ± 1°C and then maintained in a controlled room at the same temperature. Each treatment consisted of 8 replicates, each containing 12 insects, in a completely randomized design.

The following parameters were evaluated: percentage of larvae mortality; mass of dead larvae (g) per replicate; total occlusion bodies (Total OB): calculated as the number of polyhedra counted in the Neubauer chamber multiplied by the sample volume (10 mL); occlusion bodies per larvae (OB larvae^{-1}): total OB divided by the number of larvae collected in each replicate; larvae equivalent per hectare (LE ha^{-1}): calculated as 3 x 10^{11} OB per larvae in each replicate (Note: LE ha^{-1} represents the estimated number of larvae required to produce 3 x 10^{11} OB, which is the bioinsecticide dose recommended per hectare for effective pest control (MAPA, 2015); occlusion bodies per gram (OB g^{-1}): total OB divided by the weight of larvae collected in each replicate; and occlusion bodies per 100 dead larvae (OB 100^{-1} larvae): mortality percentage multiplied by OB per larvae.

Data were analyzed using analysis of variance and means were compared using the Scott-Knott test at a 5% probability level.

Determination of crude protein content of larvae fed inoculation diet

To determine the crude protein content in *Spodoptera frugiperda* larvae fed various inoculated diets, 8-day-old larvae were used and fed for 7 days on diets D2, D7, D11, D14, and D15. After this feeding period, the larvae were frozen and then dried in an oven at 40°C for 72 hours until reaching a constant weight. Dried samples were then macerated, weighed between 0.3 g and 0.5 g each, and analyzed in triplicate using paper napkins. Samples were placed in digestion tubes along with 2 g of a catalyst mixture (copper sulfate and potassium sulfate in a 1:10 ratio) and 8 mL of sulfuric acid PA. Tubes were then heated on a hot plate, gradually increasing the temperature from 50°C in 50°C increments every half hour until reaching 350°C. This gradual heating prevented sample loss and ensured complete decomposition of organic matter, indicated by a color change from black to pale blue.

After digestion, the tubes were removed from the hot plate, allowed to cool, and 10 mL of distilled water was added. Tubes were then placed in a Kjeldahl nitrogen distiller for distillation. During this step, the solution was neutralized by slowly adding 50% NaOH until the solution turned black and a precipitate formed. This addition had to be done carefully due to the exothermic nature of the reaction, which releases intense heat.

Simultaneously, a 50 mL receptor solution containing 4% boric acid with a mixed indicator (methyl red and bromocresol green) was prepared in an Erlenmeyer flask. The ammonia in the sample, carried over in the vapor, was absorbed in this solution to form ammonium borate. The endpoint of the reaction was indicated by a color change of the solution from pink to green.

The ammonium borate solution was then titrated with 0.1 M HCl until pink. A 'blank' sample containing all reagents except the sample was also prepared and subjected to the same procedure to account for any possible interference or contamination of the reagents.

After recording the mass of the samples and the volume of HCl used in the titration, calculations were performed to determine the crude protein (CP) content in the samples:

$$CP\% = [(V' - V) \times F_c \times M \times 6.25 \times 0.014)/P] \times 100$$

CP% = Percentage of crude protein (total nitrogen content in the sample)
V' = Volume of HCl used in the sample titration
V = Volume of HCl used in the blank titration
F_c = Correction factor for 0.1 M HCl
M = Molarity
P = Mass of the sample (in grams)
6.25 = Nitrogen-to-protein conversion factor
0.014 = Milliequivalent-gram of nitrogen

Results and discussion

SfMNPV inoculation diet and larval age of *Spodoptera frugiperda*

The results from the bioassay evaluating diets for viral inoculation of 8-day-old *S. frugiperda* larvae reared at 25°C are shown in Tables 4, 5, and 6. Among the artificial diets tested in the initial selection phase (D3, D4, D5, D6, and D7), none fully matched the performance of the standard diet (D2), which is routinely used for

large-scale *S. frugiperda* rearing, across the evaluated parameters (Table 4). However, diets D4 (based on cornmeal and brewer's yeast) and D7 (based on wheat germ) showed potential for enhancing the production of viral polyhedra (Table 4).

