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BARLEY CULTIVARS AFFECTING NUTRITIONAL PERFORMANCE AND DIGESTIVE ENZYMATIC ACTIVITIES OF *EPHESTIA KUEHNIELLA* ZELLER (PYRALIDAE)

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ABSTRACT. The eggs and larvae of the Mediterranean flour moth, *Ephestia kuehniella* Zeller are routinely utilized as a substitute host for the rearing of parasitoids and predators required for biological control. Nutritional performance, digestive enzymatic activities and growth indices of the fifth instar larvae of *E. kuehniella* were evaluated on flour of seven barley (Fajr 30, Reihan 03, 5 Shoor, Dasht, Sahra, Khorram and EH-83-7) and two wheat (Bam and Sepahan) cultivars at 25±1°C, 65±5% R.H., and a photoperiod of 16:8 (L: D) h. The results show that the highest larval growth index was observed when larvae were fed barley cultivar Reihan03. The highest and lowest values of larval weight gain were on barley cultivars EH-83-7 and Sahra, respectively. The highest value of efficiency of conversion of ingested food was in larvae reared on barley cultivar EH-83-7, whereas the lowest value was on barley cultivar Sahra. Moreover, the highest relative growth rate was detected on barley cultivar EH-83-7. The highest and lowest levels of amylolytic activity were on wheat cultivar Bam and barley cultivar Khorram, respectively. The highest proteolytic activity was observed in larvae reared on barley cultivar Fajr 30, whereas the lowest activity was on barley cultivars Dasht, Khorram and wheat cultivar Bam. Finally, barley cultivars EH-83-7 and Reihan 03 can be suggested as the most suitable cultivars for laboratory rearing of *E. kuehniella*.

Additional key words: Mediterranean flour moth, feeding performance, amylolytic activity, proteolytic activity, barley cultivars

In insects, the efficiency of conversion of digested food into body biomass depends on the activity of digestive enzymes in their midgut (Lazarevic et al. 2004). Also, the activity of key digestive enzymes like proteases and α-amylases depends on the nature of the food sources (Slansky 1982, Mendiola-Olaya et al. 2000). It is generally accepted that a change in food quality can significantly change the growth rate of arthropods (Waldbauer 1968). Carbohydrate is one of the important sources of energy for most insects, especially for stored-product pest species. These insects require digestive α-amylase to hydrolyze and utilize the starch in their diet (Valencia-Jiménenz et al. 2008). For example, the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), which is known as one of the major pests of stored-grain products particularly flour or other powdered cereals (Sedlacek et al. 1996, Hill 2002, Rees 2003), needs a carbohydrate source to reach maturity (Chapman 1998). Additionally, it was reported that a diet with flours deprived of protein fractions delays *E. kuehniella* development and increases the pupal mortality (Nawrot et al. 1985). Therefore, like in most insects, balance of nutrients is crucial for growth (Thomas et al. 1999).

In addition to the importance of *E. kuehniella* as a target stored product pest, its eggs and larvae are routinely used as a substitute host for mass rearing of some parasitoids and predators, because of its availability and low-cost of rearing (Iranipour et al. 2009, Jokar & Golmohammadi 2012, Jokar & Zarabi 2012, Sighinolfi et al. 2013).

Hitherto, several researches have been done on the effect of various diets on the biology and digestive enzymatic activity of *E. kuehniella* (Locatelli et al. 2008, Pytelkova et al. 2009, Madboni & Pour Abad 2012, Jafarlu et al. 2012). Abdi et al.(2014) recently considered the nutritional indices and digestive enzymatic activity of *E. kuehniella* on the flour of different wheat, *Triticum aestivum* L., cultivars. Although barley, *Hordeum vulgare* L., may be more susceptible to stored-product insects than other grains (Baker, 1988), and it generally has a low price compared to wheat (Akar et al. 2004), no published information exists on the nutritional and digestive physiology of *E. kuehniella* in response to feeding on the flour of various barley cultivars.

