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Different Protein Sources of Larval Diet on the Rearing of *Anastrepha fraterculus* (Diptera: Tephritidae): Biological and Nutritional Analyses

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Abstract

Anastrepha fraterculus (Diptera: Tephritidae) is considered an important pest in Neotropical countries. The laboratory rearing of this species should reproduce conditions in nature; thus, special attention is required to the nutritional quality of diets for larval development. Protein components (wheat germ) are costly and account for most production costs in lab insect rearing. In this sense, this work aimed to identify ingredients to replace wheat germ, without compromising diet quality for the lab rearing of *A. fraterculus*. We tested diets composed of whole rice flour, corn flour, and a mixture of whole wheat flour + soybean flour as substitutes for wheat germ as well as a raw wheat germ diet, considered the control. The protein sources used in the larval diet influenced the biological performance of both the larval and adult stages of *A. fraterculus* during six generations. The diet containing corn flour and wheat germ showed similar results in the different developmental parameters. The diet with rice flour also provided adequate biological development for *A. fraterculus* throughout its life cycle and was nutritionally similar to the control. As it is local product, rice flour can replace wheat germ (costly imported product) in artificial diets for *A. fraterculus*, reducing production costs by roughly 30% without compromising the biological and nutritional parameters of the insects. Faced with this, the rice flour can be considered suitable for the mass rearing of *A. fraterculus* in the laboratory.

Keywords South American fruit fly · Artificial diets · Mass rearing · Biological parameters · Nutritional requirement

Introduction

Larval food quality is determinant for the health of phytophagous insects (Morelli et al. 2012). According to Chapman et al. (2013), the larval stage represents a growth phase where 90% of the adult body mass is accumulated. In this sense, some studies have examined the influence of larval food quality on health components, such as larval and adult performance (Kaspi et al. 2000).

Larval nutrition determines how adult insects cope with stress because the larval stage contributes to metabolic reserves in adults. In response to nutritional stress, insects may extend their growth periods by allocating their energy to somatic maintenance (Bernays 1985), which can affect different biological parameters, namely time extension needed to reach maturity and reduction of total body size (Oviedo et al. 2011).

The use of artificial diets to rear insects provides knowledge of their biology, behavior, and nutritional requirements. Thus, it is necessary to multiply the insects in an artificial environment that provides essential nutrients to expand insect production in the laboratory quickly and gradually, within biological quality parameters (Parra 2009). Therefore, it is essential to develop alternative nutrient-rich food sources that allow for viable large-scale production of insects in a less costly and more effective way.

Studies have investigated the most suitable food sources for fruit flies through the nutritional balance, mainly proteins (Oviedo et al. 2011), as it is a key nutrient during the larval stage and, more specifically, the influence of amino acids (Chang and Vargas 2007), since the nutritional value

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of proteins depends on the content of essential amino acids needed for insect growth and development.

Several breeding techniques have been developed and improved for the *Anastrepha fraterculus* species (Wiedemann, 1830) (Diptera: Tephritidae), South American fruit fly (Jaldo et al. 2001; Morelli et al. 2012; Nunes et al. 2013). The changes proposed by the authors show an evolution in rearing techniques and point to the viability of mass rearing, provided some adjustments are made. For the larval diet of *A. fraterculus*, many fruit fly rearing laboratories in Brazil use raw wheat germ and brewer's yeast as a protein source. However, in most cases, wheat germ is imported, which leads to an increase of production costs. Thus, replacing imported ingredients for national products could represent a major advance in the rearing of *A. fraterculus*, facilitating the process by allowing to supply insects to areas with biological control and to use of sterile insect technique (SIT) (Morelli et al. 2012; Silva Neto et al. 2012), while reducing costs, since diets and labor costs comprise the main expenses in mass rearing (Parker 2005). Like this, diets tested in the laboratory have to undergo a quality control evaluation and, therefore, some biological parameters need assessing, such as morphological, biometric, and nutritional criteria (Parra 2009).

According to studies carried out to date, *A. fraterculus* has the ability to develop on artificial diets with other food sources. However, a more careful analysis of the biological potential of the species on different protein sources has never been measured. Therefore, the present study aimed to examine the effects of a larval diet with different protein sources on the biological parameters of *A. fraterculus* over six successive generations and to characterize the amino acid profile of the diets to replace wheat germ, commonly used in mass rearing of *A. fraterculus*.

Material and Methods

The *A. fraterculus* colony and the experiments were developed at the Entomology Laboratory at Embrapa Clima Temperado (Pelotas, Rio Grande do Sul State, Brazil), in air-conditioned rooms with a temperature of 25 ± 1 °C, relative humidity (RH) of 70 ± 20%, and a 12-h photophase.

Insect Rearing

To establish the maintenance rearing of *A. fraterculus* under laboratory conditions, we collected infested araçá fruits (*Psidium cattleyanum*), belonging to Embrapa Clima Temperado (Pelotas, Rio Grande do Sul State, Brazil) (31°40′53″S, 52°26′23″W). In the laboratory, the fruits were placed in plastic trays (19 × 13.5 × 3.5 cm) containing one tray of fine vermiculite (1 cm) covered with *voile* fabric and kept in an air-conditioned room under controlled temperature conditions. At the beginning of pupation, the vermiculite was sieved daily using a fine-mesh sieve (2 mm) and the puparia were transferred to Petri dishes (9 cm in diameter), where they remained until insect emergence. Subsequently, the insects were transferred to plastic cages (50 L) (50 × 40 × 40 cm) (approximately 50 pairs), placed in a breeding room. Papaya fruits (*Carica papaya* L.) were placed in the cages to obtain eggs and to allow for larval development, which were left for 24 h, following the methodology proposed by Machota et al. (2010). After this time, the fruits were removed and placed in plastic containers (1 L) overlaid on a layer of fine vermiculite (1 cm) and closed at the top with a cover mesh to allow aeration (Machota et al. 2010). The purpose of the thin vermiculite layer was to absorb excess moisture, preventing contamination and loss of insects, in addition to serving as a pupation site. At pupation, the insects were removed and placed in Petri dishes (9 cm in diameter), containing a layer of thin vermiculite (1 cm), where they remained until insect emergence. Adults of *A. fraterculus* were kept in plastic cages (57 cm long × 39 cm wide × 37 cm high). Water and food composed of refined sugar, wheat germ, and yeast at a ratio of 3:1:1 were offered (Salles 1992; Nunes et al. 2013). The eggs were collected from oviposition screens placed on the cage sides and were transferred to Erlenmeyer-type glass containers (500 mL), where they remained for a 24-h aeration process. Afterward, the eggs were inoculated into an artificial diet for larval development. The insects were multiplied for eight generations using the rearing technique described by Salles (1992) and adapted by Nunes et al. (2013).

Ingredient Choice and Preparation of Artificial Diets

We tested a modification of the artificial diet for larvae presented by Salles (1992) to evaluate the effect of the protein ingredient on the biology of *A. fraterculus*, with the replacement of wheat germ (Treatment 1) (one of the protein sources), used as a standard in the diet, for rice flour (Treatment 2), corn flour (Treatment 3), and a mixture of corn flour + soybean flour (Treatment 4). These ingredients were selected because they showed a better larval development of *A. fraterculus* in an initial screening. The amount of each ingredient was calculated according to the nutritional balance between proteins and carbohydrates (values shown on the package labels), based on the diet containing wheat germ (Table 1). All diets (treatments) were prepared according to the methodology proposed by Salles (1992) and Nunes et al. (2013).

