

Nutritional indices, and proteolytic and digestive amylolytic activities of *Ephestia kuehniella* (Lep.: Pyralidae): response to flour of nine wheat cultivars

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Abstract

The eggs and larvae of the Mediterranean flour moth, *Ephestia kuehniella* (Zell.) are widely used to rear parasitoids and predators for biological control programs. In this study, nutritional responses, proteolytic and digestive amylolytic activities of the fifth instar larvae as well as some biological parameters of *E. kuehniella* were studied on flour of nine wheat cultivars under laboratory conditions. The longest larval period of the fifth instar was on the cultivars Back cross Roshan and Sepahan and the shortest was on cultivar Tajan. The highest daily and total fecundity was observed on N-86-7. The fifth larval instar reared on Pishtaz showed the highest efficiency of conversion of ingested food. The highest general proteolytic activity was determined on the cultivar N-86-7 and the lowest was on N-80-19. Maximum and minimum digestive amylolytic activity was determined on the cultivars Kuhdasht and Khoshki line 9, respectively. According to the results obtained, Pishtaz and N-86-7 were the most suitable cultivars for laboratory rearing of *E. kuehniella* as the alternative host to use in the mass production of natural enemies.

Key words: *Ephestia kuehniella*, nutritional responses, proteolytic activity, amylolytic activity, wheat cultivars

چکیده

شاخص‌های تغذیه‌ای و فعالیت پروتئولیتیک و آمیلولیتیک گوارشی *Ephestia kuehniella* (Lep.: Pyralidae) واکنش به آرد نه رقم گندم

عارفه عبدی، بهرام ناصری و سید علی اصغر فتحی

نخ‌ها و لاروهای شب‌پره مدیترانه‌ای آرد، *Ephestia kuehniella* (Zell.) به‌طور وسیعی در برنامه‌های کنترل بیولوژیک به‌منظور پرورش پارازیتوئیدها و شکارگرها مورد استفاده قرار می‌گیرند. در این تحقیق، واکنش‌های تغذیه‌ای، فعالیت پروتئولیتیک و آمیلولیتیک گوارشی لاروهای سن پنجم همراه با برخی پارامترهای زیستی *E. kuehniella* روی آرد نه رقم گندم تحت شرایط آزمایشگاهی بررسی شدند. طولانی‌ترین دوره‌ی لاروی سن پنجم روی ارقام Back cross Roshan و Sepahan و کوتاه‌ترین آن روی رقم Tajan بود. بیش‌ترین باروری روزانه و کل روی رقم N-86-7 مشاهده شد. لاروهای سن پنجم پرورش‌یافته روی رقم Pishtaz بیش‌ترین کارایی تبدیل غذای خورده‌شده را دارا بودند. بیش‌ترین فعالیت پروتئولیتیک کل روی رقم N-86-7 و کم‌ترین آن روی رقم N-80-19 بود. همچنین، بیش‌ترین و کم‌ترین فعالیت آمیلولیتیک گوارشی به‌ترتیب روی ارقام Kuhdasht و Khoshki line 9 بود. براساس نتایج به‌دست آمده، Pishtaz و N-86-7 ارقام مناسبی برای پرورش آزمایشگاهی *E. kuehniella* به‌عنوان میزبان جایگزین برای استفاده در تولید انبوه دشمنان طبیعی بودند.

واژگان کلیدی: *Ephestia kuehniella*، واکنش‌های تغذیه‌ای، فعالیت پروتئولیتیک، فعالیت آمیلولیتیک، ارقام گندم

Introduction

The Mediterranean flour moth, *Ephestia kuehniella* (Zell.), is one of the major pests of stored products, particularly flour (Sedlacek *et al.*, 1996; Hill, 2002; Rees, 2003), and is likely to be found in any mills or warehouses in which flour or other processed cereal products are stored (Sedlacek *et al.*, 1996). Although *E. kuehniella* is known as a destructive pest, the eggs and larvae of this species are widely utilized as an alternative host for the mass rearing of several parasitoids including *Habrobracon hebetor* (Say) (Hym.: Braconidae) (Shonouda & Nasr, 1998) and *Trichogramma ostrinia* (Peng & Chen) (Hym.: Trichogrammatidae) (Hoffmann *et al.*, 2001), and

predators including *Adalia bipunctata* (L.) (Col.: Coccinellidae) (Specty *et al.*, 2003), *Harmonia axyridis* Pallas (Col.: Coccinellidae) (De Clercq *et al.*, 2005), *Orius albidipennis* Reuter (Hem.: Anthracoridae) (Gonzalez-Zamora *et al.*, 2007), *Franklinothrips orizabensis* Johansen (Thys.: Aeolothripidae) (Hoddle *et al.*, 2001) and *Chrysoperla carnea* (Steph.) (Neu.: Chrysopidae) (Jokar & Golmohammadi, 2012; Jokar & Zarabi, 2012).

It is noticeable that species of *Trichogramma* Westwood are widely used in biological control against insect pests with over 15 million ha of agricultural crops being annually treated (van Lenteren, 2000; Wang *et al.*, 2014). The large numbers of

Trichogramma, from parasitized eggs of stored product moths, are produced by commercial facilities (Bernardi *et al.*, 2000). Furthermore, it would be useful to store large numbers of adult host to achieve the acceptable number of host eggs when planning parasitoid releases. Also, the nutritional quality of host eggs and the subsequent survival of *Trichogramma* and other egg parasitoids depend on the diet of host (Hunter, 2003).

