Research Article

Effect of different barley cultivars on nutritional physiology of Tribolium castaneum (Coleoptera: Tenebrionidae)

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Abstract: Red flour beetle, Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan and serious pest of cereal grains and their products in storage. In this research, nutritional indices and digestive enzymatic activity of T. castaneum fourth instar and adults were studied on ten barley cultivars (Fajr30, Behrokh, Sahra, Makuyi, Neek, Lout, Bahman, Nosrat, Abidar, and Sahand) at 30 ± 1 °C, relative humidity of 75 ± 5% and darkness conditions. The results showed that fourth instars and adults reared on cultivars Makuyi and Fajr30 had the lowest weight gain, efficiency of conversion of ingested food (ECI), relative growth rate and growth rate (GR) values. However, T. castaneum fed on cultivar Lout showed the highest weight gain, ECI and GR. The highest amylolytic activity of larvae was on cultivar Bahman, whereas the lowest activity was on cultivars Makuyi and Neek. Amylolytic activity of adults was the highest on cultivar Makuyi, and the lowest on cultivar Lout. Moreover, proteolytic activity of fourth instar was the highest when larvae were fed on cultivar Sahra and the lowest when they were fed on cultivars Behrokh and Makuyi. The highest proteolytic activity of adults was on cultivar Abidar, and the lowest on cultivar Bahman. The results of this study indicated that cultivars Fajr30 and Makuyi were less nutritive and cultivar Lout was more nutritive to T. castaneum. Therefore, more attention should be paid to manage the pest on cultivar Lout as a susceptible cultivar.

Keywords: Nutritional index, digestive enzyme, barley, red flour beetle

Introduction

Barley, Hordeum vulgare L., is one of the main cereals used for feed and malt production in Iran and worldwide (Khodabandeh, 2003; Schulte et al., 2009; Kordan and Gabrys, 2013; Houshyar, 2017). Red flour beetle, Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), is known as a widespread and serious pest of cereals and their products in storage (Campbell and Abrogast, 2004; Shafique et al., 2006, Javed et al., 2016; Naseri et al., 2017). Both adult and larvae are able to exploit different stored products, and cause economic damages by contaminating food stuffs and decreasing their nutritional value. Extensive grain losses could occur by T. castaneum when susceptible cultivars are stored for a long time under favorable conditions (Sarwar, 2013, 2015).

Synthetic insecticides are often effective to control stored product pests such as T.
castaneum. Since these insecticides may have dangerous side effects to humans, non-target organisms and environment (Iram et al., 2013; Shweta and Prakash, 2013), it is crucial to develop control approaches that are safe and eco-friendly. To accomplish this goal, understanding the nutritional physiology of T. castaneum on various host cultivars may be practical to select host cultivars resistant to this pest (Fabres, 2014; Naseri et al., 2017).

The effect of different seed inhibitors on digestive amylolytic activity of T. castaneum larvae was evaluated by Khan et al. (2012), who reported that the inhibitors extracted from wheat, maize and kidney bean showed higher inhibitory effects than the others. Kheradpir (2014) studied the food preference by T. castaneum among different flour types and found the susceptibility of wheat flour to this insect. Also, Sagheer et al. (2014) investigated the effect of wheat, corn and barley flours on nutritional indices of T. castaneum and noted that barley flour was unsuitable diet for this species. Feeding efficiency and digestive enzymatic activity of T. castaneum on different food commodities were evaluated by Naseri et al. (2017), who stated that soybean flour was the most unsuitable food for its development.

Despite the economic losses caused by T. castaneum larvae and adults on stored barley seeds (Javed et al., 2016), no published articles are available regarding the nutritional physiology of this important pest on various barley cultivars. Therefore, the aim of this research was to evaluate the nutritional indices and activity of two main digestive enzymes (amylase and protease) of T. castaneum when fed on various barley cultivars. The results from this study will be helpful to improve better pest management strategies on stored barley cultivars.

Materials and Methods

Chemicals
Substrates, dinitrosalicylic acid (DNS) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, USA). Bovine serum albumin (BSA) was purchased from Roche Co. (Germany). Electrophoresis chamber was obtained from Bio-Rad Laboratories (Richmond, CA), and materials used to prepare the Electrophoresis gel were purchased from Merck Co. (Germany).

