Self-Selection of Two Diet Components by *Tenebrio molitor* (Coleoptera: Tenebrionidae) Larvae and Its Impact on Fitness

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**ABSTRACT** We studied the ability of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) to self-select optimal ratios of two dietary components to approach nutritional balance and maximum fitness. Relative consumption of wheat bran and dry potato flakes was determined among larvae feeding on four different ratios of these components (10, 20, 30, and 40% potato). Groups of early instars were provided with a measured amount of food and the consumption of each diet component was measured at the end of 4 wk and again 3 wk later. Consumption of diet components by *T. molitor* larvae deviated significantly from expected ratios indicating nonrandom self-selection. Mean percentages of dry potato consumed were 11.98, 19.16, 19.02, and 19.27% and 11.89, 20.48, 24.67, and 25.97% during the first and second experimental periods for diets with 10, 20, 30, and 40% potato, respectively. Life table analysis was used to determine the fitness of *T. molitor* developing in the four diet mixtures in a no-choice experiment. The diets were compared among each other and a control diet of wheat bran only. Doubling time was significantly shorter in groups consuming 10 and 20% potato than the control and longer in groups feeding on 30 and 40% potato. The self-selected ratios of the two diet components approached 20% potato, which was the best ratio for development and second best for population growth. Our findings show dietary self-selection behavior in *T. molitor* larvae, and these findings may lead to new methods for optimizing dietary supplements for *T. molitor*.

**KEY WORDS** nutrition, behavior, insect rearing, yellow mealworm, dietary self-selection

The ability of some insects to regulate the intake of nutrients has been well documented and has been referred as dietary self-selection (Waldbauer and Friedman 1991). The term dietary self-selection specifically refers to the consumption of different types of food items by selection to balance the optimal (or near optimal) levels of nutrients required by an organism (Waldbauer and Friedman 1991, Behmer 2009). Nutrient regulation by dietary self-selection has been demonstrated experimentally in a variety of insects including *Tribolium confusum* Jacquelin du Val (Tenebrionidae) (Waldbauer and Bhattacharya 1973), *Supella longipalpa* F. (Blattellidae) (Cohen et al. 1987), *Helicoverpa zea* (Boddie) (Noctuidae) (Waldbauer et al. 1984, Schiff et al. 1988), *Locusta migratoria* L. (Acrididae) (Raubenheimer and Simpson 1993, Trumper and Simpson 1993), *Anastrepha obliqua* (Macquart) (Tephritidae) (Cresoni-Pereira and Zucoloto 2001), *Spodoptera littoralis* (Boisdouval) (Noctuidae) (Lee et al. 2002), *Spodoptera exempta* (Walker) (Noctuidae) (Lee et al. 2004), *Harmonia axyridis* Pallas (Coccinellidae) (Soares et al. 2004), *Agnomus dorsale* (Pontoppidan) (Carabidae) (Mayntz et al. 2005), *Helicoverpa virescens* F. (Noctuidae) (Lee et al. 2006), and *Ectatomma ruidum* Roger (Formicidae) (Cook and Behmer 2010).

Most dietary self-selection studies have shown that insects regulate their food choices so a species-specific balance of digestible carbohydrate and protein can be attained. For instance, *T. confusum* feeding on a three component diet mix developed faster than when feeding on pure diets of each of the individual components (Waldbauer and Bhattacharya 1973). Last instars of *H. zea* self selected an 80:20 protein:carbohydrate ratio when offered two diets of identical and complete nutritional content except that one lacked casein and the other lacked glucose. The self selected ratio supported the best food utilization and food conversion when compared with each of the individual diets and a diet with a 1:1 casein to glucose ratio (Waldbauer et al. 1984).

Self selected diets can also be stage specific. For example, *S. longipalpa* developed better on a two-component diet of casein and glucose when allowed to self-select the ratio of the two components than on a single component diet or a diet with 1:1 casein:glucose. However, when forced to feed on a diet with the same component ratio as the self selected diet, the development rate was slower than the 1:1 ratio diet. The consumption ratio of the two components changed from high carbohydrate at the beginning to...
an equal ratio with protein by the end of the stadium (Cohen et al. 1987).

