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Biological and Biochemical Characteristics for Quality Control of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) Reared on a Liver-Based Diet

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Biological and biochemical parameters of a flightless strain of *Harmonia axyridis,* fed on a pork liver–based artificial diet and on *Ephestia kuehniella* eggs as controls, were compared. The diet-grown larvae showed a significantly longer developmental time and a lower adult emergence rate compared to control larvae. The weights of the newly emerged adults were significantly higher for adults fed *E. kuehniella* eggs during their larval stages than fed the artificial diet. In contrast, larval food source had no effect on the duration of the pre-oviposition period or adult longevity. For adults fed on *E. kuehniella* eggs as larvae, a significantly longer pre-oviposition period, lower daily weight gain and fecundity were found for the diet-fed females compared to those fed on *E. kuehniella* eggs throughout the life span. The adult food source had no significant effect on longevity and fertility. Lower amino acid and fatty acid contents (in particular C16:1 and C18:3n-3) were found for the prepupae and newly emerged females obtained from diet-reared larvae compared to controls. Deficiencies in fatty acids C16:1 and C18:3n-3 were also observed in females obtained from *E. kuehniella* egg-reared larvae and fed on diet from adult emergence. The analyses of the foods showed deficiencies in artificial diet, especially for some amino and fatty acids. The results suggest a non-optimal composition of the artificial diet and some possibilities for its improvement. However, this polyphagous predator could be reared from first instar larvae to fully reproductive adults on a pork liver–based artificial diet. Arch. Insect Biochem. Physiol. 68:26–39, 2008. © 2008 Wiley-Liss, Inc.

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INTRODUCTION

The multicolored Asian ladybeetle *Harmonia axyridis* (Pallas) is a polyphagous predator that prefers aphids (Hukusima and Kamei, 1970), but also feeds on coccids (McClure, 1987; Hodek and Honêk, 1996), lepidopteran eggs (Schanderl et al., 1988), as well as other insects (Koch, 2003). *H. axyridis* was introduced into the United States in 1978 and France in 1982 for the biological control of aphids (Ferran et al., 1997). It is a very active and highly efficient predator of different species of aphids and scale insects (Krafsur et al., 1997). This species has expanded into many states in the United States and Canada where it has become established successfully and has reached high local population levels, contrary to France where no important populations have been detected so far.

A natural flightless mutation was selected in a laboratory population (Ferran et al., 1998; Tourniaire

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et al., 2000) to increase the efficiency of *H. axyridis* in biological control and limit its ability to become established during winter. *H. axyridis* can be reared in large numbers with lepidopteran eggs (*Ephestia kuehniella* Zeller) as a substitute for prey (Schanderl et al., 1988). Nevertheless, the main problem limiting the commercial use of *H. axyridis* as a biological control agent is the cost of the current large-scale rearing procedure. Mass production of this coccinellid could be enhanced by the availability of an artificial, inexpensive diet that is more flexible to use than *E. kuehniella* eggs. *H. axyridis* has been reared with partial success on some artificial diets (Okada et al., 1971; Matsuka and Okada, 1975; Niijima et al., 1986; Obrycki and Kring, 1998; Dong et al., 2001), but none is currently used at a commercial level. In addition to their mass production, quality control of artificially reared entomophagous insects, including *H. axyridis*, is very important, because the objective of in vitro culture of beneficial insects is not only to obtain high yields of larvae and/or adults, but also to obtain insects that satisfy the objectives for which they were produced (Grenier and De Clercq, 2003).

For the mass production and use of *H. axyridis* as a biocontrol agent, two developmental stages are critical: the young larvae (second and third instars), which are used for releases, and the adults, which are used to maintain stock cultures. Recently, some artificial diets were developed to feed second through fourth instar larvae, and adults. These diets were tested and improved with an analytical approach and subsequent interactions, by comparing the biochemical composition of the ladybeetle larvae reared on the artificial diets vs. those fed on *E. kuehniella*. With the best diets, adult emergence was near 80% for insects starting as second instar larvae (Specty, 2002).

In the present work, we compared the biochemical composition and some biological parameters of the ladybeetles reared on a liver-based diet vs. those fed on *E. kuehniella* eggs as the control. To focus the present study on post-larval development, the biochemical analyses concerned the body content of amino acids and fatty acids of prepupae and adults at emergence, and at first oviposition. As suggested by Grenier and De Clercq (2003) and Cohen (2004), carcass analyses may provide information about the nutritional needs of the predator as well as a necessary reference for deficiency and imbalance corrections in the diet composition. The methods used in this study could be considered as a model approach to develop artificial diets for other coccinellids and predatory entomophagous insects.

MATERIALS AND METHODS

Stock Culture

All the ladybeetles, originating from INRA Antibes (France), were maintained in a growth chamber at 26 ± 1°C, 60–70% RH and a 16L:8D photoperiod, for stock cultures and experiments. The laboratory colony of the flightless strain of *H. axyridis* (Ferran et al., 1998; Tourniaire et al., 2000) was started in 2002. The coccinellids were maintained in Plexiglas cages and fed on *E. kuehniella* eggs as described by Schanderl et al. (1988). The UV-irradiated *E. kuehniella* eggs were purchased from Biotop (Valbonne, France). They were stored at –20°C until use.