Barley sources
Tested eight barley cultivars (Fajr30, Behrokh, Sahra, Makuyi, Neek, Lout, Bahman, and Nosrat) were provided by Agricultural and Natural Resources Research Center of Isfahan, Iran. Seeds of two other barley cultivars (Abidar and Sahand) were obtained from Agricultural and Natural Resources Research Center (Ardabil, Iran). The selected cultivars are the most commonly cultivated in barley-growing regions of Iran. Before beginning of the experiments, all grains were ground to fine flour. Moisture level of tested cultivars ranged from 2.83% in cultivars Behrokh, Sahra and Makuyi to 8.76% in cultivar Sahand.

Insect rearing and experimental conditions
Adults of T. castaneum were collected from the laboratory colony from Department of Plant Protection, University of Mohaghegh Ardabili, (Ardabil, Iran), and maintained for two generations on a flour-baking yeast mixture (10% yeast) as a food substrate. After rearing on various barley cultivars for two generations, 20 pairs of male and female adults (1-5 days old) were each transferred into plastic containers (18 × 12 × 6 cm) containing various tested cultivars, and allowed to mate and lay eggs for 7 days. Then, the adults were removed by sieving, and the eggs laid were kept for offspring emergence. One-day old larvae were used to start the experiments. All experimental insects were reared at 30 ± 1 °C, relative humidity of 75 ± 5%, and darkness conditions (Naseri et al., 2017).

Nutritional indices
A gravimetric method described by Waldbauer (1968), based on dry weight, was used to
determine the nutritional indices of *T. castaneum* larvae and adults on tested cultivars. Seven groups of 10 fourth instars or adults were weighed and transferred to Petri dishes (diameter 6 cm, depth 1 cm) containing barley flour (≈ 200 mg) of each cultivar. Each larva and adults were weighed again after 3 and 6 days to achieve the mean changes in the body weight. To obtain the percentage dry weight of food and insects (larvae and adults), 5 specimens of each were weighed, and dried in an oven (at 60 °C for 48 h), then re-weighed. Nutritional indices of *T. castaneum* were calculated via the formulae described by Waldbauer (1968) and Farrar *et al.* (1989) as follows:

- Efficiency of conversion of ingested food (ECI) = P/E; relative consumption rate (RCR) = E/A × T; relative growth rate (GR) = P/A × T; and growth rate (GR) = P/T; where *A* = mean dry weight of larvae or adults over unit time, *E* = dry weight of food consumed, *P* = dry weight gain of larvae or adults, and *T* = duration of feeding period (days).

### Pupal mass and growth indices

To determine pupal weight of *T. castaneum*, seven groups of 10 pupae were weighed 48 hours after pupation. The larval growth index (LGI), standardized insect-growth index (SII) and fitness index (FI) of *T. castaneum* were determined on various barley cultivars using formula described by Pretorius (1976) and Itoyama *et al.*, 1999:

- LGI = *l*<sub>x</sub> / *L*
- SII = *P<sub>n</sub>/L
- FI = (*P* × *P<sub>n</sub>)/(*L* + *P<sub>0</sub>)

where, *lx* = survival rate of larvae, *L* = larval period, *P<sub>n</sub>* = pupal weight, *P* = percentage of pupation and *P<sub>0</sub>* = pupal period.

### Preparation of digestive enzymes

*Tribolium castaneum* fourth instars fed on each barley cultivar were ice-immobilized and dissected in distilled water under stereomicroscope (Stemi SV6 ZEISS, Germany). The guts (100 larvae for each cultivar) were then cleaned of adhering unwanted tissues and homogenized into 300 µl of distilled water. The whole body of 100 adults fed on various barley cultivars were collected into 300 µl of distilled water and homogenized with a handheld glass grinder on ice. The homogenates were then centrifuged at 12,000 g at 4 °C for 15 min. The obtained supernatants were collected in new micro tubes and stored in aliquots (at -20 °C) until further use (Borzou and Bandani, 2013; Naseri *et al.*, 2017).