Several researchers have determined the effects of temperature and humidity (Siddiqui & Barlow, 1973), as well as the type of diet (Locatelli & Limonta, 1998) on the development of the Mediterranean flour moth. Locatelli *et al.* (2008) studied the effect of particle size of soft wheat flour on the development of *E. kuehniella*. Also, Pytelkova *et al.* (2009) investigated the molecular adaptation of amylolytic enzymes of *E. kuehniella* to alkaline environment. Recently, the midgut amylolytic activity of the Mediterranean flour moth was evaluated by Jafarlu *et al.* (2012), who reported the highest amylolytic activity in the 5th instar female larvae. Madboni & Pourabad (2012) considered the effect of different wheat cultivars on some of the developmental parameters of this pest, and showed that the fecundity of adults increased with increasing amounts of dietary protein.

Since wheat cultivars have different suitability as host to stored product insects (Amos *et al.*, 1986; Sinha *et al.*, 1988; McGaughey *et al.*, 1990; Cortez-Rocha *et al.*, 1993), and there is no published research article about nutritional responses, enzymatic activity and biological parameters of the Mediterranean flour moth on different wheat cultivars, therefore the present study was conducted to consider the proteolytic and digestive amylolytic activity, nutritional indices and life history aspects of *E. kuehniella* on the flour of different wheat cultivars. Due to the probable differences in nutritional values of wheat cultivars tested in this study and their effect on the enzymatic activity of *E. kuehniella* larvae, it was hypothesized that the larvae will accumulate biomass more efficiently when reared on some wheat cultivars than the others. It is further hypothesized that the adults who emerge from the larvae fed on the high-

nutritious cultivar will have high reproductive activity patterns. It is expected that the obtained results will be useful to introduce suitable wheat cultivar for the successful laboratory and mass rearing of *E. kuehniella* for its natural enemies.

Materials and methods

Wheat cultivars and insect rearing

Five wheat cultivars including Bam, Pishtaz, Sepahan, Khoshki line 9 and Back cross Roshan were obtained from Agricultural and Natural Resources Research Center of Isfahan, Iran. Four wheat cultivars including Tajan, Kuhdasht, N-86-7 and N-80-19 were obtained from Agricultural and Natural Resources Center, Moghan, Ardabil, Iran. Before starting the experiments, all wheat cultivars were milled and used with bran. The experiments were conducted during the morning to afternoon from July to December 2012.

The rearing stock of *E. kuehniella* was obtained from laboratory colony from Agricultural and Natural Resources Research Center of Isfahan, Iran. The larvae of *E. kuehniella* were reared on flour of nine wheat cultivars under a growth chamber at a temperature of 25 ± 1 °C, a relative humidity of $65 \pm 5\%$ and a 16: 8 h light: dark photoperiod. The insects examined on different wheat cultivars had already been reared for two generations on the same cultivars.

Larval development and growth indices

Duration of last instar larvae, pupal period, their mortality, and the weights of larvae and pupae were recorded. Larval growth index (*LGI*) and standardized insect-growth index (*SII*) of *E. kuehniella* were calculated on different wheat cultivars using the following formulae (Itoyama *et al.*, 1999): $LGI = l_x/L$ and $SII = P_w/L$; where, l_x = survival rate of larvae, L = larval period and P_w = pupal weight.

Oviposition period, fecundity and longevity

After emergence of the adults, a pair of male and female moths (50 replicates for each cultivar) were transferred into egg-laying plastic cylinder (diameter 8

cm, depth 9 cm) covered by mesh net for aeration. The egg-laying containers were inversely placed on the paper sheets (as an oviposition surface), and the eggs were daily collected and counted. In the current research, the adults (males and females) were given neither food nor water (Xu *et al.*, 2008). Oviposition period, daily fecundity (eggs / reproduction day), total fecundity (eggs during the reproductive period) and the longevity of adults were recorded until the death of the last adult.

Nutritional indices

To start the experiment, 1 g of newly laid eggs was added in 750 g of 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 plastic plate (diameter 8 cm, depth 1 cm), containing 1 g of flour of each examined cultivar. Nutritional indices were simply quantified using one-day-old fifth instar larvae as they were more measurable than the primary instars. The larvae were daily weighed, and the quantity of food ingested was daily calculated (by subtracting the diet remaining at the end of each experiment from the total weight of food given). To find the dry weights of the food and larvae, 100 g of wheat flour of the examined cultivars and 20 larvae that were reared on each cultivar were weighed, oven-dried (48 hours at 60 °C) and then reweighed to establish the percentage of their dry weight. The pupae were also weighed 24 hours after pupation.

Nutritional indices were calculated, based on dry weight, via formulae described by Waldbauer (1968): $CI = E / A$; $ECI = P / E$; $RCR = E / (A \times T)$; and $RGR = P / (A \times T)$; where, CI = consumption index, ECI = efficiency of conversion of ingested food, RCR = relative consumption rate, RGR = relative growth rate, A = mean dry weight of insect over unit time, E = dry weight of food consumed, P = dry weight gain of insect and T = duration of feeding period.