Table 1 Components used in the preparation of artificial diets for the rearing of *Anastrepha fraterculus*

Ingredient	Quantidade[1]			
	Wheat germ[2]	Rice flour	Corn flour	Corn flour + soybean flour
Brewer's yeast	90 g	130 g	130 g	100 g
Refined sugar	90 g	30 g	40 g	40 g
Agar	4.5 g	4.5 g	4.5 g	4.5 g
Sodium benzoate	1.5 g	1.5 g	1.5 g	1.5 g
Nipagin (methylparahydroxybenzoate)	12 mL	12 mL	12 mL	12 mL
Hydrochloric acid (37%)	10 mL	10 mL	10 mL	10 mL
Distilled water	1000 mL	1000 mL	1000 mL	1000 mL
Raw wheat germ	90 g	-	-	-
Brown rice flour	-	110 g	-	-
Cornflour	-	-	100 g	-
Wheat flour + soybean meal	-	-	-	100 + 30 g

[1]Composition to prepare 1500 mL of artificial diet

[2]Diet ofSalles (1992)

Nutritional Characterization of the Artificial Diets Evaluated

Samples of the four wheat germ diets (Walmon®, Walmon Comercial Ltda, São Paulo, São Paulo State, Brazil) (Treatment 1), brown rice flour (Volkman®) (Treatment 2), corn flour (Tordilho®) (Treatment 3), and wheat flour (Panfácil®) + soybean flour (Walmon®) (Treatment 4) were placed in falcon tubes (50 mL) in two replicates for each treatment and sent to the Liquid Cromatography Laboratory at Embrapa Agroindústria de Alimentos (Rio de Janeiro, Brazil). The nutritional characterization of the artificial diets used in this work was obtained from the information obtained on the centesimal composition and amino acid profile of the samples. The centesimal analysis consisted of the contents of protein, moisture, ash, fat, and carbohydrate. The moisture content was determined using the gravimetric method. The samples were weighed and placed in an oven at 100 °C for at least 4 h. After this time, the samples were removed from the oven, placed in a desiccator to cool, and then weighed. The operation was repeated for approximately 1 h until constant weight was reached. The method used was 931.04 (AOAC – Association of Official Analytical Chemists, 18 ed., 3ª rev, 2000). The fixed mineral residue or ash, in turn, consisted of the destruction of organic matter (OM) by burning in a muffle furnace at a temperature of 550 °C, with subsequent weighing of the residue using Method 923.03 (AOAC). The protein content was quantified by determining total nitrogen (N), using the Kjeldahl method, which is based on digesting the sample with concentrated sulfuric acid until carbon and hydrogen are oxidized. The N in the protein content was reduced and transformed into ammonium sulfate. Sodium hydroxide was added and heated to release the ammonia into a known volume of boric acid solution. The ammonium borate formed was dosed with 0.05 M sulfuric acid. The N value obtained had to be multiplied by a matrix-specific factor to transform it into the respective protein value obtained by method 2001.11 modified (AOAC). Fat was determined using automatic equipment that extracts fat from the sample using a solvent (petroleum ether). This determination was based on the official AOAC method Am 5–04 (automatic fat extractor). The carbohydrate value was calculated by difference: 100 − protein + moisture + ash + fat resulting in total carbohydrates. The amino acid profile was analyzed according to the following method 994.12/2000 (AOAC) and Liu et al. (1995).

Biological Parameters of *A. fraterculus* on Different Artificial Diets Over Generations

After preparation, the diet was distributed in plastic trays (400 mL) (24 cm long × 15 cm wide × 6 cm high) 150 mL and then closed with the respective lid (Nunes et al. 2013). After 24 h, approximately 2340 eggs (0.2 mL of a homogeneous mixture of water and *A. fraterculus* eggs up to 24 h old) were inoculated on a filter paper (0.8 cm wide × 8 cm long) using a 30-µL micropipette and the diet was superimposed to allow for larval development. The trays were then closed with their lids and placed in an air-conditioned room. The experimental design was completely randomized with 10 replicates (trays) per diet (treatments). Afterward, 3rd instar larvae of *A. fraterculus* were separated from the diet by washing in running water using a sieve (0.15 mm mesh) and placed in acrylic vials (50 mL) containing vermiculite for pupation. After pupation, 100 24-h-old puparia from each repetition were weighed on a precision analytical

balance (Shimadzu, AUY 220). They were then placed in new plastic containers (60 mL) to assess emergence rate, pupal viability (%), and sex ratio (rs). The sex ratio was calculated using the formula described by Silveira Neto et al. (1976). In addition, the lengths (days) of the egg-larva, pupa, egg-adult, and pre-oviposition periods were assessed.

To determine the biological parameters of the adult stage, 50 couples of *A. fraterculus* up to 48 h old from each treatment were placed in transparent plastic cages (50L) (26.2 × 17.7 × 14.7 cm) with a panel of red *voile* fabric on one side and covered with a thin layer of silicone to stimulate oviposition (Nunes et al. 2013). Water and food composed of refined sugar, wheat germ, and yeast were offered at a ratio of 3:1:1 (Nunes et al. 2013). Upon oviposition, all eggs were collected daily for a period of 10 days and placed on a filter paper (strips 5 cm long by 2 cm wide) using a Pasteur pipette and digitally recorded using photographs to allow egg counting using the *Paint* program to determine fecundity (number of eggs per female). To assess egg viability, a sample of 50 eggs was taken from each cage at the 5th oviposition using a brush (0.5 mm). Subsequently, the eggs were placed on a filter paper (5 × 5 cm length × width) and laid on the piece of sponge cloth (Spontex™) inside an acrylic dish (4 cm in diameter) and kept in an air-conditioned chamber to record the number of hatched larvae. During the same period, from the 5th day until the 7th day of oviposition, eggs from both the experimental cages and the maintenance rearing cages were collected and inoculated into the artificial diets corresponding to each treatment for evaluation during the following six generations. All the biological parameters mentioned above were evaluated in each generation (F1, F2, F3, F4, F5, and F6). To assess longevity (days) under stress, four cages were set up (1000-mL beakers with holes in the top cover of *voile* fabric) containing 20 males (24 h old). Four cages containing 20 females in the same condition were set up for each treatment. The insects were kept without food or water. Mortality was assessed daily to determine the longevity of the insects in the absence of food.

Flight Test

After obtaining the adults in the respective treatments (diets), the flight test was carried out on each generation according to the criteria established by FAO/IAEA/USDA (2003). To that end, three replicates were evaluated, totaling 300 pupae of *A. fraterculus* 24 h before emergence for each treatment. The puparia were placed on a Petri dish (9 cm in diameter) and placed inside a black plastic tube (9 cm in diameter × 10 cm in height) and closed at the top with tulle-like fabric. The inside of the tube was coated with a thin layer of neutral talc and a yellow adhesive strip (3 cm long by 3 cm wide) was hung from the cage top to attract and capture the hoverflies and prevent them from returning or falling into the tubes again. The insects that left the tubes were counted as the flies and the insects that remained inside the tubes were classified into the following categories proposed by FAO/IAEA/USDA (2003): (1) non-emerged (full pupae); (2) semi-emerged (part of the adult attached to the puparium); (3) deformed (flies with deformed wings); and (4) non-flying (flies that look normal but are unable to fly). The results were expressed as a percentage.

Statistical Analyses

The experiments were conducted in a completely randomized design, in a two-factor scheme. Treatment factor A was the protein source used to replace wheat germ, with three levels [brown rice flour, corn flour, a mixture of whole wheat flour + soybean flour], in addition to raw wheat germ as a control. Treatment factor B comprised the generations, with six levels. Data on the development of *A. fraterculus* was tested for normality using the Shapiro–Wilk test, for homoscedasticity using the Hartley test, and for independence of the residuals using the graphical analysis. The data was then submitted to the analysis of variance using the *F* test ($p \leq 0.05$). If statistical significance was found, the effects of protein sources and generations were evaluated using the Waller-Duncan test ($p \leq 0.05$). A joint analysis of all the evaluations was carried out by the multivariate analysis using the principal components analysis (PCA) to compare the performance of the protein sources. The PCA was extracted from a correlation matrix of the groups of dependent variables. Therefore, the information on the original variables was projected into a smaller number of underlying variables called principal components (PCs). The criterion to discard variables (PCs) used was recommended by Jolliffe (2002). This criterion states that a number of PCs that cover at least between 70 and 90% of the total variation should be retained. After selecting the number of PCs, their respective eigenvalues and eigenvectors were obtained. The graphical procedure adopted was the biplot, based on the scores and loadings of the PCs selected. The presence of correlations between the dependent variables in the study was analyzed using Pearson's correlation coefficient (*r*) ($p \leq 0.05$).