### Activity assessment of enzymes

Digestive amylolytic activities of *T. castaneum* fourth instar and adults fed on various barley cultivars were estimated according to DNS method using 1% starch as substrate (Bernfeld, 1955). Enzyme extract (20 µl) was mixed with 50 mM acetate buffer (500 µl) at pH 5.0. Then, the mixture was incubated, after addition of 1% starch solution (40 µl), at 37 °C for 30 min. The reaction was stopped by adding 100 µl of DNS and heating in boiling water for 10 min., and the absorbance was read at 540 nm after cooling on ice. All assays were carried out in three replicates with blanks containing no enzyme extract.

General proteolysis of *T. castaneum* fourth instar and adults was assayed using 1.5% (w/v) solution of azocasein substrate in 50 mM acetate buffer (pH 5.0). Enzyme extract (20 µl) was added to 80 µl substrate and incubated at 37 °C for 50 min. Proteolysis was stopped by addition of 100 µl of 30% TCA. For each assay, appropriate blanks in which TCA was added firstly to the substrate were prepared. Precipitation was achieved by cooling at 4 °C for 30 min and centrifugation at 15,000 g for 10 min. The supernatant (100 µl) was added to 100 µl of 2 M NaOH and the absorbance was recorded at 440 nm. Assays were carried out in three replicates with blanks containing no enzyme extract (Elpidina *et al.*, 2001).

General protein concentrations in the crude enzyme extract of fourth instar and adults were
determined using BSA as a standard according to the method of Bradford (1976).

Activity assessment of enzymes in gel
Native polyacrylamide gel electrophoresis (PAGE) was used to fractionate α-amylase on 10% polyacrylamide gel (Laemmli, 1970). Enzyme extracts from fourth instar and adults fed on various barley cultivars were loaded on zymogram gel, and electrophoresed at a voltage of 120 V for 2 h. After electrophoresis, the gel was rinsed twice with distilled water and left in a solution of 2.5% (v/v) Triton X-100 for 15 min. Then the gel was dipped into 1% starch substrate prepared in 50 mM acetate buffer (pH 5.0) containing 2 mM CaCl₂ and 10 mM NaCl and incubated at 37 °C for 1.5 h. Finally, to stop the reaction and stain the un-reacted starch background, the gel was treated with a solution of 1.3% I₂ and 3% KI. Zones of amylolytic activity appeared as light bands against dark background. General proteolysis in the electrophoretic gel was determined using 1% gelatin as substrate (Laemmli, 1970; Saadati et al., 2011). Enzyme extracts from fourth instar and adults fed on various barley cultivars were loaded on zymogram gel and electrophoresed at a voltage of 80 V for 4 h. After electrophoresis, the gel was rinsed twice with distilled water and put in a solution of 2.5% (v/v) Triton X-100 for 30 min. Subsequently, the gel was dipped into 50 mM acetate buffer (pH 5.0) and incubated at 37 °C overnight. Finally, the gel was stained in 40% methanol, 7% glacial acetic acid and 0.05% Coomassie Brilliant Blue R and destained until proteolytic activity appeared as light bands against dark background.

Statistical analysis
Normality of the data was checked using the Kolmogorov-Smirnov test before analysis and all of data were normal. The result of each experiment was analyzed by one-way analysis of variance (ANOVA) using statistical software Minitab 16.0. Statistical differences among the means were assessed at 5% level by Tukey’s test. The Pearson correlation coefficient was used to evaluate the correlations between the nutritional indices of larvae and adults with their enzymatic activity when fed on various barley cultivars using Minitab 16.0.

Results
Nutritional indices of fourth instar and adult
Nutritional indices of *T. castaneum* fourth instar were significantly different on tested barley cultivars (Table 1). The lowest weight gain (F = 294.15; df = 9, 60; P < 0.01), ECI (F = 161.54; df = 9, 60; P < 0.01), RGR (F = 184.98; df = 9, 60; P < 0.01) and GR (F = 298.39; df = 9, 60; P < 0.01) values were on cultivars Makuyi and Fajr30. The highest food consumed (F = 219.88; df = 9, 60; P < 0.01) and RCR (F = 165.24; df = 9, 60; P < 0.01) values were on cultivar Sahand and the lowest values were on cultivar Nosrat.

