Lipid And Protein Loads in Pupating Larvae and Emerging Adults as Affected by the Composition of Mediterranean Fruit Fly (Ceratitis capitata) Meridic Larval Diets

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The effects of sucrose and amino acid (aa) composition and concentration in meridic larval diets (e.g., partially defined at the chemical level) was examined on several parameters of Mediterranean fruit fly (Medfly) development. Lipid and protein levels of pupating larvae and emerging adults were examined. Different sucrose concentrations in the diet had small effects upon most of the development parameters. However, sucrose concentration significantly affected the ability of larvae to accumulate lipid reserves and proteins. Adults emerging from the different sucrose diets did not significantly differ in their lipid contents and protein loads. Specific deletions of aa from the diet, and general aa concentration, had a strong effect upon the parameters of development and pupating larvae lipids and proteins. Glycine-deletion was the most deleterious, followed by the deletion of all non-essential aa, and serine. High aa concentration in the diet has a detrimental effect upon development. Lipid contents in pupating larvae, and to some extent protein levels, were affected by aa manipulations in the diet. Lipid and protein loads in emerging adults were not significantly affected by aa manipulations. Based on the analysis of lipid frequency distribution it is suggested that the Medfly seems to regulate the level of lipid content in emerging adults within a certain range, regardless of the larval diet history or lipid contents. Proteins do not seem to be regulated as are lipids. These results point to an interesting and unexpected metabolic regulation of energetic resources during metamorphosis of the Medfly. Arch. Insect Biochem. Physiol. 56:97-109, 2004. © 2004 Wiley-Liss, Inc.

Keywords: lipid reserves; proteins; amino acids; sucrose; meridic larval diet; Medfly

INTRODUCTION

Food ingested by an insect may follow several paths. After being absorbed by the organism, transformed food molecules may be directly catabolized for the production of energy, utilized as anabolic precursors of structural components in the insect, and/or incorporated into reserve molecules of high energetic value (usually triacylglycerols and glycogen) (Downer, 1981). Incorporation of food and the nutritional value of nutrients and metabolites have been traditionally evaluated in insects by measuring the growth response of insect stadia to nutrient composition and contents of their food (Gordon, 1984). Other measurements of food value in insects include longevity, incorporation of marked precursors into body tissue, and effects of food upon parameters of insect behavior (Slansky and Scriber, 1985; Nation, 2002). Incorporation of nutrients into lipids, glycogen reserves, and proteins, while highly informative in terms of food value in quantitative nutrition, has been scantily investigated in immature insects (Gordon, 1972). Reserves are, in general, the outcome of the nutritional balance of the...
forming insect (Downer, 1981). It is expected that insects feeding on a low caloric and/or an unbalanced food source will direct most of their acquired resources into the formation and creation of structural elements, and less into the accumulation of energetic reserves. On the contrary, insects feeding on a well-balanced food substrate are expected to direct some of their acquired energy into the accumulation of reserves. Reserves are of importance for later non-feeding stages, or for survival and maintenance purposes during periods of food shortage and nutrient imbalance (Gordon, 1972; Downer and Matthews, 1976).

Nutritional studies on developing and adult fruit flies have been widely conducted (Tsitsipis, 1989). These studies are an important source of information for the development of mass rearing techniques, which are essential for programs of fruit fly SIT (Sterile Insect Technique) (Steiner and Mitchell, 1966). The nutrition of the Mediterranean fruit fly (Medfly), a highly polyphagous insect of large economic importance in the world, has been investigated by several groups. Examples include studies on diet selection by larvae (Zucoloto, 1987; Canato and Zucoloto, 1993; Fernandes-Da-Silva and Zucoloto, 1993), the effects of larval diets upon adult biology, reproductive capacity, and behavior of the Medfly (Zucoloto, 1988; Kaspi et al., 2002), the effect of adult diets on sexual behavior and performance (Shelly et al., 2002; Yuval et al., 2002), and the effect of adult diets upon the dynamics of lipid reserves (Nestel et al., 1985; Warburg and Yuval, 1996). In a recent study, Kaspi et al. (2002) investigated the effect of manipulating sucrose and brewer's yeast in an oligidic larval diet (e.g., the chemical structure of which is unknown) upon larval development, sexual maturation, and lipid and protein contents of emerging adult Medflies. Though Kaspi et al. (2002) showed some effects (e.g., upon body size, nutritional reserves, and egg production), it is unclear how the insect developed and reached sexual maturity with larval diets lacking any source of proteins, as claimed by the authors. Two of four experimental diets ("protein deprived diets") contained only sucrose but no brewer's yeast, and were based on wheat bran as a bulking agent (Kaspi et al., 2002). Previous studies have found that a complete elimination of brewer's yeast from the larval diet results in no adult Medfly production (Economopoulos et al., 1990). Since wheat bran has been reported to have important contents of amino acids (aa), minerals, and vitamins (http://nutritiondata.com/index.html), it is expected that Medfly larvae developing on the "protein deprived diets" of Kaspi et al. (2002) were able to complete development and reach reproductive maturity thanks to the unaccounted sources of nutrients in their oligidic experimental diets.

