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Beneficial Uses of Insects

Optimization of a Diet for the Greater Wax Moth (Lepidoptera: Pyralidae) Using Full Factorial and Mixture Design

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Abstract

Diet optimization is an important process to increase the efficiency of rearing insects and can be used to develop high-quality insects with specific fitness and life-history traits. *Galleria mellonella* (L.), the greater wax moth, is widely used in research, microbiology assays, as pet food, and host for biological control agents. Although artificial diets for *G. mellonella* have been researched and optimized for decades, preliminary tests indicated that the predominantly utilized *G. mellonella* diet could be improved to yield larger larvae with a short development time. We used a design of experiments (DOE) approach that incorporated multiple full factorial designs and a final mixture design to test the qualitative and quantitative effects of ingredients and their interactions on larval mass and survival. Analysis of 17 ingredient variations in 35 diet formulations yielded an optimized diet that supported high survival and 2.4-fold greater larval body mass than the standard rearing diet. This study demonstrates the importance and efficiency of statistical DOE in guiding the optimization of insect diets to improve traits that represent the quality and fitness of the reared insects.

Key words: diet optimization, design of experiments, rearing

Insects are reared for many purposes, including for release as biological control agents, hosts of entomopathogens, models for basic research, subjects for agricultural, apiculture, forest, aquatic, medical and veterinary research, for public displays, educational and outreach activities, and more (Cohen 2015, 2018, 2020; Schneider et al. 2018). Effective insect diets are pivotal in most rearing operations because they can shape the success of such programs by improving rearing production costs and enhancing the overall quality and fitness of the reared insects. Enhancing the nutritional quality of a diet through diet optimization can improve key life-history and biological fitness parameters including growth, flight, fecundity, and longevity. The desired outcomes of diet optimization vary depending on the purpose of the insect and rearing system. A few examples of potential outcomes include greater insect yield per unit of diet biomass, enhanced production of high-quality insects for programs such as sterile insect release and biological control, and reduced cost through use of alternative ingredients (Lapointe et al. 2008). To optimize a diet effectively, specific diet characteristics must be considered, including the presence of essential nutrients, the need for those nutritional components to be biologically available to the insect, and presence of proper feeding stimuli. An artificial diet must be chemically stable, nutritionally complete, palatable to the insect, provide bioavailable nutrients, and support growth, development, and reproduction (Cohen 2015). Furthermore, the amounts and proportions of diet ingredients, in addition to their nutritional value, can affect the suitability of the diet and the insect's performance.

Conventionally, insect rearing specialists varied single diet ingredients in their quest to obtain desired fitness and quality outcomes (Table 1) (Lapointe et al. 2008, Cohen 2015). However, this approach can be slow and tedious, and may fail to reveal interactions among diet ingredients. Such interactions are important to consider while developing diets because they can affect the suitability of the diet (Cohen 2015). Design of experiments (DOE) is a statistical approach used to examine the effect of multiple variables and their interactions on a desired outcome (Montgomery 2013). The DOE is particularly relevant in diet optimization because individual

Table 1. Nine *Galleria mellonella* diet-based studies conducted between 1960 and 2018

Reference	Aim of experiment	Ingredients or nutrients tested	Conclusions	Ingredients in successful diet	Highest mass achieved (days[a])
Beck (1960)	Determine suitable lab conditions and diet for growth and development	Compared natural diets containing brood comb to artificial diets	a. beeswax (5%) increased growth b. slight decrease in larval growth when yeast is absent c. during growth, larvae increased fat content and dry matter	mixed cereal Pablum, brewer's yeast, yellow beeswax, water, glycerol, honey	200 mg (15 d)
Dadd (1964)	Determine carbohydrate, lipid, and cholesterol requirements for optimal rearing	Compared artificial diets with individual components omitted. Varied proportions of casein, glucose, cellulose, dextrin, inulin, sorbose, and cholesterol	a. highest growth when casein was 15–20% of diet b. linoleic or linolenic acid is necessary c. 5–40% of glucose or digestible carbohydrate increased growth d. >40% glucose made the diet too sticky	cellulose, casein, glucose, glycerol, cholesterol, linoleic acid, salt mixture, 10 water-soluble vitamins	196 mg (12 d)
Dadd (1966)	Determine whether wax is needed, and carbohydrate substitutes can be found, for optimal growth	Compared ratios of glucose to beeswax in a basal diet (Dadd 1964). Compared glucose substitutes.	a. beeswax could replace carbohydrates or sugar b. 10–15% wax was optimal c. wax promoted growth as a source of metabolic water in low-water diets	cellulose powder, linoleic acid, cholesterol, salt mixture, glucose, casein, glycerol, vitamins	226 mg (15 d)
Marston and Campbell (1973)	A low-cost diet to produce high egg numbers for rearing *Trichogramma* spp. (parasitoids)	Compared six previous diets and three modifications of the Beck (1960) diet. Modifications included wax versus no wax and CSM and CSMA[b]	a. Beck (1960) diet was best for uniformity and vigor (physiological and pathology testing) b. CSM diet was best for mass rearing and egg production (for fish bait and parasitoid rearing)	CSM diet: honey, glycerin, water, CSM, CSMA, Wheast	Larval mass not recorded
Marston and Brown (1974)	Follow-up to Marston and Campbell (1973) with the same aim	Compared three yeast products, % liquid, concentrations of beeswax, mixtures of bran/flour/cornmeal	a. wax increased larval survival but amount was not significant b. wax can be used, but cost of labor outweighs the benefit c. development was slower on diets without yeast and females were smaller and produced smaller eggs	wheat flour, cornmeal, bran, Wheast, glycerin, water	Larval mass not recorded
Mohamed and Coppel (1983)	A rearing procedure for small-scale laboratory studies that reduces cost and produces high numbers of insects	Described the best rearing procedure	a. conclusions are the rearing procedure	water, honey, glycerin, beeswax, Polyvisol multivitamin supplement, baby food cereal (Gerber's Hi-protein)	Larval mass not recorded
Coskun (2006)	Produce large wax worms for *Pimpla turionella* rearing (endoparasitoid)	Compared proportions of added honeycomb with and without honey	a. 300 g honeycomb (with honey) added to 1 kg other diet ingredients increased mass by 118 mg b. 50 g honeycomb (with honey) added to 1 kg other diet ingredients increased mass by 131 mg c. mass lost in the absence of honey (wax only)	bran, glycerin, honeycomb, pure water, honey	191 mg (13 d)

Table 1. Continued

Reference	Aim of experiment	Ingredients or nutrients tested	Conclusions	Ingredients in successful diet	Highest mass achieved (days[a])
Metwally et al. (2012)	An economical diet for mass-rearing larvae as host for nematodes	Compared four low-cost diets to a natural diet	diets did not differ in the proportion of nematodes produced, but did differ in cost two diets were equally economical	suggested two diets: Diet 1: wheat flour, corn flour, milk powder, baking yeast, honey, glycerin Diet 2: same except glycerin is substituted with sorbitol	Larvae were weighed in groups; Diet 2: mean mass of the group was 3.71 g with an average of 19.1 surviving larvae
Jorjao et al. (2018)	A diet for rearing larvae used as infection models. Goals: decrease life cycle length, increase body mass, increase immune system	Compared three diets having high nutritional value (cost not considered)	Diet 3 decreased life cycle time, increased mass, showed high survival, increased hemolymph volume, increased immune system activation	Diet 3: corn meal, yeast extract, soy flour, powdered milk, honey, glycerol, beeswax	365 mg (35 d, from egg to largest instar)

[a]Days represent the time from early instar larva to late instar, unless otherwise noted.
[b]CSMA is a house fly medium and CSM is a human food product that contains a whey yeast product called Wheast, in place of brewer's yeast (Marston and Campbell 1973).

ingredients often do not affect insects independently (Assemi et al. 2012, Cohen 2015). In addition to revealing interactive effects among variables, the efficiency of DOE is superior to testing one variable at a time when other variables are kept constant. Full factorial and mixture designs are two design platforms available when using DOE in JMP (SAS Institute, Cary, NC) software. The full factorial design is a method of testing every combination of factors (i.e., diet ingredients) and supports both categorical and continuous variables. Mixture designs, however, test responses to relative proportions of components rather than absolute amounts in mixes. Mixture designs employ a polynomial approach that facilitates development of an optimal ingredient mixture by assessing insect responses to varying blends of ingredients (Lapointe et al. 2008). A mixture design generates a combination of ingredients whose proportions sum to unity (1). Therefore, when the amount of one ingredient changes, so do the relative amounts of other ingredients in the blend. The use of mixture designs can increase the efficiency of diet development and formulation (Ruohonen and Kettunen 2004) and has been successful in the optimization of insect diets (Lapointe et al. 2008, Pascacio-Villafan et al. 2017). Using a combination of full factorial and mixture design allows for both rigorous testing and efficient diet optimization.