The data in Table 2 shows nutritional indices of adults reared on various barley cultivars. The lowest weight gain (F = 62.86; df = 9, 60; P < 0.01), ECI (F = 161.54; df = 9, 60; P < 0.01), RGR (F = 42.87; df = 9, 60; P < 0.01) and GR (F = 62.86; df = 9, 60; P < 0.01) values were on cultivars Fajr30 and Makuyi. Moreover, food consumed by adults was the lowest on cultivar Neek. Also, the adults fed on cultivars Neek and Lout showed the lowest RCR value.

Pupal mass and growth indices
The pupal mass and growth indices of *T. castaneum* are indicated in Table 3. The heaviest pupal weight (F = 306.68; df = 9, 60; P < 0.01) was on cultivar Lout, and the lightest one was on cultivar Makuyi. Among various barley cultivars, the LGI varied from 1.65 on cultivar Fajr30 to 4.25 on cultivar Sahra. The highest SII index (F = 1559.52; df = 9, 60; P < 0.01) was on cultivar Lout, whereas the lowest value was on cultivars Fajr30 and Makuyi. Moreover, the highest value of FI (F = 2326.80; df = 9, 60; P < 0.01) was on cultivar Lout, and the lowest was on cultivars Fajr30 and Makuyi (Table 3).
Table 1 Mean (± SE) nutritional indices of *Tribolium castaneum* fourth instar on various barley cultivars.

<table>
<thead>
<tr>
<th>Barley cultivar</th>
<th>Weight gain (mg)</th>
<th>Food consumed (mg)</th>
<th>ECI (%)</th>
<th>RCR (mg/mg/day)</th>
<th>RGR (mg/mg/day)</th>
<th>GR (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abidar</td>
<td>0.334 ± 0.015d</td>
<td>4.55 ± 0.09b</td>
<td>11.72</td>
<td>0.401 ± 0.012b</td>
<td>0.047 ± 0.002b</td>
<td>0.076 ± 0.002b</td>
</tr>
<tr>
<td>Fajr 30</td>
<td>0.141 ± 0.008c</td>
<td>3.36 ± 0.24c</td>
<td>4.35</td>
<td>0.274 ± 0.019d</td>
<td>0.011 ± 0.001e</td>
<td>0.020 ± 0.001e</td>
</tr>
<tr>
<td>Behrokh</td>
<td>0.375 ± 0.006c</td>
<td>2.10 ± 0.03e</td>
<td>17.90</td>
<td>0.167 ± 0.003ef</td>
<td>0.030 ± 0.001c</td>
<td>0.053 ± 0.002c</td>
</tr>
<tr>
<td>Sahra</td>
<td>0.344 ± 0.017cd</td>
<td>1.74 ± 0.10fg</td>
<td>19.92</td>
<td>0.133 ± 0.01fg</td>
<td>0.026 ± 0.001cd</td>
<td>0.049 ± 0.002cd</td>
</tr>
<tr>
<td>Makuyi</td>
<td>0.168 ± 0.006e</td>
<td>2.97 ± 0.11cd</td>
<td>5.77</td>
<td>0.271 ± 0.010d</td>
<td>0.015 ± 0.001e</td>
<td>0.024 ± 0.001e</td>
</tr>
<tr>
<td>Neck</td>
<td>0.785 ± 0.025a</td>
<td>4.50 ± 0.12b</td>
<td>17.53</td>
<td>0.334 ± 0.008c</td>
<td>0.058 ± 0.001a</td>
<td>0.112 ± 0.003a</td>
</tr>
<tr>
<td>Loui</td>
<td>0.842 ± 0.011a</td>
<td>2.60 ± 0.10d</td>
<td>32.69</td>
<td>0.188 ± 0.009e</td>
<td>0.061 ± 0.001a</td>
<td>0.120 ± 0.001a</td>
</tr>
<tr>
<td>Bahman</td>
<td>0.292 ± 0.015d</td>
<td>1.77 ± 0.07fg</td>
<td>16.54</td>
<td>0.140 ± 0.005efg</td>
<td>0.023 ± 0.001d</td>
<td>0.041 ± 0.002d</td>
</tr>
<tr>
<td>Sahand</td>
<td>0.792 ± 0.016a</td>
<td>6.85 ± 0.07a</td>
<td>11.57</td>
<td>0.513 ± 0.008a</td>
<td>0.059 ± 0.001a</td>
<td>0.113 ± 0.001a</td>
</tr>
<tr>
<td>Nosrat</td>
<td>0.318 ± 0.019cd</td>
<td>1.29 ± 0.06g</td>
<td>24.72</td>
<td>0.108 ± 0.006g</td>
<td>0.026 ± 0.001cd</td>
<td>0.045 ± 0.001cd</td>
</tr>
</tbody>
</table>

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


Table 2 Mean (± SE) nutritional indices of *Tribolium castaneum* adult on various barley cultivars.