Since the nutritional value of barley cultivars tested in this research can compete with the nutritional value of wheat for *E. kuehniella* (Rees 2003, Akar et al. 2004), two wheat cultivars along with barley cultivars were used to compare the results. This study hypothesized that larvae fed on flour of some barley cultivars will accumulate biomass more efficiently than those fed on the wheat cultivars examined. The objective of this study, therefore, was to evaluate the nutritional performance, digestive enzymatic activity and growth indices of *E. kuehniella* on flour of various barley cultivars and to select the most suitable cultivar for laboratory rearing of *E. kuehniella* in order to optimize its mass rearing as a host for natural enemies released for biological control.

MATERIALS AND METHODS

Barley and wheat cultivars. In this research, seven barley cultivars (Fajr 30, Reihan 03, Shoor, Dasht, Sahra, Khorram and EH-83-7) and two wheat cultivars (Bam and Sepahan) were obtained from the Agricultural and Natural Resources Research Center of Isfahan, Iran (ANRRC). Whole grain of barley and wheat cultivars was milled and used for larval feeding.

Rearing of insects. The strain of *E. kuehniella* was obtained from a laboratory colony of the ANRRC. The larvae of *E. kuehniella* were reared on flour of seven barley and two wheat cultivars in a growth chamber at a temperature of 25 ± 1°C, a relative humidity of 65 ± 5% and a 16:8 h light:dark photoperiod. Nine separate stock cultures were maintained, for two generations, on various barley and wheat cultivars before being used in the experiments.

Growth indices. Larval growth index (LGI), standardized insect-growth index (SII) and fitness index (FI) of *E. kuehniella* were calculated for different tested cultivars using the formulae (Itoyama et al. 1999):

$$LGI = l_x/L$$
$$SII = Pw/L$$
$$FI = (P \times P_w)/(L + P_d)$$

Where, lx = survival rate of larvae, L = larval period, P = percentage of pupation, P_d = pupal period, and P_w = pupal weight.

Larval nutritional performance. To start the experiment, 1 g of newly laid eggs was added to 750 g of barley and wheat flour of each examined cultivar into plastic containers (diameter 20 cm, depth 8 cm) with a hole covered by a mesh net for aeration. Fifth instar larvae were collected from the containers and separated into five replicates (10 larvae in each) and transferred into a plastic Petri-dish (diameter 8 cm, depth 1 cm), containing 1 g of flour of each examined cultivar (Abdi et al. 2014). Nutritional performance was quantified using the fifth instar larvae after 12 h starvation, as they were easier to measure than the earlier instars. The larvae were weighed daily, and the quantity of food consumed was calculated by subtracting the diet remaining at the end of each experiment from the total weight of food given. To obtain the dry weights of the foods and larvae, 100 g of barley and wheat flour of the examined cultivars and 20 larvae reared on each cultivar were weighed, oven-dried (48 h at 60°C) and then re-weighed to establish a percentage of their dry weight. Nutritional performance was calculated based on dry weight, as suggested by Waldbauer (1968) to calculate consumption index (CI), efficiency of conversion of ingested food (ECI), relative consumption rate (RCR) and relative growth rate (RGR):

$$CI = \frac{E}{A}$$

$$ECI = \frac{P}{E}$$

$$RCR = \frac{E}{A*T}$$

$$RGR = \frac{P}{A*T}$$

where A is the mean dry weight of insect over unit time (mg), E is the dry weight of food consumed (mg), P is dry weight gain of insect (mg) and T is duration of feeding period (d).

Chemicals. Digestive enzymes substrate (azocasein and starch), Bradford reagent, the dinitrosalicylic acid (DNS) and maltose were bought from Sigma Chemical Co., St Louis, USA. Bovine serum albumin (BSA) and potassium iodine (KI) were respectively obtained from Roche Co., and Merck Co., Germany, whereas Iodine (I_2) was purchased from Maarssen Co., Netherlands.