The greater wax moth, *Galleria mellonella* (L.), is a serious global pest of the western honey bee, *Apis mellifera* L., and its economic importance has prompted studies of many facets of its biology, including life history, behavior, molecular biology, physiology, biochemistry, microbiology, and genetics (Beck 1970, Robertson 1978, Robertson and Dell 1981, Mala et al. 1987, Ellis et al. 2013, Büyükgüzel 2014, Büyükgüzel and Büyükgüzel 2016, Lange et al. 2018, Singkum et al. 2019). Wax moth larvae are commonly used as infection models of human microbial pathogens, including models of virus and bacterium virulence (Senior et al. 2011, Büyükgüzel and Büyükgüzel 2016, Silva et al. 2017, Pérez-Reytor and García 2018). They are a good source of protein and fat as food for captive insectivores (Finke 2015), and they also serve as hosts for mass-rearing nematodes and parasitoids (Saunders and Webster 1999, Ciche and Ensign 2003, Coskun et al. 2006).

Nutritional requirements of wax moths were studied (Dadd 1964, 1966; Jindra and Sehnal 1989) and best rearing practices (Mohamed and Coppel 1983) have been designed mostly for small-scale production in laboratories. Rearing studies have also sought to maximize numbers of moths at low cost for rearing parasitoids and nematodes (Marston and Campbell 1973, Marston and Brown 1974, Coskun et al. 2006, Metwally et al. 2012) and to identify ideal conditions for rearing them for use as in vivo infection models (Jorjão et al. 2018). Despite being investigated for more than 60 yr in many laboratories, wax moth dietary requirements remain an unsettled issue. A review of wax moth rearing studies reveals inconsistencies, contradictions about specific requirements, and conflicting information about the proportions of various components (Table 1).

A possible reason for the large variation in diet study outcomes is that wax moth response to nutrition is plastic, and the larvae can survive on both minimal and complex diets, thus attaining a range of body masses. Large wax moth larvae can be desirable as hosts for biological control agents, in the pet food industry, and as experimental insects. For example, larger *G. mellonella* hosts produced greater numbers of the entomopathogenic nematodes *Heterorhabditis zealandica* and *H. bacteriophora* (van Zyl and Malan 2015). Production of large, fast-growing larvae is often an objective for commercial production of insect food for captive insectivores (Finke 2015). Wax moth larvae have been selected in recent years as a primary in vivo model for antibacterial and novel

drug testing due to many life cycle and immune response factors. Their large size is viewed favorably because more hemolymph can be obtained from each larva and larger larvae are easier to handle in the lab (Cutuli et al. 2019). Larger wax moths are also beneficial for studying fungal virulence and provide the opportunity to harvest more tissue to evaluate fungal tissue invasion (Desalermos et al. 2012). Faster wax moth growth rates are also desirable to maximize rearing efficiency. Many current wax moth diets are derived from the Beck (1960) diet (Table 1) (Ellis et al. 2013), but this diet is likely not yet optimized for larval size and development rate.

The goal of this study was to optimize an artificial diet for *G. mellonella* to produce large, fast-growing larvae. We used an optimization strategy based on DOE to increase final larval body mass, minimize mortality, and optimize the rate of development. Using DOE techniques, we sought to test the effectiveness of several ingredients and their proportions in the diet. We optimized the amount of water, cereal, and wax, tested inclusion of pollen, and examined a mixture of cereals, including rice bran, which is not commonly used in insect diets. The use of both full factorial and mixture designs allowed us to implement sequential diet trials in which the results determined which ingredients should be tested in the subsequent experiments. This ultimately led to the development of a diet that produced consistently large wax moth larvae with fast development rates.

Materials and Methods

Wax Moth Colony, Rearing Conditions, and Experimental Arenas

The stock colony of wax moths was started from 100 larvae (Carolina Biological Supply Co., Burlington, NC). It was continuously cultured since December 2017 at 27 ± 3.0°C, 65 ± 8% RH, under constant darkness on an oat bran diet derived from Beck (1960) (Fig. 1). Prior to the currently reported research, the colony had been subjected to several modifications of the original Beck diet, including substitution of torula yeast (*Candida utilis* (Henneberg)) for brewer's yeast (*Saccharomyces cerevisiae* Meyen ex. E. C. Hansen) and several grain substitutions to replace Pablum (a mixture of wheat, oatmeal, corn meal, bone meal, brewer's yeast, alfalfa leaf, and iron), which was no longer available. To produce experimental larvae, 15 pupae from the colony were placed in each of four 0.95-liter glass canning jars (Ball, Broomfield, CO) with organza mesh covering the top. When adults emerged, a clear ~57-ml cup containing the oat bran colony diet (Beck-derived) was inverted and placed on top of the mesh of each jar to allow for egg laying through the mesh into the diet. Three days after the appearance of neonates, each of the four cups containing the colony diet and larvae was individually placed in four 473-ml white plastic bins with an aluminum mesh window in the snapped-on lid that allowed for gas exchange and prevented escape. The diet placed in each bin contained eggs and neonates. After several days, when an excess of second instars needed for each trial were present (i.e., Trial 1: >320, Trial 2: >420, Trial 3: >320, Trial 4: >280), experimental larvae were removed for the trial, and the remaining larvae were discarded. The colony was continuously reared, and production of test insects was repeated for each of the four diet trials.

Diet Preparation, Modifications, and Relative Cost

The first step in diet preparations was weighing the dry ingredients, combining them in a clean 473-ml plastic bin and stirring by hand using a plastic stirring rod until well mixed. Next, the wet ingredients were weighed in a glass beaker. This mix was heated in a microwave oven (700 kW, high power setting) for 30 s to facilitate mixing, stirred by hand with a plastic stir rod, and added to the dry ingredients. The entire diet was stirred by hand until the wet ingredients were fully incorporated and no clumps of dry ingredients remained. No salts, vitamins, or minerals were added and the total mass of each diet varied based on the amount of each ingredient being tested. All diet mixtures are presented in Supp Table S1 (online only). Dry ingredients tested included: brewer's yeast (Sigma, St. Louis, MO), torula yeast (Lallemand, Montreal, QC, Canada), pollen pellets (CC Pollen Co., Phoenix, AZ), yellow wax pellets (Natures Oil, Aurora, OH), multigrain baby food (Gerber, Arlington, VA), oat bran, wheat bran (Bob's Red Mill Natural Foods, Inc., Milwaukie, OR), and stabilized rice bran (NutraBio Labs, Inc., Middlesex, NJ). Water amount was the