Chemicals

The general proteolytic substrate azocasein, Bradford reagent, the dinitrosalicylic acid (DNS), maltose and the amylolytic substrate starch were obtained from Sigma Chemical Co., St Louis, USA. Bovine serum albumin (BSA) and potassium iodine (KI) were purchased from Roche Co., and Merck Co., Germany, respectively. Iodine (I₂) was obtained from Maarsse Co., Netherlands.

Preparation of digestive enzymes

The rearing methods of larvae were similar to those mentioned in "insect rearing" section. After 12 h starvation, the whole body of the fifth instar larvae of *E. kuehniella*, which were reared for 24 h on flour of different wheat cultivars, were collected into a known volume of distilled water (after cold-immobilizing), 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 in new micro tubes, stored at -20 °C in aliquots for further use.

Protein quantification

Protein concentrations in the fifth instar larvae of *E. kuehniella* were determined using bovine serum albumin (BSA) as standard according to the method of Bradford (1976).

Proteolytic activity assay

General proteolytic activity of one-day-old fifth instar larvae of *E. kuehniella* fed on different wheat cultivars was determined using azocasein substrate at the optimal pH. 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) and 50 µL of crude enzyme was incubated at 37 °C for 50 min. The reaction was terminated by the addition of 100 µL 30% trichloroacetic acid (TCA), continued by cooling

at 4 °C for 30 min and centrifugation at $16000 \times g$ for 10 min. The supernatant (100 μ L) was added to 100 μ L of 2 M NaOH and the absorbance was read at 440 nm. Appropriate blanks, which TCA had been added prior to the substrate, were prepared for each treatment. One unit of protease activity was defined as an increase in optical density per milligram protein of the tissue min^{-1} due to azocasein proteolysis (Elpidina *et al.*, 2001). All experiments were carried out in triplicates.

Amylolytic activity assay

Dinitrosalicylic acid (DNS) method (Bernfeld, 1955), with 1% soluble starch as substrate at the optimal pH was used to assay digestive amylolytic activity of the fifth instar larvae of *E. kuehniella* fed on different wheat cultivars. A quantity of 20 μ L of the enzyme extract was incubated with 500 μ L of universal buffer (pH 10) and 40 μ L of soluble starch for 30 min at 37 °C. The reaction was stopped by adding 100 μ L DNS and heating in boiling water for 10 min. The absorbance was read at 540 nm after cooling on ice. All experiments were carried out in triplicates.

Protein and starch determination of flour

Protein content of flour of wheat cultivars was quantified using BSA as standard according to Bradford (1976). A quantity of 200 mg of flour of each wheat cultivar was homogenized in 10 mL of distilled water, and then 100 μ L of the homogenate was added to 3 mL of Bradford reagent. The samples were incubated in darkness at 37 °C for 60 min, and absorbance was read at 595 nm (Bouayad *et al.*, 2008). Starch content of flour of wheat cultivars was determined by the method of Bernfeld (1955) using starch as standard. A quantity of 200 mg of flour of each wheat cultivar was homogenized in 35 mL of distilled water and heated to boiling point. One hundred microliters of each sample was added into 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).

Data analysis

Nutritional indices, enzymatic activity, and some biological parameters of *E. kuehniella* reared on flour of different wheat cultivars were analyzed using one-way ANOVA followed by comparison of the means with LSD test at $\alpha = 0.05$ using statistical software Minitab 16.0. All data were tested for normality before analysis. A dendrogram of nutritional indices, enzymatic activities and biological parameters of *E. kuehniella* on flour of different wheat cultivars was created after cluster analysis by Ward's method using SPSS 16.0 statistical software.

Results

Larval development and growth indices

The results showed that the larval period of the fifth instar was significantly different on nine wheat cultivars ($F = 41.31$; $df = 8, 36$; $P < 0.01$), the longest being on Back cross Roshan (12.00 ± 0.32 days) and Sepahan (12.00 ± 0.45 days) and the shortest on Tajan (5.60 ± 0.40 days). The heaviest and the lightest larval weights ($F = 5.63$; $df = 8, 36$; $P < 0.01$) were observed on Sepahan (19.22 ± 0.93 mg) and N-86-7 (14.05 ± 0.89 mg), respectively. The longest pupal period ($F = 24.84$; $df = 8, 18$; $P < 0.01$) was on Khoshki line 9 and Back cross Roshan (16.33 ± 0.33 days) and the shortest on N-80-19 (7.00 ± 0.58 days). The heaviest and the lightest pupal weights ($F = 61.07$; $df = 8, 36$; $P < 0.01$) were detected in the larvae fed on N-86-7 (88.40 ± 4.04 mg) and Bam (12.82 ± 0.16 mg), respectively. The results also showed that the highest larval growth index (LGI) was on cultivars Tajan, Kuhdasht and N-80-19 (16.66) and the lowest was on Sepahan (5.16). The standardized insect-growth index (SII) of *E. kuehniella* showed significant difference ($F = 185$; $df = 8, 36$; $P < 0.01$) among the wheat cultivars, the highest being on N-86-7 (25.82 ± 0.96 mg/day) and the lowest on Bam (1.19 ± 0.03 mg/day) (table 1).