Insects feeding on diverse diets also self-select to balance nutrients other than carbohydrate and protein (Waldbauer and Friedman 1991, Behmer 2009). Dietary self-selection for lipids and vitamins has been documented in H. zea. Schiﬀ et al. (1988) reported that last instar larvae of H. zea self selected a superior mix of two incomplete diets. This experiment was similar to the one done by Waldbauer et al. (1984), except that the lipid and vitamin content were changed in the two diets. Trumper and Simpson (1993) demonstrated that salt intake was regulated by self-selection in nymphs of L. migratoria when using incomplete artiﬁcial diets. However, regulation of protein and carbohydrate was stronger since nymphs of L. migratoria were driven to consume a 12-fold sub optimal salt intake to balance protein and carbohydrate intake in a two-choice experiment with incomplete diets (Trumper and Simpson 1993).

Nutritional requirements of T. molitor, especially protein and amino acids, have been studied in some detail (Davis 1969, 1970, 1971a, 1971b, 1974, 1975, 1978). However, no studies have been reported on the detail (Davis 1969, 1970, 1971a, 1971b, 1974, 1975, 1978). However, no studies have been reported on the ability of T. molitor to regulate their nutrient intake or demonstrate that dietary self-selection occurs in this species. Dietary self-selection has been observed in the tenebrionid T. confusum (Waldbauer and Bhattacharya 1973), which has feeding habits similar to T. molitor. Thus, it is reasonable to assume that T. molitor also may have the ability to self-select for optimization of nutrition. Large quantities of T. molitor are needed because of their important commercial importance for feeding captive and wild birds and other pets including reptiles and tarantulas (W.L. Tedders unpublished data). This insect also may become an important source of protein for aquaculture (Ng et al. 2001), aviculture (Ramos-Elorduy et al. 2003), and even for human consumption (Ramos-Elorduy 1997). Addi tionally, T. molitor has been suggested as a host for in vivo mass production of entomopathogenic nematodes (Shapiro-Ilan et al. 2002, 2008) and for some heteropterous predators such as Brontocoris tabidus (Signoret) (Zanuncio et al. 1996), Podisus maculiventris (Say) (DeClercq et al. 1998) P. nigrispinus (Dallas) (Zanuncio et al. 2001), and Pristhesanctus plagiennis (Walker) (Grundy et al. 2000). Self-selection studies could improve our understanding of T. molitor nutrition and allow the development of optimal diet mixtures, which would beneﬁt the capabilities for mass rearing. Consequently the objectives of our research were to determine if T. molitor has the ability to selectively feed in a two-component diet mix and if the selected ratios of the two components approach the optimum for population growth of this insect.

Materials and Methods

Colony Maintenance. A laboratory colony was established from a biological stock of T. molitor obtained from Southeastern Insectaries (Perry, GA). Larvae and adult beetles were fed exclusively with food grade wheat bran (P. No. 18704, BulkFoods.com, Toledo, OH). The colony was kept in a dark environmental room at 27 ± 2°C, 75 ± 10% RH. Water was provided twice a week by sprinkling ~50 ml in each rearing box. Adults were maintained in groups of 300–500 inside modiﬁed Rubbermaid boxes (350 × 230 × 100 mm). Two nested boxes were used; the top box containing the adults was modiﬁed by replacing the bottom with a nylon screen standard number 20 (850 µm). Hatched ﬁrst instars moved through the screen openings and fell to the second nested box at the bottom. Young larvae were collected weekly and placed in clean plastic boxes for a period of 30 d. Older larvae were kept in nested Rubbermaid boxes similar to those used for the adults but with a smaller screen size (standard number 35, 500 µm), which allowed the passing of frass pellets to the box at the bottom. The oldest instars were transferred to larger plastic pans (550 × 430 × 100 mm) where they were inspected daily for pupation. Pupae were removed to a separated pan lined with paper towels and inspected daily for adult emergence.

Experimental Treatments. Four different ratios of wheat bran and dry potato were tested. These two ingredients were chosen because of their differences in nutritional content (U.S. Department of Agriculture [USDA] 2010) (Table 1). The carbohydrate content is much higher in potato than in bran, and protein and lipid contents are very low in potato relative to bran. In addition, bran contains more minerals while potato has more vitamin C (Table 1).

