J. Crop Prot. 2017, 6 (2): 273-285

Research Article

Type of cereal flours as factors affecting biological and physiological characteristics of *Ephestia kuehniella* (Lepidoptera: Pyralidae) larvae

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Abstract: The Mediterranean flour moth, *Ephestia kuehniella* Zeller is one of the conventional hosts for rearing of natural enemies to be used in biological control programs. In this study, the effects of three cereal flours (wheat, corn and barley) on some biological parameters, nutritional responses, as well as proteolytic and amylolytic digestive activities of the fifth instar larvae of *E. kuehniella* were studied to determine suitability of the cereals for optimum growth and reproduction of *E. kuehniella*. The highest rate of larval survival (0.88) and larval growth index (9.77) were obtained in larvae which fed on corn flour. The relative growth rate (9.17 ± 0.33 mg/mg/day) and the efficiency of conversion of ingested food (12.08 ± 1.11%) were the highest rate for fifth instar larvae which fed on corn flour. The highest protein concentration in midgut of larvae (63.77 ± 2.31 mg/ml) and consequentially, proteolytic activity including tryptic (0.0012 ± 0.00 Umg^{-1}) and chymotryptic (0.543 ± 0.001 Umg^{-1}) were observed in larvae that fed on corn flour. According to the results obtained, corn (Var. 704) was the most suitable cereal for laboratory rearing of *E. kuehniella*, resulting in the highest rate of biological and physiological parameters.

Keywords: Mediterranean flour moth, cereal, nutritional indices, proteolytic and amylolytic activity

Introduction

The Mediterranean flour moth, *Ephestia kuehniella* Zeller, is one of the important stored products pests, which is found on grains, particularly powdered cereal products (Cox and Bell, 1991; Hill, 2002; Rees, 2004; Tarlack *et al*., 2015). The powdered food is preferred over the whole grains although they can feed on the whole grains too. *E. kuehniella is* not only known as an important pest, but also, the eggs and larvae of this species are widely used as a conventional host for the mass rearing of several parasitoids such as Braconidae and Trichogrammatidae (Hoffmann *et al*., 2001; Shonouda and Nasr, 1998) as well as, predators including *Adalia bipunctata* (L.) (Col.: Coccinellidae) (Shonouda and Nasr, 1998; Specty *et al*., 2003), *Harmonia axyridis* Pallas (Col.: Coccinellidae) (De Clercq *et al*., 2005), *Orius albidipennis* Reuter (Hem.:Anthocoridae) (González-Zamora *et al*., 2007), *Franklinothrips orizabensis* Johansen (Thys.: Aeolothripidae) (Hoddle *et al*. 2001) and *Chrysoperla carnea* (Steph.) (Neu.: Chrysopidae) (Jokar and Zarabi, 2012).

Due to the fundamental importance of host plant quality on tri-trophic nutritional system (e.g.

Handling Editor: Saeid Moharramipour

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Received: 20 April 2017, Accepted: 24 June 2017
Published online: 18 July 2017

host plant- herbivore- natural enemies), it would be better to study the effects of different cereals on nutritional requirement aspects of *E. kuehniella* (Price *et al*., 1980; Mostafazadeh and Mehrkhou, 2016). In other words, quantities and types of food eaten and consequentially, the efficiency of them affect the availability of nutrients for maintenance and growth during developmental time (Browne and Raubenheimer, 2003; Slansky, 1990; Waldbauer, 1968). These physiological indices can be used to determine whether food plant affects insect behavior or metabolism (Slansky, 1990). It is also important to understand target insect physiology and midgut enzymes activity (Kazzazi *et al*., 2005).

Some biological characteristics including growth, survival and reproduction are affected by feeding and digestion processes. The digestion process takes place by digestive enzymes, in which the macrometabolites (such as carbohydrates, lipids and proteins) are broken down into smaller molecules to be absorbed by cells in the midgut tissue (Terraand Ferreira, 1994). This process is perfectly controlled by digestive enzymes that depend on their site of activity in the insect gut (Klowden, 2013). In addition to the important role of the enzymes, they can also act as elicitors of the defense mechanism in plants when they are encountered with the feeding damage of the insect pests (Mattiacci *et al.*, 1995; Zibaee, 2012), as well as in plant–herbivore co-evolution because of their importance in the breakdown of secondary metabolites of plants (Hemming and Lindroth, 2000). The major digestive enzymes in the midgut of insects consist of amylases and proteases, which act on *α*-D-(1,4)-glucan linkages in carbohydrates and peptide bonds, respectively (Terra and Ferreira, 1994).