Table 4. Parameters of viral polyhedra production in alternative diets of the first stage for inoculation of SfMNPV in 8-day-old larvae of *Spodoptera frugiperda* developed at a temperature of 25 ± 1°C. Photophase: 12 hours and RH: 70 ± 10%.

Diets	Viral polyhedron production parameters [1]				
	LE*	OB total^{-1} (× 10^8)[1]	OB lag^{-1} (× 10^6)	OB 100 lag^{-1} (× 10^8)	OB g^{-1} (× 10^8)
D2	113.34 ± 11.30 a	177.50 ± 16.97 a	2944.78 ± 449.81 a	1479.17 ± 141.43 a	70.2 ± 5.59 a
D3	44883.74 ± 9306.38 d	1.17 ± 0.30 d	9.83 ± 2.46 d	9.74 ± 2.47 d	2.64 ± 0.56 d
D4	298.01 ± 16.97 b	113.75 ± 5.25 b	1030.41 ± 60.13 b	947.92 ± 43.76 b	59.93 ± 2.77 b
D5	823.13 ± 95.38c	38.28 ± 6.18 c	402.27 ± 48.63 c	320.69 ± 50.71 c	23.87 ± 3.36 c
D6	922.28 ± 102.25 c	40.97 ± 4.51 c	354.15 ± 39.08 c	345.50 ± 38.10 c	28.61 ± 3.12 c
D7	234.98 ± 32.10 b	122.03 ± 20.80 b	1445.70 ± 184.07 b	1059.32 ± 181.11 b	51.31 ± 3.16 b

[1]Means followed by the same letter in the column do not differ at 5% probability level by Scott-Knott test. *LE: log (x) transformed data and the other parameters root (x).

Table 5. Parameters of viral polyhedron production in alternative diets of the second stage for inoculation of SfMNPV in 8-day-old larvae of *Spodoptera frugiperda* developed at a temperature of 25 ± 1°C. Photophase: 12 hours and RH: 70 ± 10%.

Diets	Viral polyhedron production parameters [1]*				
	LE	OB total^{-1} (× 10^8)[1]	OB lag^{-1} (× 10^8)	OB 100 lag^{-1} (× 10^8)	OB g^{-1} (× 10^8)
D2	274.21 ± 23.33 a	134.90 ± 11.35 a	11.56 ± 1.07 a	1116.01 ± 98.19 a	84.34 ± 3.30 a
D8	76601.41 ± 9460.26 d	0.55 ± 0.09 d	0.05 ± 0.007 d	4.55 ± 0.78 d	4.16 ± 0.48 d
D9	71868.08 ± 7926.16 d	0.56 ± 0.08 d	0.05 ± 0.007 d	4.68 ± 0.69 d	4.42 ± 0.76 d
D10	23545.38 ± 5628.98 b	2.26 ± 0.57 b	0.18 ± 0.004 b	18.82 ± 4.73 b	13.54 ± 2.84 b
D11	14065.05 ± 1697.22 b	3.01 ± 0.59 b	0.25 ± 0.05 b	25.05 ± 4.93 b	14.21 ± 2.15 b
D12	114853.24 ± 29511.87 d	0.49 ± 0.11 d	0.04 ± 0.009 d	4.07 ± 0.93 d	3.01 ± 0.64 d
D13	33284.48 ± 5468.69 c	1.31 ± 0.21 c	0.11 ± 0.01 c	10.91 ± 1.74 c	7.90 ± 1.42 c

[1]Means followed by the same letter in the column do not differ at 5% probability level by Scott-Knott test. *Data were transformed into log (x).

Table 6. Parameters of viral polyhedron production in alternative diets of the third stage for inoculation of SfMNPV in 8-day-old larvae of *Spodoptera frugiperda* developed at a temperature of 25 ± 1°C. Photophase: 12 hours and RH: 70 ± 10%. And crude protein percentage (CP [%]) of the larvae fed with the inoculation diets.