Preparation of digestive enzymes. After 12 h starvation, the fifth instar larvae of *E. kuehniella* fed for 24 h on the flours of various barley and wheat cultivars were immobilized on ice for several minutes and quickly dissected under a stereo-microscope. The midguts were cleaned by removal of unwanted tissues, collected into a known volume of distilled water and homogenized with a handheld glass grinder on ice. The homogenates were then centrifuged at 16000 × g for 10 min at 4°C and the resulting supernatants were collected into new micro tubes, stored at -20°C in aliquots for further use.

Protein quantification of larvae. Protein concentrations in *E. kuehniella* fifth instar larvae were determined by Bradford's method (Bradford 1976) using BSA as a standard.

Amylolytic activity assay. Dinitrosalicylic acid (DNS) procedure (Bernfeld 1955), with 1% soluble starch as substrate at the optimal pH (pH 10), was used to assay the digestive amylolytic activity of the fifth instar larvae of *E. kuehniella* fed with various barley and wheat cultivars. Briefly, the enzyme extract (20 µL) was incubated with soluble starch (40 µL) in 10 mM universal buffer (500 µL) at pH 10 and at 37 °C for 30 min. The reaction was stopped by adding 100 µL DNS and heating in boiling water for 10 min. The absorbance was read at 540 nm (spectrophotometer JENWAY 6705 UV/Vis, USA) after cooling on ice. One amylase unit was expressed as the amount of enzyme required to release 1 mg of maltose equivalent per minute under the above conditions. All experiments were carried out in triplicates (with three different supernatants).

Proteolytic activity assay. The general proteolytic activity of *E. kuehniella* fifth instar larvae was determined using the azocasein digestion method. The universal buffer system (50 mM sodium phosphate borate) was used to assay the optimal pH of proteolytic activity (Elpidina et al. 2001). To evaluate the azocaseinolytic activity, the reaction mixture containing 80 µL of 1.5% azocasein solution in 50 mM universal buffer (pH 12 as an optimal pH) and 50 µL of crude enzyme was incubated at 37 °C for 50 min. The enzyme activity was stopped by adding 100 µl 30% trichloroacetic acid (TCA), continued by cooling at 4 °C for 30 min and centrifugation at 16000 × g for 10 min. The supernatant (100 µl) was added to 100 µL of 2 M NaOH and the absorbance was read at 440 nm (Microplate reader anthos 2020, England). Appropriate blanks, which TCA had been added prior to the substrate, were prepared for each treatment. One protease activity unit was defined as an increase in optical density mg^{-1} protein of the tissue min^{-1} due to azocasein proteolysis (Elpidina et al. 2001). All experiments were done in triplicates (with three different supernatants).

Starch and protein determination of flour of barley and wheat cultivars. The starch content of barley and wheat cultivars flour was evaluated by the method of Bernfeld (1955), with some modifications, using starch as standard. A quantity of each cultivar flour (200 mg) was homogenized in distilled water (35 ml),heated to boiling point, and centrifuged at 13000 × g for 10 min. One-hundred microliters of each sample was added to 2.5 ml of iodine reagent (0.02% I_2 and 0.2% KI) and absorbance was read at 580 nm (Bouayad et al. 2008).

The protein content of flour of barley and wheat cultivars was quantified using BSA as a standard according to Bradford (1976) with minor modifications. A quantity of flour of each cultivar (200 mg) was homogenized in distilled water (10 ml), centrifuged at 13000 × g for 10 min, and then 100 µl of the homogenate was added to 3 ml of Bradford reagent. The samples were incubated in darkness at 37°C, and absorbance was read at 595 nm (Bouayad et al. 2008).