Results

All the essential and non-essential amino acids were present in the four diets tested, but at different proportions (Table 2). In the PCA, the first two PCs were used because they accounted for 70% of the variation. The new set of three orthogonal variables (PCs) was generated by the PCA, where PC1 had the highest eigenvalue of 12.98 and accounted for 72.11% of the variability in the data set. PC2 had an eigenvalue of 4.04 and accounted for 22.43% of the

Table 2 Nutritional characterization of four artificial diets used for the larval development of *Anastrepha fraterculus*

Componente	Wheat germ1	Rice flour1	Corn flour1	Corn flour + soybean flour1
Ashes	0.790	0.825	0.730	0.640
Total nitrogen	0.545	0.725	0.675	0.675
Ethereal extract	ND	ND	0.05	ND
Humidity	82.520	81.620	82.930	81.395
Carbohydrate	16.145	16.830	15.615	17.290
Aspartic acid	0.200	0.380	0.320	0.2300
Serina	0.110	0.195	0.175	0.130
Glutamic acid	0.310	0.525	0.515	0.370
Glycine	0.090	0.160	0.150	0.105
Histidine	0.055	0.085	0.085	0.060
Arginine	0.180	0.300	0.270	0.190
Threonine	0.110	0.210	0.185	0.135
Alanine	0.140	0.255	0.230	0.160
Proline	0.150	0.255	0.280	0.190
Tyrosine	0.095	0.150	0.145	0.115
Valina	0.115	0.210	0.190	0.135
Lysine	0.165	0.310	0.250	0.190
Isoleucine	0.090	0.170	0.155	0.110
Leucine	0.145	0.270	0.255	0.180
Phenylalanine	0.100	0.180	0.170	0.120
Tryptophan	0.260	0.240	0.245	0.215
Methionine	0.008	0.009	0.006	0.008
Cysteine	0.006	0.007	0.006	0.008

[1]All quantities were expressed in g/100 g

ND = value below detection limit

data variance. PC3, on the other hand, produced progressively lower eigenvalues (0.98) and did not significantly explain the data variability. The first two PCs explained a large proportion of the total variation, that is, 94.54%, which allowed to plot the component scores and the loadings for the levels of the treatment factor studied (protein sources). All the other results and correlations obtained can be seen in Fig. 1.

The biological parameters of *A. fraterculus* on artificial diets with different protein sources showed significant differences for the egg-adult periods (Fig. 2) and fecundity (Fig. 3). As for pupal weight, larvae fed a diet based on wheat flour + soybean flour had the lowest weights (Table 3). In contrast, puparia from larvae fed rice flour and corn flour had similar pupal weights over the six generations evaluated (Table 3). On the other hand, in general, larvae fed on wheat flour + soybean flour showed the lowest egg viability during the generations, ranging from 27% (1st generation) to 50% (5th generation) (Table 4). However, the pupal stage displayed a significant variation in pupal viability during the generations, including wheat germ (control). However, pupal viability was considered low in all treatments, mainly in the 2nd (< 17%) and 3rd generations (< 52%) (Table 4).

Longevity of males and females of *A. fraterculus* under feeding stress showed significant differences among the treatments (Fig. 4). However, adults from larvae fed both diets showed an average longevity longer than 48 h, reaching 120 h in adults from the rice flour–based diet. As proposed by FAO/IAEA/USDA (2003), there were significant differences in the percentage of *A. fraterculus* classified as unemerged ($F = 23.31$; df = 15; $p < 0.0001$), semi-emerged ($F = 2.89$; df = 15; $p = 0.0027$), deformed ($F = 22.23$; df = 15; $p < 0.0001$), non-flying ($F = 4.19$; df = 15; $p < 0.0001$), and flying ($F = 26.73$; df = 15; $p < 0.0001$) when evaluating the interaction between the treatment factors tested (protein source and generations) (Table 5).

Regarding production costs, the use of rice flour, a product produced locally, not only provided good biological performance for *A. fraterculus*, but also reduced production costs by approximately 30% compared to the wheat germ-based diet (Table 6). The same was observed for corn flour and a mixture of corn flour + soybean flour.

Discussion

The results obtained in the present study represent an advance for mass rearing of *A. fraterculus*. This is the first study to consider the chemical and biological profile of different artificial diets for the development of *A. fraterculus*. The amino acid profile allowed to verify that some amino acids were present in greater quantities in the rice flour and corn flour diets when compared to the wheat germ and wheat flour + soybean flour diets. These include glutamic acid, glycine, arginine, threonine, alanine, proline, valine, lysine, and leucine. This factor may explain the improvement in different biological developmental parameters of the species under study. In holometabolous insects, changes in diet quality during the development stages have major effects on many characteristics throughout the insect life (Chapman et al. 2013), and amino acids are closely related to this process. However, it is not only the presence or absence of amino acids that influences insect performance. The imbalance in the number of amino acids can significantly affect the development and fitness of insects subjected to nutritionally precarious diets (Dadd 1985). Previous studies have evaluated different yeasts for mass rearing of *Anastrepha ludens* (Loew, 1873), *A. obliqua*, and *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) and indicated that the yeast contents tested, with some variations, contained the

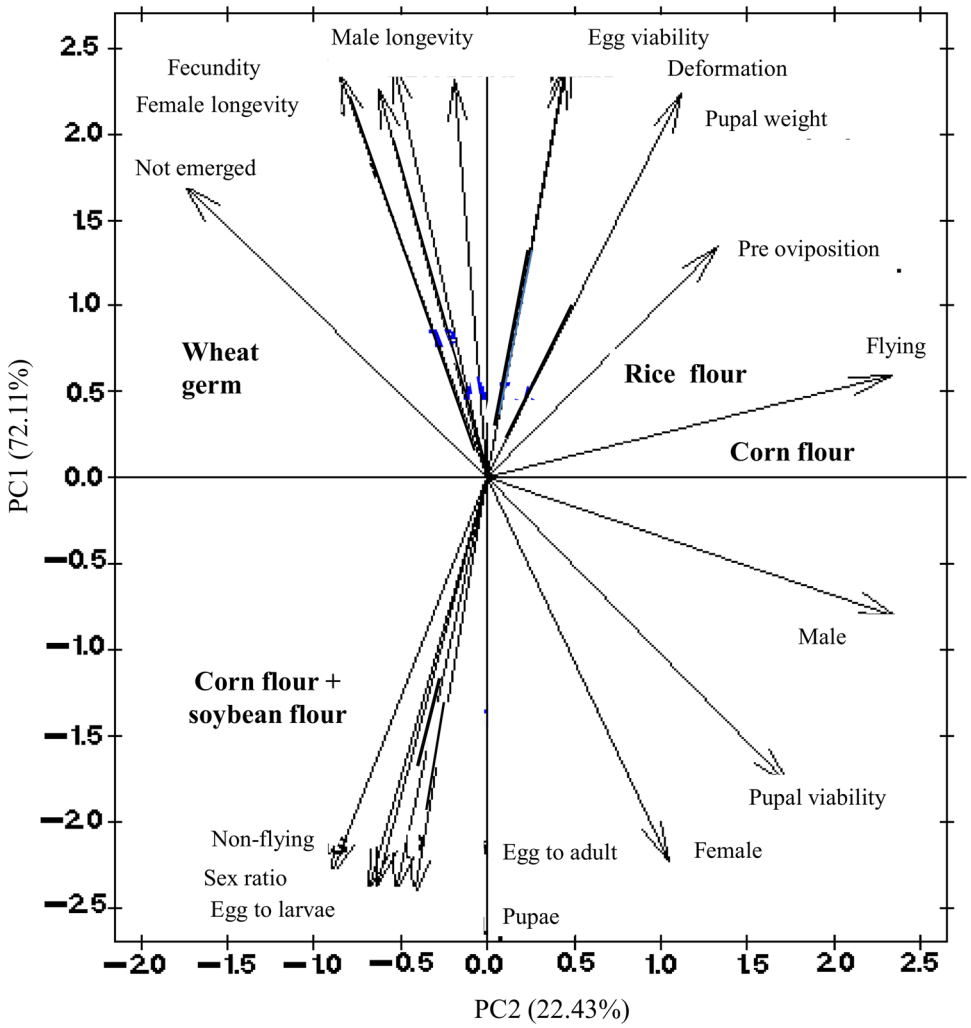

Fig. 1 Plot of PC1-PC2 scores and loadings for the dependent variables analyzed considering all generations of *Anastrepha fraterculus* from artificial diets with different protein sources (germ: wheat germ; rice: rice flour; corn: corn flour; and corn flour + soybean flour). Dependent variables: pupal weigh; sex ratio; percentage of *Anastrepha fraterculus* classified as non-emerged, deformation, non-flying, and flying; egg viability, and pupal viability; duration of the egg to larva, pupae, egg to adult, pre-oviposition, fecundity, female longevity and male longevity

