Dietary Protein and Carbohydrate Levels Affect Performance and Digestive Physiology of *Plodia interpunctella* (Lepidoptera: Pyralidae)

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Abstract

In this study, life history and nutritional indices of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) was evaluated on six food commodities: dried fig, dried wheat germ, dried white mulberry, groundnut, pistachio, and raisin, compared with artificial diet. The influence of dietary macronutrient content on digestive α-amylase was also assessed. A delay in the developmental time of *P. interpunctella* immature stages was detected when larvae were fed on raisin. The highest survival rate of immature stages was on the artificial diet, and the lowest was on raisin. The highest realized fecundity and fertility were recorded for the females reared on artificial diet. Overall, fourth instar *P. interpunctella* reared on artificial diet had the highest relative consumed and growth rate, and lowest rates were observed in larvae fed raisin. Amylolytic activity and isoform patterns varied depending on larval instar and diets, but were higher for larvae fed artificial diet with moderate carbohydrate and protein. Zymograms showed the presence of three isoforms of α-amylase in midgut extracts of *P. interpunctella* fed different diets. Larvae fed dried white mulberry, fig, and raisin had one (A2) α-amylase isoform. The data suggest that dietary carbohydrate and protein content induce changes in nutritional efficiency, development, and α-amylase activity. A survey of the differences in digestive enzyme activity in response to macronutrient balance and imbalance highlight their importance in the nutrition of insects.

Key words: dietary protein and carbohydrate, digestive enzyme, life span, nutritional index, the Indianmeal moth

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Insecta: Lepidoptera: Pyralidae), is a cosmopolitan pest of storage products such as cereal and legume grains, nuts, dried vegetables and dried fruits in temperate and tropical areas of the world (Mohandass et al. 2007, Burks et al. 2011, Razazzian et al. 2015, Harrison et al. 2016). In serious infestation of stored products by *P. interpunctella* larvae, quality and quantity of food commodities decrease through feeding and contamination with spin webs that can make it unpleasant, indigestible, or unmarketable (Mohandass et al. 2007).

The destructive activity of *P. interpunctella* and other storage pests have been adequately subdued by chemical pesticides comprising fumigation of stored commodity with methyl bromide, chlorine disulfide, and several other chemicals, but their negative effects, such as pest resistance and environmental pollution, limit their use by farmers (Borzou et al. 2015, Han et al. 2016). As part of the quest for alternative control methods to chemical insecticides, it is essential to understand the biological aspects and nutritional physiology of *P. interpunctella* in response to feeding on different host diets.

The nutritional value of food an animal eats has profound effects on its lifespan and fitness and is determined by multiple factors, such as the quantity and quality of macronutrients and proteinaceous inhibitors (Deans 2014). Previous studies have shown that high quality food generally resulted in higher larval growth rates, and these rates were primarily the result of higher consumption rates (Grandison et al. 2009, Borzou et al. 2017, Suits et al. 2017). Na and Ryoo (2000) studied the effects of different dried vegetables on the developmental period of *P. interpunctella* and reported that the shortest developmental period and the highest survival was on green onion. A study by Bouayad et al. (2008) on the effect of different food commodities on fitness-related parameters of *P. interpunctella* found that sorghum was the suitable host diet for this pest.

There is growing evidence that at least some insects attempt to regulate intake of multiple nutrients independently, rather than maximizing energy intake subject to constraints (Ko et al. 2017). In the complex diets of insects, the consumption of nutritionally imbalanced foods is sometimes inevitable, forcing trade-offs between eating too much of nutrients present in the foods in relative excess...
Materials and Methods

Laboratory Mass Culture of *P. interpunctella*

Colony of *P. interpunctella* was established from wild moths captured from stored walnut in Karaj, Iran. The insects were maintained on walnut at 29 ± 1°C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h, as described by Bouayed et al. (2008). After colonization of *P. interpunctella* for five generations, adults were transferred to different host diets and artificial diet was based on the previously described methods (Borzoui and Bandani 2013). Larvae were chilled on ice, and lumens were extracted following removal of the posterior and anterior ends and immediately placed into ice-cold solution of 10 mM NaCl per 50 midguts. The midguts were homogenized using a precooled Teflon pestle and the homogenate was centrifuged at 15,000×g for 15 min at 4°C. The resulting supernatant was stored at −20°C until use.