The four tested ratios of bran:potato (9:1, 4:1, 7:3, and 3:2) were equivalent to 10, 20, 30, and 40% dry potato flakes (Idahoan, Idahoan Foods, Lewisville, ID) evenly mixed with wheat bran. The control diet consisted of only wheat bran. Diet treatments were selected based on preliminary tests, which showed that T. molitor was unable to complete development on a diet of exclusively dry potato flakes and mortality was extreme in diet mixtures equal to or higher than 50% potato.
Two different types of experiments were designed using the food ratios described above: no-choice diet ratio experiments and self-selection experiments. The difference between the two types of experiments was on the ability of the larvae to choose the consumption ratio of the two components. In the no-choice experiments new food was added to the larvae groups only when existing food supply had been depleted forcing the larvae to consume the ratios of the two ingredients as provided. In the self-selection experiments food was replenished after a 4 wk period before the existing supply was consumed and then again at the end of a 3 wk period, allowing the larvae to selectively consume the food ingredients so the ratios selected by the larvae could be determined.

**Self-Selection Experiments.** Early instars of *T. molitor* were obtained from the laboratory colony by separating small larvae using sieves. Larvae that passed through a standard number 25 sieve (600 μm openings) and failed to pass through a standard number 35 sieve (500 μm openings) were selected for the experiment. Instar morphometrics of the head capsule showed that head capsules between 500–600 μm correspond to those of fifth and sixth instars (J.A. Morales-Ramos unpublished data) and heads are as wide as the body or wider. Bypassing earlier instars provided a more efficient way to measure food consumption because it takes from 25 to 32 d to reach fifth and sixth instars, respectively, at 27°C (Morales-Ramos et al. 2010). Food consumption rates during this period were measured in micrograms, but the rates of food consumption and growth increased dramatically after the end of the fifth stadium (J.A. Morales-Ramos unpublished data).

Selected larvae were separated into 150 groups of 10 larvae each. Groups were weighed, their initial weight was recorded, and they were placed in petri dishes (15 mm height × 55 mm diameter). Groups were randomly assigned to each of the four treatments and control (N = 30 groups per treatment) described above and provided with 1 g of the corresponding food mix. Dishes of each treatment were stacked in six groups of five (position 1–5), placed in a tray and maintained in an environmental chamber for a 4 wk period at the same conditions described above. Positions of the dishes within the stacks were maintained through the study and treated as repetitions (5). Larval groups were provided with 5 μl of water twice a week.

At the end of the 4 wk period, the weight of the larvae groups was determined and recorded. The frass was separated from the remaining food mix using a number 35 sieve. The remaining food was separated in its two components manually with the aid of number seven forceps and a stereo microscope. Potato flakes were easily distinguished from wheat bran flakes by their color and consistency. The weight of each food component in each dish was recorded. The larval groups were returned to their corresponding dishes and provided with 2 g of new food containing the appropriate mix. The dishes were returned to the environmental chamber where they were kept for an additional 3 wk period. Larvae were provided with 5 μl of water twice a week and additional food (weighed) of the corresponding treatment if needed. At the end of this period the process was repeated to obtain a second data set at a later larval age. The weight of each of the consumed diet components was determined for each of the dishes by subtracting the remaining weight of the food components from the initial weight. These data were used to calculate actual percentages of potato consumed by each of the larval groups.

**Data Analysis.** Data consisting of larval weight gained and total food consumed per group were analyzed using ANOVA. Means were compared among treatments and repetitions using the Tukey-Kramer honestly significant difference (HSD) test. Percentages of potato consumed were arcsine transformed (p’ = ArcSin √(p)) (Zar 1999), analyzed, and compared among treatments and repetitions using ANOVA and Tukey–Kramer’s HSD test (as described for larval weight). Contingency table analysis was used to determine deviations in the percentages of potato consumption, that is, observed versus expected consumption percentages using the Chi-square likelihood ratio test using JMP software (SAS Institute 2008). Food conversion efficiency was calculated using the formula of Waldbauer (1968) FCE = weight gain/weight of food eaten. Arcsin transformations of FCE were analyzed using ANOVA and means were compared using the Tukey–Kramer HSD test.