While of great importance, oligidic diets, which have been highly efficient in the development of mass rearing systems for fruit flies, provide restricted information on the nutritional requirements of the flies. The determination of nutritional necessities requires that the investigations be conducted on holidic diets (e.g., chemically defined, where all the constituents are chemically known) (Tsitsipis, 1989). These types of diets for larvae fruit flies seldom have been developed (Tsitsipis, 1989). Recently, Chang et al. (2000) reported on the development of a meridic larval diet for the Medfly, in which all of the ingredients but one are chemically characterized. The new diet is based on corn cob as a bulking agent that, despite some trace nutrient elements, is basically a nutritionally inert substance (Chang et al., 2001). The meridic diet was used to manipulate essential nutrients and investigate the effect of these nutrient manipulations upon parameters of development (e.g., development periods of immature flies, pupal recovery, and weight, etc.) and adult behavior (Chang et al., 2000, 2001). We took advantage of the meridic diet to investigate the effect of diet constitution on the ability of Medfly larvae and emerging adults to accumulate lipids and proteins.

**MATERIALS AND METHODS**

**Establishment of Feeding Units and General Procedures**

Investigated diets (see below) were freshly prepared before each experiment, and 30 g of diet were...
dispensed into 5-cm disposable Petri dishes (experimental unit or replicate). For each diet treatment, 3 replicate Petri dishes were simultaneously prepared. On each Petri dish, 100 fresh Medfly eggs were seeded by placing eggs on a sterile black filter paper previously wetted with a 0.05% methylparaben water solution, and then placing the filter paper on the diet. *Ceratitis capitata* eggs ("Sade" strain) were collected from several adult cages on the same day of the experiment from the colony of the Institute of Biological Control of the Citrus Marketing Board, Israel. Eggs were incubated until hatching and the number of hatched eggs per Petri dish counted. Uncovered Petri dishes were then placed inside closed plastic containers (0.5 L), where developed larvae pupated. Pupae were then removed and sorted as needed for further treatments. For each Petri dish, we studied the following parameters: (1) % egg hatch, (2) larval development time (from the time of egg hatching until the first larvae started to pupate in the Petri dish), (3) pupal development time (from the onset of pupariation of the foremost jumping larvae till the emergence of the first adults), (4) % pupal recovery (total amount of pupae produced from the number of hatched eggs), (5) pupal fresh weight at day 5 after pupariation, (6) % adult emergence, (7) dry weight of newly emerged adult males and females, (8) % flyers (see below), (9) lipid content in pupating larvae (e.g., immobilized pupae or onset of prepupal stage [see Nestel et al., 2003]), (10) soluble protein content in pupating larvae, (11) lipid content in newly emerged adults (e.g., within the first 4 h of adult eclosion), and (12) soluble protein content in newly emerged adults. All the experiments were incubated in a temperature-controlled room, at 27°C, and 14 h photoperiod.

**Total Lipid and Protein Determination.** Lipids and protein contents were determined from individual organisms. For each replicate, 10–20 specimens were sampled (in a few cases, less than 10 individuals were used due to poor recovery from certain diets). For lipid determination, individuals were homogenized in 0.2 ml 2% Na<sub>2</sub>SO<sub>4</sub>. A solution of 1.3 ml chloroform: methanol (1:2) was added to the homogenate, mixed, and centrifuged (5,200g) for 10 min. After centrifugation, an aliquot (30 µl) of the supernatant was used for lipid determination using the vanillin reagent method (Warburg and Yuval, 1996). Lipid content was colorimetrically determined at 490 nm in an ELISA reader spectrophotometer, using Triolein (Sigma, St. Louis, MO) as a standard.

Soluble protein was determined by homogenizing single individuals in 1.2 ml of Phosphate Buffer Saline (PBS). After vortexing and centrifuging (5,200g) for 4 min, an aliquot (25 µl) was taken and diluted in 0.775 ml fresh PBS. This solution then reacted with Bradford Reagent (Bio-Rad Laboratories, Richmond, CA), and the amount of protein estimated from a standard (bovine Serum Albumin; Sigma) by measuring absorbance at 595 nm in an ELISA reader spectrophotometer.