Life History Variables

Development and survival

Newly emerged larvae (within 24 hr) were allowed to feed on dried fig, dried wheat germ, dried white mulberry, groundnut, pistachio, and raisin. Sixty neonates were transferred into Petri dishes (diameter 8 cm, depth 2 cm), containing each of the above-mentioned food commodities and also to a wheat bran-based artificial diet. Petri dishes were visited daily, and the duration of immature stages, larval weight, adult longevity, and their survival were recorded.

Realized fecundity and egg fertility

To determine daily realized fecundity, newly emerged adults (one male and one female) were transferred to plastic vials (diameter 5 cm, depth 10 cm) that were covered by a net cloth for collecting eggs. The oviposition vials were then inversely placed on the black paper sheets as an oviposition surface (Borzoui and Naseri 2016). The paper sheets were removed daily and the number of deposited eggs was recorded until the female’s death. All eggs collected in this study were maintained for 15 d to determine the percentage of hatched eggs (fertility).

Nutritional indices

To determine nutritional indices of *P. interpunctella* larvae fed on different food commodities and artificial diet, a gravimetric method was used according to Waldbauer (1968), Manuwoto and Scriber (1982), and Farrar et al. (1989) with some modifications by Nouri-Ganbalani et al. (2016). After molting to the fourth stadium, groups (*n* = 5) of 10 larvae that had been starved for at least 12 hr were weighed on a microbalance (Sartorius, GCA8035) and they were set up on each diet. After 5 d, the larvae and food remaining were weighed again to determine the change in weight. The mass of the fecal pellets (following oven drying for 48 hr at 60°C) was also determined. The dry weight of the different diets (5 g wet weight) and larvae (10 randomly chosen larvae) was determined after they were oven-dried for 48 hr at 60°C. The nutritional indices of the insect were calculated by using following formulas (Waldbauer 1968, Manuwoto and Scriber 1982, Farrar et al. 1989):

- **Relative consumption rate** (RCR) = \( \frac{E}{A \times T} \);
- **Relative growth rate** (RGR) = \( \frac{P}{IA \times T} \);
- **Efficiency of conversion of ingested food** (ECI) = \( \frac{P}{E} \).

where *A* is mean dry weight of larvae over the feeding period (mg), *E* indicates dry weight of food consumed (mg), *P* is dry weight gain of larvae (mg), and *T* is duration of feeding period (day).

Crude midgut extract preparation

The enzyme preparation of *P. interpunctella* fourth instar fed on different food commodities and artificial diet was based on the previously described methods (Borzoui and Bandani 2013). Larvae were chilled on ice, and lumens were extracted following removal of the posterior and anterior ends and immediately placed into ice-cold solution of 10 mM NaCl per 50 midguts. The midguts were homogenized using a precooled Teflon pestle and the homogenate was centrifuged at 15,000 g for 15 min at 4°C. The resulting supernatant was stored at −20°C until use.

Qualitative and quantitative analyses of α-amylase

Amylolytic activity of midgut extracts was assayed according to the method described by Bernfeld (1955) with some modifications. Starch (1% w/v) dissolved in 20 mM Tris–HCl, pH 8.5, was used as substrate. Equalized volumes of midgut extracts (10 μl) were used for carrying out in vitro α-amylase assays. In brief, the enzyme preparation was mixed with 165 μl of 20 mM Tris–HCl, pH 8.5, at 37°C. Reaction was started by the addition of 25 μl of 1% starch and stopped 30 min later by adding 100 μl of dinitrosalicylic acid, and heating in boiling water for 10 min. The absorbance was read at 540 nm after cooling on ice.

Zymogram analysis of α-amylase was performed using the method described by Kazzazi et al. (2005). The midgut extracts (10 μl) were run at 4°C on 10% SDS-polyacrylamide gel electrophoresis (PAGE). Following electrophoresis, SDS was subsequently eluted by washing the gel in 1% (w/v) Triton X-100 in distilled water, for 15 min at room temperature. After renaturation, the gel was transferred to a substrate/buffer solution (1% (w/v) starch, 20 mM Tris–HCl 20 mM NaCl–0.2 mM CaCl₂, pH 8.5), for 1.5 hr at 37°C. After briefly rinsing the gel in distilled water, amylolytic activity was stopped by transferring the gel to the staining solution [1.5% (w/v) *I₃, 3% (w/v) KI]. After coloration, amylolytic activity was revealed as a zone of white clearing in a dark brown background.