Artificial Diet

The composition of the artificial diet used in the current study was described by Specty (2002). This diet was prepared by blending a portion of fresh pork liver sold as human food (35.8 g), Isio 4 oil (Lesieur®) (2.4 g), olive oil (3.0 g), sucrose (12.0 g), glycerin (3.0 g), aqueous amino acid solution (40.0 g), yeast extract (3.5 g), and Vanderzant's vitamin mixture (Sigma®) (0.3 g). The water amino acid solution contained Tyr (0.25 mg/g), His (0.62 mg/g), Arg (0.94 mg/g), and Ethanolamine (0.19 mg/g). The diet was stored at –20°C until used. Contrary to Specty (2002), the diet was supplied to the larvae or adults as 0.3–0.5-cm-diameter drops that were deposited on Bristol paper, dried at room temperature (22–24°C) for 18–24 h, and subsequently stored at 4°C until used. Before use, the drops were detached from the substrate. An Eppendorf tube (1.5 ml) filled with distilled water and plugged with cotton was provided to satisfy water needs.

Biological Parameters

Efficacy of the artificial diet for rearing *H. axyridis* from the first instar larva through emergence. Egg clusters from conventional rearing were collected and placed in 5.5-cm-diameter plastic Petri dishes. Newly hatched larvae were placed individually in Plexiglas cylindrical chambers (diameter 4.5 cm, height 2.5 cm) to avoid cannibalism. The larvae were supplied artificial diet ad libitum (number of larvae = 220) or *E. kuehniella* eggs (number of larvae = 150) every other day. Mortality was recorded daily throughout the pre-imaginal development up to emergence. Newly emerged adults were weighed using a Mettler microbalance (0.1 mg sensitivity) and, subsequently, one male and one female were paired and reared together in Plexiglas cylindrical chambers (diameter 8.0 cm, height 8.0 cm). Paired adults obtained from both larval food regimens were reared ad libitum on *E. kuehniella* eggs. The following parameters were evaluated: (1) development time in days from first instar to adult emergence; (2) number of emerged adults and % emergence calculated over the original number of first instar larvae; (3) pre-oviposition duration in days; (4) female daily weight gained from emergence to first oviposition in mg/day, i.e. (weight at first oviposition–weight at emergence)/pre-oviposition duration; (5) number of eggs/female laid during the 10 days following the first egg laying (= E_{10}), as a fecundity expression (Ferran et al., 1998; Evans et al., 1999), and number of eggs laid per mg of female weight (= E_{10}/female weight at the first egg laying); (6) % first instar larvae hatched from eggs as a criterion to evaluate fertility; (7) adult male and female longevity in days, calculated from emergence to death. For each treatment, every larva (pre-imaginal parameters) or couple (adult parameters) was considered as a replicate. Only the couples including females, which oviposited at least one fertile egg, were considered in the final result evaluation.

Efficacy of the artificial diet for rearing *H. axyridis* adults. Larvae were individually reared and supplied *E. kuehniella* eggs ad libitum every other day, as in the stock culture. After emergence, one male and one female were paired. Each couple was placed in a Plexiglas cylindrical chamber (diameter 8.0 cm, height 8.0 cm) and fed with *E. kuehniella* eggs as the control (= *E. kuehniella*/*E. kuehniella*), or artificial diet (= *E. kuehniella*/artificial diet). The reproductive performance of adults was evaluated on 15 couples for each treatment. The results were expressed in terms of the parameters number (3–7) described previously. As above, only the couples including females, which oviposited at least one fertile egg, were considered in the result evaluation.

Biochemical Parameters

The analyses were performed on food offered (*E. kuehniella* eggs and artificial diet), and on insects, as prepupae (late fourth instar larvae, before fixing to a substrate for pupation), newly emerged females, and females at the first egg laying. All the total amino acid analysis samples were hydrolyzed, and analyzed by ion exchange chromatography in an automatic amino acid analyzer (Beckman 6300, Roissy, France), as described in Specty et al. (2003). Total lipids were extracted from the food offered or from the whole insect body according to the modified method of Folch et al. (1957), and analyzed using gas chromatography (Hewlett Packard 5890, Wilmington, DE), as described in Specty et al. (2003) and modified by Sighinolfi (2005).

Statistical Analyses

Pre-imaginal mortality and adult emergence were analyzed by 2 × 2 contingency tables. All the other biological parameters were analyzed by one-way analysis of variance, or the Kruskal-Wallis non-parametric procedure (when variance heterogeneity occurred). For the biochemical analyses, the data were expressed as nmol or µg per mg of fresh weight or as percentages, and were analyzed by one-way analysis of variance or the Kruskal-Wallis test. All per-

TABLE 1. Developmental Times (in Days) From Egg to Adult Emergence, Adult Emergence (%), and Male and Female Adult Weight (mg) of *H. axyridis* Reared Either on *E. kuehniella* Eggs or on an Artificial Diet*

Larval food	Developmental duration (day)	Adult emergence rate (%)	Adult weight (mg) Males	Adult weight (mg) Females
E. kuehniella	15.3 ± 0.04a (n = 129)	91.5b (n = 141)	33.1 ± 0.3b (n = 58)	38.5 ± 0.3b (n = 69)
Artificial diet	25.5 ± 0.3b (n = 53)	27.6a (n = 192)	19.0 ± 0.7a (n = 21)	21.0 ± 0.6a (n = 32)

*Number of replicates is given in parentheses below the means (± SE). Values followed by different letters in a column are significantly different, using the Kruskall-Wallis test (developmental duration and female weight), the one-way analysis of variance (male weight), or the 2 × 2 contingency tables (adult emergence rate) ($P < 0.05$).

centage values were transformed for the analyses using an arcsine transformation. Statistical tests were done with STATISTICA 6.0 (StatSoft Inc., 2001); results were given as F and *P* values for ANOVA, and as H and *P* values for Kruskal-Wallis tests.