Data analysis. The nutritional performance and digestive enzymatic activity of *E. kuehniella* reared on the flours of various barley and wheat cultivars were analyzed using one-way analysis of variance (ANOVA) followed by comparison of the means with LSD test at $\alpha = 0.05$, using statistical software Minitab 16.0. All data were checked for normality prior to analysis.

RESULTS

Growth indices. The results show that the larval growth index of *E. kuehniella* ranged from 1.527 when larvae were fed with the barley cultivar Khorram, to 2.214 when they were fed with the barley cultivar Reihan 03. The standardized insect-growth index (F= 13.40, df= 200, $P<0.01$) and fitness index (F= 25.57, df=

TABLE 1. Nutritional performance of fifth instar larvae *Ephestia kuehniella* on flour of barley and wheat cultivars

	Index (mean ± SE)						
Host (cultivar)	**P[a] (mg)**	**A[b] (mg)**	**FC[c] (mg/larva)**	**CI[d]**	**ECI[e] (%)**	**RCR[f] (mg/mg/day)**	**RGR[g] (mg/mg/day)**
Barley (Fajr)	39.65±7.28bcd	245.90±2.21c	22.168±0.99ab	1.352±0.057bc	12.09±2.39bcd	0.243±0.010b	0.030±0.006bcd
Barley (Reihan)	43.15±7.01abcd	227.98±7.39d	17.49±2.92bc	1.127±0.167bc	18.87± 4.67ab	0.211±0.033bc	0.036±0.006bc
Barley (Shour)	55.35±0.88ab	247.92±0.36c	21.12±1.03b	1.305±0.086bc	15.14±2.61abc	0.242± 0.017b	0.043±0.001ab
Barley (Dasht)	21.67±7.04cde	185.77±2.68e	26.48±2.16a	2.133±0.156a	6.06± 2.24de	0.438±0.060a	0.022±0.006cd
Barley (Sahra)	4.91±0.34e	188.66±4.10e	17.51±0.97bc	1.395±0.083b	1.93± 0.06e	0.231±0.009b	0.004±0.000e
Barley (Khorram)	19.00±4.25de	182.68±3.63e	15.96±1.92c	1.324±0.184bc	8.03± 1.70cde	0.217±0.032bc	0.017±0.004de
Barley (EH-83-7)	66.60±6.30a	237.41±4.55cd	20.38±1.53bc	1.284±0.080bc	21.79±0.99a	0.250±0.018b	0.055±0.005a
Wheat (Bam)	44.30±14.80abc	404.93±6.94a	20.35±1.16bc	0.757±0.051d	13.88±4.40abcd	0.135±0.011c	0.021±0.007cd
Wheat (Sepahan)	26.15±8.52cde	273.56±6.66b	19.47±1.32bc	1.076±0.093c	8.56±2.23cde	0.190±0.020bc	0.018±0.007de

Means followed by different letters in the same column are significantly different (LSD, $P<0.01$).

[a] Dry weight gain of insect
[b] Mean dry weight of insect over unit time
[c] Dry weight of food consumed
[d] Consumption index
[e] Efficiency of conversion of ingested food
[f] Relative consumption rate
[g] Relative growth rate