Oviposition period, fecundity and longevity

Wheat cultivars tested showed significant effects on pre-oviposition period ($F = 6.35$; $df = 8, 126$; $P <$

0.01) of *E. kuehniella*, with the longest on Sepahan (2.80 ± 0.20 days) and the shortest on N-80-19 (1.33 ± 0.13 days). The longest oviposition period ($F = 11.08$; $df = 8, 126$; $P < 0.01$) on flour of different wheat cultivars was on Kuhdasht (5.93 ± 0.32 days), and the shortest on Pishtaz (3.73 ± 0.18 days). There was a significant difference in the effect of different cultivars of wheat as larval food on the number of eggs laid per reproduction day ($F = 8.65$; $df = 8, 126$; $P < 0.01$). The highest number of eggs laid per reproduction day was on N-86-7 (37.05 ± 2.50 eggs), and the lowest one was on N-80-19 (14.30 ± 0.93 eggs). There was also, a significant difference in the total number of eggs laid per individual of *E. kuehniella* on nine wheat cultivars ($F = 11.18$; $df = 8, 126$; $P < 0.01$), with the highest on N-86-7 (126.80 ± 9.47 eggs) and the lowest on N-80-19 (47.67 ± 2.86 eggs) (table 2). Differences between the wheat cultivars showed no significant effect on the longevity of male *E. kuehniella*, but the longest female longevity ($F = 12.86$; $df = 8, 126$; $P < 0.01$) was on

Khoshki line 9 (9.60 ± 0.31 days) and the shortest on Bam (5.73 ± 0.23 days) (table 2).

Nutritional indices

The results of the nutritional responses of the fifth instar *E. kuehniella* larvae are shown in table 3. The highest food consumption (FC) ($F = 15.40$; $df = 8, 36$; $P < 0.01$) was observed in the larvae fed on N-80-19 (72.91 ± 6.30 mg/larva) and the lowest on Pishtaz (8.30 ± 1.55 mg/larva). The highest and the lowest values of larval weight gain ($F = 3.02$; $df = 8, 36$; $P < 0.05$) were observed on N-80-19 (6.52 ± 1.08 mg) and Back cross Roshan (1.61 ± 0.37 mg). Nutritional indices of the fifth instar larvae of *E. kuehniella* were significantly different on flour of wheat cultivars. The larvae reared on cultivars N-80-19 (9.83 ± 2.14) and Pishtaz (0.48 ± 0.08) showed the highest and the lowest values of *CI* ($F = 13.30$; $df = 8, 36$; $P < 0.01$). The larvae reared on Pishtaz cultivar showed the highest value of *ECI* ($F = 9.77$; $df = 8, 36$; $P < 0.01$) ($42.89 \pm 9.19\%$), while the

Table 1. Duration of immature stages, larval and pupal weight and growth indices of *Ephestia kuehniella* on the flour of different wheat cultivars.

Wheat cultivar	Index (mean \pm SE)					
	Fifth instar larval period (day)	Larval weight (mg)	Pupal period (day)	Pupal weight (mg)	LGI	SII (mg/day)
Bam	10.80 ± 0.37 ab	16.54 ± 0.60 cd	7.33 ± 0.33 c	12.82 ± 0.16 e	8.80	1.19 ± 0.03 e
Pishtaz	10.60 ± 0.51 b	16.76 ± 0.46 cd	11.00 ± 0.58 b	26.47 ± 1.78 d	6.54	2.50 ± 0.19 de
Sepahan	12.00 ± 0.45 a	19.22 ± 0.93 a	14.33 ± 0.33 a	41.66 ± 1.68 c	5.16	3.57 ± 0.10 cd
Khoshki line 9	11.60 ± 0.25 ab	17.08 ± 0.31 bcd	16.33 ± 0.33 a	42.30 ± 1.38 c	5.33	3.52 ± 0.11 cd
Back cross Roshan	12.00 ± 0.32 a	18.96 ± 0.40 ab	16.33 ± 0.33 a	45.77 ± 2.35 c	5.83	3.80 ± 0.20 cd
Tajan	5.60 ± 0.51 d	15.33 ± 0.76 de	12.00 ± 1.15 b	26.72 ± 1.78 d	16.66	4.91 ± 0.55 c
Kuhdasht	6.00 ± 0.32 d	17.31 ± 0.74 abc	12.00 ± 0.58 b	61.36 ± 3.87 b	16.66	10.22 ± 0.65 b
N-86-7	7.40 ± 0.51 c	14.05 ± 0.89 e	12.00 ± 1.15 b	88.40 ± 4.04 a	10.00	25.82 ± 0.96 a
N-80-19	6.40 ± 0.51 cd	17.65 ± 0.78 abc	7.00 ± 0.58 c	60.50 ± 5.30 b	16.66	9.55 ± 1.04 b

The means followed by different letters in the same columns are significantly different (LSD, $P < 0.01$). LGI = larval growth index, SII = standardized insect-growth index.

Table 2. Oviposition period, fecundity and longevity of *Ephestia kuehniella* on the flour of different wheat cultivars.