Carbohydrate and protein determination of tested food commodities

Carbohydrate concentration of tested food commodities and artificial diet was quantified by the method of Jood et al. (1993) using
glucose as a standard. Briefly, 100 mg of diet was homogenized on ice in 400 µl of 2% Na2SO4. An additional 1,300 µl of chloroform–methanol (1:2) was added to the homogenate to extract the carbohydrate of the diet. Sample homogenate was centrifuged for 10 min at 8,000xg. Then, 30 µl of the supernatant was taken and mixed with 20 µl of distilled water. The sample was reacted for 15 min at 90°C with 100 µl of anthrone reagent (50 mg of anthrone dissolved in 50 ml of concentrated H2SO4). The absorbance was read at 630 nm after cooling on ice. This experiment was replicated five times.

Protein concentration of tested food commodities was quantified by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Briefly, 100 mg of diet was homogenized on ice in 1 ml of 50 mM sodium phosphate buffer (pH 7) using a pre-cooled Teflon pestle. Following the homogenization, 20 µl of the homogenate was added to 100 µl of Lowry reagent (Ziets Chem. Co., Tehran, Iran) and the mixture was incubated for 30 min at room temperature prior to absorbance measurement at 545 nm.

Statistical Analysis
Results are presented as mean ± SE. Before analysis, all the data were examined for normality using Kolmogorov–Smirnov test (PROC GLM; SAS Institute 2011). Since the data were normally distributed, no data transformation was conducted. Statistical analyses were done with SAS ver 9.2 program (PROC GLM; SAS Institute 2011) for Windows. Life history and digestive α-amylase activity were analyzed, based on a completely randomized design, by one-way analysis of variance (ANOVA). When F values were significant (P < 0.05), means were compared with Tukey’s test (Tukey 1949).

Results
Effects of Food Commodities and Artificial Diet on Life History
The results of the effect of different food commodities and artificial diet on developmental time of P. interpunctella are given in Table 1. The incubation period was longest in the insects reared on dried wheat germ and raisin, which was significantly different from other foods (F4,36 = 24.27; P < 0.001). Also, the longest larval period (F4,36 = 118.03; P < 0.001) was detected on raisin, and the shortest on artificial diet. Similarity, pupal period was the longest in the insects reared on raisin, and was shortest in the insects reared on artificial diet (F4,36 = 108.15; P < 0.001). Moreover, the longest developmental time of immature stages (from egg to adult stage; F4,36 = 131.96; P < 0.001) was detected on raisin. Different food commodities showed a significant effect on the adult longevity of P. interpunctella (F4,36 = 34.65; P < 0.001). The records for the longest longevity of adults were for the insects reared on pistachio and artificial diet. In contrast, the record for the shortest longevity of adults was on groundnut (Table 1).

Statistical analysis showed that there was a highly significant difference on survival rate of larvae fed different food commodities and artificial diet (F4,36 = 46.38; P < 0.001; Fig. 1). The survival rate throughout the immature stage ranged from 27.5 to 93.7% with the higher and lower survival rate observed on artificial diet and raisin, respectively.

The results of the effect of different food commodities and artificial diet on realized fecundity and egg fertility of P. interpunctella are given in Table 2. The highest realized fecundity (F4,36 = 143.73; P < 0.001) were recorded for P. interpunctella females developed from larvae reared on artificial diet (107.8 ± 3.7 eggs/female), and the lowest values of this parameter was recorded on raisin (33.3 ± 2.0 eggs/female). Also, females developed from larvae fed with artificial diet and dried wheat germ recorded the highest and lowest rates of fertility, respectively (F4,36 = 49.69; P < 0.001).

Nutritional Indices of Larvae on Different Food Commodities and Artificial Diet
Nutritional indices (RGR, RGR, and ECI) of fourth instar P. interpunctella were significantly different on different tested food commodities and artificial diet (F4,20 = 7.15; P < 0.001 for RCR; F4,20 = 18.74; P < 0.001 for RGR; F4,20 = 12.76; P < 0.003 for ECI; Table 3). The highest value of RCR was found on artificial diet, while larvae fed on dried fig and raisin exhibited the lowest. Also, the highest value of RGR was found on artificial diet, while the lowest value was on dried wheat germ and raisin. The values of ECI, however, were not statistically different for larvae fed on tested food commodities and artificial diet.