**Percent adult emergence and flyers.** To estimate adult emergence and flying ability (see Boller et al., 1981), 20 pupae per replicate per diet were pooled together (a total of 60 pupae) and placed on a 9-cm Petri dish. Pupae (approximately 2 days before adult emergence) were centered on the Petri dish and encircled with a small strip of black filter paper (1 cm width) that served to maintain the pupae inside the ring and as a flying platform for the flies. Afterwards, a grey Plexiglas tube (8.85 cm in diameter, 10 cm height) was fitted into the Petri dish. The inside of the tube was lightly coated with talcum powder to prevent flies from climbing out. The whole system (Petri dish, talcum-Plexiglas cylinder, and pupae) was placed inside a large (20 x 30 x 40 cm) insect cage kept in an incubator room at 27°C. Tests continued for 5 days after adult emergence. Cages were then inspected and the following information obtained: (1) flies remaining in the Petri dish (non-emerged, partial emergence, and non-fliers), and (2) flies that were able to leave the Petri dish and cylinder and died outside the dish (e.g., % fliers).

**Investigated Diets**

**Reference diet.** The reference diet was an oligidic formulation based on corn cob as a bulking agent.
The diet served as a reference for the viability and development potential of the batch of eggs used in the individual experiments. We did not determine lipid and protein loads for individuals developing on this diet. The contents of the reference diet was as follows: 77% (weight/weight-w/w) corn cob 30/80 (Mt. Pulaski Products, IL), 12% (w/w) sucrose (ICN Pharmaceuticals, Costa Mesa, CA), 8% (w/w) brewer's yeast powder (Rold Shaprin, France), 2% (w/w) citric acid (ICN Pharmaceuticals), and 1% (w/w) methylparaben (ICN Pharmaceuticals). Water constituted around 60–70% of whole diet fresh weight. The brewer's yeast powder contains approximately 50% protein, 31.5% carbohydrates, and 6% fats.

Experimental diet. The experimental diet was an adaptation of Chang's meridic diet (Chang et al., 2000). All the elements used for the preparation of these diets, except corn cob (see above) were obtained from ICN Pharmaceuticals. The original Chang et al. (2000) larval meridic diet for the Medfly (e.g., C. capitata no. 1 diet) did not perform well in the laboratories of the Institute of Plant Protection, Volcani Center, Israel, mainly due to poor egg hatch. We investigated the factors involved in this poor performance by serially deleting the antibiotics and reducing the antimicrobial agents in the diet. The resulting modified Chang's meridic diet is presented in Table 1.

1. Manipulation of sucrose. This experiment consisted of manipulating the sucrose concentration in the modified Chang's meridic diet (Table 1). Three treatments, besides the reference diet, were established: low sucrose (6.5% w/w), regular (see Table 1) sucrose (12.3% w/w), and high sucrose (21.9% w/w). The remaining constituents in the diet were kept at more or less the same proportions as in Table 1.

2. Manipulation of amino acids (aa). Two types of treatments were performed: those where aa were deleted from the diet, and those where the concentration of all the aa in the diet was manipulated. The classical method for determining aa requirements consists of deleting one aa at a time from the diet (Nation, 2002). Previously Chang et al. (2001) and Chang (2002) showed that diets lacking one of the essential aa (Table 2) are unable to sustain Medfly larval development. They also showed that the removal of any or all the non-essential aa (Table 2) delayed development and decreased pupal weight and recovery, and that the deletions of glycine and serine had a major impact upon larval development.

| TABLE 1. Constituents in Modified Chang et al.'s (2000) Meridic Larval Diet* |
|-----------------------------|--------|
| Constituents                | mg     |
| Ribonucleic acid            | 100    |
| Inositol                    | 10     |
| Choline chloride            | 20     |
| Cholesterol                 | 40     |
| Vitamin mixture (thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, folic acid, p-amino benzoic acid, biotin) | 6.35 |
| McCollum & Davis Salt Mixture No. 185 | 100 |
| Methylparaben               | 64     |
| Citric acid                 | 333    |
| Sucrose                     | 2,000  |
| Amino acid mixture          | 1,600.8 |
| Corn-Cob 30/80              | 12,000 |
| Total dry weight            | 16,274.15 |

*Modification consisted of removing all antibacterial agents (streptomycin and tetracyclic) and sodium benzoate, and reducing methylparaben and citric acid concentrations. Provided are the concentrations of chemical constituents in dry weight and percentage. Water content was variable between 60–75% of the final diet weight.