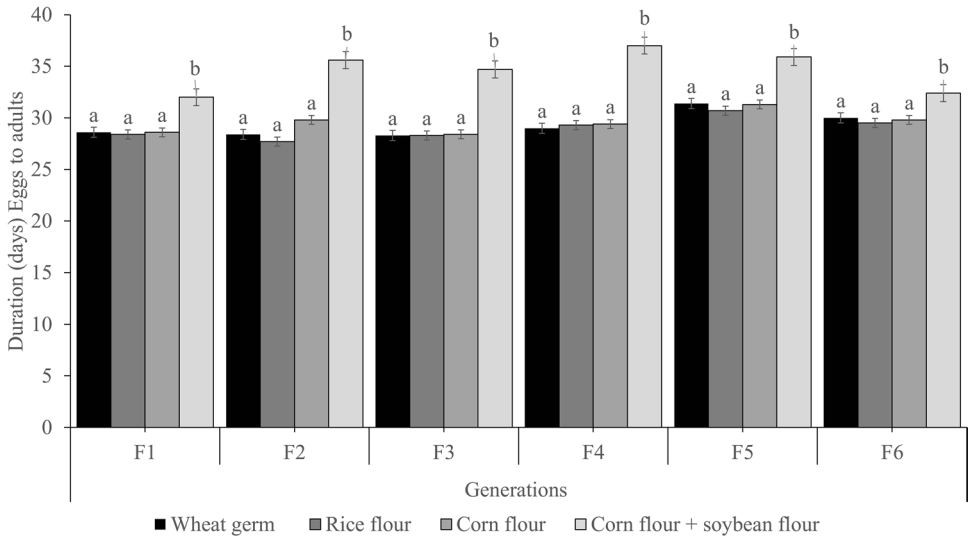

Fig. 2 Duration (days) of the egg-adult of *Anastrepha fraterculus* from artificial diets with different protein sources for six generations. Means (± standard error) followed by the same letter within each generation evaluated do not differ from each other using the Waller-Duncan test ($p \leq 0.05$)

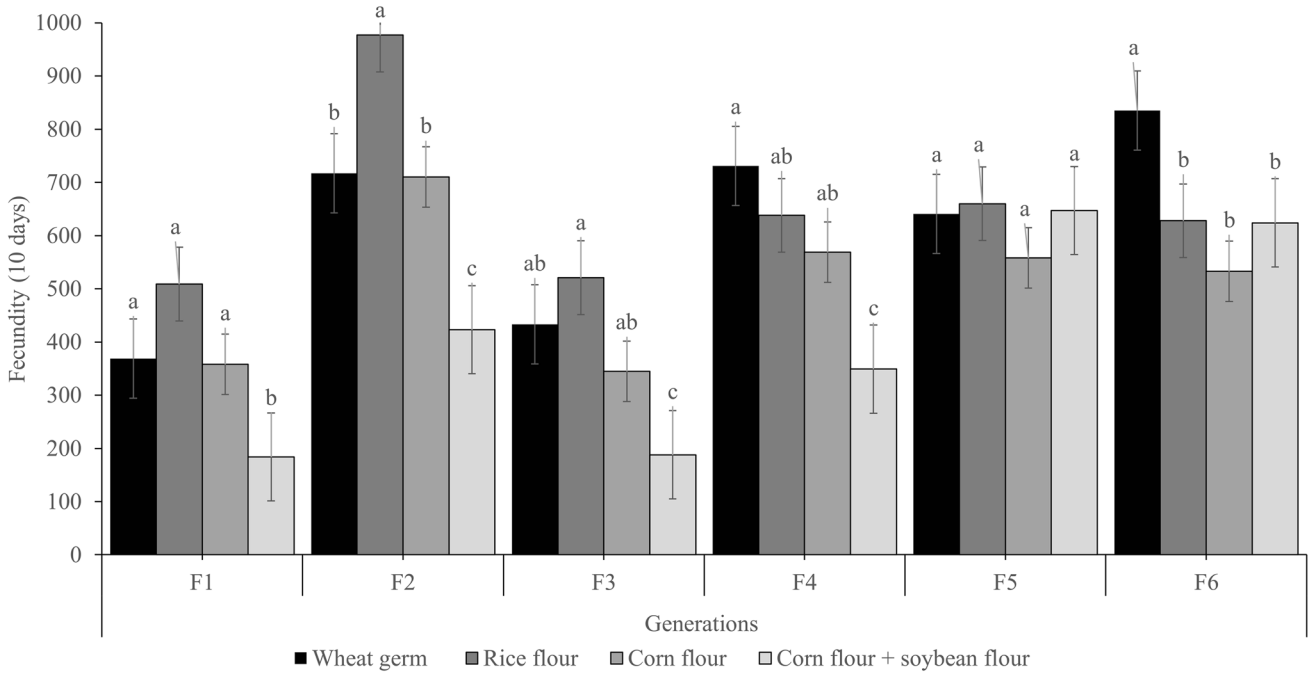

Fig. 3 Fecundity of *Anastrepha fraterculus* from artificial diets with different protein sources for six generations. Means (± standard error) followed by the same letter within each generation evaluated do not differ from each other using the Waller-Duncan test ($p \leq 0.05$)

same amino acids; however, it was necessary to determine the differences in availability and digestibility (Hernández et al. 2016). Thus, digestible amino acids provide a more accurate estimate of the amount of usable protein by the insect.

The evaluation of the biological parameters showed that during the six generations of *A. fraterculus*, the protein sources wheat germ and rice flour formed a group with similar characteristics, despite significant variations between the generations. The diet containing rice flour as a protein source provided satisfactory results for most biological parameters assessed throughout the life cycle of *A. fraterculus*, demonstrating that the amount and the quality of the food ingested by the larva strongly influence the storage of resources allocated to reproduction and to other biological parameters in the adult life (Slansky Junior and Scriber 1985). Thus, rice flour can be considered a viable source to replace wheat germ in larval diets. Although the diet containing rice flour was suitable for several biological parameters, little is known about its use in the formulation of larval diets for the development of fruit fly species, mainly because it is a locally manufactured product as in the case of our study.

The results obtained showed constant variations for some quality parameters over the generations evaluated in the different diets, possibly due to the species adaptation to the change in food source. *Anastrepha fraterculus* showed phenotypic plasticity in the different developmental environments with distinct ingredients over the generations. However, the protein quality and the possible difference in the assimilation of amino acids in the larval stage often alter the developmental parameters of both larvae and adult insects. However, these problems can be minimized over the generations with the development or with the use of rearing techniques and methodologies adjusted to improve the quality of the insects produced. According to Souza et al. (1988), other species of fruit flies, such as the Mediterranean fruit fly *C. capitata*, require at least 10 consecutive generations to adapt and completely recolonize on different larval diets.