Wheat cultivar	Index (mean \pm SE)					
	Pre-oviposition period (day)	Oviposition period (day)	Daily fecundity	Total fecundity	Male longevity (day)	Female longevity (day)
Bam	1.73 ± 0.18 cd	5.73 ± 0.23 ab	18.47 ± 1.64 de	49.67 ± 4.25 ef	8.27 ± 0.15 a	5.73 ± 0.23 d
Pishtaz	1.67 ± 0.23 cd	3.73 ± 0.18 f	26.11 ± 2.91 bc	82.60 ± 6.13 cd	9.31 ± 0.22 a	7.13 ± 0.24 cd
Sepahan	2.80 ± 0.20 a	5.07 ± 0.28 bc	27.63 ± 3.31 bc	111.33 ± 11.00 ab	10.63 ± 0.49 a	8.33 ± 0.54 abc
Khoshki line 9	2.13 ± 0.27 bc	4.47 ± 0.29 cde	21.83 ± 2.85 cd	83.53 ± 10.20 cd	11.31 ± 0.25 a	9.60 ± 0.31 a
Back cross Roshan	1.47 ± 0.17 d	5.47 ± 0.24 ab	21.53 ± 2.10 cd	96.13 ± 12.50 bc	10.38 ± 0.30 a	8.60 ± 0.35 abc
Tajan	2.07 ± 0.12 c	3.80 ± 0.22 ef	22.75 ± 1.02 cd	70.73 ± 5.38 de	9.94 ± 0.27 a	7.80 ± 0.22 bc
Kuhdasht	1.80 ± 0.20 cd	5.93 ± 0.32 a	29.26 ± 1.72 b	102.53 ± 3.70 bc	11.63 ± 0.32 a	9.33 ± 0.29 ab
N-86-7	2.67 ± 0.25 ab	4.13 ± 0.22 def	37.05 ± 2.50 a	126.80 ± 9.47 a	10.13 ± 0.32 a	8.13 ± 0.35 abc
N-80-19	1.33 ± 0.13 d	4.67 ± 0.21 cd	14.30 ± 0.93 e	47.67 ± 2.86 f	9.13 ± 0.32 a	8.40 ± 0.27 abc

The means followed by different letters in the same columns are significantly different (LSD, $P < 0.01$).

lowest value was on Back cross Roshan ($5.68 \pm 0.73\%$). The highest and the lowest values of *RCR* ($F = 22.5$; $df = 8, 36$; $P < 0.01$) were in the fifth instar larvae fed on N-80-19 (1.43 ± 0.22 mg/mg/day) and Pishtaz (0.03 ± 0.01 mg/mg/day) cultivars. Among different wheat cultivars, the *RGR* value ($F = 2.57$; $df = 8, 36$; $P < 0.05$) was the highest on Tajan (0.11 ± 0.06 mg/mg/day) compared to other cultivars.

General proteolytic activity

General proteolytic activity data ($P < 0.01$) from *E. kuehniella* fifth instar larvae reared on flour of different wheat cultivars are indicated in table 4. The highest proteolytic activity of the fifth instars was in the larvae reared on N-86-7 ($F = 79.80$; $df = 8, 18$; $P < 0.01$) (1.98 ± 0.01 U mg^{-1}), while the lowest was on N-80-19 (1.10 ± 0.01 U mg^{-1}).

Amylolytic activity

Table 4 indicates digestive amylolytic activity ($P < 0.01$) from *E. kuehniella* fifth instar larvae reared on flour of different wheat cultivars. The larvae reared on Kuhdasht ($F = 12.98$; $df = 8, 18$; $P < 0.01$) showed the highest levels of amylolytic activity (2.22 ± 0.32 mU mg^{-1}), whereas the lowest activity was in the larvae reared on cultivar Khoshki line 9 (0.52 ± 0.05 mU mg^{-1}).

Protein and starch determination of flour

Statistical tests indicated significant differences in the protein and starch contents among flour of different

wheat cultivars tested ($P < 0.01$) (table 5). The highest level of protein was in Sepahan ($F = 35.71$; $df = 8, 18$; $P < 0.01$) (1.571 ± 0.005 mg mL^{-1}), whereas the lowest level was in N-80-19 (1.474 ± 0.009 mg mL^{-1}). The highest and the lowest starch contents were found in flour of Kuhdasht ($F = 261.34$; $df = 8, 18$; $P < 0.01$) (0.368 ± 0.012 mg mL^{-1}) and Bam (0.032 ± 0.002 mg mL^{-1}), respectively (table 5).

Discussion

Growth, development, fecundity and physiological process of insects can be affected by the quality and the quantity of ingested food (Na & Ryoo, 2000; Musa & Ren, 2005). Current research demonstrated that different wheat cultivars had significant effect not only on the nutritional and enzymatic responses of *E. kuehniella*, but also on some of biological parameters of this insect.

The results showed that the larval period of the fifth instar was significantly different on nine wheat cultivars, which is in agreement with the findings of several researchers on other stored product moth pests. Locatelli & Limonta (1998) studied the development time of *E. kuehniella*, *Plodia interpunctella* (Hübner) and *Corcyra cephalonica* (Stainton) on different diets, and reported that the development time of these insects can be influenced by the different types of food consumed. In another study, Bouayad *et al.* (2008) demonstrated that the larval development time of *P. interpunctella* was significantly different on various food commodities.