There have been a number of studies on the biology of *E. kuehniella* (Brindley, 1930; Hill, 2002; Rees, 2004), its reproductive behavior (Xu, 2010), as well as the efficiency of *E. kuehniella* as a host in breeding of biological control agents (De Clercq *et al*., 2005; Kim and Riedl, 2005; Hamasaki and Matsui, 2006; Paust *et al*., 2006), on different host plants under different environmental conditions. Although some related studies have been conducted on the physiological aspects of *E. kuehniella* on different host plants (Abdi *et al*., 2014; Jafarlu *et al*., 2012; Pytelkova *et al*., 2009), not all of these studied the effects of the same host plants on proteolytic, amylolytic activity and nutritional indices of *E. kuehniella* under the same environmental conditions. Abdi *et al*. (2014) investigated the suitability of nine wheat flour cultivars on physiological processes of *E. kuehniella*, who reported, 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. Bidar *et al*. (2016) reported that barley cultivars affect nutritional performance and digestive enzymatic activities of *Ephestia kuehniella* Zeller (Pyralidae).

Although, there are some documents regarding the study of ecophysiological parameters of *E. kuehniella* on wheat (Abdi *et al*., 2014) or barley (Bidar *et al*., 2016) cultivars, but there is no study evaluating these parameters synchronously on cereal flour as studied in this research. Thus, the objectives of this study were to determine (a) some biological parameters, namely survival rate, developmental time, (b) food utilization and consumption and (c) proteolytic and amylolytic activity of the fifth instar larvae of *E. kuehniella* on three host plants (wheat: Var. Zareh; barley, Var. Makooi and corn Var. 704). It is expected that the obtained results will be useful to introduce suitable cereal host for the successful laboratory and mass rearing of *E. kuehniella*, as well as natural enemies.

Materials and Methods

Chemicals

N-*α*-benzoyl-DL-arginine-*p*-nitroanilide (BA*p*NA), *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (SAA PF*p*NA), phenylmethylsulfonyl fluoride (PMSF), N-α-p-tosyl-L-lysine choloromethyl ketone (TLCK), N-tosyl-L-phenylalanine choloromethyl ketone (TPCK), dinitrosalicylic acid (DNS) and Bovine serum albumin (BSA) were used in physiological experiments, which were obtained from Sigma Chemical Co., St Louis, MO. BSA was purchased from Roche Co., Germany.

Cereal flour and insect rearing

Seeds of the three cereals; wheat (Var. Zareh), corn (Var. 704) and barley (Var. Makooi) were obtained from Seed and Plant Breeding Institute in Urmia, Iran. Selection of the cereals was based on their importance as cultivated crops in West - Azarbaijan province (Iran). Seeds were powdered by electrical grinder and kept in refrigerator (4 ºC) to avoid any contaminations. The rearing stock of *E. kuehniella* was obtained from Insectarium of Agricultural Research Center in Urmia, Iran. The larvae of *E. kuehniella* were reared in a rectangular container (14 × 10 × 14 cm) on each cereal flour until pupal stage in a growth chamber at a temperature of 25 ± 1 °C, a relative humidity of 65 ± 5% and a 10: 14 h (L: D) photoperiod. Then, emerged adults were released form containers into plastic funnel (10 cm diameter and 15 cm height) for egg laying. A piece of wax paper was set at the bottom of containers for gathering the eggs. The insects examined on different cereals had already been reared for two generations on the hosts, before being used in experiment.

Biological and nutritional indices

Some biological parameters including larval and pupal periods and their weights, as well as survival rate of fifth instar larvae were recorded. For this reason, 100 one-day-old fifth instar larvae were collected from the colony containers and the experimental units were checked daily. Development of each individual larva was followed until its death or pupa emergence. Development and survivorship were recorded for each immature stage daily.

Larval growth index (*LGI*) and standardized insect-growth index (*SII*) of *E. kuehniella* were calculated on different cereals using the following formulae (Itoyama *et al*., 1999):

$$LGI = lx / L, \qquad SII = Pw / L$$

Where, lx = survival rate of larvae, L = larval period and Pw = pupal weight.

For nutritional indices studies, 100 one-day-old fifth instar larvae were collected from the containers and separated into 10 replicates (10 larvae in each replicate) and transferred into plastic plate (diameter 8 cm, depth 1 cm), containing 1g of flour of each examined cereal. 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 hosts and 10 larvae reared on each cereal 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 based on their fresh weights. Nutritional indices were calculated using the formulae described by Waldbauer (1968):

$$CI = E / A, \qquad ECI = P / E$$

$$RCR = E / (A \times T), \qquad RGR = P / (A \times T)$$

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.