The geometric framework (Raubenheimer and Simpson 1993) was used to graphically present the self-selected consumed ratios of protein and carbohydrates. The geometric framework is a multidimensional analysis of balance consumption of nutrients by self selection (Behmer 2009). The number of axes depends on the number of fitness affecting nutrients that are analyzed. In this study we analyzed the contents of protein versus carbohydrate in the self-selected food mix and compare it to the contents of individual food components. Lips were not in high content in the individual food components studied. Future research will be focus on *T. molitor* self selection by lipid content.

**No-Choice Diet Ratios Experiments.** Population growth statistics from life tables were used to determine the effect of the diet treatments on fitness of *T. molitor*. Immature survival, development rate, and age-dependent fecundity were determined for groups of beetles fed each of the food treatments (10, 20, 30, and 40% potato) and the control (bran only). These parameters were used to calculate life and fertility tables using the methods reported by Carey (1993).

**Immature Development and Survival.** First instars were obtained from 10 groups of 40 adults (1:1 sex ratio) each maintained in a separate pair of stacked boxes (140 × 100 × 37 mm) as described above. Adult groups were randomly assigned to one of the food treatments (two adult groups per treatment) or the control and provided with an equivalent diet mix. Adults oviposited in the flakes provided as food and
first instars dropped through the screen openings to the lower box.

First instars were collected daily for 30 d and grouped by eclosion date and food treatment (same as parental food treatment). Each eclosion date constituted a replication for a total of 30 replications. The number of first instars eclosing was different each day \( n \), but the total numbers \( N \) were 3,008; 3,435; 2,313; 2,114; and 2,234 for treatments 10–40% potato and control, respectively. Within each replication and treatment, first instars were divided in groups of 18 and placed in petri dishes (15 mm height \( \times \) 55 mm diameter), provided with 500 mg of the appropriate food mix, and placed in an environmental chamber at 27 ± 1°C, 70 ± 5% RH, and 14:10 (L:D) h photoperiod. Each dish was provided with 5 μl of water twice a week. Dishes were inspected every 2 wk for mortality by counting all live larvae remaining in each dish. Because the density of larvae is known to have an effect on the development rate of \( T. \) molitor (Tschinkel and Willson 1971, Weaver and McFarlane 1990), efforts were made to maintain a consistent larval density. This was accomplished by transferring smaller groups of larvae to smaller petri dishes (10 mm height \( \times \) 35 mm diameter) and by merging groups of the same age and treatment. Groups from 9 to 18 larvae were kept in the larger dishes and groups from 1 to 8 larvae were kept in the smaller size dishes. This limited density to a maximum of one larva per 1.20 cm². This method was also used to adjust larval density during pupation as pupae were removed. Pupae were weighed and transferred to individual small petri dishes as described above, labeled with eclosion date, pupation date, and treatment and allowed to complete development at the same conditions described above. Each pair was also provided with 10 μl of water twice a week.

Progeny were counted daily using the same method described above for first instar selection for a maximum period of 70 d. Adult dishes were inspected daily for first instars, which were removed, counted, and recorded. Dead adult males were replaced, but female deaths signaled the end of data collection for that particular dish.

Data Analysis. Survival proportions were statistically compared using contingency tables and Chi-square test. Development time, pupal weight, and total progeny per female were compared using ANOVA and the Tukey-Kramer HSD Test using JMP software (SAS Institute 2008). Life tables were calculated by using immature survival data and age dependent progeny from adult pairs. Net reproductive rate \( (R_o) \) was compared among treatments as mean female progeny per female obtained from experimental observations \( (N = 24) \) as reported by Maia et al. (2000). Intrinsic rate of increase \( (r_m) \) was calculated by iteration using the Euler-Lotka equation (Lotka 1907, Carey 1993). Multiple values of \( r_m \) were obtained for each treatment group \( (N = 25) \) by using the jackknife method (Efron and Gong 1983), which allows multiple calculations of the parameter by sequential deletion of individual observations. Doubling time \( (D_t) \) was then obtained by \( D_t = \ln(2)/r_m \) (Carey 1993) for each individual value and compared among treatments using ANOVA and the Tukey-Kramer HSD test.