<table>
<thead>
<tr>
<th>Barley cultivar</th>
<th>Weight gain (mg)</th>
<th>Food consumed (mg)</th>
<th>ECI (%)</th>
<th>RCR (mg/mg/day)</th>
<th>RGR (mg/mg/day)</th>
<th>GR (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abidar</td>
<td>0.118 ± 0.005b</td>
<td>1.64 ± 0.06b</td>
<td>7.22</td>
<td>0.123 ± 0.005b</td>
<td>0.0089 ± 0.0003cd</td>
<td>0.017 ± 0.001b</td>
</tr>
<tr>
<td>Fajr 30</td>
<td>0.080 ± 0.003c</td>
<td>2.12 ± 0.08a</td>
<td>3.77</td>
<td>0.179 ± 0.007a</td>
<td>0.0067 ± 0.0002f</td>
<td>0.011 ± 0.000c</td>
</tr>
<tr>
<td>Behrokh</td>
<td>0.115 ± 0.004b</td>
<td>2.27 ± 0.06a</td>
<td>5.11</td>
<td>0.169 ± 0.005a</td>
<td>0.0086 ± 0.0003de</td>
<td>0.017 ± 0.001b</td>
</tr>
<tr>
<td>Sahra</td>
<td>0.168 ± 0.005a</td>
<td>1.57 ± 0.07bc</td>
<td>10.78</td>
<td>0.108 ± 0.004bc</td>
<td>0.0115 ± 0.0003b</td>
<td>0.024 ± 0.001a</td>
</tr>
<tr>
<td>Makuyi</td>
<td>0.081 ± 0.002c</td>
<td>2.14 ± 0.06a</td>
<td>3.82</td>
<td>0.187 ± 0.006a</td>
<td>0.0071 ± 0.0002ef</td>
<td>0.012 ± 0.000c</td>
</tr>
<tr>
<td>Neck</td>
<td>0.111 ± 0.003b</td>
<td>1.20 ± 0.03d</td>
<td>9.32</td>
<td>0.080 ± 0.003d</td>
<td>0.0074 ± 0.0002def</td>
<td>0.016 ± 0.000b</td>
</tr>
<tr>
<td>Loui</td>
<td>0.184 ± 0.004a</td>
<td>1.32 ± 0.04cd</td>
<td>13.89</td>
<td>0.077 ± 0.002d</td>
<td>0.0106 ± 0.0002bc</td>
<td>0.026 ± 0.001a</td>
</tr>
<tr>
<td>Bahman</td>
<td>0.110 ± 0.003b</td>
<td>1.54 ± 0.03bc</td>
<td>7.07</td>
<td>0.117 ± 0.001b</td>
<td>0.0083 ± 0.0003def</td>
<td>0.016 ± 0.001b</td>
</tr>
<tr>
<td>Sahand</td>
<td>0.117 ± 0.004b</td>
<td>1.48 ± 0.03bcd</td>
<td>7.87</td>
<td>0.093 ± 0.002cd</td>
<td>0.0074 ± 0.0002def</td>
<td>0.017 ± 0.001b</td>
</tr>
<tr>
<td>Nosrat</td>
<td>0.181 ± 0.008a</td>
<td>1.58 ± 0.08bc</td>
<td>11.47</td>
<td>0.119 ± 0.006b</td>
<td>0.0136 ± 0.0006a</td>
<td>0.025 ± 0.001a</td>
</tr>
</tbody>
</table>

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


Table 3 Pupal weight (mg), larval growth index (LGI), standardized insect-growth index (SII), and fitness index (FI) of *Tribolium castaneum* on various barley cultivars.