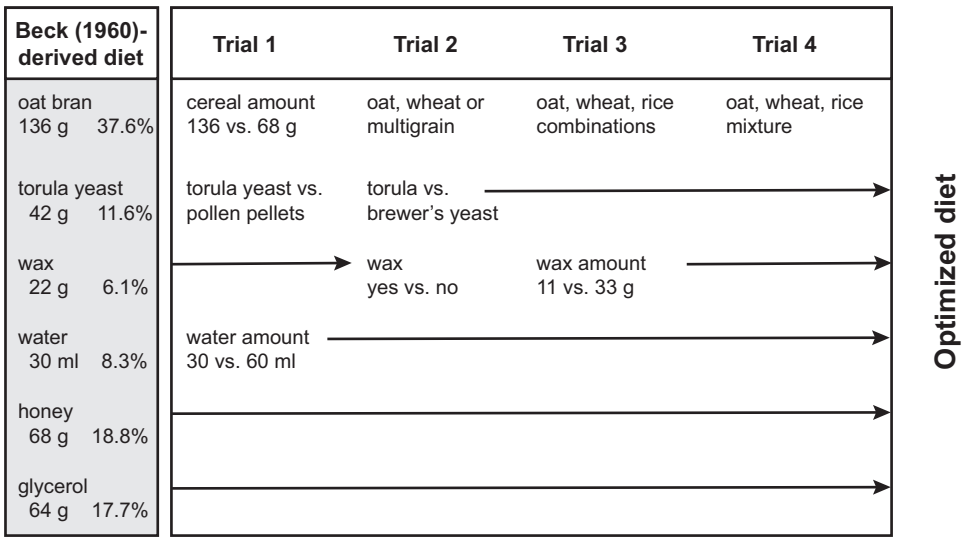

Fig. 1. The design and progression of trials, showing the standard colony diet derived from Beck (1960) on the left and the progression of ingredients tested from Trial 1 to Trial 4. The weight and proportions of ingredients tested in each trial are shown in the column under the Trial number. The arrows through a trial indicate that the ingredients remained unchanged from the previous trial. Percentages in the left column are calculated from total diet.

only wet ingredient tested. The amounts of honey (Clover Honey, Harris Teeter, Matthews, NC) and glycerol (99.7%, Lot Number 05017-EA, ChemWorld, Atlanta, GA) were not varied in any of the diets. The diets were covered with plastic lids, allowed to rest for 24 h at ambient conditions, and an aliquot of each batch (60 g) was used for experiments. Relative cost of ingredients in 1,000 g of each prepared diet is also presented in Supp Table S1 (online only).

Experimental Protocol

Experimental diet (15 g) was gently and evenly pressed into the bottom of a 59-ml plastic Solo cup (Dart Container Corporation, Mason, MI) and 10 second instars were added using a soft paintbrush. Because first instar larvae could easily be damaged during transfer, we started all trials with second instars. To allow for gas exchange, 10 punctures were made in the lid of the cup using a teasing needle. Larvae were reared for 10 d under the same environmental conditions as the colony. After 10 d, live larvae were counted and individually weighed to 0.001 g using an OHAUS Pioneer balance (Parsippany, NJ). The trial period ended after 10 d because most larvae matured to seventh instar but had not pupated yet.

Diet Optimization Using Full Factorials and Mixture Design

We tested the presence or absence and proportion of seven ingredients in four trials, resulting in 17 ingredient variations and 35 total diets (Supp Table S1 [online only]). The proportions of dry diet ingredients and water varied, but honey and glycerol remained constant. Each diet formulation was replicated four times by cup and each cup contained 10 larvae. All cups were set up on the same day. After 10 d, 40 larvae were weighed individually per diet. Except for the first trial, the ingredients and proportions tested were contingent on the results of the previous trial (Fig. 1).

Full factorial design

The first three trials were conducted utilizing a full factorial design, which allows for analysis of the main effects of each factor (i.e., diet ingredient) and the interaction among multiple factors. A full factorial model (JMP 13, SAS Institute) was used to assess the effect of each ingredient and the interactions among ingredients on survival and larval mass. When significant interactions were present, the main effect of a single ingredient was not interpreted directly from the *P*-value. Instead, the main effect was contingent on the interaction. Therefore, interactions were assessed first and then used to determine the impact of the main effect of ingredient on survival and mass. The results of each analysis informed our decision to incorporate or abandon tested ingredients and to maintain or vary their proportion in the next trial. Thus, the control diet differed for each trial and the hypothesis was related to the specific ingredients being tested in each trial. In Trial 1, the control diet was the Beck-derived diet and we hypothesized that wax moth larvae would attain greater body mass when fed natural diet components that included pollen and beeswax (Nielsen and Brister 1979), but the optimal amounts were unknown. The results from Trial 1 led to testing of yeast species and cereal type in Trial 2, the control diet was 'torula yeast, oat bran with wax' (Diet 11, Supp Table S1 [online only]), and we hypothesized that torula yeast would promote greater larval mass than brewer's yeast because it has higher protein, amino acid, and mineral content than brewer's yeast (Bekatorou et al. 2006). The results of Trial 2 led us to test wax quantity and addition of rice bran. The control in Trial 3 was '100% oat and low wax' (Diet 26, Supp Table S1 [online only]) and we hypothesized that the higher fat content in rice bran would lead to higher larval mass.

Mixture design

The final diet trial was conducted utilizing Optimal Mixture Design (JMP 13, SAS Institute) to estimate the proportion of each cereal that would yield the highest larval mass and survival. The three cereals—oat bran, wheat bran, and rice bran—were entered into the DOE platform with defined constraints based on the previous full factorial findings. The program generates experiments with factors (diet ingredients) that are components in a mixture. The limiting proportions (of total cereal mass) of rice bran, wheat bran, and oat bran were set at 0–0.3, 0.2–0.5, and 0.2–0.5, respectively. These values constituted proportions of 68 g of total cereal, which was determined to be the optimal amount of cereal in 283 g of prepared diet. Therefore, the tested weight ranges for rice bran, wheat bran, and oat bran were 0–20, 14–34, and 14–34 g, respectively. Because a diet made with 100% rice bran as the sole cereal component was not palatable for the larvae, possibly due to its 'gummy' texture, the proportion of rice bran was limited to 0.3. Trial 3 showed that wax moth larvae attained higher body mass when oat or wheat were mixed with rice bran, but it was still unknown if a diet formulation that included oat and wheat bran alone was beneficial. Therefore, the 0.2–0.5 constraints were applied to each of the oat and wheat brans. This design optimizes the proportion of the selected ingredients, and a change in one ingredient is not independent of the others.

Macronutrient Analysis of Larvae

To compare the macronutrient contents of wax moth larvae reared on the final optimized diet and the Beck-derived diet, we freeze-dried surviving larvae in each of the four cups of diet per treatment in a lyophilizer (VirTis Lyo-Centre, 3.5-liter, Gardiner, NY) for 24 h to less than 5% moisture content (OHAUS MB45 Moisture Analyzer, Parsippany, NJ). To avoid the possibility of lack of independence of larval data within each cup, only one randomly selected larva from each cup (n = 4) was used for nutritional analysis. Percent contents of proteins, lipids, carbohydrates, and amino acids were determined in individual larvae. The tests used were: Acid Orange for protein, chloroform–methanol–water Folch extraction for lipids (Folch et al. 1957), anthrone test for carbohydrates (Method 61 in Keleti and Lederer (1974)), and ninhydrin for amino acids (Method 38 in Keleti and Lederer (1974)). Each dried larva was weighed, placed in a 2-ml centrifuge tube, and ground in 0.5 ml of deionized water (DIW) for 5 min using a disposable polypropylene pellet pestle. We added 1 ml DIW to the ground insect, vortexed the tube for 5 s at 3,000 RPM (VWR, pulsing vortex mixer, Radnor, PA), and aliquoted the 1.5 ml sample into four 1.5-ml centrifuge tubes as follows: 0.5 ml each for lipids and proteins, and 0.1 ml each for amino acids and carbohydrates. Acid Orange, a dye-binding procedure, quantifies the soluble and insoluble proteins by binding with proteins containing lysine, arginine, and histidine, and quantifies total protein colorimetrically at 482 nm. The Folch extraction method generates a suspension from which the lipid-containing chloroform layer is removed and dried, and total lipid content is determined gravimetrically (Van Handel 1985). The anthrone carbohydrate test measures total carbohydrates by colorimetrically quantifying the anthrone-reacted carbohydrates in the sample at 625 nm (Dreywood 1946, Cohen 2015). The ninhydrin test colorimetrically quantifies amino acids from their reaction with ninhydrin at 570 nm. Colorimetric assays were analyzed on a single-beam

UV/visible spectrophotometer (Cole Parmer, Vernon Hills, IL) and new standards were quantified before each nutrient test and diet trial. The total protein, amino acids, lipids, and carbohydrates (in mg) were adjusted based on each aliquot to represent the total sample. The mass of each macronutrient was divided by the freeze-dried mass of the larva to yield its percentage representation in the larva.