In terms of the duration of the developmental periods of *A. fraterculus*, the diet containing wheat flour + soybean flour prolonged the egg-larva, pupa, and egg-adult periods. At specific times throughout the generations evaluated, there were significant differences of between 3 and 10 days for the egg-adult development period between insects fed rice flour and wheat flour + soybean flour during the larval stage. This is not desirable for mass rearing of insects, possibly because the mixture of these ingredients consists of a nutrient source that may be difficult for the larvae to assimilate, either due to the texture and/or the difficulty of swallowing, which may have affected the biological development of insects in our study. This mixture also reduced the overall average fecundity, as only the pre-oviposition period was not affected, since wheat flour + soybean flour reduced the egg-adult period compared to the other protein sources tested.

Table 3 Puparium weight (mg) and sex ratio of *Anastrepha fraterculus* from artificial diets with different protein sources for six generations

Source of protein	Generation												*p*-values
	F_1		F_2		F_3		F_4		F_5		F_6		
	Puparium weight (mg)												
Wheat germ	18.55 ± 0.08	aB[1/]	17.42 ± 0.06	abC	16.78 ± 0.06	cC	17.95 ± 0.06	aC	18.36 ± 0.19	aB	19.30 ± 0.17	aA	$F = 4.11$; df. = 5, 92; < 0.0001
Rice flour	18.13 ± 0.06	aB	18.01 ± 0.06	bBC	18.73 ± 0.06	bA	17.35 ± 0.06	aD	17.91 ± 0.06	bC	18.64 ± 0.06	bA	$F = 6.32$; df. = 5, 92; < 0.0001
Corn flour	18.09 ± 0.06	aC	18.65 ± 0.05	aB	19.04 ± 0.05	aA	17.95 ± 0.09	aC	18.95 ± 0.05	aA	19.14 ± 0.23	aA	$F = 8.32$; df. = 5, 92; < 0.0001
Corn flour + soybean flour	16.05 ± 0.08	bB	15.11 ± 0.06	cCD	16.68 ± 0.13	cA	14.95 ± 0.07	bD	15.26 ± 0.22	cCD	15.45 ± 0.16	cC	$F = 4.11$; df. = 5, 92; < 0.0001
F	7.15		7.02		9.11		5.75		8.22		12.89		
d.f	3, 60		3, 60		3, 60		3, 60		3, 60		3, 60		
p	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		
	Sex ratio												
Wheat germ	0.56 ± 0.01	ns	0.52 ± 0.10	ns	0.51 ± 0.09	ns	0.53 ± 0.03	ns	0.57 ± 0.02	ns	0.58 ± 0.02	ns	$\chi 2 = 11.69$; d.f. = 5, 54; $p = 0.3403$
Rice flour	0.53 ± 0.02		0.54 ± 0.03		0.65 ± 0.05		0.54 ± 0.02		0.53 ± 0.01		0.52 ± 0.03		$\chi 2 = 23.11$; d.f. = 5, 54; $p = 0.2145$
Corn flour	0.54 ± 0.01		0.61 ± 0.07		0.52 ± 0.02		0.52 ± 0.02		0.53 ± 0.01		0.58 ± 0.02		$\chi 2 = 10.54$; d.f. = 5, 54; $p = 0.3124$
Corn flour + soybean flour	0.49 ± 0.03		0.52 ± 0.14		0.55 ± 0.02		0.61 ± 0.02		0.58 ± 0.02		0.64 ± 0.01		$\chi 2 = 14.45$; d.f. = 5, 54; $p = 0.4310$
χ^2	40.11		54.10		45.08		40.12		34.19		39.05		
d.f	3, 38		3, 38		3, 38		3, 38		3, 38		3, 38		
p	= 0.3403		= 0.1134		= 0.2018		= 0.3421		= 0.5617		= 0.3678		

[1/]Means (± standard error) followed by the same lowercase letter in the column and capital letter in the row do not differ from each other using the Waller-Duncan test ($p \leq 0.05$). [ns]not significant in the column and row, according to the Dunnett test ($p \leq 0.05$)

Table 4 Viability (%) of egg and pupal stages of *Anastrepha fraterculus* from artificial diets with different protein sources for six generations. Temperature 25 ± 1 °C, relative humidity 70 ± 20%, and a photophase of 12 h

Source of protein	Generation												*p*-values
	F_1		F_2		F_3		F_4		F_5		F_6		
	Egg stage (%)												
Wheat germ	66.25 ± 1.91	a A[1/]	53.71 ± 5.80	aB	65.75 ± 6.72	aA	69.75 ± 4.06	aA	68.00 ± 2.56	aA	51.25 ± 4.31	aB	$F = 6.89$; df. = 5, 84; < 0.0001
Rice flour	62.50 ± 3.20	aB	48.00 ± 3.85	aC	71.50 ± 4.15	aA	66.75 ± 3.42	aAB	65.25 ± 2.10	aAB	37.25 ± 2.03	bD	$F = 7.09$; df. = 5, 84; < 0.0001
Corn flour	56.50 ± 2.94	aB	44.25 ± 2.52	aC	68.25 ± 3.73	aA	68.75 ± 2.33	aA	69.00 ± 4.84	aA	48.25 ± 5.31	aBC	$F = 8.22$; df. = 5, 84; < 0.0001
Corn flour + soybean flour	27.00 ± 4.28	bC	29.25 ± 6.14	bC	31.00 ± 2.85	bBC	31.00 ± 4.90	bBC	50.00 ± 3.07	bA	43.25 ± 3.14	aAB	$F = 6.87$; df. = 5, 84; < 0.0001
F	5.68		7.19		8.17		9.10		5.44		7.20		
d.f	3, 54		3, 54		3, 54		3, 54		3, 54		3, 54		
p	> 0.0001		> 0.0001		> 0.0001		> 0.0001		> 0.0001		> 0.0001		
	Pupal stage (%)												
Wheat germ	83.60 ± 1.89	aA[1/]	8.20 ± 3.76	bC	6.60 ± 2.78	dC	48.90 ± 6.75	bB	57.30 ± 6.02	cB	77.20 ± 2.41	abA	$F = 514$; df. = 5, 84; < 0.0001
Rice flour	77.40 ± 1.23	aA	17.60 ± 2.99	aD	21.90 ± 4.83	cD	54.70 ± 2.64	bC	58.00 ± 3.18	cBC	75.60 ± 5.74	aB	$F = 8.11$; df. = 5, 84; < 0.0001
Corn flour	63.70 ± 2.22	bC	11.90 ± 2.15	aE	38.60 ± 2.49	bD	66.20 ± 2.48	aC	82.70 ± 1.62	aA	73.30 ± 2.44	aB	$F = 6.45$; df. = 5, 84; < 0.0001
Corn flour + soybean flour	68.70 ± 6.87	bB	1.60 ± 0.58	cD	52.40 ± 6.36	aC	46.80 ± 7.99	bC	74.10 ± 3.42	bB	87.90 ± 2.16	aA	$F = 7.10$; df. = 5, 84; < 0.0001
F	6.78		8.10		9.14		7.16		4.55		6.78		
d.f	3, 60		3, 60		3, 60		3, 60		3, 60		3, 60		
p	> 0.0001		> 0.0001		> 0.0001		> 0.0001		> 0.0001		> 0.0001		

[1/]Means (± standard error) followed by the same lowercase letter in the column and capital letter in the row do not differ from each other using the Waller-Duncan test ($p \leq 0.05$)