<table>
<thead>
<tr>
<th>TABLE 2. Amino Acid Composition in Modified Chang et al.'s (2000) Meridic Diet for Medfly Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids mg per 16,274 mg of dry diet</td>
</tr>
<tr>
<td>Essential amino acids</td>
</tr>
<tr>
<td>L-arginine</td>
</tr>
<tr>
<td>L-histidine</td>
</tr>
<tr>
<td>L-isoleucine</td>
</tr>
<tr>
<td>L-leucine</td>
</tr>
<tr>
<td>L-lysine</td>
</tr>
<tr>
<td>L-methionine</td>
</tr>
<tr>
<td>L-phenylalanine</td>
</tr>
<tr>
<td>L-threonine</td>
</tr>
<tr>
<td>L-tryptophan</td>
</tr>
<tr>
<td>L-valine</td>
</tr>
<tr>
<td>Non-essential amino acids (NEaa)</td>
</tr>
<tr>
<td>L-alanine</td>
</tr>
<tr>
<td>L-aspartic acid</td>
</tr>
<tr>
<td>L-cysteine</td>
</tr>
<tr>
<td>L-glutamic acid</td>
</tr>
<tr>
<td>L-glycine</td>
</tr>
<tr>
<td>L-proline</td>
</tr>
<tr>
<td>L-serine</td>
</tr>
<tr>
<td>L-tyrosine</td>
</tr>
</tbody>
</table>

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Based on their results with non-essential aa, we decided to perform 3 treatments in which aa deletions were involved: (1) glycine deletion (e.g., glycine deficient), (2) serine deletion (e.g., serine deficient), and (3) the deletion from the diet of all non-essential aa (e.g., non-essential aa deficient). This was carried out in order to study how these deletions affect lipid and protein loads in the developing insect. In addition, we manipulated the concentration of all the set of aa (essential and non-essential) (Table 2) to investigate the effects of the "diet balance" (Gordon, 1984) on lipid and protein contents of developing Medflies, and on other developmental parameters of the insect. We established 3 more treatments: low aa (5.2% w/w), regular aa (9.8% w/w), and high aa (17.9% w/w). The rest of the diet constituents were maintained at approximately the concentration shown in Table 1. This experiment also included a reference diet treatment.

**Statistical Analysis**

Average and variance for all the parameters investigated were calculated from the 3 replicate Petri dishes per treatment. Pupal fresh weight was determined from 20 individual pupae, and adult weights were estimated from approximately 10 individual flies (when available), per replicate Petri dish. Lipid and protein contents, and weights, were averaged for each Petri dish (or replicate). Effect of sex upon lipid and protein contents in adults was investigated with parametric ANOVA; results showed no differences between sexes in any of the treatments (see Results), supporting the pooling of these data per replicate. These pooled averages per Petri dish for lipid and protein contents and weights were then used to obtain the mean and variance for each treatment, based on 3 replicate Petri dishes. In addition, average lipid and protein contents per replicate were standardized by the average pupal weight for that specific replicate. Differences between treatments were inferred from a non-parametric analysis of variance (Kruskal-Wallis) (Siegel, 1956). Medians were separated by the median-notch method (Statgraphics, 2000).

An additional analysis included the pooling together of all the individual data on lipid and protein content in pupating larvae and in emerging adults reared on all the different diets. These data were used to calculate frequency distribution plots for lipid and protein content in the two developmental stages of the Medfly, and investigate some of the parameters of the distributions (e.g., skewness and kurtosis) (Sokal and Rohlf, 1981). This was conducted in order to evaluate the tendency of the Medfly to regulate lipid contents in emerging adults towards a certain range, regardless of the larval diet or the original larval-pupal lipid content.