Diets	Viral polyhedron production parameters [1]*					CP (%)
	LE	OBtotal (× 10^8)[1]	OB lag^{-1} (× 10^8)	OB 100 lag^{-1} (× 10^8)	OB g^{-1} (× 10^8)	
D2	218.05 ± 9.77 a	158.34 ± 7.87 a	13.96 ± 0.65 a	1330.03 ± 44.19 a	67.12 ± 3.76 b	28.54 ± 3.58 b
D15	282.30 ± 28.19 a	134.75 ± 0.09 a	11.42 ± 1.20 a	1122.92 ± 105.44 a	90.12 ± 6.07 a	27.98 ± 2.99 b
D7	399.99 ± 22.39 b	90.72 ± 4.37 b	7.64 ± 0.37 b	764.35 ± 36.74 b	79.35 ± 5.04 ab	24.55 ± 2.38 b
D14	2159.01 ± 196.60 c	17.24 ± 2.14 c	1.50 ± 0.16 c	142.34 ± 17.93 c	7.16 ± 0.52 d	19.56 ± 2.11 b
D11	3360.17 ± 343.94 d	11.36 ± 12.65 d	0.94 ± 0.07 c	94.64 ± 7.69 d	41.57 ± 2.45 c	42. 25 ± 2.37 a

[1]Means followed by the same letter in the column do not differ at 5% probability level by Tukey test. *Data were transformed into log (x).

The results indicate that larvae inoculated with the D7 diet at 25°C at 8 days of age achieved productivity of approximately 1.059 × 10^{11} OB mL^{-1} for the parameter OB 100^{-1} larvae, while the standard diet (D2) yielded around 1.479 × 10^{11} OB mL^{-1} (Table 4). Despite the statistical difference between diets D2 and D7, D7 presents a cost advantage over the standard D2 diet routinely used for large-scale multiplication (Table 1). Even though the larvae equivalents (LE) were 113 and 234 larvae (Table 4) or 218 and 399 larvae (Table 6) for diets D2 and D7, respectively, the cost of producing the diet to inoculate these larvae is balanced, suggesting that D7 could be a viable option for viral inoculation.

The D3 diet, which relies solely on cornmeal as its energy source, showed the lowest viral polyhedron production (Table 4). In the second selection step, additional artificial diets (D8, D9, D10, D11, D12, and D13) were tested for viral inoculation (Table 6). None of these diets equaled the performance of the standard D2 diet across the evaluated parameters. However, diet D11, based on powdered milk and a vitamin solution, produced viral polyhedra at levels statistically closer to those achieved with the standard multiplication diet (Table 5). The D11 treatment produced approximately 2.505 × 10^9 OB mL^{-1} for OB 100^{-1} larvae, compared to 1.116 × 10^{11} OB mL^{-1} for the standard diet (Table 5).

Diets based on casein and albumin (D8, D9, and D12) yielded the lowest viral polyhedron production, ranging from 4.07 to 4.55 × 10^8 OB mL^{-1} (Table 5).

The third phase of diet selection included D7 and D11, which had performed best in the first and second rounds, respectively, along with two additional diets: D14, containing 4 g of sorbic acid and excluding formaldehyde, nipagin, and tetracycline, and D15, which contained half the ingredients of D14 (Table 6). In this final selection phase, D15 matched the standard D2 diet in supporting *S. frugiperda* multiplication. However, D15 had higher contamination rates than D7, leading to its exclusion from further consideration. The D11 diet exhibited the lowest viral productivity in this stage (Table 6).

The artificial diet D1 has been successfully used for mass rearing of *S. frugiperda* in the laboratory (Silva & Parra, 2013; Pinto et al., 2019; Machado et al., 2022; Stinguel et al., 2022), while diet D2 (without formaldehyde) has been used specifically for SfMNPV viral inoculation on the diet surface (Machado et al., 2022; Stinguel et al., 2022). Diets D1 and D2 are identical, except that D1 contains formaldehyde, which inactivates viral polyhedra. During viral inoculation, infected larvae tend to consume less food, leaving a surplus of unused substrate. This observation suggests that a simpler, lower-cost diet could be beneficial for the virus production process.