Statistical Analysis

Statistical analyses were performed using JMP version 13 (SAS Institute). Larval body mass data for each diet were analyzed using ANOVA, and post hoc comparisons of means were conducted with Tukey's honestly significant difference test ($\alpha = 0.05$). The ANOVA tests were used to examine the effect of the whole diet on larval body mass, instead of assessing effects of individual ingredients within the diet. Larval mass data were normally distributed (Shapiro–Wilk test, JMP, Version 13) and treatments had equal variance (Brown–Forsythe test, JMP Version 13), but survival data were not normally distributed. Therefore, a Kruskal–Wallis test and post hoc nonparametric multiple comparisons of means tests ($\alpha = 0.05$) for proportion survival were used to identify differences between pairs. Following the ANOVA tests, a full factorial model, which included the main effects of each ingredient and first-order interactions (between two ingredients), was fitted for Trials 1–3 to test whether individual ingredients had a significant effect on larval body mass and survival. A mixture design was used for Trial 4. Mann–Whitney *U* nonparametric test was used to determine differences in macronutrient content of larvae on different diets ($\alpha = 0.05$). Means ± SEM are presented in all graphs.

Results

Effects of Diets and Ingredients

Four sequential trials were performed, comparing 17 ingredient variations across 35 diets (Supp Table S1 [online only]). Wax moth larval mass after 10 d of feeding was significantly affected by diet in all trials (Table 2); survival, however, was unaffected by diet and was high across all diets (mean ± SEM for all diets was 89.58 ± 0.1%, $n = 143$). Table 2 shows the statistics for each trial's main effects (ingredients) and first-order interaction effects (between two diet ingredients) on larval mass and survival. The following combinations of ingredients and levels resulted in generally higher body mass: low oat bran with yeast supplement and low water (Fig. 2); oat bran regardless of wax presence, and multigrain cereal in the absence of wax (Fig. 3); oat bran regardless of yeast species, and multigrain cereal in presence of brewer's yeast (Fig. 4); oat bran combined with rice bran, regardless of wax level (Fig. 5); wheat or oat bran with addition of 20% rice bran (Fig. 6). In three tests, mean larval mass approached or equaled 400 mg, the highest mass achieved (Figs. 3, 4, and 7). These included a combination of the three cereals using a mixture design, at a ratio of 34 wheat bran to 20 rice bran to 14 oat bran (Fig. 7).

Because the ingredient interactions were critical to selection of ingredients to be tested in subsequent trials, we present results of the interactions (Figs. 2–6) among ingredients and how they affected mass and survival, instead of their main effects in Trials 1–3. The mixture-design response surface analysis in Trial 4 predicted the proportions of cereal ingredients that maximized larval mass (Fig. 7).

Table 2. Statistical analysis using a full factorial design for body mass and survival, showing the main effects of each factor (diet ingredient) and first-order interactions (two diet ingredients)

Test	Main effects and interactions	Body mass *P*-value[a]	Survival *P*-value[a]
Trial 1 2 × 2 × 2	Oat (high or low)	0.2140	0.2866
	Nutritional supplement (pollen or yeast)	**0.0001**	0.0540
	Water (high or low)	0.1687	0.0540
	Oat * supplement	**0.0005**	0.1738
	Oat * water	0.2640	0.6448
	Supplement * water	0.5310	0.6448
ANOVA Trial 1	Diet (body mass)	**<0.0001**	
Kruskal–Wallis	Diet (survival)		0.2016
Trial 2 3 × 2 × 2	Cereal (oat bran, wheat bran, multigrain cereal)	**0.0001**	0.0879
	Wax (presence or absence)	0.6474	0.1907
	Yeast (brewer's or torula)	**0.0013**	0.7602
	Cereal * wax	**<0.0001**	0.9292
	Cereal * yeast	**<0.0001**	0.5218
	Wax * yeast	**0.002**	0.3622
ANOVA Trial 2	Diet (body mass)	**<0.0001**	
Kruskal–Wallis	Diet (survival)		0.5555
Trial 3 4 × 2	Cereal (wheat bran only, oat bran only, 80:20 wheat:rice bran, 80:20 oat:rice bran)	**<0.0001**	0.2457
	Wax (high or low)	0.4485	0.1516
	Cereal * wax	**0.0118**	0.7522
ANOVA Trial 3	Diet (body mass)	**<0.0001**	
Kruskal–Wallis	Diet (survival)		0.2008

Significant main effects ($P < 0.05$) show that the ingredient tested affected body mass and/or survival. Significant first-order interactions ($P < 0.05$) show that the effect of one ingredient is significantly affected by the presence of the other ingredient in the interaction term.

[a]Statistical analysis of body mass based on ANOVA and survival based on Kruskal–Wallis tests comparing diets within each trial. *P*-values < 0.05 are shown in bold.

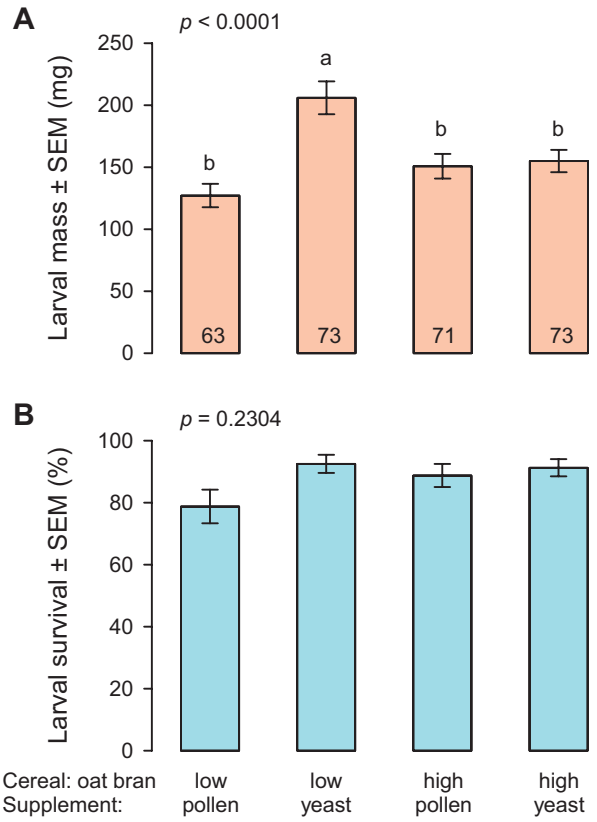

Fig. 2. Average larval growth (A) and survival (B) on diets in Trial 1, comparing low (68 g) and high (136 g) oat bran in combination with pollen or torula yeast. Larval mass was significantly affected by diet: ANOVA: $F_{3,276} = 9.5514$, $P < 0.0001$. Means (± SEM) that do not share letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$). Survival was not significantly affected by diet: Kruskal–Wallis test: $H_3 = 4.3050$, $P = 0.2304$. In (A) the number of individually weighed larvae in each treatment ($n = 80$) is indicated within each bar. Survival (B) represents the mean of survival values across eight replicates. Low oat with yeast and low water resulted in higher larval mass.