RESULTS

Biological Parameters

Efficacy of the artificial diet for rearing *H. axyridis* from first instar larva through emergence. The artificial diet allowed the development of *H. axyridis* from first instar larvae to adults. However, all the pre-imaginal parameters were significantly affected by the food source (Table 1). Compared to those grown on *E. kuehniella* eggs, the larvae grown on the artificial diet showed significantly longer developmental times (H = 163; n = 182; $P = 0.001$) and lower adult emergence rates (27.6% emerged adults on the artificial diet vs. 91.5% on *E. kuehniella* eggs, $\chi^2 = 134$; $P = 0.001$). Most individuals reared on the artificial diet died either as third instar larvae or at emergence (38.0 and 16.7% respectively, relative to first instar larvae). The weights of the newly emerged adults were significantly greater for the control coccinellids (for females H = 65; n = 101; $P = 0.001$ and for males $F_{1,77} = 454$; $P = 0.01$) as compared with the artificial diet group.

Data concerning adults obtained from larvae reared either on *E. kuehniella* eggs or artificial diet, are shown in Table 2. The daily weight gains and the numbers of eggs laid by females (E_{10}) reared as larvae on the artificial diet were significantly lower as compared with the control insects ($F_{1,19} = 7.8$; $P = 0.01$, and H = 15; n = 21; $P < 0.01$, respectively). In contrast, the larval food sources had no effect on the duration of the pre-oviposition period ($F_{1,19} = 0.3$; $P = 0.59$), on the number of eggs laid per mg of female weight ($F_{1,19} = 2.8$; $P = 0.16$), and on adult longevity, (for males $F_{1,28} = 0.55$; $P = 0.46$ and for females $F_{1,28} = 1.2$; $P = 0.17$). Fertility (% hatched eggs) was higher for females fed with the artificial diet in the larval stages ($F_{1,19} = 5.5$; $P = 0.03$).

Efficacy of the artificial diet for rearing *H. axyridis* adults. Egg laying occurred in 13 of the 15 couples fed on the artificial diet (Table 3). The adult food source had no significant effect on fertility ($F_{1,15} = 0.6$; $P = 0.47$) and adult longevity (males $F_{1,30} = 1.4$; $P = 0.25$ and females $F_{1,30} = 2.7$; $P = 0.11$). However, for females fed the artificial diet, the pre-oviposition period was much longer (H = 10.5;

TABLE 2. Daily Weight Gain (mg/day), Pre-Oviposition Duration (Day), Total Eggs Laid in 10 Days (= E_{10}), Fertility (% Hatched Eggs), Number of Eggs Per mg of Female Weight (n/mg), and Adult Longevity (Day) of *H. axyridis* Adults Fed on *E. kuehniella* Eggs*

Larval/adult foods	Daily weight gain (mg/day)	Pre-oviposition (day)	Total eggs laid in 10 days (= E_{10})	Fertility (% hatched eggs)	No. eggs/ female weight (n/mg)	Longevity (day) Males	Longevity (day) Females
E. kuehniella/*E. kuehniella*	2.7 ± 0.2b (n = 10)	7.8 ± 0.3a (n = 10)	546 ± 32b (n = 10)	63.0a (n = 10)	9.1 ± 0.6a (n = 10)	71.1 ± 9.5a (n = 15)	87.7 ± 9.4a (n = 15)
Artificial diet/*E. kuehniella*	2.1 ± 0.1a (n = 11)	8.1 ± 0.4a (n = 11)	303 ± 16a (n = 11)	73.5b (n = 11)	8.0 ± 0.5a (n = 11)	79.9 ± 6.8a (n = 15)	108.5 ± 11.3a (n = 15)

*The larvae were fed on either *E. kuehniella* or artificial diet. Number of replicates is given in parentheses below the means (± SE). Values followed by different letters in a column are significantly different, using the Kruskall-Wallis test (E_{10}) or the one-way analysis of variance (other parameters) ($P < 0.05$).

TABLE 3. Biological Parameters of *H. axyridis* Adults Fed on *E. kuehniella* Eggs or Artificial Diet: Daily Weight Gain (mg/day), Pre-Oviposition Duration (Day), Total Eggs Laid in 10 Days (E_{10}), Fertility (% Hatched Eggs), Number of Eggs Per mg of Female Weight (n/mg), and Adult Longevity (Day)*

Adult food	Daily weight gain (mg/day)	Pre-oviposition (day)	Total eggs laid in 10 days (= E_{10})	Fertility (% hatched eggs)	No. eggs/ female weight (n/mg)	Longevity (day) Males	Longevity (day) Females
E. kuehniella	3.0 ± 0.3b (n = 4)	6.0 ± 0.0a (n = 5)	483 ± 38b (n = 5)	34.5a (n = 5)	8.5 ± 0.5b (n = 5)	67.9 ± 24.4a (n = 12)	49.6 ± 14.4a (n = 12)
Artificial diet	1.1 ± 0.1a (n = 13)	13.5 ± 0.9b (n = 13)	47 ± 7a (n = 13)	29.7a (n = 13)	0.9 ± 0.1a (n = 13)	79.3 ± 28.9a (n = 20)	60.9 ± 21.4a (n = 20)