Diets evaluated in this study showed promising results for viral replication. Although there was a statistical difference between diets D2 and D7, with D2 achieving approximately 1.479×10^{11} OB mL^{-1} and D7 reaching 1.059×10^{11} OB mL^{-1} for the parameter OB 100 $larvae^{-1}$, diet D7 had a financial advantage, costing around 35% less than D2. Reducing the quantity of ingredients can significantly decrease preparation costs (Truzi et al., 2021), and in some cases, a single ingredient substitution can cut diet costs by up to 60% (Alfazairy et al., 2012; Truzi et al., 2021). Additionally, diet D7 exhibited low microbial contamination, an essential factor for viral inoculation (Subramanian et al., 2006).

Research into cost-effective diet modifications that support OB production has also been pursued for other pathosystems, such as *Spodoptera littoralis* (Lepidoptera: Noctuidae) and its nucleopolyhedrosis virus (SpliNPV) (Alfazairy et al., 2012; Sayed et al., 2021). These studies found that replacing agar with a more affordable ingredient significantly reduced diet costs per kilogram.

However, substituting ingredients, such as starch or gelatin, did not support pupal development in the diet (Alfazairy et al., 2012; Sayed et al., 2021). Nonetheless, these substitutions resulted in higher insect mortality rates when exposed to the SpliNPV virus compared to larvae fed an agar-based diet. According to the authors, gelling agents can influence viral pathogenicity, especially at lower viral concentrations. Elvira et al. (2010b) hypothesized that the release of ions or water-soluble materials associated with gelling agents could negatively impact OB activity.

This finding underscores the importance of the current study, as altering diet composition for viral inoculation may also affect viral production. Therefore, it is essential that ingredient substitutions or removals for cost reduction still preserve the characteristics needed for effective viral polyhedra production.

Research has shown that larvae of *Trichoplusia ni* (Lepidoptera: Noctuidae) exhibit varying susceptibility to *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV) based on diet during the viral inoculation period (Chen et al., 2018). The lethal dose (LD_{50}) was found to be 3.1 OBs for larvae reared on artificial diet, 16.6 OBs on cabbage leaves, and 61.6 OBs on potato leaves (Chen et al., 2018). The authors noted differences in the peritrophic membrane structure of larvae fed different diets, which affected pathogen susceptibility. Besides physical changes, diet can also influence humoral and cellular defenses or immune suppression through plant defense chemicals (Shikano et al., 2010).

Larvae reared on artificial diets had thinner, more fragile peritrophic membranes, offering little barrier to viral infection, while larvae fed cabbage and potato leaves developed thicker membranes and microvilli, which inhibited viral infection (Chen et al., 2018). In this study, since all treatments used artificial diets, physical changes in the peritrophic membrane were likely not responsible for differences in susceptibility to SfMNPV and subsequent OB production.

The main differences between treatments lie in the nutritional composition of diets. For instance, while both D15 and D14 diets included 4 g of sorbic acid, the amount of ascorbic acid in D14 was double that in D15. Ascorbic acid can affect gut pH, making it slightly more acidic, as observed by Chen et al. (2018) in *T. ni* larvae fed cabbage leaves. This may explain the difference in viral productivity, with D15 yielding 1.12×10^{11} OB 100^{-1} larvae and D14 yielding only 1.42×10^{10} OB 100^{-1} larvae, despite its richer nutritional profile. Generally, lepidopteran midgut pH ranges from 9 to 11, and an alkaline environment (above pH 8) can disrupt viral polyhedra (Chen et al., 2018). Therefore, any reduction in midgut pH may interfere with viral infectivity and OB production.

Determination of crude protein content in larvae fed inoculation diet

The crude protein content of larvae fed different inoculation diets is presented in Table 6. Diet D11 showed the highest percentage of crude protein content at 42.25%, differing from the other diets, which ranged from 19.56 to 28.54% in protein content.

Since viral polyhedra are primarily composed of a lipoprotein membrane, it was initially hypothesized that protein-rich diets would support better viral production (Lacey et al., 2015; Grzywacz & Moore 2017; Hussain et al., 2021). However, as shown in Table 6, higher crude protein levels in larvae tissues actually resulted in lower OB production.

This observation may be explained by the concept of macronutrient-based self-medication (Povey et al., 2009), where infected invertebrates alter their feeding behavior to seek out nutrients that help them fight infections. Recent studies have shown that macronutrients influence immune responses, and pathogen-challenged invertebrates often select diets higher in protein and lower in carbohydrates (Povey et al., 2009). Additionally, higher protein concentrations have been linked to increased resistance in *T. ni* larvae against nucleopolyhedrovirus (SNPV) (Shikano et al., 2010).