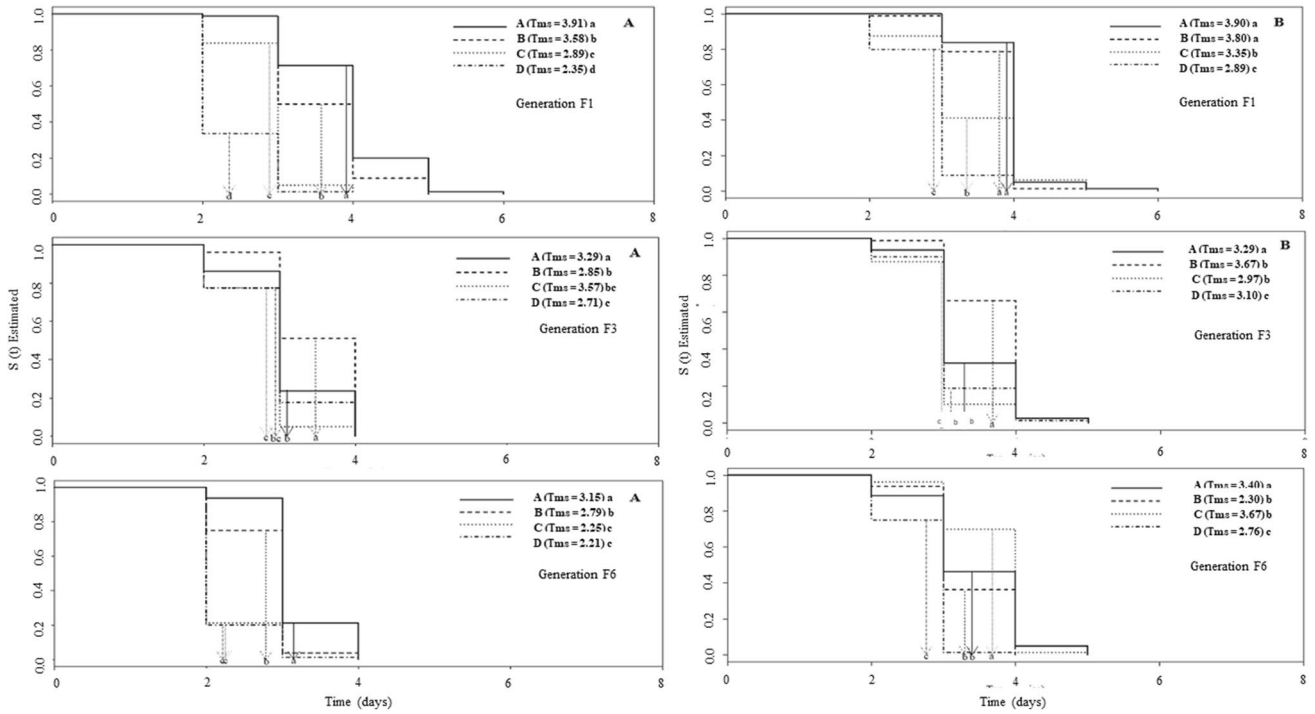

Fig. 4 Survival curves of females (**A**) and males (**B**) of the first, third, and sixth generations of *Anastrepha fraterculus* from artificiais diets with different sources of protein (A = wheat germ; B = rice flour; C = flour corn; and, D = wheat flour + soybean meal). Curves followed by the same letters, for each sex, do not differ from each other using the log-rank test (Tms—average survival time)

The pupal weights observed in this study using diets containing rice flour and corn flour are close to those found by González (1971) who reported values in the range of 18.0 mg. Flores et al. (2012), using a diet adapted from *A. ludens* and *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae), obtained pupae weight values of 16.7 and 14.2 mg, respectively, for the diet containing different proportions of corn flour for *A. fraterculus*. These values were lower than those obtained for the diet containing corn flour in the present study. Silva Neto et al. (2012) found that *C. capitata* females showed a preference for copulation with larger males, indicating that size can reflect on the reproductive competitiveness of the adult. Besides, heavier pupae tend to result in females of higher fecundity, an important and desirable factor for insect mass rearing.

Regarding egg viability, the average hatch rate was relatively low throughout the generations, with the highest value of 71.50% observed using rice flour in the 3rd generation. The negative effect on egg viability may be related to the practices used in the rearing process, such as egg collecting or even sample preparations to check egg viability, since the values were also low using wheat germ (control treatment). According to Morelli et al. (2012), the viability of *A. fraterculus* eggs showed two significant increases over several generations of study, one between the 13th and 18th and the other between the 25th and 30th generations. The first increase was obtained by adding raw wheat germ to the adult diet, while the second was acquired by replacing the protein source in the adult diet. An average value above 75% was reached only from the 43rd generation onward. Resilva et al. (2014) working with *Bactrocera philippinensis* (Drew and Hancock 1994) (Diptera: Tephritidae) on a liquid diet for 12 generations also reported wide ranges of variations, around 41.6 and 86.6% for egg hatch percentage.

Pupal viability was also greatly influenced by the diets, showing great variation throughout the study. In the 1st generation, the percentages were above 60% for all the diets tested, which can be considered desirable. However, in the 2nd generation, all treatments showed problems with emergence, including the control treatment, possibly because of some external factor, such as assessment made by different people. The same fact was also observed in the flight tests, where the percentage of flying insects was low in the 2nd generation. In some cases, feedback has been detected between environmental temperature and nutritional outcomes in developing insects (Coggan et al. 2011). Finally, in the 5th and 6th generations, the average values for pupal viability increased, with percentages above 50% (5th generation) again and close to 90% (6th generation) as occurred in the artificial diet using corn flour + soybean flour. Some studies have reported delays in the development of *C. capitata* larvae due to variation in the amino acid profile, which

Table 5 Percentage of *Anastrepha fraterculus* classified as non-emerged, semi-emerged, deformed, non-flying, and flying from artificial diets with different protein sources for six generations. Temperature 25 ± 1 °C, relative humidity 70 ± 20%, and a photophase of 12 h

Fonte de proteína	Generation												Valores de *p*
	F_1		F_2		F_3		F_4		F_5		F_6		
	Non-emerged (%)												
Wheat germ	10.33 ± 0.88	cC[1/]	77.33 ± 0.88	cA	84.67 ± 2.03	aA	36.67 ± 2.96	aB	27.67 ± 3.84	aC	11.00 ± 3.21	aC	$F = 8.11$; df. = 5, 177; < 0.0001
Rice flour	16.67 ± 1.76	bCD	75.00 ± 1.53	cB	89.33 ± 2.03	aA	18.00 ± 2.08	cCD	20.00 ± 1.53	bC	13.00 ± 1.73	aD	$F = 7.89$; df. = 5, 177; < 0.0001
Corn flour	29.67 ± 3.84	bC	83.33 ± 0.88	bA	48.33 ± 2.18	bB	23.67 ± 1.45	bCD	17.67 ± 3.33	bD	15.67 ± 4.48	aD	$F = 8.10$; df. = 5, 177; < 0.0001
Corn flour + soybean flour	35.67 ± 9.61	aB	96.33 ± 0.33	aA	18.00 ± 6.35	cC	44.33 ± 1.20	aB	14.33 ± 7.69	bC	12.00 ± 1.73	aC	$F = 6.18$; df. = 5, 177 < 0.0001
F	6.78		4.15		6.11		10.12		8.17		9.01		
d.f	3, 117		3, 117		3, 117		3, 117		3, 117		3, 117		
p	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		
	Semi-emerged (%)												
Wheat germ	0.33 ± 0.33	bB[1/]	6.33 ± 0.67	aA	4.00 ± 1.15	aA	3.33 ± 0.88	aA	4.33 ± 1.33	aA	0.33 ± 0.33	bB	$F = 7.20$; df. = 5, 177; < 0.0001
Rice flour	2.33 ± 0.33	aAB	6.67 ± 2.73	aA	2.67 ± 1.33	aAB	0.00 ± 0.00	bB	2.00 ± 1.00	bAB	1.00 ± 0.58	aB	$F = 7.45$; df. = 5, 177; < 0.0001
Corn flour	1.67 ± 0.88	aA	4.67 ± 1.76	aA	3.33 ± 0.33	aA	1.33 ± 0.33	abA	2.33 ± 0.88	bA	2.00 ± 0.00	aA	$F = 8.19$; df. = 5, 177; < 0.0001
Corn flour + soybean flour	1.00 ± 0.58	aB	0.67 ± 0.67	bB	0.33 ± 0.33	bB	4.67 ± 1.85	aA	1.67 ± 0.88	bAB	2.33 ± 0.88	aAB	$F = 7.10$; df. = 5, 177; < 0.0001
F	5.11		6.75		4.56		6.98		6.35		6.54		
d.f	3, 117		3, 117		3, 117		3, 117		3, 117		3, 117		
p	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		
	Deformed (%)												
Wheat germ	10.00 ± 0.00	aA[1/]	1.67 ± 0.88	aB	0.67 ± 0.33	bC	2.67 ± 0.88	abB	3.67 ± 0.33	aB	4.67 ± 1.76	aB	$F = 8.23$; df. = 5, 177; < 0.0001
Rice flour	10.33 ± 2.40	aA	0.67 ± 0.67	bC	0.00 ± 0.00	bC	1.00 ± 0.58	bC	3.33 ± 1.20	aBC	5.33 ± 0.88	aB	$F = 7.15$; df. = 5, 177; < 0.0001
Corn flour	7.33 ± 2.33	aA	2.00 ± 0.58	aB	2.33 ± 0.33	aB	3.33 ± 0.33	aAB	2.67 ± 1.33	aB	4.33 ± 1.45	aAB	$F = 8.90$; df. = 5, 177; < 0.0001
Corn flour + soybean flour	5.00 ± 1.00	bA	0.00 ± 0.00	bC	1.00 ± 0.00	aBC	4.00 ± 2.08	aAB	3.67 ± 0.88	aAB	2.00 ± 0.58	bABC	$F = 5.13$; df. = 5, 177; < 0.0001
F	9.10		12.34		6.74		15.69		6.90		6.85		
d.f	3, 117		3, 117		3, 117		3, 117		3, 117		3, 117		
p	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		
	Non-flying												
Wheat germ	25.00 ± 1.15	aA[1/]	5.33 ± 2.03	aC	2.67 ± 0.33	cC	4.00 ± 1.15	bC	13.33 ± 2.90	abB	4.67 ± 0.33	bC	$F = 4.87$; df. = 5, 177; < 0.0001
Rice flour	17.67 ± 4.18	bA	7.33 ± 0.33	aBC	2.33 ± 1.20	bC	12.67 ± 1.76	aAB	9.67 ± 1.20	bB	7.00 ± 1.15	bBC	$F = 7.86$; df. = 5, 177; < 0.0001
Corn flour	13.68 ± 1.85	bA	4.67 ± 0.88	bB	10.00 ± 2.00	bAB	8.33 ± 2.03	aAB	6.67 ± 3.18	bB	7.67 ± 0.33	bAB	$F = 9.23$; df. = 5, 177; < 0.0001
Corn flour + soybean flour	23.33 ± 11.35	aAB	0.67 ± 0.33	cC	35.67 ± 5.33	aA	11.00 ± 1.15	aBC	14.33 ± 6.57	aBC	11.00 ± 3.60	aBC	$F = 5.16$; df. = 5, 177; < 0.0001
F	6.77		11.44		5.68		10.03		9.11		6.54		
d.f	3, 117		3, 117		3, 117		3, 117		3, 117		3, 117		
p	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		
	Flying (%)												