<table>
<thead>
<tr>
<th>Barley cultivar</th>
<th>Pupal weight (mg)</th>
<th>Index (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GI</td>
</tr>
<tr>
<td>Abidar</td>
<td>1.872 ± 0.008de</td>
<td>3.990</td>
</tr>
<tr>
<td>Fajr 30</td>
<td>1.725 ± 0.010f</td>
<td>1.646</td>
</tr>
<tr>
<td>Behrokh</td>
<td>1.914 ± 0.012d</td>
<td>4.231</td>
</tr>
<tr>
<td>Sahra</td>
<td>2.020 ± 0.012c</td>
<td>4.250</td>
</tr>
<tr>
<td>Makuyi</td>
<td>1.622 ± 0.008g</td>
<td>1.753</td>
</tr>
<tr>
<td>Neck</td>
<td>2.248 ± 0.033b</td>
<td>3.323</td>
</tr>
<tr>
<td>Loui</td>
<td>2.401 ± 0.011a</td>
<td>4.033</td>
</tr>
<tr>
<td>Bahman</td>
<td>1.898 ± 0.005de</td>
<td>4.168</td>
</tr>
<tr>
<td>Sahand</td>
<td>2.250 ± 0.012b</td>
<td>3.612</td>
</tr>
<tr>
<td>Nosrat</td>
<td>1.820 ± 0.007e</td>
<td>3.877</td>
</tr>
</tbody>
</table>

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Tukey’s test (*P* < 0.01).
Activity assessment of enzymes

Amylolytic activities of *T. castaneum* fourth instar and adults reared on various barley cultivars are shown in Fig. 1. The highest amylolytic activity of fourth instar (F = 47026.65; df = 9, 20; \( P < 0.01 \)) was in larvae reared on cultivar Bahman, whereas the lowest activity was observed in larvae fed on cultivars Makuyi and Neek (Fig. 1A). The highest amylolytic activity of adults was on cultivar Makuyi (F = 12302.93; df = 9, 20; \( P < 0.01 \)), and the lowest activity was on cultivar Lout (Fig. 1B).

Figure 1 Mean (± SE) amylolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) (n = 3) fed on various barley cultivars. The means followed by different letters are significantly different (Tukey’s test, \( P < 0.01 \)).
Figure 2 indicates general proteolytic activity of *T. castaneum* fourth instar and adults reared on various barley cultivars. The fourth instar (*F* = 2480.49; df = 9, 20; *P* < 0.01) reared on cultivar Sahra showed the highest level of proteolytic activity, whereas the lowest activity was in larvae reared on cultivars Behrokh and Makuyi (Fig. 2A). Our data showed that the highest proteolytic activity of adults (*F* = 2395.03; df = 9, 20; *P* < 0.01) was on cultivar Abidar, whereas the lowest activity was on cultivar Bahman (Fig. 2B).

**Figure 2** Mean (± SE) general proteolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) (*n* = 3) fed on various barley cultivars. The means followed by different letters are significantly different (Tukey's test, *P* < 0.01).

**Activity assessment of enzymes in gel**

Results from polyacrylamide gel test nearly agreed with those from the quantitative assays. Fourth instar reared on various barley cultivars showed two strong bands of amylolytic activity (isoenzymes 2 and 3). According to the
amylolytic activity pattern, larvae fed on cultivars Sahra, Neek and Lout exhibited a weak intensity of band (isoenzyme 1) as compared to the others (Fig. 3A). By contrast, the adults fed on barley cultivars showed two bands on cultivars Sahra and Makuyi, and one band on the other cultivars which nearly showed the same activity pattern (Fig. 3B).

According to the zymogram of general proteolytic activity, larvae fed on cultivar Makuyi had only one band of proteolytic activity (isoenzyme 3). However, larvae that were fed on the other cultivars showed three bands (isoenzymes 1-3) (Fig. 4A). The general proteolytic activity of adults fed on various barley cultivars, except for cultivars Abidar and Fajr30, showed one proteolytic band (isoenzyme 3). However, three bands of proteolytic activities were detected on cultivars Abidar and Fajr30 (Fig. 4B).