Amylolytic Activity During Larval Development on Different Food Commodities and Artificial Diet
Fig. 2 shows changes in values of amylolytic activity contained within the larvae midgut from first instar until fifth instar for P. interpunctella feeding on different food commodities and artificial diet (F4,20 = 9.83; P < 0.001 for pistachio, F4,20 = 17.65; P < 0.001 for dried white mulberry, F4,20 = 55.74; P < 0.001 for groundnut, F4,20 = 35.65; P < 0.001 for artificial diet, P4,20 = 9.83; P < 0.001 for raisin). The α-amylase levels of larvae fed on all the food commodities and artificial diet progressively increased with the instars. The activity pattern showed one sharp peak in fourth instar larvae and a marked decrease of activity in fifth instar larvae.

Fig. 3(A–G) shows the results of zymogram image analysis of amylolytic activity during larval stages of P. interpunctella feeding on different food commodities and artificial diet. Overall the

Table 1. Mean (±SE) duration (days) of immature stages and adult longevity of Plodia interpunctella fed different food commodities compared to artificial diet

<table>
<thead>
<tr>
<th>Food commodities</th>
<th>n</th>
<th>Egg</th>
<th>n</th>
<th>Larva</th>
<th>n</th>
<th>Pupa</th>
<th>n</th>
<th>Immature stages</th>
<th>n</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial diet</td>
<td>58</td>
<td>5.0 ± 0.0 b</td>
<td>57</td>
<td>17.2 ± 0.3 e</td>
<td>56</td>
<td>6.1 ± 0.2 d</td>
<td>56</td>
<td>27.2 ± 0.2 c</td>
<td>56</td>
<td>8.1 ± 0.2 a</td>
</tr>
<tr>
<td>Dried fig</td>
<td>58</td>
<td>5.0 ± 0.0 b</td>
<td>36</td>
<td>35.3 ± 1.7 a</td>
<td>21</td>
<td>14.2 ± 0.7 a</td>
<td>21</td>
<td>30.8 ± 2.8 b</td>
<td>21</td>
<td>7.0 ± 0.3 c</td>
</tr>
<tr>
<td>Dried wheat germ</td>
<td>44</td>
<td>7.0 ± 0.0 a</td>
<td>34</td>
<td>19.5 ± 0.6 cd</td>
<td>28</td>
<td>9.4 ± 0.3 b</td>
<td>28</td>
<td>34.6 ± 0.3 d</td>
<td>28</td>
<td>7.6 ± 0.3 b</td>
</tr>
<tr>
<td>Dried white mulberry</td>
<td>46</td>
<td>4.0 ± 0.0 c</td>
<td>40</td>
<td>20.6 ± 0.5 c</td>
<td>29</td>
<td>9.6 ± 0.4 b</td>
<td>29</td>
<td>32.9 ± 0.7 de</td>
<td>29</td>
<td>7.1 ± 0.4 bc</td>
</tr>
<tr>
<td>Groundnut</td>
<td>58</td>
<td>5.0 ± 0.0 b</td>
<td>49</td>
<td>24.2 ± 0.4 b</td>
<td>47</td>
<td>13.5 ± 0.2 a</td>
<td>47</td>
<td>41.5 ± 0.5 c</td>
<td>47</td>
<td>6.3 ± 0.2 d</td>
</tr>
<tr>
<td>Pistachio</td>
<td>50</td>
<td>4.0 ± 0.0 c</td>
<td>49</td>
<td>18.6 ± 0.3 de</td>
<td>47</td>
<td>8.9 ± 0.2 c</td>
<td>47</td>
<td>30.3 ± 0.2 e</td>
<td>47</td>
<td>8.2 ± 0.3 a</td>
</tr>
<tr>
<td>Raisin</td>
<td>45</td>
<td>7.0 ± 0.0 a</td>
<td>31</td>
<td>38.9 ± 1.7 a</td>
<td>16</td>
<td>15.1 ± 1.0 a</td>
<td>16</td>
<td>61.4 ± 3.4 a</td>
<td>16</td>
<td>6.8 ± 0.2 c</td>
</tr>
</tbody>
</table>

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Turkey’s test (P < 0.01).
patterns and intensities of individual α-amylase isoforms varied by instar and diet. The pattern of increasing α-amylase bands during the larval development showed that in the first instar, only the main bands were present. For the all diets, the intensity of bands in the fourth instar larvae was the greatest. In the fifth instar larvae, the number and intensity of the bands sharply decreased.