Results

Self-Selection. The consumption ratios of bran to dry potato showed little variation among diet treatments. Mean (±SD) percentage potato consumed during the first 4 wk was 11.98 ± 1.60, 19.16 ± 1.02, 19.02 ± 3.47, and 19.27 ± 4.09% for 10–40% potato treatments, respectively, and during the next 3 wk was 11.89 ± 1.54, 20.48 ± 1.48, 24.67 ± 2.84, and 25.97 ± 3.59% for 10–40% potato treatments, respectively. Analysis of variance (ANOVA) showed significant differences in the percentage potato consumed among the diet treatments during both experimental periods, \( (F = 55.39; df = 3, 116; P < 0.0001) \) for the first 4 wk period) and \( (F = 225.37; df = 3, 116; P < 0.0001) \) for the next 3 wk period) (Fig. 1). However, there were no significant differences in the percentage of potato consumed among the 20, 30, and 40% potato diets during the first 4 wk period and among the 30 and 40% potato diets during the next 3 wk period (Fig. 1). Thus ANOVA results indicate that \( T. \) molitor larvae fed selectively; if \( T. \) molitor larvae had fed randomly on the two ingredients, we would expect the percentage potato consumed to differ among all diet treatments.

Additionally, the Chi-square analysis indicated selective feeding. Significant differences between observed and expected (as provided) percentages of potato consumed were observed. Significantly more potato was consumed than expected in the 10% potato diet \( (X^2 = 6.05; df = 1; P = 0.0139 \) and \( X^2 = 5.48; df = 1; P = 0.0192, \) for the first 4 wk and next 3 wk periods,
respectively), and significantly less potato was consumed in the 30% potato diet ($\chi^2 = 94.34; \text{df} = 1; P < 0.0001$ and $\chi^2 = 21.47; \text{df} = 1; P < 0.0001$, for the first 4 wk and next 3 wk periods, respectively), and the 40% potato diet ($\chi^2 = 314.168; \text{df} = 1; P < 0.0001$ and $\chi^2 = 134.37; \text{df} = 1; P < 0.0001$, for the first 4 wk and next 3 wk periods, respectively). The observed percent consumption of potato in the 20% potato diet was not significantly different than the percentage provided.

The base 10 ratios of carbohydrate, protein, and lipid of wheat bran and dry potato flakes are reported to be 5.23, 3.73, 1.04 and 8.96, 1.0, 0.04, respectively (USDA 2010) (Table 1). A geometric framework plot of protein versus carbohydrate was generated for the diet treatments and their selected ratios based on the reported content of protein and carbohydrate in the two diet components. The plot of protein consumption versus carbohydrates showed that the means of relative consumption of these two basic nutrient components by $T. \text{molitor}$ larvae were closer to that of the 20% potato diet (Fig. 2A).

Some of the larvae groups feeding on the 10% potato diet depleted the diet mix of dry potato before the end of the experiments (first 4 wk and next 3 wk periods) and as a result the relative consumption of protein to carbohydrates did not reach the same levels in this treatment as in the rest of the treatments (Fig. 2B). Similarly, some larvae groups feeding on the 40% potato diet depleted the diet mix of bran before the end of the experiments showing the opposite effect as the one observed in the 10% potato diet (Fig. 2B). The mean proportional ratios of carbohydrate, protein, and lipid caloric content of the self-selected food ratios by $T. \text{molitor}$ larvae resembled those of a 20% potato mix (6.47, 2.82, and 0.7 carbohydrate, protein, and lipid, respectively) most closely (Table 2).