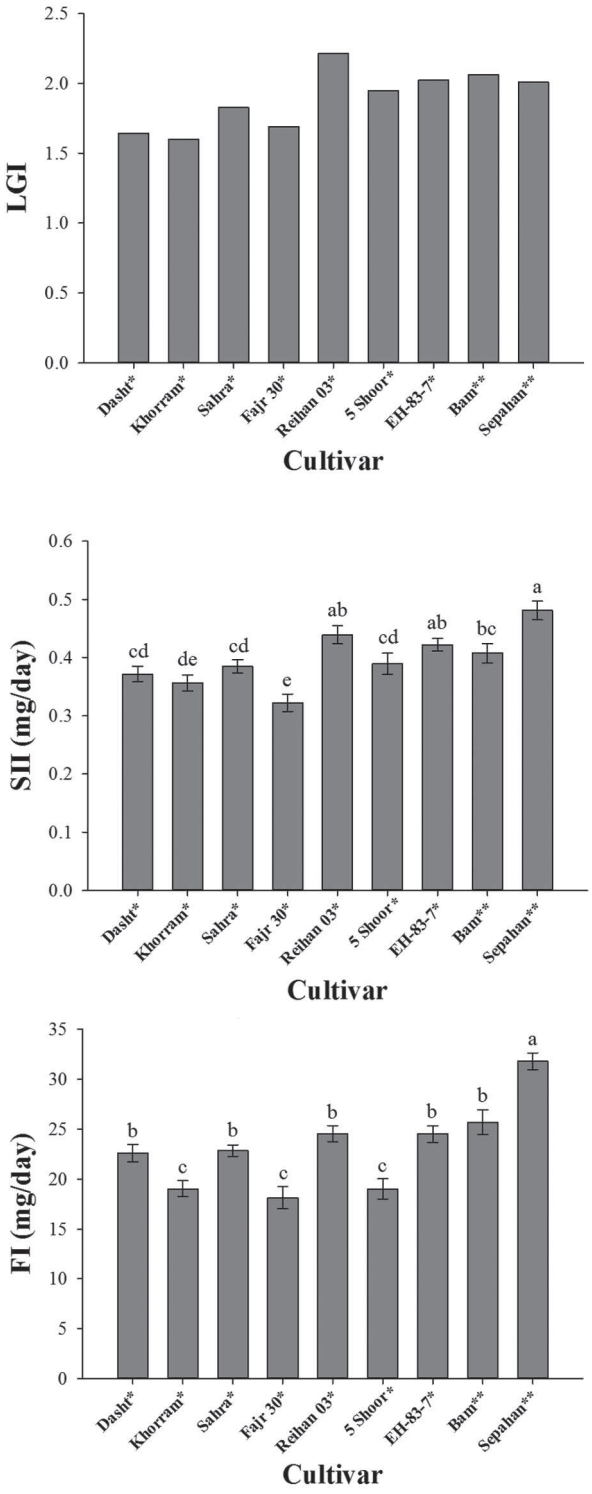

FIG. 1. Mean (±SE) larval growth index (LGI), standardized-insect growth index (SII) and fitness index (FI) of *Ephestia kuehniella* on different barley and wheat cultivars. Means followed by different letters are significantly different (LSD, P < 0.01). *Barley cultivars; **Wheat cultivars

142, $P<0.01$) of *E. kuehniella* showed significant difference, being highest on wheat cultivar Sepahan (Fig. 1).

Larval nutritional performance. The results of the nutritional performance of the fifth instar larvae are shown in Table 1. Significant differences were found for larval weight gain (F= 4.80; df = 8, 36; $P<0.01$). The highest and lowest values of larval weight gain were on the barley cultivars EH-83-7 (66.60 ± 6.30 mg) and Sahra (4.91 ± 0.34 mg). Significant differences in mean larval weight were found (F= 174.94; df = 8, 37; $P<0.01$). Mean larval weight was heaviest on wheat cultivar Bam (404.93 ± 6.94 mg) and lightest on barley cultivars Khorram (182.68 ± 3.63 mg), Dasht (185.77 ± 2.68 mg) and Sahra (188.66 ± 4.10 mg). The highest food consumption (F= 3.00; df = 8, 38; $P<0.01$) was detected in larvae fed on the barley cultivar Dasht (26.48 ± 2.16 mg/larva), while the lowest was recorded for the larvae reared on the barley cultivar Khorram (15.96 ± 1.92 mg/larva) (Table 1).