There was significant difference in amylolytic activity between the larvae fed on different diets \((F_{6,28} = 78.47; P < 0.001)\). Significantly higher amylolytic activity was found in the fourth instar feeding on artificial diet when compared with similar instar of *Plodia interpunctella* larvae feeding on other foods. In contrast, the lowest level of amylolytic activity in the fourth instar was seen on raisin (Fig. 2).

Zymogram showed the presence of three isoforms of α-amylase in different diets fed *Plodia interpunctella* midgut extracts (Fig. 4). The second (A2) α-amylase isoform was common in the midgut extracts of larvae fed on all food commodities and artificial diet while the first α-amylase isoform (A1) was present only in pistachio-fed larvae. Dried white mulberry, fig, and raisin-fed larvae had one (A2) α-amylase isoform.

### Carbohydrate and Protein Content of Tested Food Commodities and Artificial Diet

The highest carbohydrate content \((F_{6,28} = 22.46; P < 0.001)\) was measured in raisin, whereas the lowest content was in groundnut. Among different diets, the highest and lowest protein contents \((F_{6,28} = 33.76; P < 0.001)\) were in dried wheat germ and raisin (Table 4).

### Discussion

Eating a low-quality food, in terms of the ratio of protein-carbohydrate and the presence of proteinaceous inhibitors, has been associated with high mortality, reduced growth, delayed development, and low reproductive output in insect herbivores (Awmack and Leather 2002, Behmer 2009, Suits et al. 2017). Our data reveal that *P. interpunctella* did perform better on diets with moderate carbohydrate, but also moderate protein. Also, they use developmental plasticity and enzyme flexibility to minimize the variation between their optimal nutritional target and their nutritional intake in the face of difference in food quality.

The developmental time of *P. interpunctella* immature stages showed a significant difference among tested diets, with values ranging from 27.2 d on artificial diet to 61.4 d on raisin. These results agree with those found by Abdel-Rahman et al. (1968), who reported that development of *P. interpunctella* on different varieties of corn ranged from 25 to 57 d from egg hatch to adult emergence at 27°C and 70–80% RH. Artificial diet, as a nutritionally rich food, helps *P. interpunctella* to complete its life cycle early, promoting its multivoltine nature, and thus exhibiting developmental flexibility. Na and Ryoo (2000) and Borzouei et al. (2017) expressed that the variations in the nutritional quantity and presence of inhibitors can affect the length of immature stages in stored product pest.

![Graph showing food commodities](https://example.com/graph.png)

**Food commodities**

*Fig. 1.* Mean (±SE) percentage survival of *Plodia interpunctella* immature stages on different food commodities and artificial diet. The means followed by different letters are significantly different (Tukey’s test, \(P < 0.01\)).

### Table 2. Mean (±SE) realized fecundity (eggs laid per female) and egg fertility (percentage of hatched eggs per female) of *Plodia interpunctella* fed different food commodities compared to artificial diet

<table>
<thead>
<tr>
<th>Food commodities</th>
<th>(n)</th>
<th>Fecundity</th>
<th>Fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial diet</td>
<td>28</td>
<td>107.8 ± 3.7 a</td>
<td>81.8 ± 2.6 a</td>
</tr>
<tr>
<td>Dried fig</td>
<td>10</td>
<td>63.8 ± 4.8 d</td>
<td>63.6 ± 2.2 c</td>
</tr>
<tr>
<td>Dried wheat germ</td>
<td>13</td>
<td>44.2 ± 4.8 e</td>
<td>60.4 ± 3.7 c</td>
</tr>
<tr>
<td>Dried white mulberry</td>
<td>14</td>
<td>85.7 ± 6.5 bc</td>
<td>75.7 ± 1.8 b</td>
</tr>
<tr>
<td>Groundnut</td>
<td>22</td>
<td>82.0 ± 4.8 c</td>
<td>65.3 ± 3.5 c</td>
</tr>
<tr>
<td>Pistachio</td>
<td>22</td>
<td>93.5 ± 6.2 b</td>
<td>80.6 ± 3.6 ab</td>
</tr>
<tr>
<td>Raisin</td>
<td>10</td>
<td>33.3 ± 2.0 f</td>
<td>61.5 ± 2.9 c</td>
</tr>
</tbody>
</table>

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Turkey’s test \((P < 0.01)\). The \(n\) value shows the sample size for each parameter.