*During the larval stages, all coccinellids were fed on *E. kuehniella* eggs. Number of replicates is given in parentheses below the means (± SE). Values followed by different letters in a column are significantly different, using the Kruskall-Wallis test (pre-oviposition period and E_{10}) or the one-way analysis of variance (other parameters) ($P < 0.05$).

n = 18; $P < 0.01$), and the daily weight gain was much lower ($F_{1,15} = 59.2$; $P < 0.01$) compared with the controls. Also the total number of eggs laid and the number of eggs laid per mg of female weight were dramatically lower compared with those observed for females nourished on *E. kuehniella* eggs (respectively H = 10.3; n = 18; $P < 0.01$, and $F_{1,15} = 435$; $P < 0.01$).

Biochemical Parameters

Composition of the two foods tested. The total amount of amino acids in the artificial diet (mean of 3 independent analyses ± SE = 1,563 ± 59 nmol/mg) was significantly higher than that recorded for *E. kuehniella* eggs (mean of 2 independent analyses ± SE = 1,162 ± 25 nmol/mg) ($F_{1,3} = 37.1$; $P < 0.01$). Moreover, the artificial diet contained a significantly higher total amount of fatty acids than *E. kuehniella* eggs ($F_{1,8} = 583$; $P < 0.001$) (mean of 4 or 5 independent analyses ± SE = 158.1 ± 2.6 nmol/mg for the artificial diet and = 91.4 ± 1.2 nmol/mg for *E. kuehniella* eggs). The patterns of relative amino acid and fatty acid content (respectively expressed as % of total amino acids and fatty acids) are shown in Figure 1. Significant differences were observed for twelve amino acids, but values in percentages showed less than two units of divergence, except for Glu. Compared to *E. kuehniella* eggs, the artificial diet showed significantly higher proportions of Thr, Pro, Ala, Val, Ile, Leu, Phe, Lys, and significantly lower proportions of Glu, Gly, Met, and Tyr (Fig. 1A). With regard to the pattern of relative fatty acid content, the artificial diet showed significantly lower proportions of C16:0, C16:1, and C18:3n-3 and significantly higher proportions of C18:0, C18:1, and C18: 2n-6 (Fig. 1B).

Efficacy of the artificial diet for rearing *H. axyridis* from first instar larva through emergence. Data concerning the biochemical composition of *H. axyridis* are shown in Table 4. In the prepupae of *H. axyridis*, the total amounts of amino acids and fatty acids were higher when the larvae were reared on *E. kuehniella* eggs than on artificial diet (H = 6; n = 9; $P < 0.05$ for the amino acids; and $F_{1,7} = 54.6$; $P < 0.01$ for the fatty acids). Similar results were observed in the newly emerged females fed in the larval stages on the two food sources (total amino acid contents $F_{1,7} = 8.9$; $P < 0.05$; total fatty acid contents $F_{1,8} = 259$; $P < 0.01$).

The patterns of relative amino acid content (expressed as % of total amino acids) were similar for newly emerged females of *H. axyridis* grown on *E. kuehniella* eggs or artificial diet. Significant differences were observed for ten amino acids, although values in percentages showed less than three units of divergence (Fig. 2A). In contrast, the patterns of fatty acids were very different between newly-emerged females grown on the two food sources, with significantly higher proportions of C18:0, and significantly lower of C16:0, C16:1, and C18:3n-3 for the artificial diet group. The latter two fatty acids were totally lacking in females grown on artificial diet (Fig. 2B). Myristic acid (C14:0) was present in trace amounts (<0.5 µg/mg) in female adults reared as larvae on both food sources.