Table 3. Nutritional responses of the fifth instar larvae of *Ephesia kuehniella* on the flour of different wheat cultivars.

Wheat cultivar	Index (mean \pm SE)					
	FC (mg/larva)	WG (mg)	CI	ECI (%)	RCR (mg/mg/day)	RGR (mg/mg/day)
Bam	13.38 \pm 2.27 e	3.73 \pm 0.72 bc*	0.79 \pm 0.11 c	34.37 \pm 8.14 a	0.07 \pm 0.01 d	0.02 \pm 0.00 b*
Pishtaz	8.30 \pm 1.5 5e	4.77 \pm 0.69 ab	0.48 \pm 0.08 c	42.89 \pm 9.19 a	0.03 \pm 0.01 d	0.02 \pm 0.01 b
Sepahan	32.58 \pm 2.92 cd	2.75 \pm 0.64 bc	1.79 \pm 0.11 bc	8.41 \pm 1.03 b	0.13 \pm 0.01 d	0.01 \pm 0.00 b
Khoshki line 9	32.85 \pm 2.70 cd	3.26 \pm 0.59 bc	2.01 \pm 0.18 bc	10.75 \pm 1.83 b	0.14 \pm 0.01 d	0.01 \pm 0.00 b
Back cross Roshan	29.58 \pm 3.64 d	1.61 \pm 0.37 c	1.56 \pm 0.17 bc	5.68 \pm 0.73 b	0.11 \pm 0.01 d	0.01 \pm 0.00 b
Tajan	45.89 \pm 8.07 bc	4.34 \pm 1.26 ab	3.02 \pm 0.56 b	11.71 \pm 1.78 b	0.59 \pm 0.15 b	0.11 \pm 0.06 a
Kuhdasht	32.48 \pm 4.57 cd	2.79 \pm 0.93 bc	1.89 \pm 0.28 bc	10.15 \pm 1.51 b	0.27 \pm 0.04 cd	0.02 \pm 0.01 b
N-86-7	47.44 \pm 7.22 b	3.19 \pm 0.70 bc	3.42 \pm 0.54 b	7.45 \pm 0.63 b	0.43 \pm 0.07 bc	0.03 \pm 0.01 b
N-80-19	72.91 \pm 6.30 a	6.52 \pm 1.08 a	9.83 \pm 2.14 a	9.09 \pm 1.56 b	1.43 \pm 0.22 a	0.03 \pm 0.01 b

The means followed by different letters in the same columns are significantly different (LSD, $P < 0.01$ and $P < 0.05$).

FC = food consumed, WG = weight gain, CI = consumption index, ECI = efficiency of conversion of ingested food, RCR = relative consumption rate, RGR = relative growth rate.

The present study indicated that the heaviest and the lightest larval weights were on Sepahan and N-86-7, respectively (table 1). It would be concluded that the larvae fed on diet rich in protein content (Sepahan) showed the highest larval weight. It is generally recognized that the ultimate instars of lepidopteran larvae have greater needs for protein (Simpson *et al.*, 1988), and the larvae fed on diets rich in protein demonstrate higher body weight (Sarate *et al.*, 2012). Also, the pupal period and pupal weight of *E. kuehniella* were significantly affected by different wheat cultivars. The pupal period differed from 7.00 ± 0.58 days on N-80-19 to 16.33 ± 0.33 days on cultivars Khoshki line 9 and Back cross Roshan, indicating that the differences in the nutrients or secondary compounds among the wheat cultivars can influence the pupal period. Leuck & Perkins (1972) noted that the pupal weight is an indicator of lepidopteran fitness. Since the heaviest pupal weight was in the larvae fed on N-86-7, and it has been previously reported that lepidopteran larvae fed on nutritive diets have a high pupal mass (Awmack & Leather, 2002), the highest standardized insect-growth index (25.82 ± 0.96 mg/day) was observed on this cultivar, indicating that N-86-7 was a nutritive wheat cultivar for feeding and growth of *E. kuehniella*. The results also showed that the highest value of the larval growth index was on Tajan, Kuhdasht and N-80-19, demonstrating that the larvae fed on these cultivars had higher survival rate than the larvae reared on other cultivars.

Because the types of diet consumed by the larval stages can influence the pre-oviposition and oviposition periods in the adult (Jallow *et al.*, 2001; Shayesteh *et al.*, 2010; Farahani *et al.*, 2011), the wheat cultivars tested in the current study showed significant effects on pre-oviposition and oviposition periods of *E. kuehniella*. Several authors have previously reported that the fecundity of moths can be affected by the types of different diets (Mbata, 1985; Kumral *et al.*, 2007; Fathipour & Naseri, 2011; Madboni & Pourabad, 2012; Arghand *et al.*, 2014). Accordingly, the fecundity of *E. kuehniella* was significantly influenced by the wheat

cultivars eaten by the larvae. Daily and total number of eggs per individual of the Mediterranean flour moth on nine wheat cultivars was highest on N-86-7, and lowest on N-80-19 (table 2). Since proteins are essential for egg production in the adult moth (Sorge *et al.*, 2000), and cultivar N-80-19 was poor in protein content (table 5), the lowest fecundity was detected for the insects reared on this cultivar. Consequently, the highest fecundity detected on N-86-7 can be correlated with the highest pupal weight on this cultivar (Daryaei *et al.*, 2007). Differences of wheat cultivars showed no significant effect on the longevity of male *E. kuehniella*, but the female longevity on different wheat cultivars differed from 5.73 ± 0.23 days on Bam to 9.60 ± 0.31 days on Khoshki line 9 (table 2). Higher fecundity of *E. kuehniella* reared on Khoshki line 9 than on Bam cultivars was probably attributed to longer female longevity on Khoshki line 9. Because the