### Table 3. Mean (±SE) nutritional indices of *Plodia interpunctella* larvae fed different food commodities compared to artificial diet

<table>
<thead>
<tr>
<th>Food commodities</th>
<th>(n^*)</th>
<th>RCR (mg/mg/d)</th>
<th>RGR (mg/mg/d)</th>
<th>ECI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial diet</td>
<td>5</td>
<td>1.5 ± 0.1 a</td>
<td>0.28 ± 0.1 a</td>
<td>19.3 ± 2.0 ab</td>
</tr>
<tr>
<td>Dried fig</td>
<td>5</td>
<td>0.8 ± 0.0 c</td>
<td>0.15 ± 0.01 cd</td>
<td>19.0 ± 2.2 b</td>
</tr>
<tr>
<td>Dried wheat germ</td>
<td>5</td>
<td>0.93 ± 0.1 bc</td>
<td>0.14 ± 0.00 d</td>
<td>15.7 ± 0.8 c</td>
</tr>
<tr>
<td>Dried white mulberry</td>
<td>5</td>
<td>1.0 ± 0.06 b</td>
<td>0.21 ± 0.00 bc</td>
<td>21.4 ± 1.5 a</td>
</tr>
<tr>
<td>Groundnut</td>
<td>5</td>
<td>1.0 ± 0.1 b</td>
<td>0.19 ± 0.01 bcd</td>
<td>18.5 ± 2.1 b</td>
</tr>
<tr>
<td>Pistachio</td>
<td>5</td>
<td>1.2 ± 0.1 ab</td>
<td>0.23 ± 0.01 ab</td>
<td>19.7 ± 1.7 ab</td>
</tr>
<tr>
<td>Raisin</td>
<td>5</td>
<td>0.8 ± 0.1 c</td>
<td>0.14 ± 0.01 d</td>
<td>18.5 ± 0.5 b</td>
</tr>
</tbody>
</table>

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Turkey’s test \((P < 0.01)\).

AD approximate digestibility; RCR relative consumption rate; RGR relative growth rate; ECI efficiency of conversion of ingested food; ECD efficiency of conversion of digested food.

\(^*\)The \(n\) value shows the sample size for each parameter.
In agreement with other researches (Nay and Perring 2008, Borzouei et al. 2015), the results of present study showed that tested diets had significant effect on the adult longevity of *P. interpunctella*. The longest longevity of *P. interpunctella* adults on pistachio and artificial diet suggested that these diets contained the ingredients necessary for the better development of *P. interpunctella* immature stages, which led to the emergence of adults with longer longevity.

Survivorship of *P. interpunctella* immature stages was higher on artificial diet than on other food commodities tested, suggesting that artificial diet is a more suitable food than other commodities for feeding of this pest. Usually, artificial diets are complete foods designed for high performance of insects and considered to be better than natural diets (Hari et al. 2007). It is well known that dietary factors, such as protein, carbohydrates, vitamins, enzyme inhibitors, can affect the ability of moths to survive on diets (Bouayad et al. 2008, Behmar et al. 2009, Borzouei et al. 2017).

In many insect groups, it has been well established that larval diet has a considerable impact on realized fecundity and egg fertility (Bin et al. 2011, Majd-Marani et al. 2017). Fecundity was greatest among those fed the artificial diet (107.8 eggs/female; Table 2), and it is similar to data on fecundity of *P. interpunctella* females reared in similar conditions reported by Nansen and Phillips (2004) (≈100 eggs/female), but smaller than that reported by Masoumzadeh et al. (2014) (147.2 eggs/female). Similarly, fertility increased significantly when larvae were fed on the artificial diet. High fecundity and fertility in the artificial diet-fed insects might be due to nutritionally balanced composition, low level of inhibitors in this food or both.

We found that the ECI and RGR of *P. interpunctella* fourth instar were increased on artificial diet (Table 3), indicating that the larvae reared on artificial diet had more efficacy in the conversion of ingested food into growth (Hemati et al. 2012). Differences in the amount of food ingested could be because *P. interpunctella* perform better on artificial diet with carbohydrate and protein content optimized for its digestive physiology. In contrast, low values of ECI and RGR lead to delay in immature stages development and formation of smaller pupae that have a direct effect on the adult longevity and fecundity (Khosravi et al. 2010), which are observed in raisin-fed *P. interpunctella*. Similarly, Borzouei et al. (2015) reported that differing nutrient levels in tested diets affect nutritional indices of *Trogoderma granarium* Everts (Coleoptera: Dermestidae).