The fifth instar larvae reared on barley cultivar Dasht (2.133 ± 0.156) and wheat cultivar Bam (0.757 ± 0.051) show the highest and lowest values of consumption index (F=9.79; df = 8, 38; $P<0.01$), respectively. The highest value of efficiency of conversion of ingested food (F= 4.09; df = 8, 37; $P<0.01$) was in larvae reared on barley cultivar EH-83-7 (21.79 ± 0.99%), whereas the lowest value was on barley cultivar Sahra (1.93± 0.06%). Moreover, the larvae fed on the barley cultivar Dasht (0.438 ± 0.060 mg/mg/day) and wheat cultivar Bam (0.135 ± 0.011 mg/mg/day) had the highest and lowest values of relative consumption rate (F= 8.57; df = 8, 38; $P<0.01$). The highest value for relative growth rate (F= 6.20; df = 8, 36; $P<0.01$) was recorded for the fifth instar larvae reared on barley cultivar EH-83-7 (0.055 ± 0.005 mg/mg/day), while the lowest value was for those fed on the barley cultivar Sahra (0.004 ± 0.000 mg/mg/day) (Table 1).

Amylolytic activity. Significant differences in the digestive amylolytic activity for the fifth instar larvae reared on flour of various barley and wheat cultivars were found (F =4.46; df = 8, 18; $P<0.01$) (Table 2). Larvae fed on the wheat cultivar Bam (0.0262 ± 0.0016 mU mg^{-1}) showed the highest levels of amylolytic activity, while the lowest activity was in larvae fed on the barley cultivar Khorram (0.0146 ± 0.0014 mU mg^{-1}).

General proteolytic activity. General proteolytic activity data ($P<0.01$) from *E. kuehniella* fifth instar larvae reared on flour of various barley and wheat cultivars are shown in Table 2. The highest proteolytic activity was for the larvae reared on the barley cultivar Fajr 30 (F = 5.10; df = 8, 18; $P<0.01$) (3.256 ± 0.250 U mg^{-1}), whereas the lowest activity was recorded for larvae

fed on the barley cultivars Dasht (2.301 ± 0.095 U mg^{-1}) and Khorram (2.249 ± 0.210 U mg^{-1}) as well as wheat cultivar Bam (2.253 ± 0.152 U mg^{-1}).

Starch and protein determination of flour of barley and wheat cultivars. Statistical tests indicated significant differences in the content of starch and protein among the flours of the various barley and wheat cultivars tested ($P<0.01$) (Table 3). The highest starch content was found in the flour of the wheat cultivar Sepahan (F = 78.71; df = 8, 18; $P<0.01$) (11.375 ± 0.240 mg mL^{-1}), while the lowest content was in the flour of the barley cultivars EH-83-7 (4.287 ± 0.324 mg mL^{-1}), Khorram (4.456 ± 0.213 mg ml^{-1}) and Reihan 03 (4.471 ± 0.153 mg mL^{-1}). The highest content of protein was in the wheat cultivar Sepahan (F =9.20; df = 8, 18; $P<0.01$) (0.0343 ± 0.0032 mg ml^{-1}) and barley cultivar Dasht (0.0341 ± 0.0044 mg ml^{-1}), however, the lowest content was in the barley cultivar Fajr 30 (0.0085 ± 0.0011 mg ml^{-1}).

Discussion

This study shows that different barley cultivars and two wheat cultivars had a significant effect not only on the nutritional indices of *E. kuehniella*, but also on the enzymatic activities, as well as growth indices of this insect.

Although the highest standardized insect-growth index was observed when larvae were fed wheat cultivar Sepahan, however, no significant difference was detected between this cultivar and the barley cultivars Reihan 03 and EH-83-7, suggesting that Sepahan, Reihan 03 and EH-83-7 are suitable nutritious cultivars for the feeding and growth of *E. kuehniella*. Also, the results indicate that the highest larval growth index was on cultivar Reihan 03, showing that the larvae fed on this cultivar had a higher survivorship compared to larvae reared on other cultivars tested.