Larvae consumed more food and grew faster in the two-component diet mixes than in a pure bran diet regardless of the mix ratio. Food consumption and larval weight gained were significantly lower in the control treatment (bran only) than in the 10 and 20% potato during the first 4 wk experiment ($F = 9.31; \text{df} = 4, 145; P < 0.0001$; and $F = 5.43; \text{df} = 4, 145; P = 0.0004$, respectively) and in the 10, 20, 30, and 40% potato diets during the next 3 wk experiment ($F = 22.43; \text{df} = 4, 145; P < 0.0001$; and $F = 20.99; \text{df} = 4, 145; P < 0.0001$, respectively). There was no significant difference in food consumption and larval weight gained among the diet mix treatments in both experimental periods. Food conversion efficiency (wet weight) for the 7 wk period of both experiments was significantly lower in the control than in the 10 and 20% potato diets ($F = 5.58; \text{df} = 4, 145; P = 0.0003$) (Fig. 3).

Fig. 1. Self-selected percentages of dry potato by larvae of $T. \text{molitor}$. Symbols represent means and brackets represent standard deviation. The straight line represents the expected consumed percentages if consumption was random. Means with the same letter are not significantly different (Tukey-Krammer HSD test analysis of arcsine-transformed data, $a = 0.05$) lower case letters first 4 wk period and upper case letters next 3 wk period.

Fig. 2. Geometric framework plot of digestible carbohydrate versus protein in experimental diet mixtures, wheat bran, and dry potato flakes (lines) based on data in Table 1. Self-selected ratios for four diet treatments (symbols) by groups of 10 $T. \text{molitor}$ larvae: (A) treatment means and (B) group by group. Circles represent the first 4 wk period and triangles the next 3 wk period. Colors represent different diet treatments: green diet 1, yellow diet 2, orange diet 3, and red diet 4. Notice that the ‘Y’ axis has been adjusted to compensate for a carbohydrate bias in all diets.
only) \((163.1 \pm 23.12 \, \text{d} = \text{mean} \pm \text{SD})\) than in all the other treatments followed by the 40% potato treatment \((142.97 \pm 19.36 \, \text{d})\). The development time was significantly shorter in the 20% potato treatment \((131.74 \pm 16.00 \, \text{d})\) than in the 10% potato \((134.57 \pm 57 \, \text{d})\) and 40% potato \((142.97 \pm 19.36)\), but it was not significantly different than the one observed in the 30% potato treatment \((133.51 \pm 21.10 \, \text{d})\) (Fig. 4A).

Development time of the 10% potato group was significantly longer than that of the 20% potato treatment, but not significantly different than that of the 30% potato treatment (Fig. 4A). Survival rate from first instar to adult was also significantly affected by the potato to bran diet ratio \((\chi^2 = 2178.97; \, N = 7725; \, \text{df} = 4; \, P < 0.0001)\). Immature proportion survival was significantly higher in the 10 and 20% potato \((0.5645 \, \text{and} \, 0.5406\), respectively) than in all the other groups (Fig. 4B). The lowest proportion immature survival was observed for 40% potato \((0.141)\) followed by that for 30% potato \((0.1985)\). The control group had a survival rate of 0.4443, which was significantly different than that of all the treatments. Pupal weight did not differ significantly among the control group (bran only) and the 10 and 20% potato treatments (Fig. 4C). However, the 40% potato group showed significantly lower pupal weight \((F = 7.24; \, \text{df} = 4, 203; \, P < 0.0001)\) than all other groups except for 30% potato \((0.5406)\) (Fig. 4C). The ANOVA showed significant effect of bran to potato ratios on pupae per female \((F = 2.66; \, \text{df} = 4, 115; \, P = 0.0358)\); however, treatment means did not differ significantly among each other after Tukey-Kramer HSD test. Females fed on higher ratios of potato to bran \((30 \, \text{and} \, 40\%)\) produced less progeny, but these differences were not statistically significant (Fig. 4D). Immature stages were impacted the most by potato to bran diet ratios (Fig. 4A and B).