Sample preparation

Final instar larvae were cold- immobilized and dissected to remove the whole midgut. Only actively feeding larvae with food filling the gut tracts were chosen for dissection. After the removal of the whole gut, the unwanted tissues were removed, the gut tissue was homogenized with a handheld glass grinder on ice and transferred to 1.5ml micro tubes and centrifuged at 13,000 rpm for 20 min at 4 °C. The clear supernatant was transferred to a pre-chilled microtube. The samples were stored at -20 ºC until further use.

The protein quantification of cereal and larval midgut

BSA was used as a standard to determine the protein concentration of both cereal flour and larval midguts. For protein content of cereal flours, briefly 200mg of each host plant flour was dissolved in distilled water (10 ml), centrifuged at 13000 × g for 10 min, and then 100µl of the homogenate was added to 3 ml of

Bradford reagent. The samples were incubated in darkness at 37 °C for 30 min, then absorbance was read at 595 nm (Bouayad *et al*., 2008). Protein concentration of fifth instar larvae midgut was determined by BSA as standard (0.1, 0.3, 0.5, 0.7, 0.9, 1.0 and 1.2 mg/ml) (Bradford, 1976).

Protease and α-amylase activity assays

General proteinase activity of larval guts was determined using azocasein as substrate at broad pH range (pH 6–12). The universal buffer system (50mM sodium acetate–phosphate–borate) was used to determine the optimal pH of proteolytic activity (Elpidina *et al*., 2001). For azocaseinolytic activity, the reaction mixture consisted of 30 µl of 2% azocasein solution in 90 mM universal buffer of specified pH and 15 µl enzymes. The reaction mixture was incubated at 37ºC for 60 min. Proteolysis was stopped by addition of 30 µL of 30% trichloroacetic acid (TCA). Appropriate blanks were prepared by TCA which was added before the substrate for each assay. Precipitation was achieved by cooling at 4 ºC for 60 min and the reaction mixture was centrifuged at 16000 × g for 10 min. An equal volume (75 µl) of NaOH 1M (1N) was added to the supernatant and the absorbance was recorded at 440 nm using a microplate reader (BioTek Synergy HT).

The α-amylase activity of fifth instar larvae of *E. kuehniella* was assayed by DNS method (Bernfeld, 1955) with 1% soluble starch as substrate at broad pH range (5-12) on different cereal flours. Fifty µl of the enzyme extract was incubated with 250 µl of universal buffer and 20 µl of soluble starch for 30 min at 35 ºC. The reaction was stopped by adding 50 µl DNS and heating in boiling water for 10 min prior to read absorbance at 540nm. One unit of *α*-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35 °C. All experiments were carried out in triplicates.

Trypsin and chymotrypsin assays

Trypsin- and chymotrypsin-like activities (as two sub-classes of serine proteases) were assayed using BApNA and SAAPFpNA, respectively. A reaction mixture consisted of 10 µl enzyme, 85 µl of universal buffer at optimum pH, and 5 µl of the above mentioned substrates. The reaction mixture was incubated at 37 °C for 10 min before adding 30% acetic acid to terminate the reaction. The absorbance of the resulting mixture was measured spectrophotometrically at 405 nm by p-nitroaniline release.

Inhibitors activity assay

The effect of different inhibitors on proteolytic activities of midgut enzyme extract was determined. The inhibitors and their concentration were: general serine protease inhibitor, 1mM PMSF; trypsin inhibitor (TLCK), 0.1 mM and chymotrypsin inhibitor (TPCK), 0.1 mM. To determine the effect of these compounds on enzyme activities, the enzymes were preincubated with the appropriate inhibitors at room temperature for 15 min, then substrate was added and the assays were carried out as described in the enzyme assay section.

Data analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by comparison of the means with the Tukey's test at $p < 0.05$ using the software SPSS (V. 20).

Results

Biological and nutritional indices

The survival rate of larvae which were reared on corn flour (0.88) and barley flour (0.65) had the highest and lowest rate, respectively. The results showed that the larval period of the fifth instar was significantly different on cereal flour ($F = 31.11$; $df = 2, 97$; $P < 0.01$), the longest and shortest being on barley (9.00 ± 0.44 d) and corn (7.00±0.10 d), respectively. The heaviest and the lightest larval weight gain ($F = 3.63$; $df = 2, 97$; $P < 0.05$) were observed on corn (8.50 ± 0.75 mg) and barley flour (0.93 ± 0.17 mg), respectively. The longest pupal period ($F = 21.84$; $df = 2, 97$; $P < 0.01$) was on barley (9.51 ± 0.23 days) and the shortest on corn flour (7.93 ± 0.10 days). The heaviest pupal weights (F =

21.07; df = 2, 97; $P < 0.05$) were detected in the larvae fed on corn flour (23.30 ± 1.50 mg). The results also showed that the highest larval growth index (*LGI*) was on corn flour (12.57 ± 2.51) and the lowest was on barley flour (7.22 ± 1.22). The standardized insect-growth index (*SII*) of *E. kuehniella* showed significant difference (F = 115; df = 2, 97; $P < 0.05$) among the cereals, the highest and the lowest being on corn (3.32 ± 0.54 mg/day) and on barley flour (1.83 ± 0.18 mg/day) (Table 1).