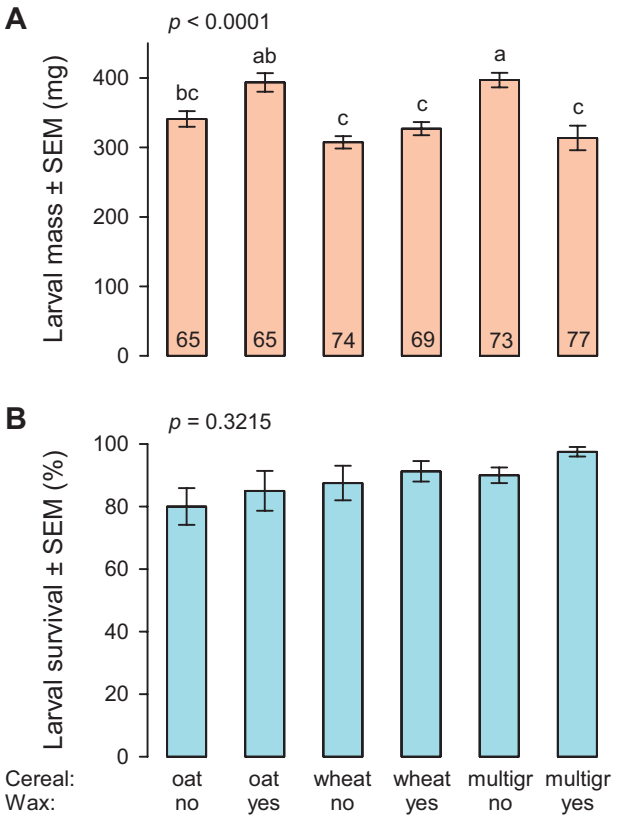

Fig. 3. Average larval growth (A) and survival (B) in Trial 2 on diets that included individual cereals (oat bran, wheat bran, or multigrain baby food cereal) with and without beeswax. Larval mass was significantly affected by diet: ANOVA: $F_{5,417} = 10.0262$, $P < 0.0001$. Means (± SEM) that do not share letters are significantly different (Tukey's honestly significant difference test, $P < 0.05$). Survival was not significantly affected by diet: Kruskal–Wallis test: $H_5 = 5.8463$, $P = 0.3215$. In (A) the number of individually weighed larvae in each treatment ($n = 80$) is indicated within each bar. Survival (B) represents the mean of survival values across eight replicates. Oat bran, regardless of beeswax presence, and multigrain cereal without beeswax resulted in higher larval mass.

Optimization of Diets for Larval Growth and Survival Using DOE

Trial 1

In Trial 1 we tested the effects of high and low amounts of oat bran (136 g vs 68 g), pollen versus torula yeast as nutritional supplements, and high and low amounts of water (60 ml vs 30 ml) on larval mass. The main effect of the nutritional supplement was significant, with larger body mass attained on yeast than on pollen (Table 2), so yeast was used in all subsequent trials. Within the tested ranges, high or low amounts of water and oat bran did not significantly affect larval mass. However, the outcome of water level was marginally significant, leading us to select the more beneficial levels of this ingredient in subsequent trials; lower water content tended to result in higher survival ($P = 0.0540$), so the low level was selected. Because varying the amount of oat bran did not significantly affect larval body mass ($P = 0.2140$), we examined which level of oat bran supported higher growth in the presence of yeast, which significantly increased larval mass. A significant interaction between the amount of oat bran and the yeast nutritional supplement ($P = 0.005$) led us to use low proportion of oat in diets (Fig. 2A). Therefore, we maintained low amount of cereal (68 g), yeast as a supplement, and low (30 ml) water in Trials 2–4.

Trial 2

This trial tested the effect of yeast species (brewer's vs torula), cereal type (oat bran, wheat bran, or multigrain baby cereal), and wax (presence vs absence) on larval mass. Survival was unaffected by any of these manipulations (Table 2). Wax did not affect larval body mass, but cereal and yeast did. Highly significant first-order interactions of cereal * wax, cereal * yeast, and yeast * wax suggested that these interactions should guide the selection of ingredients to be used in the following diet optimization trials.

The cereal * wax interaction ($P < 0.0001$) showed that larvae attained a higher mass when wax was present in diets containing oat bran or wheat bran, and lower body mass when wax was combined with multigrain cereal (Fig. 3A). The multigrain cereal diets became strongly cohesive and formed a 'clay-like' ball, which appeared to inhibit the typical tunneling behavior of the larvae. Therefore, we eliminated multigrain cereal from consideration in subsequent diets. Further testing was then required to understand the effect of wax presence in wheat or oat bran diets, as these outcomes were not significant (Fig. 3A).

The cereal * yeast interaction ($P < 0.0001$) showed that larvae attained marginally higher mass when fed torula yeast and wheat bran, compared to other cereals. However, larvae also attained higher mass

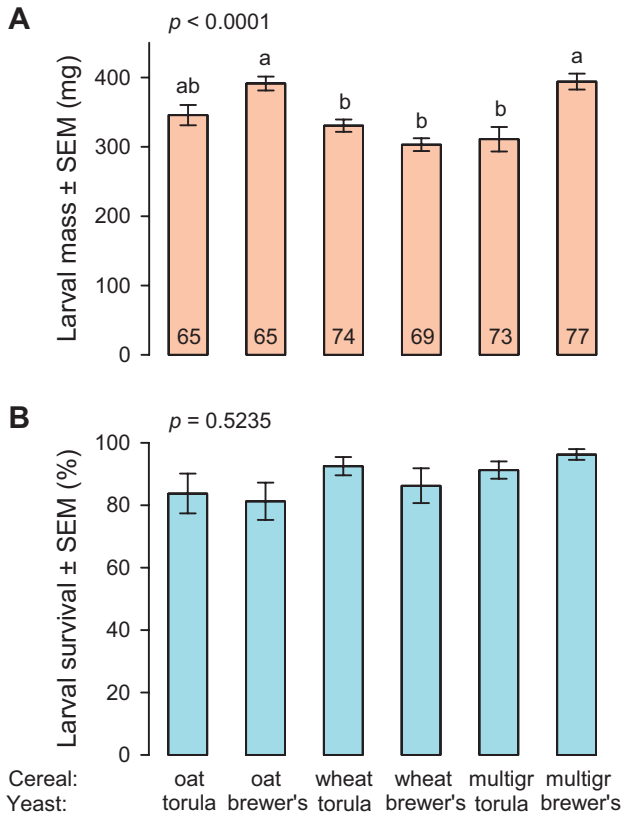

Fig. 4. Average larval growth (A) and survival (B) in Trial 2 on diets that contained various cereals (oat bran, wheat bran, and multigrain baby food cereal) combined with brewer's or torula yeast. Larval mass ANOVA: $F_{5,417}$ = 9.8379, P < 0.0001. Means (± SEM) that do not share letters are significantly different (Tukey's honestly significant difference test, P < 0.05). Survival Kruskal–Wallis test: H_5 = 4.1823, P = 0.5235. In (A) the number of individually weighed larvae in each treatment (n = 80) is indicated within each bar. Survival (B) represents the mean of survival values across eight replicates. Oat bran, regardless of yeast species, and multigrain cereal in presence of brewer's yeast resulted in higher larval mass.

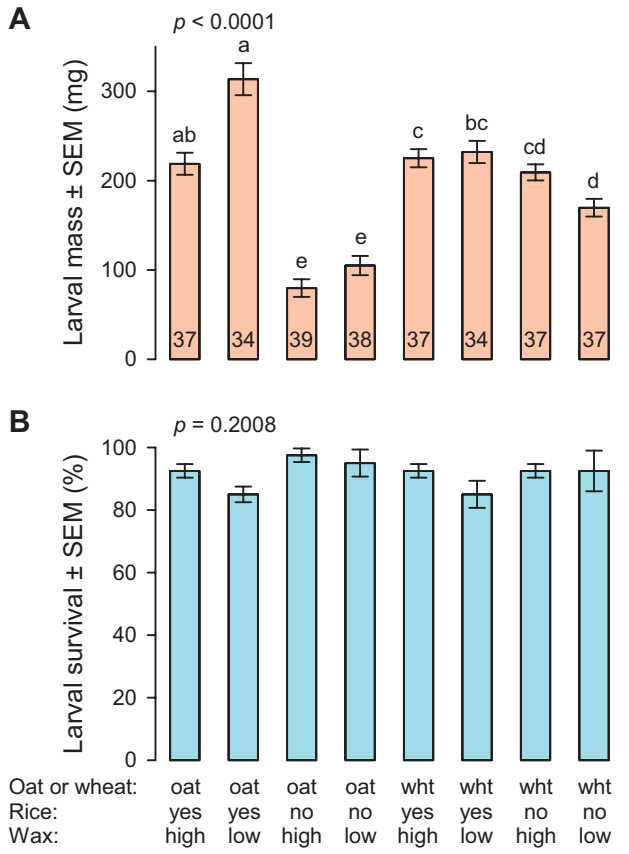

Fig. 5. Average larval growth (A) and survival (B) in Trial 3 on diets that contained wheat bran or oat bran alone or in combination with 20% rice bran, combined with high or low beeswax levels. Larval mass ANOVA: $F_{7,285}$ = 47.1447, P < 0.0001. Means (± SEM) that do not share letters are significantly different (Tukey's honestly significant difference test, P < 0.05). Survival Kruskal–Wallis test: H_7 = 9.7897, P = 0.2008. In (A) the number of individually weighed larvae in each treatment (n = 40) is indicated within each bar. Survival (B) represents the mean of survival values across four replicates. Oat bran combined with rice bran, regardless of beeswax level, resulted in higher larval mass.

in the presence of brewer's yeast in diets containing multigrain cereal, and marginally more in diets containing oat bran (Fig. 4A). Although brewer's yeast in a multigrain cereal diet yielded superior larval mass in Trial 2, we eliminated multigrain cereal from further trials due to its adverse effects in the cereal * wax interaction. Because multigrain cereal was eliminated, the significant body mass attained when brewer's yeast was added was no longer a factor in decision-making for subsequent trials. Therefore, we selected torula yeast for inclusion in the following two trials, but cereal type and wax amount were still undecided.