This phenomenon could explain why larvae fed diet D11 produced fewer OBs (Table 6). The high protein levels in these larvae may have supported a self-medicative response, reducing OB productivity. Moreover, protein resources likely enhanced cellular activity, potentially aiding in the rapid clearance of infected midgut cells (Shikano et al., 2010).

In addition, during the SfMNPV infection cycle, internal body cells, particularly adipose cells, are the primary sites of occlusion-derived virus (ODV) production, where these viruses are encapsulated into OBs (Reid et al., 2023). Since adipocytes typically require carbohydrates, this finding suggests that carbohydrate-rich diets may be more suitable for enhancing virus production. Therefore, the results of this study highlight the potential of carbohydrate-rich diets to improve OB yield and guide future research to optimize dietary composition for effective virus production.

Conclusion

The evaluated diets did not match the viral production performance of the diet currently used for mass rearing of *S. frugiperda*. However, diet D7 demonstrated similar cost efficiency to diet D2 in producing a viral inoculation dose per hectare.

Additionally, *S. frugiperda* larvae with higher crude protein content exhibited lower production of viral polyhedra.

References

Alfazairy, A. A., Alfy, H., Quirguis, G. Z., & Karam, H. H. (2012). An agar-free insect rearing artifical diet: A new approach for the low cost mass rearing of the egyptian cotton Leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Life Science Journal, 9*(4), 4646-4653.

Barrera, G., Simón, O., Villamizar, L., Williams, T., & Caballero, P. (2011). *Spodoptera frugiperda* multiple nucleopolyhedrovirus as a potential biological insecticide: Genetic and phenotypic comparison of field isolates from Colombia. *Biological Control*, *58*(2), 113-120. https://doi.org/10.1016/j.biocontrol.2011.04.009

Chen, E., Kolosov, D., O'Donnell, M. J., Erlandson, M. A., McNeil, J. N., & Donly, C. (2018). The effect of diet on midgut and resulting changes in Infectiousness of AcMNPV Baculovirus in the cabbage looper, *Trichoplusia ni*. *Frontiers in Physiology, 9*, 1-11. https://doi.org/10.3389/fphys.2018.01348

Claus, J. D., Gioria, V. V., Micheloud, G. A., & Visnovsky, G. (2012). Production of insecticidal baculoviruses in insect cell cultures: potential and limitations. In S. Soloneski, & M. Larramendy (Ed.), *Insecticides – basic and other applications* (pp. 127-152). In Tech. https://doi.org/10.5772/29551

Costa, V. H. D., Soares, M. A., Dimate, F. A. R., Sá, V. G. M., Zanuncio, J. C., & Valicente, F. H. (2019). Genetic identification and biological characterization of baculovirus isolated from *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *Florida Entomologist*, *102*(1), 59-64. https://doi.org/10.1653/024.102.0109

Elvira, S., Williams, T., & Caballero, P. (2010a). Juvenile hormone analog technology: effects on larval cannibalism and the production of *Spodoptera exigua* (Lepidoptera: Noctuidae) nucleopolyhedrovirus. *Journal of Economic Entomology, 103*(3), 577-582. https://doi.org/10.1603/ec09325

Elvira, S., Gorría, N., Muñoz, D., Williams, T., & Caballero, P. A. (2010b). Simplified low-cost diet for rearing *Spodoptera exigua* (Lepidoptera: Noctuidae) and its effect on *S. exigua* nucleopolyhedrovirus production. *Biological and Microbial Control, 103*(1), 17-24. https://doi.org/10.1603/EC09246

García-Banderas, D., Tamayo-Mejía, F., Pineda, S., Rosa, J. I. F., Lasa, R., Chavarrieta-Yáñez, J. M., Gervasio-Rosas, E., Zamora-Avilés, N., Morales, S. I., Ramos-Ortiz, S., Valle, J. & Martinéz-Castillo, A. M. (2020). Biological characterization of two *Spodoptera frugiperda* nucleopolyhedrovirus isolates from Mexico and evaluation of one isolate in a small-scale field trial. *Biological Control*, *149*, 1-8. https://doi.org/10.1016/j.biocontrol.2020.104316