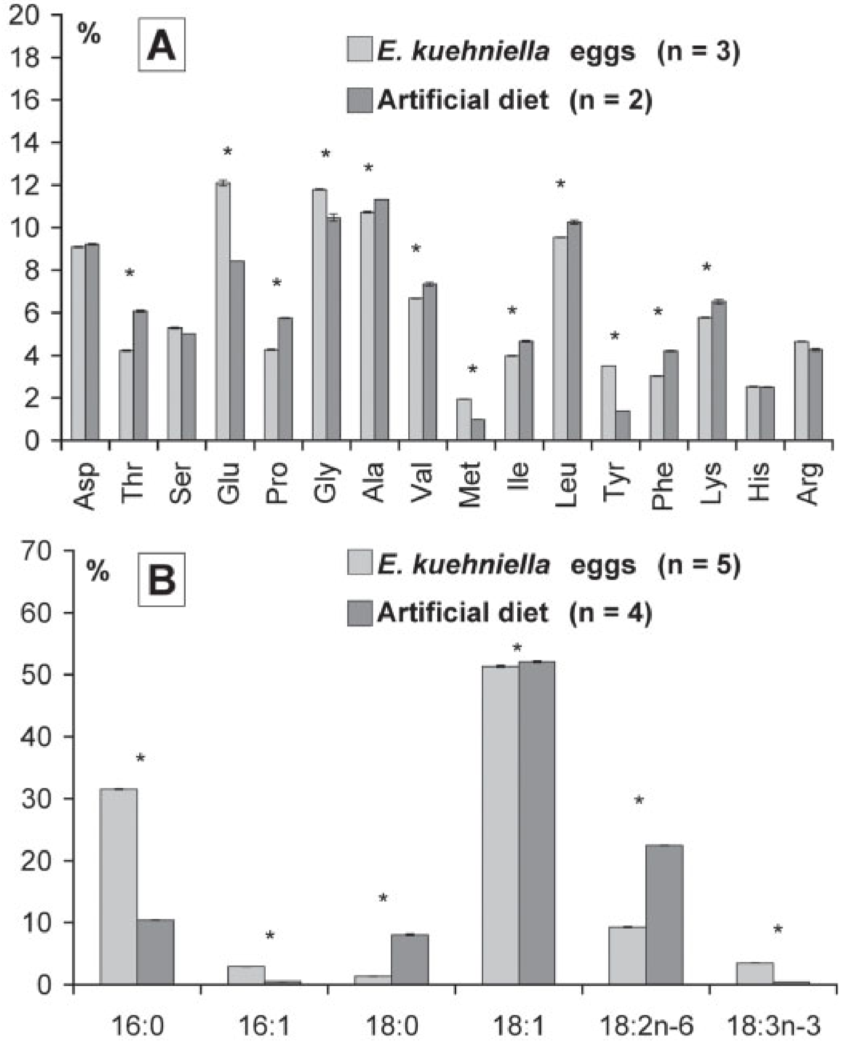

Fig. 1. Relative amino acid (**A**) and fatty acid (**B**) contents of *Ephestia kuehniella* eggs or artificial diet. The mean values of independent analyses (n = number of replicates) are given together with their confidence intervals. *Significant difference ($P < 0.05$). Abbreviations for fatty acids: 16:0 = palmitic acid, 16:1 = palmitoleic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2n-6 = linoleic acid, 18:3n-3 = α-linolenic acid.

In females at the first oviposition, when larvae and adults were fed, respectively, on artificial diet and *E. kuehniella* eggs (= artificial diet/*E. kuehniella*), the total amount of amino acids (1,937 ± 34 nmol/mg) was higher compared with the control (1,400 ± 106 nmol/mg) ($F_{1,7} = 18.8$; $P < 0.01$). The patterns expressed as % of relative amino acid contents for ladybeetles at first oviposition were, however, similar for the two foods as well as with that observed in the newly emerged females. Slight differences were observed for Thr, Val, Ile, Leu, Phe, and His, which were higher in females supplied with artificial diet in the larval stages. A more important difference was observed for

TABLE 4. Total Amino Acid (nmol/mg of Fresh Weight) and Total Fatty Acid (µg/mg of Fresh Weight) of Prepupae and Newly-Emerged Females of *H. axyridis* Grown on *E. kuehniella* Eggs or Artificial Diet*

	Prepupae		Newly-emerged females	
Larval food	Amino acid (nmol/mg)	Fatty acid (µg/mg)	Amino acid (nmol/mg)	Fatty acid (µg/mg)
E. kuehniella	1409 ± 74b	110.0 ± 3.2b	1225 ± 47b	73.4 ± 2.8b
Artificial diet	951 ± 13a	64.1 ± 5.7a	887 ± 115 a	17.3 ± 2.0a

*The mean value of 5 independent analyses are given, together with their Standard Error. Values followed by different letters in a column are significantly different, using the Kruskall-Wallis test (pre-pupae amino acids) or the one-way analysis of variance (other parameters) ($P < 0.05$).