The results of the nutritional responses of the fifth instar *E. kuehniella* larvae are shown in table 2. All of the nutritional parameters including, food consumed (F = 0.517; df = 2, 97; $P < 0.01$), *CI* (F = 21.50; df = 2, 97; $P < 0.01$), *RCR* (F = 19.93; df = 2, 97; $P < 0.01$), *RGR* (F = 27.98; df = 2, 97; $P < 0.01$) and *ECI* (F = 26.46; df = 2, 97; $P < 0.01$) were affected by type of cereal flour. The larvae reared on barley flour showed the highest values of *CI* (0.078 ± 0.005) and *RCR* (226.33 ± 5.78 mg/mg/day). The larvae reared on corn flour showed the highest value of *ECI* (12.08 ± 1.11%), while the lowest value was on barley flour (1.73 ± 0.21%). The *RGR* value varied from 3.48 ± 0.31 to 9.17 ± 0.33 (mg/mg/day) among different cereals, the highest rate being on corn flour.

The protein quantification

Statistical tests indicated significant differences in the content of protein among various cereal flours tested (F = 4.50; df = 2, 6; $P < 0.05$). The highest content of protein was obtained in larvae reared on barley flour (0.099 ± 0.001 mg ml^{-1}) followed by corn flour (0.074 ± 0.0145 mg ml^{-1}) and wheat flour (0.070 ± 0.010 mg ml^{-1}), respectively. The protein concentration of larvae midgut extracts which were reared on mentioned cereals were significantly different (F = 8.35; df = 2, 6; $P < 0.05$). The protein content of midgut larvae which fed on corn, wheat and barley were 63.77 ± 2.31, 18.73 ± 1.40 and 19.00 ± 1.33 mg ml^{-1}, respectively (Table 3).

Table 1 Biological parameters (Mean ± SE) of *Ephestia kuehniella* on flour of different cereals.

Cereals	Larvae survival	larval period (d)	LWG (mg/days)	Pupa period (d)	PW (mg)	LGI	SII (mg/day)
Corn	0.88	7.00 ± 0.10^{b}	8.50 ± 0.75^{a}	7.93 ± 0.10^{b}	23.30 ± 1.50^{a}	12.57 ± 2.51^{a}	3.32 ± 0.54^{a}
Wheat	0.78	8.11 ± 0.76^{ab}	5.00 ± 0.60^{b}	8.41 ± 0.12^{ab}	17.41 ± 0.31^{b}	9.61 ± 1.36^{ab}	2.14 ± 0.84^{ab}
Barely	0.65	9.00 ± 0.44^{a}	0.93 ± 0.17^{c}	9.51 ± 0.23^{a}	16.52 ± 0.12^{b}	7.22 ± 1.22^{b}	1.83 ± 0.18^{b}

Means within columns followed by the same letters are not significantly different (Tukey's test, $P < 0.05$). *LWG*= larval weight gain, PW= pupa weight, *LGI* = larval growth index, *SII* = standardized insect-growth index.

Table 2 Nutritional indices of the fifth instar larvae (Mean ± SE) of *Ephestia kuehniella* on flour of different cereals.

Cereals	FC (mg)	CI	ECI (%)	RCR (mg/mg/day)	RGR (mg/mg/day)
Corn	0.060 ± 0.0009^{b}	0.007 ± 0.000^{b}	12.08 ± 1.11^{a}	49.43 ± 6.93^{b}	9.17 ± 0.33^{a}
Wheat	0.065 ± 0.0009^{ab}	0.013 ± 0.001^{ab}	8.38 ± 0.64^{b}	77.87 ± 5.39^{ab}	3.65 ± 0.40^{b}
Barely	0.073 ± 0.0006^{a}	0.078 ± 0.005^{a}	1.73 ± 0.21^{c}	226.33 ± 5.78^{a}	3.48 ± 0.31^{b}

Means within columns followed by the same letters are not significantly different (Tukey's test, $P < 0.05$). FC = food consumed, WG = weight gain, *CI* = consumption index, *ECI* = efficiency of conversion of ingested food, *RCR* = relative consumption rate, *RGR* = relative growth rate.