**RESULTS**

**Effect of Sucrose Concentration**

Egg hatch was high (>90%) in all the treatments, and there were no significant differences between treatments \((H = 2.43, P > 0.05)\). Larval development time was equal in all the treatments, and pupal development time and pupal recovery did not significantly differ between treatments (Table 3). Lipid levels in pupating larvae, both per individual (Table 3) and standardized by pupal weight (Fig. 1), were significantly affected by sucrose content in the diet. Low sucrose diet produced larvae with a significantly lower level of lipids per individual (Table 3), and per pupal weight (Fig. 1, \(H = 5.7, P < 0.05\)). In emerging adults, no statistically significant differences were observed between treatments (Table 3 and Fig. 1, \(H = 5.4, P > 0.05\)), although high sucrose diets produced adults with larger loads of lipids than the other two diets. No differences were found in lipid content between adult males and females \((F = 1.2, P > 0.05)\). Sucrose concentration in the diet also significantly affected the level of soluble protein in pupating larvae (Table 3; Fig. 2, \(H = 6.5, P < 0.05\)), the protein level being larger in the high sucrose diet. In contrast, sucrose concentration in the diet did not affect the protein loads in emerging adults (Table 3; Fig. 2, \(H = 4.0, P > 0.05\)), which was also similar between sexes \((F = 0.01, P > 0.05)\). Pupal fresh weight was not affected by sucrose concentration in the diet, while high sucrose loads in the
TABLE 3. Effect of Sucrose Concentrations in “Modified” Chang et al.’s (2000) Meridic Diet on Some Developmental Parameters of the Medfly*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low sucrose</th>
<th>Regular sucrose</th>
<th>High sucrose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval development time in days</td>
<td>6 ± 0</td>
<td>6 ± 0</td>
<td>6 ± 0</td>
<td>—</td>
</tr>
<tr>
<td>Pupal development time in days</td>
<td>12.3 ± 1.2</td>
<td>13 ± 0</td>
<td>11.3 ± 0.6</td>
<td>11 ± 0</td>
</tr>
<tr>
<td>% Pupal recovery</td>
<td>81 ± 10.7</td>
<td>83.6 ± 3.5</td>
<td>91.8 ± 5.4</td>
<td>95 ± 4.5</td>
</tr>
<tr>
<td>Pupal fresh weight in mg</td>
<td>8.3 ± 0.1</td>
<td>8.7 ± 0.1</td>
<td>8.7 ± 0.2</td>
<td>8.7 ± 0.1</td>
</tr>
<tr>
<td>% Adult emergence (pooled)</td>
<td>85</td>
<td>92.5</td>
<td>90.9</td>
<td>90</td>
</tr>
<tr>
<td>% Flyers (pooled)</td>
<td>62.7</td>
<td>77.6</td>
<td>78</td>
<td>72.2</td>
</tr>
<tr>
<td>Newly emerged male dry weight in mg</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Newly emerged male dry weight in mg</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>2 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>µg Lipids/individual in pupating larvae</td>
<td>104.4 ± 61.7</td>
<td>332.5 ± 118.7</td>
<td>329.9 ± 68.6*</td>
<td></td>
</tr>
<tr>
<td>µg Lipids/individual in emerging adult</td>
<td>167.9 ± 68.6</td>
<td>159 ± 23.1</td>
<td>258.3 ± 20.2</td>
<td></td>
</tr>
<tr>
<td>µg Proteins/individual in pupating larvae</td>
<td>71.5 ± 41.2</td>
<td>98.8 ± 25.1</td>
<td>143.2 ± 47.5*</td>
<td></td>
</tr>
<tr>
<td>µg Proteins/individual in emerging adult</td>
<td>82.6 ± 26.4</td>
<td>97.8 ± 8.9</td>
<td>115.8 ± 22.6</td>
<td></td>
</tr>
</tbody>
</table>

Statistics and probability

- H = 7.6
- P > 0.05
- H = 6.9
- P > 0.05
- H = 6.3
- P > 0.05
- H = 13.9
- P < 0.05
- H = 2.6
- P > 0.05
- H = 5.7
- P < 0.05
- H = 5.4
- P > 0.05
- H = 6.5
- P < 0.05
- H = 4.0
- P > 0.05

*Values represent the Avg. ± SD. Within a row, different letters denote treatments with statistically significant means (Kruskal-Wallis).

diet produced male flies with a significantly larger dry weight (Table 3). Female dry weight was not affected by sucrose loads in the diet. Although we were unable to apply inferential statistics to the pooled data, adult emergence and % flyers seems to be similar between all the treatments (Table 3).