Summarizing the impact of the diet mixture on the biological parameters by life table analysis provides a better understanding of diet impact on the life cycle of *T. molitor*. The net reproductive rate \((R_0)\), which summarizes fecundity, immature survival, and sex ratio, was not significantly different among larval groups on the control and 10 and 20% potato. However, these three groups had a significantly higher Ro value than did groups on 30 and 40% potato diets \((F = 14.37; \, \text{df} = 4, 500; \, P < 0.0001)\) (Fig. 4E). Doubling population time \((DT)\), which summarizes all the biological traits, including development time, was significantly different among all treatment and control groups \((F = 2677.29; \, \text{df} = 4, 120; \, P < 0.0001)\). This population statistic provides the best measure of fitness and our results showed that the 10% potato diet was the best treatment followed by 20% potato, and the worst treatment was 40% followed by 30% potato (Fig. 4 F). These results showed that the optimal diet ratio of bran to potato for *T. molitor* population growth occurs between 10 and 20% potato (9:1 and 4:1).

**Discussion**

The first criteria for testing self-selection, established by Waldbauer and Friedman (1991), is nonrandomness in the consumption of two mixed food components. This condition was satisfied for *T. molitor* larvae as shown by significant deviations in consumption ratio from the expected given the ratios provided. When the food mix provided contained a lower percentage of potato than the optimal, larvae consumed more potato than expected. Similarly, when the mix provided contained more potato than optimal, larvae consumed more bran than expected. Regardless of the provided ratio, consumption of the two food ingredients had a tendency to approach 20% potato or a 4:1 bran to potato. This was more evident during the first 4 wk period when the mean percentages of potato consumed did not reach 20% in any of the treatments (11.99, 19.16, 19.02, and 19.27% for diets 1–4, respectively). Larvae consumed more potato during the next 3 wk period (11.89, 20.48, 24.67, and 25.97% for diets 1–4, respectively); however, this may have resulted as
a consequence of complete depletion of the bran component of the diet mix before the end of the experiment. Another possible explanation for the difference in self-selected ratios between the first 4 wk and the next 3 wk periods may be the result of differences in nutritional requirements among different instars. Cohen et al. (1987) observed changes in self-selected ratios of glucose and casein within stadia of the cockroach *S. longipalpa*.

Self-selecting animals not only make nonrandom choices, but the individuals of a uniform group also tend to consume major nutrients in diet mixtures in more or less consistent proportions (Waldbauer and Friedman 1991). The geometric framework plot of carbohydrate and protein relative consumption of individual groups of *T. molitor* larvae shows consistency even when variation in the quantity of food consumed was high among individual groups.

The second criteria for self-selection by Waldbauer and Friedman (1991) is that self-selectors should fare as well or better on the self-selected ratio than on other mixture ratios of the same ingredients or on a diet lacking one of the self-selected nutrients. Results showed that *T. molitor* fitness was significantly affected by changes in the ratios of two food items, wheat bran and dry potato flakes. The optimal no-choice ratio of bran to potato for population growth of *T. molitor* was 10% potato flakes (9:1). When offered a choice between these two food ingredients, *T. molitor* larvae consumed ratios approaching 20% potato (4:1), which was the second best ratio for population growth of this insect. However, the shortest development time was observed for the 20% potato diet and the development time was significantly shorter in this diet than in the 10% potato diet. The self-selected ratios observed in this study occurred during the larval stage of *T. molitor* but no information is available on adult self-selection. It is possible that optimal carbohydrate to protein ratios for reproduction differ significantly from those required for growth and devel-

Fig. 4. Response of *T. molitor* to four different no-choice ratios of wheat bran and dry potato flakes and to a wheat bran only control diet. Dots represent means and brackets represent standard deviation. Means with the same letter are not significantly different (Tukey-Kramer HSD test, α = 0.05). (A) Development time in days from first instar to adult; (B) immature survival (number of adults/number of first instars); (C) pupal weight in milligrams; (D) progeny produced per female during a period of 70 d; (E) net reproductive rate (Ro) calculated from life tables (mean female progeny per female); and (F) doubling time (DT) (time in days required to double the population).
opment. Because the no-choice ratios were provided during the whole life cycle, it is reasonable to expect that ratios selected by larvae differ from the optimal for population growth. However, self-selected ratios by larvae seem to correspond to the optimal no-choice ratios for growth and development.