Trial 3

Statistically significant interactions in Trial 2 and elimination of the multigrain cereal component prompted us to reexamine cereal and wax in Trial 3. We tested the effects on larval mass of high and low wax levels (33 g vs 11 g) and cereal type (wheat bran, oat bran, wheat and rice mix, and oat and rice mix). Survival was unaffected by any of these diet manipulations (Table 2). Wax level did not affect body mass (P = 0.4485), but there was a significant interaction between cereal type and wax amount (P = 0.0118; Table 2). The interaction indicated that the lower amount of wax promoted higher body mass on some diets (oat bran, oat and rice brans, and wheat and rice brans), but resulted in lower mass in the wheat bran-only diet (Fig. 5A). Because the lower amount of wax resulted in greater larval mass in three of the four cereal treatments, including the oat and rice bran diet, we retained the lower amount of wax (11 g) in subsequent diets.

Larvae attained higher body mass on wheat bran compared with oat bran, and replacement of 20% of wheat bran with rice bran significantly augmented mass (Fig. 6A). Oat bran resulted in low larval mass, but replacement of 20% of oat bran with rice bran resulted in a 3.2-fold increase in body mass (Fig. 6A). Larval mass attained was significantly higher on the oat–rice mix than on the wheat–rice mix.

Overall, Trials 1–3 enabled selection of yeast species and amounts of water and wax in an optimized diet, but the mixture of cereals needed further testing.

Trial 4

Using mixture design, we sought to determine the types and proportions of cereals to include in the final diet. No significant difference in survival was evident (Fig. 7B), but a highly significant difference in larval body mass was evident among diets with varying ratios of rice, wheat, and oat brans (P < 0.0001) (Fig. 7A). In general, as the proportion of rice bran increased from 0 to 0.3, larval mass also significantly increased. A mixture of 0.5 wheat bran, 0.3 rice bran, and 0.2 oat bran yielded the highest mass (Fig. 7A).

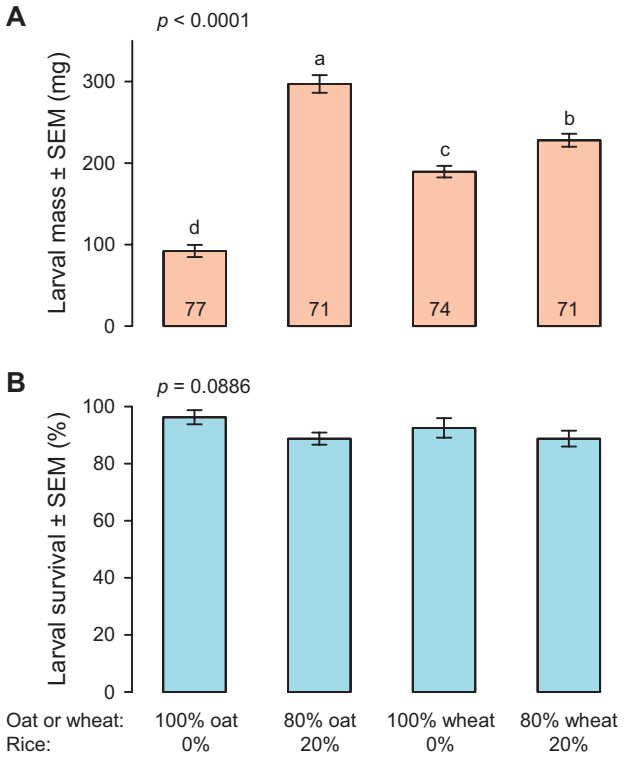

Fig. 6. Average larval growth (A) and survival (B) in Trial 3 on diets that include wheat bran or oat bran alone or in combination with 20% rice bran. Larval mass ANOVA: $F_{3,289}$ = 103.3210, P < 0.0001. Means (± SEM) that do not share letters are significantly different (Tukey's honestly significant difference test, P < 0.05). Survival Kruskal–Wallis test: H_3 = 6.5273, P = 0.0886. In (A) the number of individually weighed larvae in each treatment (n = 40) is indicated within each bar. Survival (B) represents the mean of survival values across eight replicates. The addition of 20% rice bran to oat and wheat bran resulted in higher larval mass.

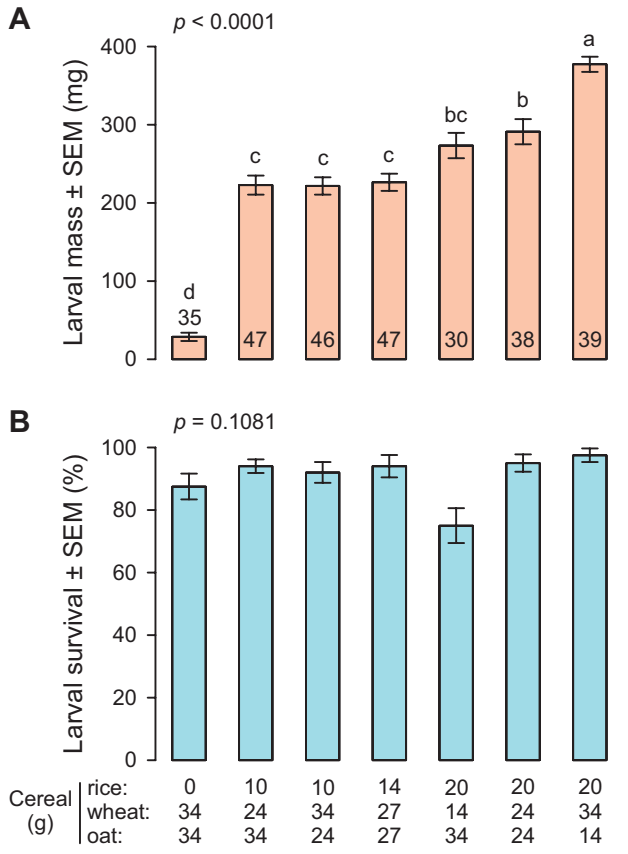

Fig. 7. Average larval growth (A) and survival (B) in Trial 4 on diets that include combinations of three cereals (wheat bran, oat bran, rice bran) in different proportions based on a mixture design. Total cereal was 68 g in each mixture. Larval mass ANOVA: $F_{6,275}$ = 68.5361, P < 0.0001. Means (± SEM) that do not share letters are significantly different (Tukey's honestly significant difference test, P < 0.05). Survival Kruskal–Wallis test: H_6 = 10.4194, P = 0.1081. In (A) the number of individually weighed larvae in each treatment is indicated within each bar. Survival (B) represents the mean of survival values in each of 4–5 replicate cups with 10 larvae in each cup. Four replicates (n = 40) were made in the 'rice:wheat:oat'; 0:34:34, 20:14:34, 20:24:24, 20:34:14 treatments and five replicates (n = 50) in the 'rice:wheat:oat'; 10:24:34, 10:34:24, 14:27:27 treatments. The optimal diet is shown in the final bar (rice: 20 g, wheat: 34 g, oat: 14 g).