The significant differences in nutritional performance of the fifth instar larvae of *E. kuehniella* on the flour of barley and wheat cultivars indicate that the cultivars tested had different nutritional values. The results show that the highest and lowest values of larval weight gain were on the barley cultivars EH-83-7 and Sahra, respectively, suggesting that these cultivars are a high and low-nutritious diet for this insect (Che Salmah 2010). The highest larval weight gain on barley cultivar EH-83-7 is higher than that reported by Abdi et al. (2014) for *E. kuehniella* on the wheat cultivar Pishtaz (a suitable host) (4.77 ± 0.69 mg). The inconsistency can be due to differences in the calculation method, host cultivar or variations in the strains of *E. kuehniella*. The highest mean larval weight was in the larvae fed on the wheat cultivar Bam, while the lowest CI was detected on this cultivar, indicating that cultivar Bam can be a proper diet for *E. kuehniella* larvae.

In insects, the amount of food consumed (FC) is one of the main characteristics that can influence the enzymatic activity, responsible for supplying energy (Sivakumar et al. 2006). It is noticeable that the ingested nutrients must meet requirements for growth and other metabolic processes. In this study, the larvae reared on barley cultivar Khorram recorded the lowest FC value, showing that the larvae fed on this cultivar had low weight gain and digestive enzymatic activity. Moreover, the highest rate of food consumed by the larvae of *E. kuehniella* was on the barley cultivar Dasht, which may be correlated with relatively low soluble starch content in this cultivar. In parallel of food consumption, the highest consumption index and relative consumption rate by larvae was recorded on the barley cultivar Dasht.

Table 2. Mean (± SE) amylolytic and proteolytic activities of midgut extracts from fifth instar larvae of *Ephestia kuehniella* on flour of barley and wheat cultivars

Host (cultivar)	Amylolytic activity (mU mg^{-1})	Proteolytic activity (U mg^{-1})
Barley (Fajr)	0.0156±0.0018ef	3.256 ± 0.250a
Barley (Reihan)	0.0206±0.0023bcde	2.568 ± 0.189cd
Barley (Shour)	0.0162±0.0018def	2.931 ± 0.187abc
Barley (Dasht)	0.0226±0.0003ab	2.301 ± 0.095d
Barley (Sahra)	0.0216±0.0011abcd	2.659± 0.073cd
Barley (Khorram)	0.0146±0.0014f	2.249 ± 0.210d
Barley (EH-83-7)	0.0218± 0.0012abc	2.738 ± 0.108bcd
Wheat (Bam)	0.0262±0.0016a	2.253 ± 0.152d
Wheat (Sepahan)	0.0168±0.0035cdef	3.225 ± 0.206ab

Means followed by different letters in the same column are significantly different (LSD,P <0.01)

TABLE 3. Mean (± SE) starch and protein contents of flour of barley and wheat cultivars used for *Ephestia kuehniella* feeding

Host (cultivar)	Starch content (mg mL^{-1})	Protein content (mg mL^{-1})
Barley (Fajr)	7.755±0.699b	0.0085 ± 0.0011c
Barley (Reihan)	4.471±0.153d	0.02219 ± 0.0007b
Barley (Shour)	7.111±0.174b	0.0201 ± 0.0022b
Barley (Dasht)	5.820±0.274c	0.0341 ± 0.0044a
Barley (Sahra)	1.954±0.104e	0.0192± 0.0011b
Barley (Khorram)	4.456±0.213d	0.0223 ± 0.0043b
Barley (EH-83-7)	4.287± 0.324d	0.0189 ± 0.0004b
Wheat (Bam)	7.241±0.058b	0.0190 ± 0.0024b
Wheat (Sepahan)	11.375±0.240a	0.0343 ± 0.0032a

Means followed by different letters in the same column are significantly different (LSD, $P < 0.01$).