Table 4. The mean (\pm SE) proteolytic (U mg⁻¹) and digestive amylolytic (mU mg⁻¹) activities of whole body extracts from fifth instar larvae *Ephestia kuehniella* on the flour of different wheat cultivars.

Wheat cultivar	Proteolytic activity	Amylolytic activity
Bam	1.61 \pm 0.05 c	0.90 \pm 0.13 de
Pishtaz	1.33 \pm 0.05 e	1.44 \pm 0.17 bc
Sepahan	1.78 \pm 0.05 b	1.47 \pm 0.07 bc
Khoshki line 9	1.30 \pm 0.01 e	0.52 \pm 0.05 e
Back cross Roshan	1.66 \pm 0.04 c	0.90 \pm 0.07 de
Tajan	1.46 \pm 0.00 d	1.05 \pm 0.10 cd
Kuhdasht	1.81 \pm 0.00 b	2.22 \pm 0.32 a
N-86-7	1.98 \pm 0.01 a	2.15 \pm 0.12 a
N-80-19	1.10 \pm 0.01 f	1.74 \pm 0.23 ab

The means followed by different letters in the same columns are significantly different (LSD, $P < 0.01$).

Table 5. The mean (\pm SE) protein and starch contents (mg mL⁻¹) of the flour of different wheat cultivars used for *Ephestia kuehniella* feeding.

Wheat cultivar	Protein content	Starch content
Bam	1.548 \pm 0.003 b	0.032 \pm 0.002 f
Pishtaz	1.508 \pm 0.005 c	0.166 \pm 0.003 d
Sepahan	1.571 \pm 0.005 a	0.236 \pm 0.010 c
Khoshki line 9	1.498 \pm 0.007 cd	0.251 \pm 0.001 c
Back cross Roshan	1.493 \pm 0.002 cde	0.283 \pm 0.005 b
Tajan	1.482 \pm 0.003 ef	0.075 \pm 0.005 e
Kuhdasht	1.492 \pm 0.004 de	0.368 \pm 0.012 a
N-86-7	1.500 \pm 0.005 cd	0.147 \pm 0.008 d
N-80-19	1.474 \pm 0.009 f	0.149 \pm 0.004 d

The means followed by different letters in the same columns are significantly different (LSD, $P < 0.01$).

larvae fed on cultivar N-86-7 showed the highest pupal weight, standardized insect-growth index, and daily and total fecundity, this cultivar can be suggested as a suitable host diet for the growth and development of *E. kuehniella*.

Scanning the available literature revealed that the amounts and types of food eaten by an insect as well as the efficiency of conversion of ingested food are the factors determining the availability of nutrients for its maintenance and growth (Waldbauer, 1968; Slansky, 1990; Barton-Browne & Raubenheimer, 2003). The observed significant differences among nutritional indices, especially for *ECI* value, of *E. kuehniella* fed on the flour of different wheat cultivars indicating that the wheat cultivars had various nutritional values. It is noticeable that, among the nutritional indices, *ECI* is a feeding index that can be different due to variations in the food digestibility and the proportional amount of digestible food converted to insect biomass and metabolized to obtain the energy (Abdel-Rahman & Al-Mozini, 2007). Moreover, this index demonstrates an insect's ability to incorporate food into the growth (Nathan *et al.*, 2005). Among the nine wheat cultivars, the highest *CI* value of *E. kuehniella* was on N-80-19 (table 3), indicating that the rate of intake relative to the mean larval weight during the feeding period was the highest on this cultivar. It is reported that the insect larvae effort to compensate for reduced *ECI* by increasing their *CI* (Price *et al.*, 1980), a response that has been reported by many other researchers for the insect nutrition (Naseri *et al.*, 2010; Mansouri *et al.*, 2013; Rahimi Namin *et al.*, 2014). In the current study, the highest *ECI* value of the Mediterranean flour moth was recorded for the larvae fed on cultivar Pishtaz, suggesting that they were more efficient at converting ingested food to body matter. The lowest *ECI* value on N-86-7 may be attributed to the deficiency of nutritional components and/or presence of some secondary chemicals in this cultivar. Nutritional requirements of an insect are correlated with body biomass and the period of immature stages. Furthermore, as the amount of food ingested is

decreased, the insect becomes lighter (Lazarevic & Peric-Mataruga, 2003). Additionally, the significantly higher *ECI* achieved by the fifth instar larvae fed on Pishtaz suggests that the larvae fed on this cultivar had higher weight gain (tables 3). Insects consume less of a special diet simply because they are able to convert it more efficiently into the growth. Also, when the larvae consume less, the food will tend to pass through their alimentary canal slowly, and it can be efficiently converted into their body matter (Soo Hoo & Fraenkel, 1966). Accordingly, the lowest *RCR* value was observed in the last instar larvae of the Mediterranean flour moth fed on Pishtaz, while the highest value of *ECI* was on this cultivar (table 3).