Table 3 Mean (± SE) protein contents (mg ml^{-1}) of cereal flours and last instar larvae midgut extract of *Ephestia kuehniella*, which fed on flour of different cereal.

Cereals	Protein content of flour (mg ml^{-1})	Protein content of midgut larvae (mg ml^{-1})
Corn	0.074 ± 0.015^{b}	63.77 ± 2.31^{a}
Wheat	0.070 ± 0.010^{b}	18.73 ± 1.40^{b}
Barely	0.099 ± 0.001^{a}	19.00 ± 1.33^{b}

Means within columns followed by the same letters are not significantly different (Tukey's test, $P < 0.05$).

Protease and α-amylase activity assays

The effect of pH on protease and amylase activity of the gut extract from *E. kuehniella* larvae is presented in Fig. 1. Proteolytic activity with azocasein as a protein substrate showed that the substrate was hydrolyzed over a broad range of alkaline pHs (6-12) with a peak at 11-12 (Fig. 1, A). Serine proteolytic activity data ($F = 11.24$; df = 2, 6; $P < 0.05$) by general substrate from *E. kuehniella* fifth instar larvae reared on various cereals are shown in Table 4. The lowest serine proteolytic activity was obtained in larvae reared on barley using azocasein (0.775 ± 0.015 U mg^{-1}), whereas the highest activities were in larvae reared on corn and wheat flour (Table 4).