Finally, the optimal diet (Diet 35, Supp Table S1 [online only]) that resulted from the four trials was compared to the standard Beck-derived diet that was fed to the colony (Fig. 1). The two diets resulted in equivalent and high survival of larvae (Fig. 8B), but the new diet supported 2.4-fold greater wax moth larval mass, from 156.8 ± 13.6 mg on the standard diet to 377.4 ± 9.7 mg on the new diet (P < 0.0001) (Fig. 8A).

Cost of diet ingredients ranged from about $20 to $32 per kg for all prepared diets (Suppl Table S1 [online only]). These figures represent cost of ingredients purchased in small quantities for experimental purposes rather than in bulk. Diets containing torula yeast were more costly than those lacking the yeast. The optimized diet contained torula yeast and was among the most expensive.

No significant differences were evident in percent protein (Mann–Whitney U, H_1 = 0.7500, P = 0.3865), lipids (Mann–Whitney U, H_1 = 0.0833, P = 0.7728), or carbohydrates (Mann–Whitney U, H_1 = 1.3333, P = 0.2482) between larvae fed on the optimized diet and the Beck-derived diet (Fig. 8C). However, the relative content of amino acids was significantly greater in larvae on the Beck-derived diet than our optimized diet (Mann–Whitney U, H_1 = 4.0833, P = 0.0433) (Fig. 8C). The difference in percent amino acid can be attributed to one larva having an exceptionally high percent amino acids (9.62%) compared to the average of the other three larvae in the Beck-derived diet treatment (5.45% ± 0.55% SE). This freeze-dried larva was also small (28.8 mg) compared to the other larvae in this treatment (96.00 ± 6.38 mg SE, n = 3), and was likely anomalous. Overall, the macronutrient data indicated that the relative macronutrient composition of larvae was not affected despite faster development and greater body mass attained when larvae fed on the optimized diet.

Discussion

Using statistical DOE, we optimized a diet to achieve substantially greater mass of wax moth larvae than reported for previous diets. Larvae reared on the optimized diet gained 2.4 times more body mass than larvae reared on the original Beck (1960)-derived diet, which is a standard diet for many current wax moth rearing programs (Ellis et al. 2013).

Comparison With Previous Diet

The standard wax moth diet supported larval growth to an average body mass of ~200 mg at 15 d (Beck 1960). Wax moth larvae reared from second instar for 10 d on the Beck (1960)-derived diet in our lab reached an average body mass of 156.8 mg in the final larval instar.

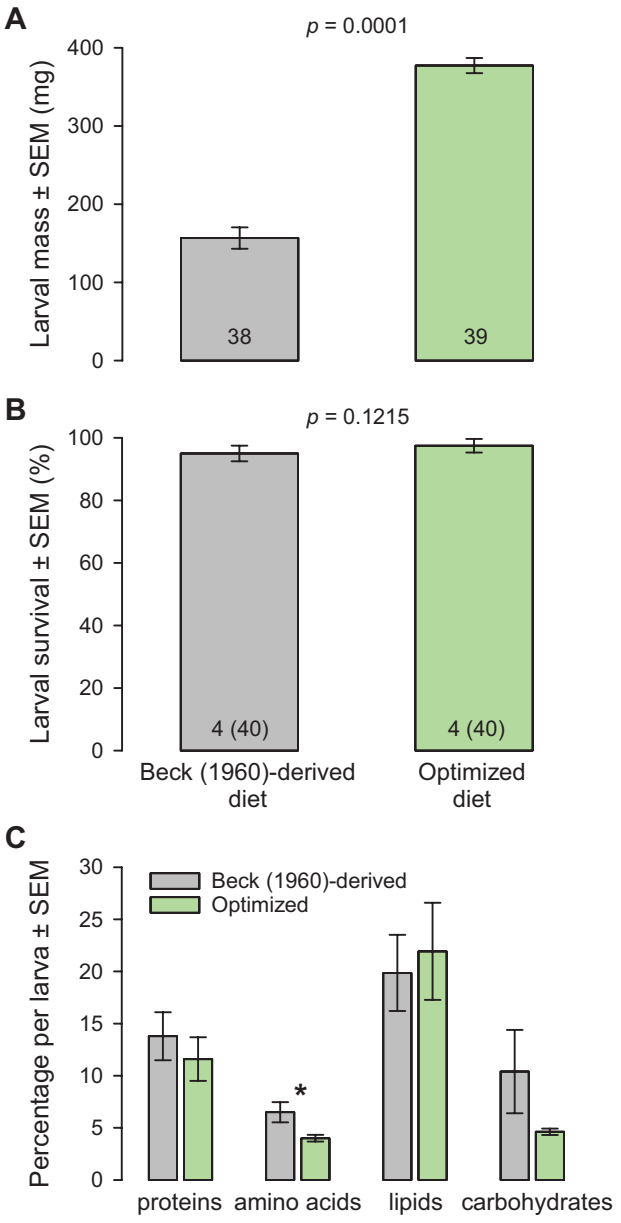

Fig. 8. Comparison of mean (± SEM) larval growth (A) and survival (B) on the standard colony diet that was modified from Beck (1960) and the optimized diet derived from our four trials. The diet was modified by using oat bran and torula yeast in place of Pablum and brewer's yeast, respectively. Larvae reared on the optimized diet gained 2.4-fold the mass gained by larvae on the standard colony diet (*t*-test, 12.99, $P < 0.0001$) and there was no significant difference in survival (Mann–Whitney *U*, $H_1 = 2.3973$, $P = 0.1215$). (C) Macronutrient composition of larvae reared on the modified Beck (1960) diet and the new diet. Ten larvae were reared per cup, but only one larva was randomly selected for analysis from each of four cups ($n = 4$). There were no significant differences between larvae raised on the two diets in percent protein (Mann–Whitney *U*, $H_1 = 0.7500$, $P = 0.3865$), lipids (Mann–Whitney *U*, $H_1 = 0.0833$, $P = 0.7728$), or carbohydrates (Mann–Whitney *U*, $H_1 = 1.3333$, $P = 0.2482$), but a marginally significant difference in amino acids (Mann–Whitney *U*, $H_1 = 4.0833$, $P = 0.0433$) is noted with *.

In contrast, our optimized diet produced larvae with an average body mass of 377.4 mg and maximum mass of 478 mg in only 10 d. Our study protocol is comparable to Beck's, but there are two noteworthy differences. First, Beck (1960) started his experiments with neonates, whereas we initiated each trial with second instars. It is plausible, though unlikely, that first instars might respond differently to our diet if we began with neonates. The second difference is that Beck's trials were conducted at 35°C, at the temperature maintained in the interior of honey bee colonies (Southwick and Heldmaier 1987), while ours were conducted at 27°C. Lower temperatures of 20°C and 25°C, irrespective of humidity, were shown to decrease larval development in wax moths, and the shortest larval development time was obtained using high relative humidity and 35°C (Hanumanthaswamy et al. 2013). Therefore, Beck's larvae would be expected to grow faster at the higher temperature. The first instar typically lasts 2 d; thus, assuming equal growth rate with Beck's larvae, ours would have matured after about 13 d. The 3-d faster development time is, therefore, strong evidence for more rapid growth on the optimized diet compared with Beck's diet. Overall, such rapid and large increases in body mass shown in our study have not been achieved with previous wax moth diets deemed by researchers to be effective (Table 1). We attribute our ability to efficiently achieve the 2.4-fold increase in larval mass, to two main factors: 1) the use of DOE to test mixtures and interactions among diet ingredients, and 2) qualitative changes that we implemented with new diet ingredients.

Optimization Strategy Using DOE

Full factorial and mixture DOE approaches are especially useful for insect rearing because full factorial designs not only test the effect of ingredients but also the interactions among diet ingredients. In all our full factorial trials, the effect of diet was significant, but significant ingredient interactions were also present (Trials 1–3). The analysis of the effects of individual ingredients and their interactions guided the development of the optimized diet. The results support the premise that interactions between and among diet components need to be assessed to achieve rearing of high-quality insects (Keena et al. 1995, Cohen 2015). In addition, the choice of diet ingredients, ingredient source, storage methods, and diet preparation need to be considered because they can also affect insect growth and development.