It is well known that the soluble carbohydrates and proteins are efficiently utilized by insects and many species obtain most of their nourishment from these nutrients (Ishaaya *et al.*, 1971). In the insects, the degree of food utilization has a direct impact on the enzymatic activity responsible for providing energy and nutrition to the growing larvae (Sivakumar *et al.*, 2006), thus, for availability of the required nutrients, regulation of digestive enzymes is important (Kotkar *et al.*, 2009). Proteases catalyze the release of amino acids from dietary proteins in an insect's alimentary canal to meet its nutritional necessities (Terra & Ferreira, 1994), and amylases play a main role in the starch digestion (Valencia-Jimenez *et al.*, 2008). In the current research, general proteolytic and digestive amylolytic activities of *E. kuehniella* fifth instar larvae were studied. According to Razavi Tabatabaei *et al.* (2011), comparison of α -amylase activity in the whole body extracts from *Ectomyelois ceratoniae* (Zeller) (Lep.: Pyralidae) fifth instar larvae with the midgut extract indicated that all amylase activity from the larval body comes from the midgut. Therefore, it could be suggested that digestive amylolytic activity in *E. kuehniella* whole larval body extracts comes from the larval midgut. The present research demonstrated that the proteolytic and digestive amylolytic activities of the Mediterranean flour moth were significantly affected by the flour of different wheat cultivars (table

4). The highest proteolytic activity of the fifth instars was in the larvae reared on N-86-7; however, this cultivar had the moderate content of protein. The lowest proteolytic activity and protein content was detected on N-80-19 (tables 4 and 5). This is in agreement with the results of other researchers, who stated that the insects can release less of the digestive enzymes for nutrients present in excess, while maintaining or increasing the enzyme levels for nutrients in deficit (Kotkar *et al.*, 2009; Lwalaba *et al.*, 2010). Since protein intake occurs absolutely during the larval stages (Sorge *et al.*, 2000), the larvae can not allow any dietary protein to pass undigested through the alimentary canal. So, it is helpful to keep a constant level of protease release in the event that even a small amount of protein is eaten (Lwalaba *et al.*, 2010).

It is generally accepted that the insects can regulate the expression of digestive amylase regarding to the substrate levels of their food (Sarate *et al.*, 2012). Because variations in the starch content in wheat cultivars can lead to differences in the amylolytic activity of *E. kuehniella*, thus, the highest amylolytic activity was detected in the larvae fed on Kuhdasht cultivar, which is attributed to the highest starch content of this cultivar. However, the larvae reared on Khoshki line 9 had the lowest levels of amylolytic activity, which was approximately four-fold lower than Kuhdasht. The mean digestive amylolytic activity of the fifth instar larvae of *E. kuehniella* on different wheat cultivars was approximately four- and five-fold higher than those reported for the last instar female and male larvae of this insect on wheat flour, respectively (Jafarlu *et al.*, 2012). Possible reasons for this inconsistency can be because of the differences of wheat cultivars, variations in experimental conditions and genetic variations as a result of laboratory rearing of *E. kuehniella*. Comparison of the relationships between enzymatic activity and protein and starch contents of various cultivars tested demonstrated that there is likely an insect mechanism to exactly determine the diet contents, and regulate the levels of digestive enzymes (Kotkar *et al.*, 2009). Higher

protease and amylase activities of *E. kuehniella* larvae on some wheat cultivars can be because of the differences in either protein and starch contents of the food or presence of enzyme-inhibitors.

The soluble protein estimations of the flour of nine wheat cultivars indicated that Sepahan had the highest protein content. In the case of Pishtaz as a high-nutritious cultivar for *E. kuehniella*, the levels of protease and amylase activity are down-regulated to metabolize the requisite energy from ingested food. Because low dietary protein can cause a boosting in the consumption rate (Slansky, 1993); the highest food consumption of the fifth instar larvae of *E. kuehniella* was observed on cultivar N-80-19.

Comparison of the enzymatic activity, biological parameters and fecundity of the Mediterranean flour moth on the wheat cultivars tested revealed that the lowest daily and total fecundity as well as the highest values of *CI* and *RCR* were in the larvae reared on N-80-19, indicating that this cultivar is the least suitable wheat cultivar for rearing of *E. kuehniella*, however it can be utilized as a resistance cultivar to design new strategies for this pest management. Because the highest pupal weight, *SII* and fecundity (daily and total) were in *E. kuehniella* reared on N-86-7, and the larvae fed on Pishtaz showed the highest value of *ECI*, these two cultivars can be introduced as suitable wheat cultivars for feeding and rearing of *E. kuehniella*. For the future, it is recommendable to study the life table parameters and specific activity of digestive proteinases and carbohydrases of *E. kuehniella* in response to feeding on different wheat cultivars.

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