**Figure 3** Mean (± SE) amylolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) in gel visualization of isozymes.
Figure 4 Mean (± SE) general proteolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) in gel visualization of isozymes.

**Correlation analysis**

Table 4 demonstrates the correlation analysis coefficients of the nutritional indices of *T. castaneum* larvae and adults with their enzymatic activity when fed on various barley cultivars. No correlations were observed between all nutritional indices of larvae with their amylolytic and proteolytic activities. However, correlation coefficients in adults showed that the food consumed ($r = 0.787$) and RCR ($r = 0.794$) values exhibited a positive correlation with amylolytic activity. There was a negative correlation between ECI values with the amylolytic activity of adults. Moreover, no significant correlations were observed between all nutritional indices of adults with their proteolytic activity.
Nutritional physiology of *T. castaneum* on barley

**Table 4** Correlation coefficients ($r$) of nutritional indices of *Tribolium castaneum* larvae and adults with their enzymatic activity when fed on various barley cultivars.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Larva</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amylolytic activity</td>
<td>Proteolytic activity</td>
</tr>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>Weight gain</td>
<td>0.191</td>
<td>0.597</td>
</tr>
<tr>
<td>Food consumed</td>
<td>-0.197</td>
<td>0.584</td>
</tr>
<tr>
<td>ECI$^1$</td>
<td>0.532</td>
<td>0.114</td>
</tr>
<tr>
<td>RCR$^2$</td>
<td>-0.294</td>
<td>0.410</td>
</tr>
<tr>
<td>RGR$^3$</td>
<td>0.142</td>
<td>0.696</td>
</tr>
<tr>
<td>GR$^4$</td>
<td>0.189</td>
<td>0.602</td>
</tr>
</tbody>
</table>

$^1$ Efficiency of conversion of ingested food, $^2$ Relative consumption rate, $^3$ Relative growth rate, $^4$ Growth rate.

**Discussion**

Since feeding requirements of the insects can change throughout their development (Barton Browne, 1995), we considered the effect of various barley cultivars not only on the nutritional physiology of *T. castaneum* larva, but also on the adult stage. In agreement with the other works (Naseri and Borzouei, 2016; Naseri *et al.*, 2017), the results of this study showed that the nutritional physiology of *T. castaneum* fourth instar and adult was significantly affected by feeding on various barley cultivars.

The insects prefer host plants that are more suitable for their growth and reproduction (Futuyma and Moreno, 1988; Jaenike, 1990; Via, 1990). Evaluation of nutritional indices can be a criterion to understand an insect’s response to its host plants (Lazarevic and Peric-Mataruga, 2003). Among barley cultivars tested in this study, the lowest weight gain of larvae fed on cultivars Fajr30 and Makuyi demonstrated their low-nutritive values for this pest. The poor ability of larvae to convert food consumed to body matter was an important reason for their lower weight gain and ECI values on cultivars Fajr30and Makuyi than the others. Moreover, the lowest ECI values on these cultivars could be correlated with the kernel hardness and/or lower moisture levels of them. The lowest survival rate of *T. castaneum* larvae was observed when they were fed on cultivars Fajr30 and Makuyi (Rahimi Namin *et al.*, 2018), which is associated with lower quality of these two cultivars.

The highest ECI and RGR values in fourth instar fed on cultivar Lout suggested the high ability of larvae to incorporate the ingested food into growth. The ECI value of the fourth instar on cultivar Lout, in this study, was nearly similar to that reported by Naseri *et al.* (2017) for *T. castaneum* fourth instar on barley (unknown cultivar) (35.33%).

The lowest ECI, RGR and GR values in the adults fed on cultivars Fajr30 and Makuyi showed that these cultivars have lower amount of some nutrients, which affected their growth and increased consumption related with changes in efficiencies (Slansky and Feeny, 1977; Rahimi Namin *et al.*, 2014). In this research, the RGR value of adults on cultivars Fajr30 and Makuyi was nearly 3.5-folds lower than Sagheer *et al.* (2014) reported for *T. castaneum* adults on barley (0.024 mg/mg/day), indicating that the barley cultivar was more suitable than those used in our research. High ECI value in the adults fed on cultivar Lout revealed that they had a high efficiency to convert eaten food to body matter on this cultivar, which can be correlated with their low food consumption (Abisgold and Simpson, 1987; Rahimi Namin *et al.*, 2014; Golizadeh and Abedi, 2017).