Insects consume less of a special diet simply because they are able to transfer it more efficiently into body growth. Furthermore, when the larvae consume less, the diet will tend to pass through their gut slowly, and it can be efficiently converted into their body biomass (Soo Hoo & Fraenkel 1966).

It is useful to note that, among the nutritional indices, ECI is a feeding index that can be different due to variations in food digestibility and the proportion of digestible food converted to insect body matter and metabolized to obtain energy (Abdel-Rahman & Al-Mozini 2007). Moreover, this index indicates an insect's ability to incorporate food into growth (Nathan et al. 2005). The ECI value increased when larvae was fed flour of the barley cultivar EH-83-7, suggesting that it was more efficient at the conversion of ingested food to biomass, evident as weight gain by the larvae (Koul et al. 2003). Also, the larvae fed with the wheat cultivar Bam had the lowest RCR value, most likely because of appropriate nutrient content.

With regards to the results of this study, the lowest RGR was recorded on the barley cultivar Sahra, which may be because of a decrease in ECI. Also, the highest RGR was recorded on the barley cultivar EH-83-7, indicating its high quality and suitability as a diet for the larvae of the Mediterranean flour moth. According to the obtained results, the flour of barley and wheat cultivars considerably influenced the digestive enzymatic activity of the *E. kuehniella* fifth instar larvae. Since the variations of starch content in the barley and wheat cultivars may lead to differences in the amylolytic activity (Lwalaba et al. 2010) of the Mediterranean flour moth, the highest amylolytic activity was detected in the larvae reared on the wheat cultivar Bam, which is attributed to the high starch content of this cultivar. In addition, the larvae fed with the barley cultivar Khorram had the lowest level of amylolytic activity, which was approximately 2-fold lower than the wheat cultivar Bam. It can be concluded that the amylolytic activity of this insect on the above-mentioned cultivars was directly proportional to the starch content. The amylolytic activity of the Mediterranean flour moth on flour of the wheat cultivar Bam is lower than the amylolytic activity reported by Abdi et al. (2014) for *E. kuehniella* on the wheat cultivar Bam (0.90 ± 0.13 mU mg-1). According to the study of Abdi et al. (2014), *E. kuehniella* whole extract body (instead of midgut extract) was used to assess the digestive enzymatic activity, and the possible reason for this discrepancy can be due to variations in experimental methods.

The fifth instar larvae of *E. kuehniella* fed on the barley cultivar Fajr 30 showed the highest proteolytic activity, while the protein content was lowest in this cultivar. Previously, it was reported that the insects release less of the enzymes for nutrients present in excess, while maintaining or increasing levels of enzymes for nutrients in shortage (Kotkar et al. 2009, Lwalaba et al. 2010). Also, because protein ingestion takes place totally during the larval stages (Sorge et al. 2000), thus, the larvae can allow no dietary protein to pass undigested through the gut.

The highest starch content was detected in the flour of the wheat cultivar Sepahan, while the larvae fed on this cultivar showed low level of amylolytic activity, which can be correlated to the presence of some amylase inhibitors. The soluble protein evaluations of the flour of barley and wheat cultivars suggest that the wheat cultivar Sepahan and barley cultivar Dasht had

the highest protein content. Although the highest protein content was detected in these cultivars, the larvae fed with them had low weight gain and ECI value, indicating the unsuitability of these diets for *E. kuehniella*. Moreover, since low dietary protein can cause an increase in consumption rate (Slansky 1993), this study observed a high food consumption by the fifth instar larvae fed with the barely cultivar Fajr 30.

In conclusion, the highest larval weight gain, efficiency of conversion of ingested food and relative growth rate as well as more regulated amylolytic activity were obtained when larvae were fed with the barley cultivar EH-83-7. Also, standardized insect-growth index and larval growth index were higher when larvae were fed with the cultivar Reihan 03, therefore, it can be suggested that EH-83-7 and Reihan 03 are the most suitable cultivars for the laboratory rearing of *E. kuehniella*.

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