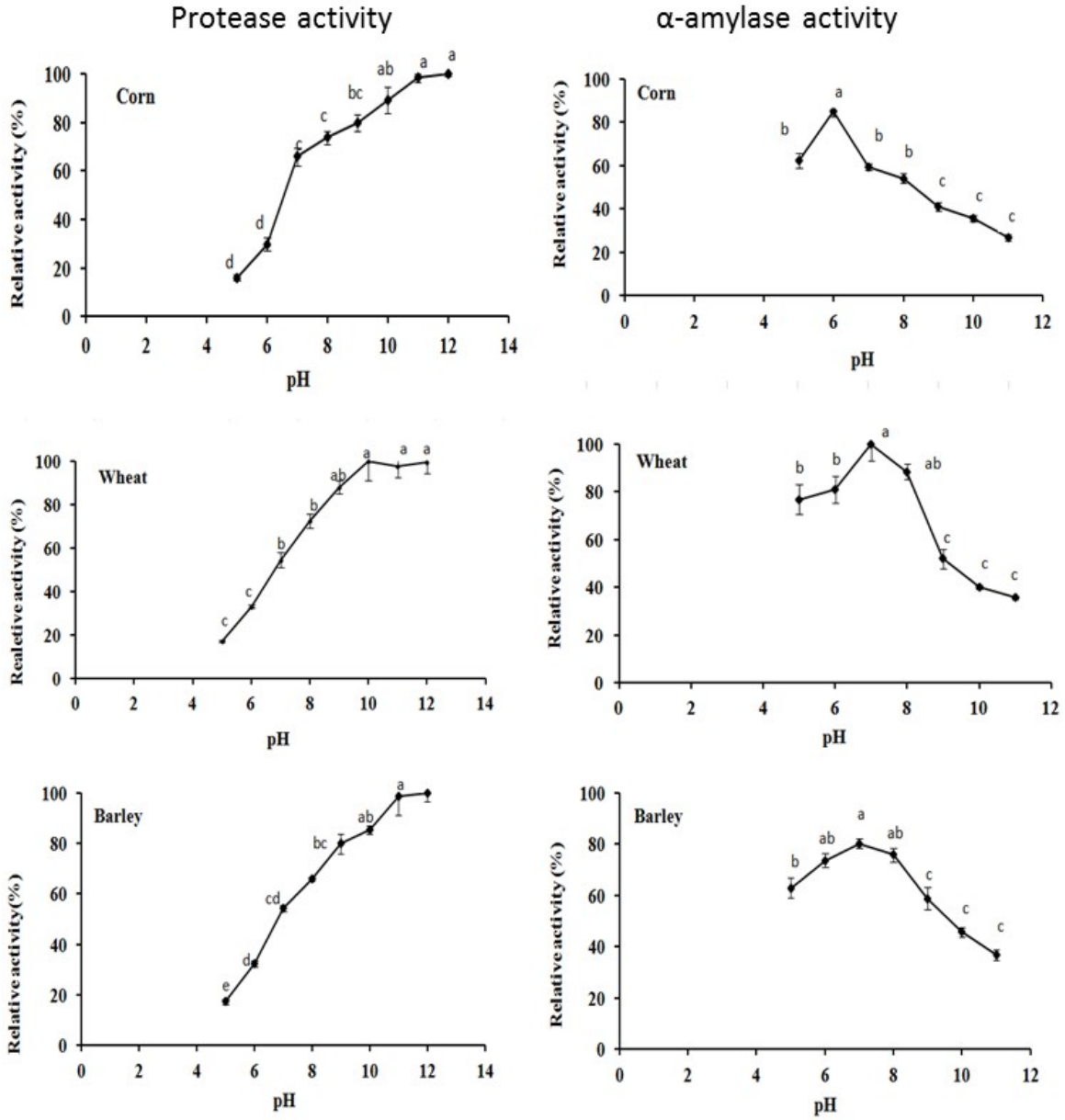

Figure 1 The effect of pH on the Protease and α-amylase activity of larval gut extract from *Ephestia kuehniella*, which fed on flour of different cereals. Means followed by the same letters are not significantly different (Tukey's test, $P < 0.05$).

Maximum α-mylase activity from larval midgut occurred at the pH 6 in larvae reared on corn and 7 on wheat and barley flour, respectively (Fig. 1, B). Enzyme activity increased steadily from pHs 5 to 7, and then decreased with increasing pH. α-amylase activity data ($F = 90.22$; df = 2,6; $P < 0.05$) by starch from larvae of *E. kuehniella* fed on various cereal are shown in Table 4. The lowest and highest α-amylase activity was in larvae reared on barley (47.09 ± 2.41 Umg^{-1}) and corn (471.21 ± 32.62 Umg^{-1}), respectively (Table 4). Results demonstrated the higher rate of α-amylase in comparison with protease activity on the three mentioned cereals.

Trypsin and chymotrypsin activity assays

The results obtained when assaying larval digestive extracts, which fed on different cereal with specific substrates showed the presence of both chymotrypsin and trypsin-like activities (Table 4). The substrates SAAPFpNA and BApNA were hydrolyzed with maximum activity at pH 10. The trypsin, and Chymotrypsin activity showed significant difference among flour of various host plants ($F = 14.36$; df = 2, 6; $P < 0.05$), so the highest rate of endoproteolytic activity was observed in midgut of larvae which fed on corn flour.

Inhibitors activity assay

The total proteolytic activity of larval gut extract was further characterized using general and specific serine proteinase inhibitors (Table 5). It was found that PMSF, as a serine protease inhibitor, significantly ($F = 28.90$; df = 2, 6; $P < 0.05$) decreased proteolytic activity. The trypsin-like ($F = 0.232$; df = 2, 6; $P < 0.05$) and chymotrypsin-like ($F = 1.140$; df = 2, 6; $P < 0.05$) activity were affected by TLCK and TPCK inhibitors, respectively (Table 5). The highest inhibition by PMSF, TLCK and TPCK inhibitors were observed in gut of larvae fed on corn flour ($65.30 \pm 0.042\%$), ($17.20 \pm 0.01\%$) and ($19.64 \pm 0.075\%$), respectively (Table 5).

Table 4 Mean (± SE) serine (U mg^{-1}), α-Amylase, Tryptic and Chymotryptic activity of the gut extracts from the fifth instar larvae of *E. kuehniella* on flour of different cereals at optimum pH..

Cereals	Serine activity (U mg^{-1})	α-Amylase activity (U mg^{-1})	Tryptic activity (U mg^{-1})	Chymotryptic activity (U mg^{-1})
Corn	2.96 ± 0.010^{a}	471.21 ± 32.62^{a}	0.0012 ± 0.00^{a}	0.5430 ± 0.001^{a}
Wheat	2.77 ± 0.001^{a}	174.66 ± 26.00^{b}	0.0003 ± 0.00^{b}	0.0005 ± 0.000^{b}
Barely	0.775 ± 0.015^{b}	47.09 ± 2.41^{c}	0.0002 ± 0.00^{b}	0.0020 ± 0.000^{b}

Means within columns followed by the same letters are not significantly different (Tukey's test, $P < 0.05$).

Table 5 The effects of some protease inhibitors on the general proteolytic activity from gut extract of *E. kuehniella* on flour of different cereals at optimal pH using general substrates.

Cereals	Inhibition (%)		
	PMSF	TLCK	TPCK
Corn	65.30 ± 0.042^{a}	17.20 ± 0.01^{a}	19.64 ± 0.075^{a}
Wheat	24.70 ± 0.004^{b}	13.20 ± 0.07^{ab}	19.10 ± 0.018^{a}
Barely	23.90 ± 0.054^{b}	9.40 ± 0.06^{b}	9.60 ± 0.048^{b}

Means within columns followed by the same letters are not significantly different (Tukey's test, $P < 0.05$).