Effect of Amino Acid Deletions and Concentration

Egg hatch was satisfactory (above 88%) and similar in all the diets (H = 4.2, P > 0.05). However, larval and pupal development time and pupal recovery were significantly affected by the
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regular aa</th>
<th>Glycine deficient</th>
<th>Serine deficient</th>
<th>Non-essential aa deficient</th>
<th>Low aa</th>
<th>High aa</th>
<th>Reference</th>
<th>Statistics and probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval development time in days</td>
<td>7 ± 0°</td>
<td>12 ± 0°</td>
<td>10 ± 0°</td>
<td>7 ± 0°</td>
<td>8 ± 0°</td>
<td>7 ± 0°</td>
<td>H = 19</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Pupal development time in days</td>
<td>11.3 ± 0.5°</td>
<td>13 ± 0°</td>
<td>9.3 ± 0.6°</td>
<td>12.3 ± 0.6°</td>
<td>11 ± 0°</td>
<td>17 ± na</td>
<td>H = 17.2</td>
<td>H &lt; 0.05</td>
</tr>
<tr>
<td>% Pupal recovery</td>
<td>80.2 ± 19.6°</td>
<td>22.1 ± 5.4°</td>
<td>61.3 ± 17.5°</td>
<td>68.2 ± 10°</td>
<td>84.4 ± 3.6°</td>
<td>42.9 ± 15.8°</td>
<td>87.6 ± 2.5°</td>
<td>H = 15.9</td>
</tr>
<tr>
<td>Pupal fresh weight in mg</td>
<td>8.3 ± 0.3°</td>
<td>8.8 ± 0.3°</td>
<td>8 ± 0.3°</td>
<td>7.8 ± 0.1°</td>
<td>7.9 ± 0.3°</td>
<td>8.8 ± 0.2°</td>
<td>H = 14.5</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>% Adult emergence (pooled)</td>
<td>83.6</td>
<td>5</td>
<td>84</td>
<td>88</td>
<td>96.7</td>
<td>75</td>
<td>90</td>
<td>—</td>
</tr>
<tr>
<td>% Flyers (pooled)</td>
<td>65.2</td>
<td>na</td>
<td>69</td>
<td>72.7</td>
<td>75.9</td>
<td>91.7</td>
<td>63</td>
<td>—</td>
</tr>
<tr>
<td>Newly emerged male dry weight in mg</td>
<td>1 ± 0.3°</td>
<td>na</td>
<td>1.3 ± 0.1°</td>
<td>1.1 ± 0.3°</td>
<td>1.6 ± 0.2°</td>
<td>1.3 ± 0.2°</td>
<td>1.2 ± 0.2°</td>
<td>H = 30.2</td>
</tr>
<tr>
<td>Newly emerged female dry weight in mg</td>
<td>1.4 ± 0.2°</td>
<td>na</td>
<td>1.3 ± 0.2°</td>
<td>1.1 ± 0.2°</td>
<td>1.2 ± 0.2°</td>
<td>1.6 ± 0.4°</td>
<td>1.2 ± 0.3°</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>µg Lipids/individual in pupating larvae</td>
<td>461.3 ± 67.7°</td>
<td>386.6 ± 118°</td>
<td>464.5 ± 66.8°</td>
<td>276.1 ± 68.6°</td>
<td>240 ± 126.4°</td>
<td>421.8 ± 145.5°</td>
<td>H = 12.8</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>µg Lipids/individual in emerging adult</td>
<td>123.7 ± 66.8</td>
<td>107.2 ± na</td>
<td>94.6 ± 9.1</td>
<td>106.1 ± 9</td>
<td>109.3 ± 23</td>
<td>123.6 ± 65.6</td>
<td>H = 8.5</td>
<td>H &lt; 0.05</td>
</tr>
<tr>
<td>µg Proteins/individual in pupating larvae</td>
<td>230.9 ± 8.8°</td>
<td>241.5 ± 1.4°</td>
<td>243 ± 14.3°</td>
<td>184.3 ± 26.2°</td>
<td>164.1 ± 52.4°</td>
<td>227.9 ± 57°</td>
<td>H = 12.0</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>µg Proteins/individual in emerging adult</td>
<td>160.9 ± 6.9</td>
<td>147.4 ± na</td>
<td>141 ± 21</td>
<td>133.1 ± 13.6</td>
<td>122.2 ± 16.8</td>
<td>158.2 ± 51.8</td>
<td>H = 9.4</td>
<td>H &gt; 0.05</td>
</tr>
</tbody>
</table>

*Values represent the Avg. ± SD. Within a row, different letters denote treatments with statistically significant means (Kruskal-Wallis). na: non-available.
deletion of aa from the diet and by aa concentration (Table 4). In general, glycine deletion from the diet was more detrimental than the removal of serine, or all non-essential aa (Table 4). Regarding the effects of aa concentration in the diet, high aa content had a detrimental effect when compared to the development time and pupal recovery of the regular aa diet (Table 4). Pupal development time was highly delayed in contrast to the regular aa diet, and pupal recovery was about half of that obtained in the regular and low aa, or reference, diets (Table 4).

Lipid content in pupating larvae, both per individual and per pupal weight, were significantly affected by the deletion and concentration of aa, in the diets (Table 4; Fig. 3, H = 13.0, P < 0.05). Larvae developing on the regular, serine-deficient, glycine-deficient and high aa concentration in the diet had the highest loads of lipids per individual (Table 4). When standardized by pupal weight, however, the glycine-deficient diet produced pupating larvae with significantly lower lipid loads/mg weight than the other three diets (Fig. 3). Non-essential aa-deficient diets produced pupating larvae with a very high variability of lipid contents, both per individual and per weight (Table 4, Fig. 3). Low aa content produced pupating larvae with a significantly lower level of lipids, both per individual and per weight (Table 4, Fig. 3). Regarding emerging adults, aa deletions and concentrations did not significantly affect lipid loads, either when measured per individual (Table 4) or when measured as a proportion of pupal weight (Fig. 3, H = 7.5, P > 0.05). Additionally, adult sex did not affect lipid loads (F = 1.2, P > 0.05). Soluble protein loads per pupating larvae (when measured per individual) were significantly affected by aa diet: glycine-deficient, serine-deficient, and high aa content diets produced pupating larvae with similar levels of protein to those of the regular diet, while low aa contents and non-essential aa deficient diets produced pupating larvae with significantly lower loads of protein (Table 4). Although showing similar trends to those found per individual, when standardized by pupal weight, protein contents per mg of weight in pupating larvae was statistically similar for all the diet treatments (Fig. 4, H = 9.8, P > 0.05). Emerging adult protein loads were not affected by diet treatment (Table 4; Fig. 4, H = 8.1, P > 0.05), nor by sex (F = 0.7, P > 0.05).