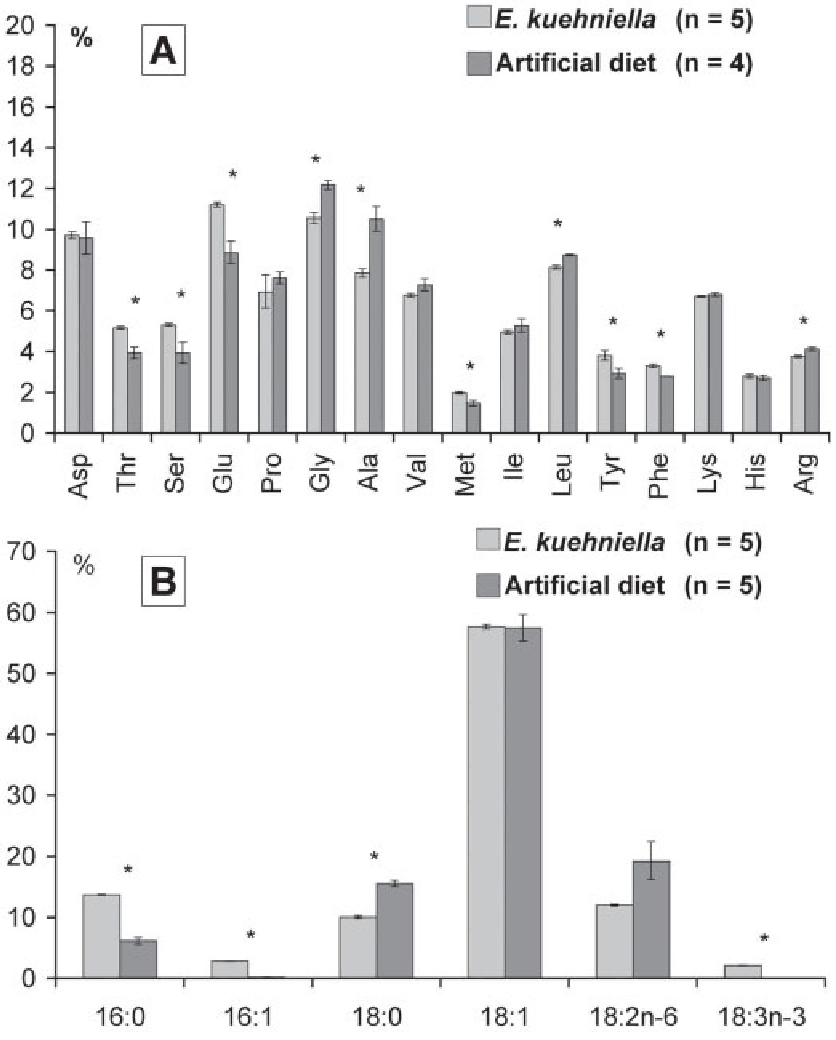

Fig. 2. Relative amino acid (**A**) and fatty acid (**B**) contents of *Harmonia axyridis* newly emerged females. Larvae were reared on control (*Ephestia kuehniella*) or artificial diet. The mean values of independent analyses (n = number of replicates) are given together with their confidence intervals. *Significant difference ($P < 0.05$). See Figure 1 for abbreviations of fatty acids.

Ala, which was higher in the control (data not shown).

No difference occurred between the total amounts of fatty acids in the controls (50.4 ± 4.2 μg/mg) and females supplied with artificial diet in the larval stages (46.9 ± 2.7 μg/mg) ($F_{1,7} = 0.5$; $P = 0.53$). When the results were expressed as relative percentages for individual fatty acids, there were significant differences for some of them (Fig. 3).

Efficacy of the artificial diet for rearing *H. axyridis* adults. The total amino acid contents in females at the first oviposition was 1,532 ± 50 nmol/mg in the control and 1,418 ± 43 nmol/mg for the artificial diet (non-significant difference, $F_{1,8} = 1.1$; $P = 0.33$), and the patterns expressed in % were similar for both groups.

Also total fatty acid amounts were not significantly different for females fed on *E. kuehniella* eggs (65.7 ± 8.3 μg/mg) vs. artificial diet (85.5 ± 9.7 μg/mg) ($F_{1,8} = 2.4$; $P = 0.16$). Differences for individual fatty acids were observed with C16:1 and C18:3n-3 completely absent in females fed on artificial diet (Fig. 4).

DISCUSSION

Biological Parameters

The artificial diet used in the present study proved to be suitable for the development of *H. axyridis* from newly hatched larvae to adults, and supported adult survival and reproduction. The

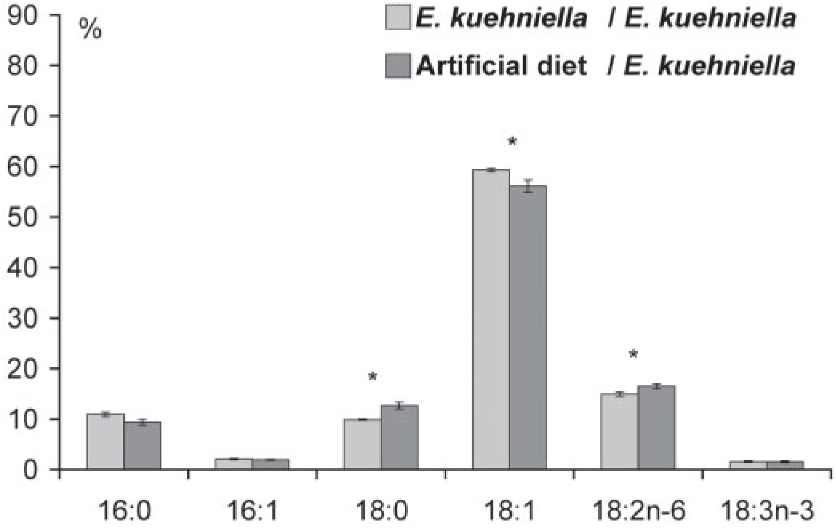

Fig. 3. Relative fatty acid content of *Harmonia axyridis* females at first oviposition. Larvae were reared on control food (*Ephestia kuehniella*) or artificial diet. Adults from each food treatment (control or artificial diet) were reared on *E. kuehniella* eggs. The mean values of 5 (*E. kuehniella*/*E. kuehniella*) or 4 (Artificial diet/*E. kuehniella*) independent analyses are given together with their confidence intervals. *Significant difference ($P < 0.05$). See Figure 1 for abbreviations of fatty acids.