Grzywacz, D., & Moore, S. (2017). Production, formulation, and bioassay of baculoviruses for pest control. In L. A. Lacey (Ed.), *Microbial control of insect and mite pests. From theory to pratice* (pp.109-124). Academic Press. https://doi.org/10.1016/B978-0-12-803527-6.00007-X

Hashimoto, Y., Hayashi, K., Hayakawa, T., Ueno, Y., Shimojo, E.-I., Kondo, A., Miyasono, M., Sano, Y., Matsumoto, T., & Granados, R. R. (2000). Physical map of a *Plutella xylostella* granulovirus genome. *Applied Entomology and Zoology, 35*(1), 45-51. https://doi.org/10.1303/aez.2000.45

Hussain, A. G., Wennmann, J. T., Goergen, G., Bryon, A., & Ros, V. I. D. (2021). Viruses of the fall armyworm *Spodoptera frugiperda*: a review with prospects for biological control. *Viruses*, *13*(11), 1-21. https://doi.org/10.3390/v13112220

Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M., & Goettel, M. S. (2015). Insect pathogens as biological control agents: back to the future. *Journal of Invertebrate Pathology, 132*, 1-41. https://doi.org/10.1016/j.jip.2015.07.009

Machado, L. C., Santos Junior, H. G., Santos, B. A., Soares, C. O., Mauri, L. V. R., & Rodrigues, J. A. (2022). Desenvolvimento de estágio larval de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) em temperaturas sob condição padronizada. *Revista em Agronegócio e Meio Ambiente, 15*(4), 1-11. https://doi.org/10.17765/2176-9168.2022v15n4e9668

Ministério da Agricultura, Pecuária e Abastecimento [MAPA]. (2015). Instrução Normativa Conjunta, n° 1, de 6 de fevereiro de 2015. *Diário Oficial da União*. http://www.in.gov.br/autenticidade.html

Pinto, J. R. L., Torres, A. F., Truzi, C. C., Vieira, N. F., Vacari, A. M., & Bortoli, S. A. (2019). Artificial corn-based diet for rearing *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Insect Science, 19*(4), 1-8. https://doi.org/10.1093/jisesa/iez052

Povey, S., Cotter, S. C., Simpson, S. J., Lee, K. P., & Wilson, K. (2009). Can the protein costs of bacterial resistance be offset by altered feeding behaviour? *Journal of Animal Ecology, 78*(2), 437-446. https://doi.org/10.1111/j.1365-2656.2008.01499.x

Ramírez-Arias, F. G., Lasa, R., Murillo, R., Navarro-de-la-Fuente, L., Mercado, G., Williams, T. (2019). Post-mortem incubation influences occlusion body production in nucleopolyhedrovirus-infected larvae of *Spodoptera frugiperda*. *Biological Control, 135*, 33-40. https://doi.org/10.1016/j.biocontrol.2019.05.003

Reid, S., Chan, L., & Oers, M. M. van (2014). Production of entomopathogenic viruses. In J. A. Morales-Ramos, M. G. Rojas, & D. I. Shapiro-Ilan (Ed.), *Mass production of beneficial organisms: invertebrates and entomopathogens* (pp. 437-482). Elsevier. https://doi.org/10.1016/B978-0-12-391453-8.00013-3

Reid, S., Malmanche, H., Chan, L., Popham, H., & Oers, M. M. van (2023). Production of entomopathogenic viruses. In J. A. Morales-Ramos, M. G. Rojas, & D. I. Shapiro-Ilan (Ed.), *Mass production of beneficial organisms: invertebrates and entomopathogens* (pp. 375-406). Elsevier. https://doi.org/10.1016/B978-0-12-822106-8.00020-8

Rios-Velasco, C., Gallegos-Morales, G., Berlanga-Reyes, D., Cambero-Campos, J., & Romo-Chacón, A. (2012). Mortality and production of occlusion bodies in *Spodoptera frugiperda* larvae (Lepidoptera: Noctuidae) treated with nucleopolyhedrovirus. *Florida Entomologist, 95*(3), 752-757. https://doi.org/10.1653/024.095.0326