Self-selected ratios of wheat bran and potato flakes provided better nutrition for development and population growth than either of the two ingredients alone. *T. molitor* was unable to complete development in potato flakes alone in preliminary tests (J.A. Morales-Ramos unpublished data) and feeding on wheat bran only resulted in significantly longer development time, lower immature survival rates, and longer doubling time than when the two components were present in ratios similar to the self-selected. The values of efficiency of conversion of ingested food were significantly higher in the groups that self-selected between the two components than in groups that consumed wheat bran alone.

Results of this study provide evidence that *T. molitor* larvae fed nonrandomly on a two component diet and that the self-selected ratios of these components approached those that provided optimal development and population growth fulfilling both conditions for nutrient self-selection behavior as established by Waldbauer and Friedman (1991). Our results indicate that *T. molitor* possesses the ability to self-select between wheat bran and dry potato flakes in a two diet mix in ratios approaching those optimal for population growth.

Potential behavioral and physiological mechanisms of dietary self selection in insects have been discussed by Waldbauer and Friedman (1991). A feedback mechanism coupled by learning in which animals respond to feeding sources according to how they feel afterwards has been named the “malaise hypothesis” (Waldbauer and Friedman 1991). Insects deprived of nutrients tend to behave in ways that enhance their likelihood to encounter and ingest food through increased locomotion activity and responsiveness to olfactory, visual, and gustatory cues provided by food (Barton Browne 1993). Colasurdo et al. (2007) observed changes in exploratory activity in *Malacosoma disstria* (Hübner) in response to different ratios of carbohydrate and protein in semidefined artificial diets. Caterpillars appeared to increase exploration when fed on a carbohydrate biased diet suggesting that protein deficiency promotes exploration in *M. disstria* (Colasurdo et al. 2007). Changes in chemoreceptor sensitivity in response to nutrient intake have been observed in *Locusta migratoria* L. Abisgold and Simpson (1988) detected changes in the electrophysiological responses of palp tip chemosensilla to protein after increase in amino acid concentration in the haemolymph of *L. migratoria*.

The effect that nutrient deficiency may have had on the exploratory activity of *T. molitor* larvae was not of relevance in this study because food components were mixed evenly and larvae had immediate access to both components. Changes in exploratory behavior do not explain dietary self selection in *T. molitor* based on our observations. Larval activity may have been affected, but an increase activity would contribute to maintain an even mix of food ingredients in the confined space of the experimental units. Changes in chemoreceptor sensitivity could better explain our observed results, but no evidence exists that sensitivity of *T. molitor* chemoreceptors is affected by food ingestion. Larvae of *T. molitor* may be able to recognize food ingredients by chemoreceptors present in their antennae. Bloom et al. (1982) described five types of sensilla (trichoid, uniporous pegs, papillate, and placoid sensilla) from the third antennal segment of *T. molitor* larvae. Trichoid sensilla (trichodea) are regarded as chemoreceptors responsible for smell and taste in other insects (Wigglesworth 1972, Frazier 1985, Zacharuk and Shields 1991). Uniporous pegs, also named sensilla basiconica, have been associated with smell responses in several insects including *Tenebrio* sp. (Wigglesworth 1972, Zacharuk 1980, Frazier 1985, Zacharuk and Shields 1991). Placoid sencilla, also known as multiporous plates, have been identified as chemoreceptors responsible for olfaction in some insect species (Zacharuk 1980, Frazier 1985). Although no studies have been done describing chemoreceptor sensilla in the mouth parts of *T. molitor*, many chemoreceptors have been identified in maxilla, labium, slypeo, and labrum of larvae of other coleopterous species (Zacharuk and Shields 1991). The presence of these sensilla in the antenna of *T. molitor* larvae suggest that they have the sensory capacity to identify and discriminate among food sources; however, no studies have been done on the effect that food intake may have on the sensitivity of chemoreceptors in *T. molitor*. Future research on *T. molitor* may provide new perspectives on the study of the mechanisms of insect dietary self selection behavior.

Dietary self selection can be exploited as a means to develop optimized diets to improve the mass production of *T. molitor*. The optimal proportions of important dietary components can be determined by allowing larvae to self select them in two-choice tests. Multiple-choice tests can be devised to allow self-selection of multiple diet components, thus eliminating the long trial and error process required for diet development. Future research will focus on the study of self-selection of multiple diet components by *T. molitor*.

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