biological parameters used here as measures of fitness were better for the food source consisting of *E. kuehniella* eggs than for the artificial diet. The lower emergence rates and adult weights obtained on artificial diet vs. *E. kuehniella* eggs were consistent with those observed for other coleopteran predators grown in vitro rather than in vivo (Racioppi et al., 1981; Hussein and Hagen, 1991; Ogura and Hosoda, 1995). Moreover, Specty et al. (2003) showed that a number of biological parameters, including emergence and adult weight, were higher for *H. axyridis* fed as larvae on *E. kuehniella* eggs than for ladybeetles reared on one of its natural prey, the pea aphid *Acyrthosiphon pisum* (Harris). In our study, adult emergence and male or female weight, obtained on *E. kuehniella* eggs, were comparable with those observed by Specty et al. (2003) for the same food source (i.e., 96.7%, 29.7 and 36.1 mg, respectively). The same parameters were lower for aphid-reared coccinellids (i.e., 76.2%, 25.9 and 29.4 mg, respectively), but higher than those reported here for the artificial diet, which proved to be less suitable than the aphid prey as a food for *H. axyridis* larvae. Moreover, the larvae grew faster on *E. kuehniella* eggs than on the artificial diet in the present study, while Specty et al. (2003) found that the developmental times were similar for insects reared on either *E. kuehniella* eggs or aphids. Hukusima and Ohwaki (1972) reported longer developmental times and lower adult weights

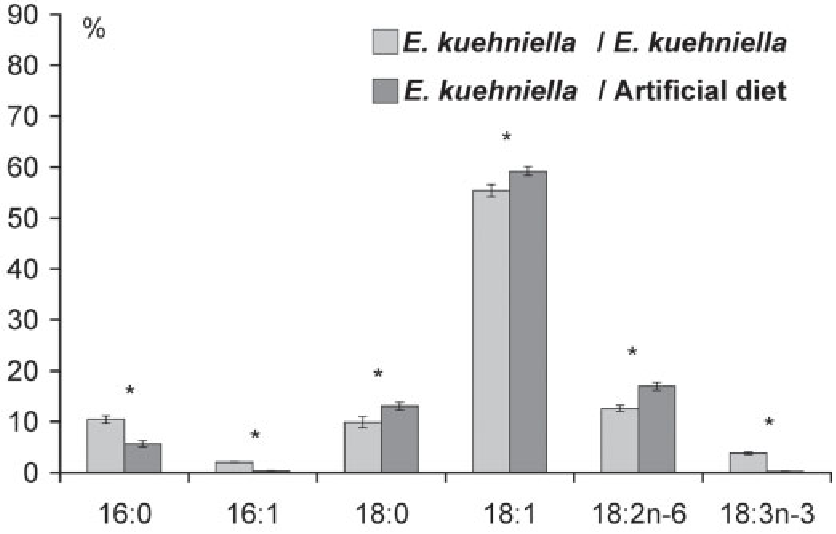

Fig. 4. Relative fatty acid content of *Harmonia axyridis* females at the first oviposition, reared in the larval stage on *Ephestia kuehniella* eggs. Adults were reared on control food (*E. kuehniella*/*E. kuehniella*) or artificial diet (*E. kuehniella*/Artificial diet). The mean values of 5 independent analyses are given together with their confidence intervals. *Significant difference ($P < 0.05$). See Figure 1 for abbreviations of fatty acids.

for *H. axyridis* nourished as larvae with *Myzus persicae* (Sulzer) in conditions of quantitative alimentary restriction. They found a negative correlation between adult weight and development time in this ladybeetle, which was later confirmed by Ueno (1994). As a consequence, it may be hypothesized that *H. axyridis* larvae fed on the artificial diet undergo a stress condition comparable to alimentary restriction with limited natural prey.

Females fed artificial diet during their larval life showed an important fitness recovery (in particular with regard to the pre-oviposition duration and the number of eggs per mg of female weight) when adults were supplied with *E. kuehniella* eggs. For these females, both the egg fertility and the pre-oviposition duration were comparable to those of females fed on *E. kuehniella* eggs their entire lives. Similarly, Evans (2000) showed that the females of *Coccinella transversalis* Fabricius fed on artificial diet as larvae and adults quickly recovered their oviposition capacity when the diet was integrated with natural prey. In the present study, the quality recovery was incomplete, and differences remained for absolute daily weight gains and the total number of eggs laid in ten days. The number of eggs laid per mg of female weight was similar for both food sources. Previous studies have shown that the weights of a number of entomophagous insects reared in vivo or in vitro were correlated with other biological parameters, including the time between adult emergence and the beginning of oviposition and the number of eggs laid (Grenier and De Clercq, 2003; Omkar and Srivastava, 2003). Weight alone cannot be considered an absolute quality assessment criterion, especially as a parameter to predict the values of other traits (Dindo et al., 2006). Brun and Iperti (1978) stated, with regard to *H. axyridis*, that the "number of eggs laid during an arc of time" is a more representative parameter for the quality evaluation of coccinellids reared in captivity.

The biological characteristics of *H. axyridis* adults were significantly affected by food source. Adult females fed artificial diet showed lower daily weight gains, a twofold increase in pre-oviposition period, and lower numbers of eggs laid compared with the controls. Problems concerning egg laying in adults fed on artificial diet have been observed by several authors, for numerous predator insects (Kariluoto, 1980; Racioppi et al., 1981). However, adult longevity of *H. axyridis* was unaffected by food source, contrary to some heteropteran predators grown on artificial diets (De Clercq et al., 1998; Adams, 2000).