In the present research, larvae reared on cultivar Fajr30 had the lightest pupal weight as compared to the others, suggesting that cultivar Fajr30 was unsuitable cultivar for *T. castaneum* larvae. Also, lower value of LGI in the larvae fed on cultivars Fajr30 and Makuyi can be correlated
with a lower survival rate of larvae and longer larval period on these cultivars. Moreover, the lowest SII value on cultivars Fajr30 and Makuyi may be related to lower pupal weight or longer larval period on these cultivars. Obtained results showed that FI was the lowest on cultivars Fajr30 and Makuyi, which can be attributed with a lower percentage of puation or lower pupal weight on these cultivars (Table 3).

This work demonstrated that various barley cultivars significantly affected the amylolytic and proteolytic activities of \textit{T. castaneum} fourth instar and adult. Although low amylolytic activity of the fourth instar was on cultivar Makuyi, the highest amylolytic activity of the adults was on this cultivar. According to Batista-Pereira et al. (2002), the amount of food consumption by an insect depends on the food digestibility. The RCR value and amount of food consumed by larvae on cultivar Makuyi (Table 4) were the main factors, which had a positive correlation with the digestive amylolytic activity (Sivakumar et al., 2006; Naseri and Borzouei, 2016).

The low proteolytic activity level in larvae fed on cultivar Makuyi (Fig. 2A), might be due to the high kernel hardiness (Rahimi Namin et al., 2018) or response of the insect to the ingested protease inhibitors (PIs). Also, high proteolytic activity of adults on cultivar Fajr30 (Fig. 2B) might be correlated with the high protein content (Rahimi Namin et al., 2018) or presence of PIs in this cultivar. The role of PIs on level of enzymatic activity in \textit{T. castaneum} is unknown, and should be investigated in future works.

The larval dietary conditions are vital for the insects whose larval food sources vary from those of adults and the effects of poor larval nutrition cannot be compensated by adult feeding (O’Brien et al., 2004; Plesnar-Bielak et al., 2017). According to Plesnar-Bielak et al. (2017), even if \textit{T. castaneum} larvae and adults live on the same food, larval diet is a main factor affecting adult life history. In the current research, higher ECI and enzymatic activity for \textit{T. castaneum} fourth instar as compared to the adults indicated a better feeding performance of larvae than adult stage. Consequently, controlling of the larvae should be targeted to improve effective management of \textit{T. castaneum} on barley cultivars.

In conclusion, the observations of the present research revealed that cultivars Fajr30 and Makuyi were less nutritive for feeding and growth of \textit{T. castaneum} owing to lower nutritional performance (especially ECI) and larval growth indices. Furthermore, results from digestive enzymes tests showed that amylolytic and proteolytic activities of larval and adults, in most cases, were relatively lowest when they were reared on cultivar Makuyi. Therefore, it is concluded that Makuyi was an unsuitable barley cultivar and can be suggested to be grown and stored in areas where the damage of \textit{T. castaneum} is greater. In the future investigations, responses of \textit{T. castaneum} to the PIs from barley and other cereals should be evaluated for selection of proper PIs, which can be utilized in transgenic expression for the insect pest resistance.

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References


تأثیر ارقام مختلف چو روی فیزیولوژی تغذیه‌ای T. castaneum (Tenebrionidae)

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چکیده: تغذیه T. castaneum (Herbst) (Coleoptera: Tenebrionidae) در حیات تجاری به عنوان آفت و کاهش کود در ادارات ذخیره در پایان اثرات مفیدی ندارد. کاهش شاخص تغذیه، آنزیم تغذیه‌ای، جو و آب مخصوصی T. castaneum در میزان شرایط سالانه سطح ۰±۳ درجه سلسیوس، رطوبت نسبی ۵±۷ درصد و شرایط تازی مطالعه شدند. نتایج نشان داد که از این نسبت افزایش وزن و رشد روزانه ECI و GR با افزایش ارزش غذایی پایین‌تر بود.

واژگان کلیدی: شاخص تغذیه‌ای، آنزیم تغذیه‌ای، T. castaneum