The full factorial experiments used in Trials 1–3 suggested that a mixture of three cereals might be superior to a binary mixture. Therefore, we implemented a mixture design to determine the proportion of each cereal ingredient needed for an optimal diet. Mixture designs are applicable when only finite ranges of the relative proportions of components produce the desired outcome (Eriksson et al. 1998, Kowalski et al. 2000). The mixture design model has been used only sparingly in insect rearing; it was used to reduce the number of ingredients in the artificial diet of the polyphagous weevil *Diaprepes abbreviates* (L.) (Lapointe et al. 2008), and to reduce the cost of rearing Mexican fruit fly, *Anastrepha ludens* (Loew), for sterile insect releases (Pascacio-Villafan et al. 2017). To our knowledge, this study is the first to incorporate both full factorial and mixture design models. The successful integration of both models shows that researchers should consider the synergistic and antagonistic responses to ingredient interactions in diet development and optimization.

Qualitative Effects of Diet Ingredients

Improved diets may result from use of ingredients that have not been previously utilized in the target insect's diet, as demonstrated in this study. It is beneficial to test natural diet ingredients in addition to new ingredients to determine if there is a preference for either food source. The high larval mass achieved in this study can be attributed to ingredients that are not part of the moth's natural diet and were not previously utilized for rearing wax moths.

We tested torula yeast against pollen as a nutritional supplement in the first factorial trial. Pollen contains protein, amino acids, starch, sterols, lipids, and nitrogen (Roulston and Cane 2000) and is a natural food for wax moth larvae, as observed in apiaries (Nielsen and Brister 1979). Torula yeast is used in other insect diets as a source of protein (Chang 2009, Pereira et al. 2009). Surprisingly, larvae attained a higher larval mass on yeast than on pollen diets, prompting us to eliminate pollen from the optimized diet. According to food labels, pollen has a higher carbohydrate content and lower fat and protein content than yeast (fdc.nal.usda.gov [bee pollen FDC ID#: 513506, brewer's yeast FDC ID#: 913208]). However, pollen varies considerably across plants and its protein content varies from 2.5 to as much as 61% (Roulston and Cane 2000, Roulston et al. 2000).

Torula yeast was the primary nutritional supplement for the optimized diet. Better performance on torula than brewer's yeast was also shown in the rice–Bermuda grass strain of fall armyworm, *Spodoptera frugiperda* (J.E. Smith); larval growth rates were about 50% lower on brewer's than torula yeast, with 40 and 90 mg/d weight gain, respectively (Whitford et al. 1992). In general, yeast species are high in B-vitamins and offer some protection against pathogenic bacteria, secondary metabolites, and xenobiotics (Bekatorou et al. 2006). Torula yeast contains B-vitamins similar to those in brewer's yeast, but it has higher protein, amino acid, and mineral content than brewer's yeast (Bekatorou et al. 2006). Its higher nutrient content may explain why torula yeast yielded significantly better results than brewer's yeast for most of the tested diets.

Our mixture analysis revealed that a combination of all three bran products produced the highest body mass at a ratio of rice, wheat, and oat brans of 20:34:14 g, respectively (29.4:50.0:20.6% of the cereal fraction). It is noteworthy that oat bran and multigrain diets also yielded high larval mass when brewer's yeast was present in Trial 2, i.e., 394 ± 21.8 and 388 ± 13.0 mg, respectively (Fig. 4). However, highly significant interactions between cereal type and yeast species were also present in Trial 2, leading to further investigation in Trials 3 and 4. Examining brewer's yeast and yeast * cereal interactions may be warranted in future studies. Nonetheless, the mixture results are an important finding for insect rearing because they demonstrate the efficacy of mixtures of complex components for optimal insect fitness.

Table 3. Composition of the optimized *Galleria mellonella* diet and nutritional profiles of rice bran, wheat bran, and oat bran

Diet ingredient	Composition (g or ml, and %) of the optimized diet		Macronutrient composition (%)[a]		
Cereal			Proteins	Lipids	Carbohydrates
Oat bran	14 g	4.9%	18	5	68
Wheat bran	34 g	12.0%	13	3	67
Rice bran	20 g	7.1%	3	25	60
Torula yeast	42 g	14.8%			
Wax	11 g	3.9%			
Honey	68 g	24.0%			
Glycerol	64 g	22.6%			
Water	30 ml	10.6%			

[a]Macronutrient composition is taken from food labels. Rice bran: NutraBio 100% Pure Stabilized Rice Bran (NutraBio Labs, Inc.). Wheat bran: Bob's Red Mill High Fiber Wheat Bran, and Oat bran: Bob's Red Mill High Fiber Oat Bran (Bob's Red Mill Natural Foods, Inc.). The remaining product contents are represented by minerals, fiber, and undisclosed components.

Rice bran had a large effect on larval mass when mixed with other cereals. In addition to the nutritional benefits of multiple cereal ingredients, rice bran appears to also have useful cohesive properties that support the consistency of the diet. The rice bran used in these experiments was modified by the supplier to consist of soluble components and has a distinctly different nutritional profile than wheat bran and oat bran; it has five times more fat (3–5%) than wheat and oat brans, and <10% protein compared with 13–18% protein in wheat and oat brans. All three brans have similar carbohydrate contents of 60–68% (Table 3). The uniquely high fat content in rice bran may be responsible for high larval mass attained by wax moth larvae.

Understanding how diet nutrient composition affects nutrient accumulation in reared insects is crucial for evaluating the success of a diet because the insect's food has been shown to affect macronutrient content (Teder et al. 2014, Oonincx et al. 2015). Storage of protein and lipid in the larval stage affects life-history traits in adults (Hahn 2005) and large alterations in insect macronutrient values can impact molting, postmolting somatic growth, reproduction, and dispersal behaviors (Boggs 1981). Because no difference was detected in proportions of lipid or other macronutrient content between larvae fed the optimized diet and the Beck-derived diet, we do not expect a negative impact on adult life-history traits when larvae feed on the optimized diet. However, higher replication and investigation into the effect of larval macronutrient accumulation on adult performance is warranted to fully test this conclusion in wax moths.

Because genetics, environment, and nutrition can all affect insect growth rates and size (Beukeboom 2018), we minimized the effects of genetic and environmental factors by rearing the wax moth colony under constant environmental conditions, utilizing larvae from the same cohort in each trial, and conducting all trials under similar conditions. Therefore, major treatment effects can be attributed to the experimental diets. Response factors such as larval mass and survival are good indicators of overall larval fitness and are useful for assessing the effectiveness of diets. Size (generally reported as body mass) correlates well with fitness for most insects, reptiles, and plants (Kingsolver and Huey 2008, Cohen 2015) and is one of the most commonly measured fitness traits in insect rearing systems. We focused on mass of the larva because this is the only wax moth life stage that feeds, and thus is responsive to diet quality. It should be noted that as adults, males are typically smaller than females in length and wingspan (Kwadha et al. 2017), and, therefore, presumably, in mass. Larval sex is indistinguishable by size or external morphology (Smith 1965) and we assumed random sampling would yield a 1:1 sex ratio in our replicates. Overall, late instar larvae vary slightly in length (25–30 mm) and diameter (5–7 mm) in field populations (Smith 1965), though it is unclear whether they vary in body mass. Because the larval stage is used in most applications, we did not consider sex variation in our study. In general, large larvae, irrespective of sex, are often beneficial for mass rearing of biocontrol agents and as food for captive insectivores. Furthermore, in our study, survival was generally unaffected by the various diet manipulations, highlighting the utility of *G. mellonella* as a resilient and highly plastic laboratory model. In conclusion, the results of this study demonstrate that DOE is a useful and efficient tool for optimizing insect diets. The importance of considering combinations of ingredients and their interactive effects on insect fitness is highlighted. This approach to diet optimization is useful to support small lab-based colonies in addition to large-scale production. DOE can also be applied to optimize rearing studies where fitness or quality metrics other than mass and survival are

used, such as fecundity, parasitization rate, flight ability, and immune competence. Finally, new, uniform, and easily obtainable ingredients were tested to optimize a diet for *G. mellonella* that produced large, fast-growing larvae. This new diet should enhance the utility of wax moths in various research disciplines and pest management programs.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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