Pupal weight was affected by aa in the diet: significantly heavier pupae were produced in the glycine-deficient, regular aa, and high aa diets than in the other treatments (Table 4). Adult dry weight did not show a clear pattern nor relation to pupal weight or other parameter. Males were significantly heavier in the low aa diet than in the other diets, while females produced on the high aa content and regular aa diets were heavier than females produced on other diets (Table 4). Percent adult emergence was very low in the glycine-deficient diet, and similar in the other diets (Table 4). Besides a high percentage of flyers in the high aa diet, the other diets showed similar values for this parameter (Table 4).

Fig. 3. Average lipid content (± SD), standardized by fresh pupal weight, in pupating larvae (shaded bars) and emerging adults (white bars) developing from meridic diets where amino acids (aa) have been deleted, singly or in group, and in diets with different concentration of all the aa (see Table 2): low aa (5.2% w/w), regular aa (9.8% w/w), and high aa (17.9% w/w). Letters stand for statistical differences between groups of pupating larvae (P < 0.05). Adult lipid contents did not differ statistically. Average for emerging adults in glycine deficient was calculated from a single replicate.
Fig. 4. Average soluble protein contents (± SD), standardized by fresh pupal weight, in pupating larvae (shaded bars) and emerging adults (white bars) developing from meridic diets where amino acids (aa) have been deleted, singly or in group, and in diets with different concentration of all the aa set (see Table 2): low aa (5.2% w/w), regular aa (9.8% w/w), and high aa (17.9% w/w). There were no statistical differences between groups at P = 0.05. Average for emerging adults in high aa was calculated from a single replicate.

**Frequency Distribution of Lipid and Protein Contents in Pupating Larvae and Emerging Adults Reared on Different Larval Diets**

Figure 5 shows the frequency distribution for lipid contents in pupating larvae and in emerging adults that developed in all the tested diets. Pupating larvae lipid content frequency distribution resulted in a negative index of kurtosis (-0.73), suggesting that the data is platykurtic (e.g., having more individual lipid contents spread throughout all the categories, and fewer at the mean as expected for a normal distribution). Platykurtic frequency distributions tend to be bimodal or multimodal (i.e., Fig. 5) and suggest that treatments have a differential effect upon lipid loads. In contrast, the resulting adult lipid content derived from all the diets in the study shows a highly leptokurtic frequency distribution (Fig. 5) with a very large positive kurtosis index (2.69). Leptokurtic distributions are obtained when most of the individuals in the population fall close to the mean. The skewness in the pupating treatments. The arrow highlights the direction of development and how a highly platykurtic frequency distribution obtained for pupating larvae gives rise, after metamorphosis, to a highly leptokurtic distribution of lipid content in emerging adults.

**Lipid Contents Classes (µg of lipids/organism)**

Fig. 5. Frequency distribution histogram and cumulative frequency distribution (line) for all the data on lipid contents in pupating larvae and emerging adults. The frequency distribution for each developing stage was calculated by pooling together all the results derived from all the diet July 2004
larvae lipid content frequency distribution was negative (-0.30), suggesting a long tail towards lower values of lipids. The level of skewness in the adult frequency distribution was positive (1.4) suggesting that the tail distended more towards the higher lipid loads (resulting from the high sucrose diet).

In contrast to lipids, protein contents both in pupating larvae and emerging adults tend to distribute in a platykurtic mode (data not shown). The kurtosis index for pupating larvae was -0.90, while for adults it was -0.76. In the two cases, skewness was negative (for pupating larvae, -0.40, and for adults, -0.33).

**DISCUSSION**

Within the tested range of concentrations, the effects of sucrose on most of the investigated parameters of development were minimal or nil. However, low sucrose content in the diet did affect the ability of larvae to accumulate lipids, suggesting that this group was probably under a negative energetic balance (Downer, 1981). Of interest is the fact that during metamorphosis, the lipid level in this group balances itself and reaches levels similar to the one in flies emerging from regular and high sucrose diets (Fig. 1). A similar tendency was found by Kaspi et al. (2002) on emerging male and female Medflies reared on "low" and "high" sucrose oligidic diets: teneral flies had similar loads of lipids. Kaspi et al. (2002) and our results indicate that Medfly lipid content tends to be regulated towards an optimal level during metamorphosis. As suggested by Figure 5, the large variability in lipid contents in pupating larvae, which results from the different larval diet constitutions, produced newly emerged flies with similar lipid loads and a steep leptokurtic distribution.