Table 5 (continued)

Fonte de proteína	Generation												Valores de *p*
	F_1		F_2		F_3		F_4		F_5		F_6		
	Non-emerged (%)												
Wheat germ	10.33 ± 0.88	cC[1/]	77.33 ± 0.88	cA	84.67 ± 2.03	aA	36.67 ± 2.96	aB	27.67 ± 3.84	aC	11.00 ± 3.21	aC	$F = 8.11$; df. = 5, 177; < 0.0001
Wheat germ	54.33 ± 1.20	aA[1/]	9.33 ± 1.45	aB	8.00 ± 1.15	cB	53.33 ± 2.02	aA	51.00 ± 1.15	bA	79.33 ± 4.05	aA	$F = 8.11$; df. = 5, 177; < 0.0001
Rice flour	53.00 ± 3.51	aC	10.33 ± 1.45	aD	5.67 ± 0.88	cD	68.33 ± 0.88	aAB	65.00 ± 1.73	aB	73.67 ± 2.33	aA	$F = 7.89$; df. = 5, 177; < 0.0001
Corn flour	47.67 ± 4.18	aB	5.33 ± 0.33	bD	36.00 ± 3.46	bC	63.33 ± 0.88	aA	70.67 ± 0.67	aA	70.33 ± 3.18	aA	$F = 8.10$; df. = 5, 177; < 0.0001
Corn flour + soybean flour	35.00 ± 2.31	bC	2.33 ± 0.67	bD	45.00 ± 2.89	aB	36.00 ± 0.58	bC	66.00 ± 2.31	aA	72.67 ± 4.18	aA	$F = 6.18$; df. = 5, 177; < 0.0001
F	6.03		12.56		7.19		4.11		6.98		6.34		
d.f	3, 117		3, 117		3, 117		3, 117		3, 117		3, 117		
p	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		

[1/]Means (± standard error) followed by the same lowercase letter in the column and capital letter in the row do not differ from each other using the Waller-Duncan test ($p \leq 0.05$)

may have contributed to a lower pupation percentage in insects reared within the same artificial diet (Chang and Vargas 2007).

As previously discussed, the class of flying insects showed large variations between values over the generations. The 2nd generation showed low values for the percentage of flying insects, due to the reduction in the percentage of insect emergence. In contrast, in the 5th and 6th generations, the percentage of flying insects reached values above 50% in all artificial diets evaluated. Resilva et al. (2014), working with *B. philippinensis* for 12 generations on a liquid diet, also obtained high rates of variation in the percentage of flying insects, from 40.2 to 93.2%. In the present study, even though radiation doses were not tested, it is possible to extract results that could be used for rearing insects using the sterile insect technique (SIT). For flight capacity testing, mainly when the aim is to breed insects for use in SIT studies, there is a standard to be followed by the FAO/IAEA/USDA. For example, the minimum post-irradiation percentages acceptable for flight capacity are 60% for *genetic sexing strains* (GSS) of *C. capitata*, 80% for *A. suspensa*, and 72% for *A. obliqua*, while for *A. fraterculus*, there are no reports in the FAO manual. Kamiya (2010), testing different irradiation doses for *A. fraterculus*, found values between 48.8 and 57.8%, similar in some situations and lower for the class of flying insect when compared to several developmental generations evaluated in the present study. On the other hand, estimating the percentage of pupae that give rise to adults with a minimum flying capacity serves as indicative of their performance when working with insect rearing to release sterile males in field conditions, allowing thus to assess their competitiveness against wild insects. The data showed that the insects produced in all artificial diets evaluated are capable of flying and dispersing, important factors in the search for a place to mate.

Another important parameter within SIT is insect longevity under stress and, in our study, both males and females showed an average survival time of over 48 h, regardless of treatment. For SIT, the minimum post-irradiation percentages of acceptable survivors, according to FAO/IAEA/USDA (2003), is 55% for *A. ludens* and 40% for *A. obliqua* after 72 h. For *A. fraterculus*, Kamiya (2010) found percentages above 55% of individuals alive after 48 h for all the treatments tested (testing radiation doses). The results of the longevity test under stress express a relative measure of the nutritional reserves to fly and the capacity of storing nutrients during the larval period, making them available to the adult at the time of emergence, and provide information on the quality of the insects to be released into the field.