Discussion

The efficiency rate of physiological processes namely enzymatic activity and nutritional indices is a reflection of the quality and quantity of food resources of host plants, which affects biology, survival, growth, and reproduction of phytophagous insects (Bernays and Chapman, 1994). The quality of a host plant is a key determinant of the fecundity of herbivorous

insects. Components such as carbon, nitrogen, and defensive metabolites of host plants directly affect a plant's potential and determine the level of fecundity (Awmack and Leather, 2002). Waldbauer's nutritional assays are applied to identify behavioral and physiological responses of insects to plant compositions.

Mohammadi and Mehrkhou (2014) have previously demonstrated that larvae of *E. kuehniella* fed on barley had a weak potential to increase their population suggesting that it was less suitable host and more resistant to the *E. kuehniella* than the other cereals tested. By combining the data from their studies on the biology and demographic parameters of the *E. kuehniella* with the findings of the present research on the nutritional indices of the larvae fed on corn, it appears that, there is coordination among these results, as the highest rate of some important nutritional indices parameters namely, *RGR*, *ECI*, larval biomass, were observed on larvae fed on corn and the efficiency of these parameters on biological and reproduction parameters were at their highest rate, and the shortest developmental time and highest rate of fecundity were reported on corn (Mohammadi and Mehrkhou, 2014), whereas the lowest rate of mentioned parameters were obtained on barley. These results may be due to the fact that, the energy gained by feeding on barley has been consumed for storage of lipids in a fatty body around the digestive system instead of increasing biological and reproductive parameters. Such lipids were observed in dissected larvae that fed on barley. Because of the insects convert the carbohydrates to fats, and may contribute to the production of amino acids and it is one of the important biochemical pathways to compensation of protein and amino acid shortages (Chapman, 1998).

The pupal weight was higher than larval weight on the three mentioned cereals, it was due to the fact that, the larval and pupal weights were determined based on dry and fresh weight, respectively. The survival rate affects the larval growth index, directly. Because the highest survival rate was recorded for larvae which fed on corn flour and so it lead to the highest rate of *LGI*. Regardless of insect feeding habits, most insects must digest proteins, carbohydrates and lipids and therefore they have similar array of enzymes in the midgut. Nevertheless, enzymes function efficiently only within limited ranges of temperature, pH and redox potential. The pH of gut contents is a major factor that affects digestive enzymes (Chapman 1998; Terraand Ferreira 1994; Nation 2008). Enzymes are most active only within a limited range of pH. Most insects have enzymes with optima at pH 6-7 (Chapman, 1998), which coincides with our data, so the highest relative enzyme activity occurs in midgut larvae of Mediterranean flour moth with neutral (6-7), which shows the presence of amylase activity, although the proteases were also active in their midguts which happens at optimal pH 11-12, but the amylolytic activity was higher than proteolytic activity. Insects feeding on stored products, rich in carbohydrates, generally have higher amylase activities than those feeding on wool or plants, but the latter usually have higher proteolytic activity (Chapman, 1998). Similar situation has been reported for other lepidopteran and coleopteran pests of stored products such as *Plodia interpunctella* Hübner (Chapman, 1998), *Phthorimaea operculella* Zeller (Mansouri *et al*., 2013), *E. kuehniella (Abdi et al.* 2014), *Trogoderma granarium* Everts (Hosseininaveh *et al*., 2007), and *Ectomyelois ceratoinae* Zeller (Razavi Tabatabaei *et al*., 2011). Polysaccharides, especially starch, is one of the major nutrients in plant tissues that must initially be digested by α-amylase to smaller parts for absorption via epithelial cells (Zibaee, 2012). Our results showed amylolytic activity were greater than proteolytic in all of cereals, which coincides with other studies. Abdi *et al*. (2014) reported that some wheat cultivars (Kuhdasht, N-86-7, N-80- 19 and Pishtaz) had more α-amylase activity than proteolytic, and that 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.

The results of the current study revealed several digestive enzymes including α-amylase, proteases namely trypsinase and chymotrypsinase in the midgut of *E. kuehniella*, which has been confirmed using related substrates and inhibitors. According to Mohammadi and Mehrkhou (2014) and Mostafazadeh and Mehrkhou (2016), the larvae of *E. kuehniella* which were reared on corn had the shortest developmental time and highest rate of fecundity and that coen was the most suitable host among cereal for mass rearing of *E. kuehniella*, as well as natural enemies (e.g. *Habrobracon hebetor* Say) in tri-trophic interaction system whichagrees with our study specially in the case of proteolytic activity. As can be seen, with increasing the protein content of larval midgut serine protease, trypsin-like and chymotrypsin-like activities increased, which were involved in digestive process.