Moreover, previous observations in which diets were manipulated (e.g., concentrations of brewer's yeast and other sources of proteins such as powdered milk and larval diet decay) showed that pupating larvae lipid loads are significantly affected by diet (Nestel, unpublished data). However, adults tend to emerge with similar average lipid contents regardless of larval diet history (Nestel, unpublished data). While still requiring to be investigated, it seems that larvae reared on the high sucrose diets were on a strong positive energetic balance. Therefore, the lipid contents in the emerging adult are in the upper quartile of the frequency distribution, resulting in a positively skewed lipid frequency pattern (Fig. 5).

Protein contents in pupating larvae were affected by sucrose concentration in the diet: low and regular sucrose produced larvae with significantly lower soluble protein than those reared on a high sucrose content diet; (Table 3 and Fig. 2). In emerging adults, no statistically significant differences were found between sucrose treatments. However, average protein levels in adult flies were slightly elevated when raised on high sucrose diets compared to regular and low diets, and corresponded with the protein contents in pupating larvae. Moreover, the average protein contents in the pupating larvae originating from the different diets significantly correlated with the level of protein contents in emerging adults originating from the same Petri dish ($r = 0.60$, $P < 0.01$). In contrast, average lipid contents per diet in pupating larvae did not significantly correlate with the level of emerging adults ($r = -0.27$, $P > 0.05$). This last point strengthens the notion that adult lipid loads are regulated towards a certain range, which may be genetically determined (Gordon, 1972).

Deletion of non-essential aa from the meridic diet delayed larval and pupal development and reduced pupal recovery. These results are similar to those of Chang (2002). In addition, and in accord with Chang (2002), glycine deletion had the most harmful effect upon all the parameters (Table 4). Glycine deletion was even more detrimental than removing the whole set of 8 non-essential aa from the diet, suggesting that the fly symbiont metabolic complex may be able to cope with the lack of all the non-essential aa better than with the absence of a single non-essential, and possibly significant, aa from the diet. The metabolic mechanisms involved in this unanticipated type of regulation are currently unknown.

The effect of glycine absence in the diet upon all the measured parameters suggests that glycine
has a more significant role in the development of the Medfly than the rest of the non-essential aa. Glycine was reported by Dadd (1978) as a very important "non-essential" aa in Diptera, which seems to complement the essential aa to support full development. In addition, glycine has been reported as an important element of pupal cuticle proteins in several insects (Schopf, 1981; Okot-Kotber et al., 1994; Hopkins et al., 2000). Moreover, glycine is a key substrate for the entry of single carbon compounds into many constituents through its transformation into serine (Febvay et al., 1995). This could also be the case in the Medfly. The fact that the diet effect is not completely expressed when all the 8 "non-essential" aa are lacking (including glycine) is at present unclear and requires further research.

The results obtained with the manipulation of aa concentrations in the diet suggest that not only the presence of individual aa is of importance in the development of the fly, but also their relative contents (Gordon, 1972). Reduced general levels of aa in the diet did not significantly affect larval and pupal development time nor pupal recovery when compared to the recommended level of aa (e.g., regular aa) (Table 4). In contrast, low aa contents (50% of the recommended) have a strong reduction effect upon lipid and protein loads in the pupating larvae (Table 4, Figs. 3 and 4). These results suggest that aa concentration in the larval diet is important in the determination of reserves during larval development. On the other hand, high general levels of free aa in the diet (e.g., twice the recommended concentration) appear, as shown with other fruit flies (Manoukas, 1981), to be detrimental to the developing Medfly. This detrimental effect may be related to possible toxic effects of the "imbalanced" diet (Gordon, 1984), or to a possible feeding deterrent effect of the diet, which reduces the rate of food ingestion (Nation, 2002). This suggested adverse effect, however, did not affect the ability of developing insects to accumulate similar lipid and protein loads to those of flies developing on regular diets, and to produce heavier pupae than the other diets. This effect of excess aa upon pupal weight could be related to the conversion of surplus aa loads to keto-acids and ammonia, which is used for chitin synthesis (Gordon, 1972). This chitin may wind up as pupal exo-cuticle and serve as a disposal mechanism for the excessive ammonia.

Amino acids in the larval diet, more than carbohydrates, seem to be the limiting factor for the optimal development of the Medfly. This has been previously suggested by Zucoloto (1987) through behavioral experiments in which larvae from the Medfly were shown to prefer foraging in areas of the food substrate where the protein is located. The importance of aa for the optimal development of the larval fruit flies can also be inferred from the outcome of the known association between symbiotic bacteria and larval flies: symbionts enrich larval food substrates both in quantity and quality of aa (White, 1993). Expected, also, are the results of this study in which aa and sucrose contents affected the ability of the developing larvae to accumulate lipid reserves and proteins. Unexpected, was the observed tendency of adult flies reared on different aa and sucrose diets to emerge with a more or less similar load of lipid reserves, and a low variability in soluble proteins. Therefore, there is a highly developed, possibly unique, regulation of metabolism during the larval-adult transition of the Medfly, which deserves to be further investigated.

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LITERATURE CITED


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