Given the nutritional and biological evaluations throughout the developmental period, the diet containing corn flour showed satisfactory results, but in specific developmental parameters and stages, for example, puparium weight and

Table 6 Cost of ingredients used to prepare the artificial diet for the larval development of *Anastrepha fraterculus*. Composition for the preparation of 1.5 L of artificial diet

Ingredient	Unid	Price (R$)*	Price (US$)*	Total to prepare 1.5 L of diet			
				Wheat germ	Rice flour	Corn flour	Corn flour + soybean flour
Brewer's yeast	kg	35.00	6.91	3.15	4.55	4.55	3.5
Refined sugar	kg	2.80	0.55	0.25	0.08	0.11	0.11
Bacteriological agar	Container (500 g)	285.00	56.32	2.56	2.56	2.56	2.56
Sodium benzoate	Container (500 g)	55.00	10.86	0.17	0.17	0.17	0.17
Nipagin	Container (500 g)	58.00	11.4	0.14	0.14	0.14	0.14
Hydrochloric acid (37%)	litro	28.75	5.68	0.29	0.29	0.29	0.29
Raw wheat germ	kg	59.9	11.83	5.39	-	-	-
Rice flour	kg	7.00	1.38	-	0.77	-	-
Corn flour	kg	2.27	0.44	-	-	0.23	-
Wheat flour	kg	2.97	0.58	-	-	-	0.3
Soybean bran	kg	29.95	5.91	-	-	-	0.9
Total (R$)				11.95	8.56	8.05	7.97
Total (US$)				2.36	1.69	1.59	1.57

Contact value in December 2023

egg viability. However, rice flour could effectively replace the imported wheat germ used in the larval diet of *A. fraterculus* (and other fruit flies), reducing costs without causing negative effects on the quality parameters of the insects produced. According to Parra (2009), in addition to providing good biological suitability and performance for insects, an artificial diet should also contain ingredients that are low in cost and easy to acquire. Therefore, we should consider the cost/benefit ratio of the ingredients (wheat germ and rice flour) and the prices in the local market. For instance, the average price of 1 kg of wheat germ is US$11.83, while 1 kg of rice flour costs US$1.38 (US$1.00 = R$5.06), indicating that in addition to the biological parameters, the use of rice flour reduces the costs generated by the use of the artificial diet. Based on the biological results and on the nutritional estimates and production costs, rice flour can be considered suitable for the mass rearing of *A. fraterculus* in the laboratory.

Author Contribution SO, KP, RMB, RSG, SDN, and DB conducted, analyzed, and wrote the manuscript. DEN and DB revised the manuscript.

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Declarations

Conflict of Interest The authors declare no competing interests.

References

AOAC (2000) Association of Official Analytical Chemists. Offcial Methods of Analysis. Section 994.12.14th Ed. Association of Official Analytical Chemist. Washington D.C. USA https://www.aoac.org/officialmethods-of-analysis/?gad_source=1&gclid=CjwKCAjw1920BhA3EiwAJT3lSdHK6e3dIj9iBuOf2Alw6KsbbcPFHJ1N8FiXc1s2T-XrvFBDbClp-BoCcxoQAvD_BwE. Accessed 17 July 2024

Bernays E (1985) Regulation of feeding behavior. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry and pharmacology. Pergamon Press, London, pp 1–45

Chang CL, Vargas RI (2007) Wheat germ oil and its effects on a liquid larval rearing diet for oriental fruit flies (Diptera: Tephritidae). J Econ Entomol 100:322–326. https://doi.org/10.1093/jee/100.2.322

Chapman RF, Simpson SJ, Douglas AE (2013) The insects: structure and function. Press, Cambridge University, p 929

Coggan N, Clissold FJ, Simpson SJ (2011) Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. Proc Biol Sci 278:2745–2752. https://doi.org/10.1098/rspb.2010.2675

Dadd RH (1985) Nutrition: organisms. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry and pharmacology. Pergamon Press, London p, pp 313–389

FAO/IAEA/USDA (2003) Manual for product quality control and shipping procedures for sterile mass-reared Tephritid fruit flies. Version 5.0. Vienna, Austria: IAEA, 84 p

Flores HS, Hernández E, Toledo J (2012) Dessarrollo de un sistema de cría artificial para *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae). Acta Zoo Mex 28:321–340

González JB (1971) Crianza masal de *Anastrepha fraterculus* (Wiedemann). Rev Peru Entomol 14:71–76

Hernández E, Rivera P, Aceituno-Medina M, Aguilar-Laparra R, Quintero-Fong L, Orozco-Dávila D (2016) Eficiencia de levaduras para la cría masiva de *Anastrepha ludens*, *Anastrepha obliqua* y *Ceratitis capitata* (Diptera: Tephritidae). Acta Zool Mex 32:240–252

Jaldo HE, Gramajo MC, Willink E (2001) Mass rearing of *Anastrepha fraterculus* (Diptera: Tephritidae): a preliminary strategy. Fla Entomol. Gainesville, 84(4):716–718

Jolliffe IT (2002) Ed. Principal component analysis, 2th ed. New York: Springer-Verlag, 487 p

Kamiya AC (2010) Criação massal em dieta líquida e radioesterilização da mosca-sul-americana *Anastrepha sp. 1 aff. fraterculus* (Wiedemann, 1830)(Diptera: Tephritidae). 2010. 71 p. Dissertação (Mestrado em Ciências) - Universidade de São Paulo, USP, Piracicaba, São Paulo

Kaspi R, Taylor PW, Yuval B (2000) Diet and size influence sexual advertisement and copulatory success of males in mediterranean fruit fly leks. Ecol Entomol 25:1–6. https://doi.org/10.1046/j.1365-2311.2000.00266.x

Liu HJ, Chang BY, Yan HW, Yu FH, Lu XX (1995) Determination of amino acids in food and feed by derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and reversed-phase liquid chromatographic separation. J AOAC Int 78:736–744. https://doi.org/10.1093/jaoac/78.3.736

Machota Jr. R, Bortoli LC, Tolotti A, Botton M (2010) Técnica de criação de *Anastrepha fraterculus* (Wied., 1830) (Diptera: Tephritidae) em laboratório utilizando hospedeiro natural. Embrapa Uva e Vinho, Bento Gonçalves, 23 p

Morelli R, Costa KZ, Fagioni KM, Costa MDLZ, Nascimento ASD, Pimentel RMDA, Walder JMM (2012) New protein sources in adults diet for mass-rearing of *Anastrepha fraterculus* (Diptera: Tephritidae). Braz J Biol Sci 55:827–833. https://doi.org/10.1590/S1516-89132012000600004

Nunes AM, Costa KZ, Faggioni KM, Costa MLZ, Gonçalves RS, Walder JMM, Garcia MS, Nava DE (2013) Dietas artificiais para a criação de larvas e adultos da mosca-das-frutas sul-americana. Pesqui Agropecu Bras 48:1309–1314. https://doi.org/10.1590/S0100-204X2013001000001

Oviedo A, Nestel D, Papadopoulos NT, Ruiz MJ, Prieto SC, Willink E, Vera MT (2011) Management of protein intake in the fruit fly *Anastrepha fraterculus*. J Insect Physiol 57:1622–1630. https://doi.org/10.1016/j.jinsphys.2011.08.013

Parker AG (2005) Mass rearing for sterile insect release. In: Hendrichs J, Robinson AS, Dyck VA (eds) Sterile insect technique: principles and practice in area wide integrated pest management. Springer, Dordrecht, pp 209–232

Parra JRP (2009) A evolução das dietas artificiais e suas interações em ciência e tecnologia. In: Panizzi AR (ed) Bioecologia e nutrição de insetos: Base para o Manejo Integrado de Pragas. Embrapa, Brasília, pp 91–174

Resilva SS, Obra GB, Chang CL (2014) Suitability of a liquid larval diet for rearing the Philippines fruit fly *Bactrocera philippinensis* (Diptera: Tephritidae). Int J Trop Insect Sci 34:53–58. https://doi.org/10.1017/S1742758414000083

Salles LAB (1992) Metodologia de criação de *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) em dieta artificial em laboratório. An Soc Entomol Bras 21:479–486

Silva Neto AM, Dias VS, Joachim-Bravo LS (2012) Comportamento reprodutivo de *Ceratitis capitata* Wiedemann (Diptera Tephritidae): efeito do tamanho dos machos sobre o seu sucesso de cópula. EntomoBrasilis 5:190–197. https://doi.org/10.12741/ebrasilis.v5i3.182

Silveira Neto S, Nakano O, Barbin D, Villa Nova N (1976) Manual de ecologia dos insetos. Ceres, São Paulo, p 419

Slansky Junior F, Scriber JM (1985) Food consumption and utilization. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry and pharmacology. Pergamon, Oxford, pp 87–163

Souza HML, Matioli SR, Souza WN (1988) The adaptation process of *Ceratitis capitata* to the laboratory analysis of life-history traits. Entomol Exp Appl 49:195–201. https://doi.org/10.1111/j.1570-7458.1988.tb01180.x