Sayed, W. A. A., El-Helaly, A., Jamal, Z. A., & El-Bendary, H. (2021). Effect of a low cost diet on the cotton leaf worm, *Spodoptera littoralis* nucleopolyhedrosis virus pathogenicity and sterile insect technique. *Egyptian Journal of Biological Pest Control, 31*(117), 1-8. https://doi.org/10.1186/s41938-021-00464-9

Shikano, I., Ericsson, J. D., Cory, J. S., & Myers, J. H. (2010). Indirect plant-mediated effects on insect immunity and disease resistance in a tritrophic system. *Basic and Applied Ecology, 11*(1), 15-22. https://doi.org/10.1016/j.baae.2009.06.008

Silva, C. S. B. & Parra, J. R. P. (2013). New method for rearing *Spodoptera frugiperda* in laboratory shows that larval cannibalism is not obligatory. *Revista Brasileira de Entomologia, 57*(3), 347-349. https://doi.org/10.1590/S0085-56262013005000029

Souza, A. M. L., Ávila, C. J., & Parra, J. R. P. (2001). Consumo e utilização de alimento por *Diatraea saccharalis* (Fabr.) (Lepidoptera: Pyralidae), *Heliothis virescens* (Fabr.) e *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) em duas temperaturas. *Neotropical Entomology, 30*(1), 11-17. https://doi.org/10.1590/S1519-566X2001000100003

Stinguel, P., Paiva, C. E. C., Zuim, V., Azevedo, A. C. T., Valicente, F. H., & Santos Júnior, H. J. G. (2022) Optimization of *in vivo* production of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV). *Neotropical Entomology, 51*(1), 122-132. https://doi.org/10.1007/s13744-021-00917-8

Subramanian, S., Santharam, G., Sathiah, N., Kennedy, J. S., & Rabindra, R. J. (2006). Influence of incubation temperature on productivity and quality of *Spodoptera litura* nucleopolyhedrovirus. *Biological Control, 37*(3), 367-374. https://doi.org/10.1016/j.biocontrol.2006.01.011

Truzi, C. C., Vieira, N. F., Souza, J. M., & Bortoli, S. A. (2021). Artificial diets with different protein levels for rearing *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Insect Science, 21*(4), 1-7. https://doi.org/10.1093/jisesa/ieab041

Valicente, F. H., Tulher, E. S., & Barros, E. C. (2010). *Processo de produção comercial de baculovírus em grande escala* (Circular Técnica, 157). EMBRAPA-CNPMS. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/30164/1/circ-157.pdf

Valicente, F. H., Tuelher, E. S., Pena, R. C., Andreazza, R., & Guimarães, M. R. F. (2013). Cannibalism and virus production in *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae fed with two leaf substrates inoculated with *Baculovirus spodoptera*. *Neotropical Entomology*, *42*(2), 191-199. https://doi.org/10.1007/s13744-013-0108-6

Velasco, E. A., Molina-Ruíz, C. S., Gómez-Díaz, J. S., Williams, T. (2022). Properties of nucleopolyhedrovirus occlusion bodies from living and virus-killed larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biological Control, 174*, 1-8. https://doi.org/10.1016/j.biocontrol.2022.105008

Vieira, C. M., Tuelher, E. S., Valicente, F. H., Wolff, J. L. C. (2012). Characterization of a *Spodoptera frugiperda* multiple nucleopolyhedrovirus isolate that does not liquefy the integument of infected larvae. *Journal of Invertebrate Pathology, 111*(2), 189-192. https://doi.org/10.1016/j.jip.2012.07.010

Zamora-Avilés, N., Martínez, A. M., Pineda, S., Bravo-Patiño, A., Figueroa, I., & Lasa, R. (2017). Cool-textured diets for use in baculovirus production. *Biocontrol Science and Technology, 27*(11), 1327-1338. https://doi.org/10.1080/09583157.2017.1397598

Zanella-Saenz I., Herniou E. A., Ibarra J. E., Huerta-Arredondo I. A., & Del Rincón-Castro M. C. (2022). Virulence and genetic characterization of six baculovirus strains isolated from different populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Archives of Microbiology*, *204*(108), 1-11. https://doi.org/10.1007/s00203-021-02722-8