Our finding regarding the eco-physiological parameters demonstrated that, the lowest rate of seine protease and amylase activity in larvae which fed on barley lead to the reducing of biological, nutritional indices, as well as demographic parameters and life history periods (Mohammadi and Mehrkhou, 2014), which might be due to the presence of some plant proteinase inhibitors in barley. In fact, lower specific proteases activity in the midgut of insects indicates that proteins are not efficiently utilized due to the presence of allelochemicals and plant inhibitors, which leads to the reducing of growth rate and fecundity, as can be seen in the case of barley. The adaptation of some insects to various host plants could be due to their ability in altering the specificities of their gut proteases in response to qualitative changes in dietary protein content (Gatehouse *et al.*, 1997). Mechanisms of insect resistance to protease inhibitors (PIs) include the upregulation of enzymes that degrade the PIs (Yang *et al*., 2009), the induction of enzymes that resist inactivation by PIs (Broadway, 1996), and overproduction of enzymes to maintain normal levels of gut proteolysis (Brioschi *et al*., 2007).

Conclusion

As is known, proteins have fundamental roles in fecundity rate of insects, which are supplied by proteases. The highest rate of protein resources occurred in midgut of larvae which were reared on corn flour resulting in adults with highest rate of fecundity and which is supported by earlier researchers. These results could be used in mass rearing of *E. kuehniella* and natural enemies on corn flour as a suitable cereal flour. On the other hand, the Mediterranean flour moth is an important pest in stored products, determination of barley as an unsuitable and resistant hosts among mentioned cereal could be used to management of *E. kuehniella* in stored products by producing transgenic plants, which confirmed by other researchers.

Acknowledgements

We would like to thank Maryam Frouzan Seed and Plant Breeding Institute for preparing of tested cereals and Maryam Alikhani at the Organization of Plant Protection in West Azerbayjan-Urmia for preparing insect colony.

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تأثیر آردهای غلات مختلف بر ویژگی‌های بیولوژیکی و فیزیولوژیکی لاروهای شب‌پره مدیترانه‌ای آرد *Ephestia kuehniella* (Lepidoptera: Pyralidae)

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چکیده: شب‌پره مدیترانه‌ای آرد *Ephestia Kuehniella* Zeller یکی از میزبان‌های مرسوم برای پرورش دشمنان طبیعی در برنامه‌های کنترل بیولوژیک می‌باشد. در این تحقیق تأثیر آردهای غلات مختلف (گندم، جو و ذرت) روی برخی پارامترهای بیولوژیکی، شاخص‌های تغذیه‌ای و فعالیت آنزیم‌های گوارشی لاروهای سن پنجم *E. kuehniella* جهت تعیین تناسب میزبانی آنها برای لاروها مورد مطالعه قرار گرفت. بیش‌ترین میزان نرخ بقای لاروی (۰/۸۸) و شاخص رشدی (۹/۷۷) در لاروهای تغذیه شده از ذرت به‌دست آمد. بیش‌ترین میزان شاخص‌های نرخ رشد نسبی (۰/۳۳ ± ۹/۱۷ میلی‌گرم/میلی‌گرم/روز) و کارایی تبدیل غذای خورده شده (۱/۱۱ ± ۱۲/۰۸ درصد) لاروی روی ذرت مشاهده شد. بیش‌ترین میزان پروتئین (۲/۳۱ ± ۶۳/۷۷ میلی‌گرم/میلی‌لیتر) و متعاقب آن فعالیت تریپسینی (۰/۰۰ ± ۰/۰۰۱۲ واحد فعالیت آنزیمی/میلی‌گرم) و کیمو تریپسینی (۰/۰۰۱ ± ۰/۵۴۳ واحد فعالیت آنزیمی/میلی‌گرم) در لاروهای تغذیه شده با آرد ذرت به‌دست آمد. نتایج حاصله نشان داد که به‌دلیل تأثیر بالای آرد ذرت بر پارامترهای بیولوژیکی و فیزیولوژیکی لاروهای شب‌پره مدیترانه‌ای آرد، می‌تواند به‌عنوان میزبان مناسبی برای پرورش آزمایشگاهی بید آرد مورد استفاده قرار گیرد.

واژگان کلیدی: شب‌پره مدیترانه‌ای آرد، غلات، شاخص‌های تغذیه‌ای، فعالیت آمیلازی و پروتئازی