We observed that early instars of *P. interpunctella* have lower levels of amylolytic activity, which progressively increase until the fourth instar and decreased in the fifth instar. Results of SDS–PAGE are in close agreement with those obtained by using test tube assays. In the case of *P. interpunctella*, maximum intake of food is during the fourth instar and feeding of larvae slow down or stops in the fifth instar. Probably, the fourth larvae instars need to gain more energy from the diet, which might be the reason for the expression of additional α-amylase level. Similar patterns in specific activity of digestive α-amylase were also reported throughout the larval development of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Dastranj et al. 2013) and *Mamestra configurata* Walker (Lepidoptera: Noctuidae) (Hegedüs et al. 2003).

When phytophagous insects are forced to feed on various diets—highly variable in terms of micro- and macromolecular compositions—they actively regulate the release of their digestive enzymes in order to cope up with the larva’s diverse intake (Silva et al. 2001). According to our data, food commodities rich in carbohydrate (raisin, dried fig and dried white mulberry) induce a down-regulation of the amylolytic activity in the *P. interpunctella* midgut. This result can be explained in such a way that when carbohydrates are high even lower amounts of digestive α-amylases seem to be sufficient for metabolism. Also, highest amylolytic activity in the larvae fed on artificial diet (with moderate carbohydrate content) might be due to its nutritionally balanced composition. Hari et al. (2007) reported that artificial diets are usually complete foods designed for high performance of insects and usually considered to be better than natural foods. This supports earlier studies that herbivores release less of the digestive enzymes for nutrients present in excess, while maintaining or boosting levels of enzymes for nutrients in deficit (Behmer 2009, Parde et al. 2010).

In the present study, the results of zymogram reveal the existence of diverse specificities of amylolytic isofoms present...
in the *P. interpunctella* midgut fed on different food commodities and artificial diet. Differences in expression levels among the midgut α-amylase isoforms were also detected in the larvae feeding on different diets. These data suggest the potential of *P. interpunctella* larvae to express midgut α-amylase of different specificities in response to dietary protein and carbohydrate and also ingestion of proteinaceous inhibitors. It has been shown by earlier studies that insects rapidly alter their midgut composition by up- and down-regulation of digestive enzyme(s) in response to dietary factors.

Table 4. Carbohydrate and protein contents (n = 5) of food commodities and artificial diet used for feeding of *Plodia interpunctella* larvae

<table>
<thead>
<tr>
<th>Food commodities</th>
<th>Carbohydrate (mg/ml)</th>
<th>Protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial diet</td>
<td>1.35 ± 0.11 c</td>
<td>0.16 ± 0.01 c</td>
</tr>
<tr>
<td>Dried fig</td>
<td>2.12 ± 0.24 b</td>
<td>0.02 ± 0.00 d</td>
</tr>
<tr>
<td>Dried wheat germ</td>
<td>1.45 ± 0.10 c</td>
<td>1.73 ± 0.03 a</td>
</tr>
<tr>
<td>Dried white mulberry</td>
<td>2.34 ± 0.09 ab</td>
<td>0.02 ± 0.00 d</td>
</tr>
<tr>
<td>Groundnut</td>
<td>0.37 ± 0.01 e</td>
<td>1.61 ± 0.06 a</td>
</tr>
<tr>
<td>Pistachio</td>
<td>0.73 ± 0.06 d</td>
<td>1.02 ± 0.01 b</td>
</tr>
<tr>
<td>Raisin</td>
<td>2.65 ± 0.16 a</td>
<td>0.01 ± 0.00 d</td>
</tr>
</tbody>
</table>

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Turkey's test (P < 0.01).
to physico-chemical composition of food (Kotkar et al. 2009, Borzouei et al. 2015).

The Indianmeal moth larvae are known to be able to develop on a great number of diets. The results of this study showed that development cycle of this pest is influenced by the biochemical composition of the ingested food. The quantitative and qualitative difference in midgut α-amylase of *Plodia interpunctella*, depending upon the diet composition and larval developmental stages, facilitates its adaptation to the quality and quantity of ingested food. It would be interesting to focus on the quantitative and qualitative analysis of *Plodia interpunctella* digestive proteases and their interactions with the diet composition of different food commodities. Also, the insect’s response to the proteinaceous inhibitors should be essentially reviewed for the selection of appropriate inhibitors of digestive enzymes and their transgenic expression for resistance to this stored product pest.

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**References cited**