Biochemical Parameters

In the artificial diet, the total amount of amino acids was higher than in *E. kuehniella* eggs, and the patterns, in percentages, showed lower values for four amino acids, including two important essential ones (Met and Tyr). The compositions of proteins are a characteristic of the species and the patterns of total amino acids in body contents do not greatly vary according to the food consumed by the larvae. The differences could result from the variations of the composition of the free amino acid pool (Chen, 1985). These assessments are supported by our results showing patterns of relative amino acid content similar for newly emerged females fed as larvae on the two kinds of foods. Nevertheless, in the artificial diet, the significantly lower percentages of some components, key for protein synthesis, such as sulphur (Met) and aromatic amino acids (Tyr), could be limiting factors for normal growth. The important deficiency in Tyr in the artificial diet was not completely compensated by the great amount of Phe (its precursor) leading to deficiencies in both aromatic amino acids (Tyr and Phe) in newly emerged females fed as larvae on artificial diet.

Females at the first egg laying, transferred from artificial diet on *E. kuehniella* eggs at emergence, were richer in total amino acid content than the controls. But the patterns of amino acids expressed as % of total amino acid for ladybeetles were similar for the two kinds of foods. This higher concentration expressed per fresh weight could be due to the smaller size of the artificial diet-reared females obtained, and/or the accumulation of free amino acids in hemolymph. In many insects, hemolymph, functioning as a storage reservoir for many materials, is characterized by a high level of free amino acids, often occurring in excess related to protein synthesis (Chen, 1985). This level reflects the metabolic state of the insect and, in this regard, further investigations on the hemolymph compartment will be required to better adjust amino acid supplies from artificial diet to ladybeetle needs for anabolism.

The total amount of fatty acids was also higher in artificial diet than in *E. kuehniella* eggs. Most of the fatty acids consumed are stored as energy sources in neutral lipids, with limited metabolic changes leading to similar patterns in fatty acids between food ingested and insect carcass, as observed for parasitoid insects (Delobel and Pageaux, 1981). Some other fatty acids have a key role as structural elements incorporated in membrane phospholipids or as signal molecules (Downer, 1985; Stanley, 2000). The patterns of fatty acids were very different between the two foods, with lower percentages of three fatty acids (C16:0, C16:1, and C18:3n-3) in the artificial diet. In the newly emerged females, fed as larvae with artificial diet, the two later unsaturated fatty acids were completely absent, while they were present in significant percentages in *E. kuehniella*–fed females. Thus, C16:1 and C18:3n-3 could be important limiting factors for the normal development of the coccinellids fed on artificial diet. It is well accepted that specific lipids are necessary dietary requirements for all insects, particularly the immature stages (Reinecke, 1985).

Higher levels of these two fatty acids were observed at first oviposition when females fed artificial diet as larvae were transferred on *E. kuehniella* eggs from emergence. Similarly, smaller amounts of the same fatty acids occurred in females at first oviposition obtained from *E. kuehniella*–reared larvae subsequently fed on artificial diet after emergence, thus suggesting a non-optimal composition of the artificial food, with too low concentrations in C16:1 and C18:3n-3, which could be improved. Concerning the lower fecundity of the diet-reared *H. axyridis*, we may hypothesize a correlation with the observed fatty acid deficiency. Many studies suggest that predators require a complete and well-balanced diet to ensure maximal reproductive potential (Thompson and Hagen, 1999; Cohen, 2004). The polyunsaturated di- and tri-enoic C18 fatty acids (linoleic and linolenic) were found to be essential for a number of Lepidoptera and Orthoptera (Sivapalan and Ganapragasam, 1979; Reinecke, 1985). Coleopterans also showed slow growth and decreased adult fecundity in response to deficiency in these polyunsaturated fatty acids (Earle et al., 1967). Moreover, these authors showed that omission of triglycerides from the diet of larvae influenced fecundity more than their omission from the diet of adults. This could explain why only partial qualitative recovery of the biological parameters was observed for *H. axyridis* in the present study, after switching artificial diet–fed larvae to *E. kuehniella* eggs as adults.

When larvae were reared on the artificial diet rather than *E. kuehniella* eggs, the prepupae and newly emerged females showed lower total amounts of amino acids and fatty acids. These differences occurred despite the fact that the total amounts of amino acids and fatty acids in the artificial diet were significantly higher than those recorded for *E. kuehniella* eggs. The deficiencies found in diet-obtained ladybeetles may be either attributed to food-intake or conversion difficulties from their part. Specty (2002) showed that the food intake of fourth instar larvae of *H. axyridis* was lower on artificial diet compared to *E. kuehniella* eggs or the natural prey *A. pisum*. In ladybeetles reared both on control food and artificial diet in the larval stages, the fatty acid content (µg/mg) dramatically dropped in newly emerged females compared with prepupae. Moreover, the difference of weight between prepupae and newly emerged females (loss during pupation) was more important in predators reared on artificial diet, possibly related to a fatty acid storage in the artificial diet–reared prepupae not well fitted with the needs. This may be also explained by a disparity of biochemical conversions during the pupal stage between the coccinellids reared on the two food sources, emphasized by unbalances in the main nutrient classes (proteins, lipids, and carbohydrates) of the artificial diet. Thus, we can hypothesize that this high catabolism of fatty acids could occur during pupation when energy sources such as carbohydrates are limiting. Only synthetic diets with well-adapted proportions of the essential nutrients could support normal growth in many insects (Dadd, 1985). It has, however, to be noted that adults reared on aphids were also less rich in total amino acids (20%) and total fatty acids (50%) than those reared on *E. kuehniella* eggs (Specty et al., 2003). Considering this aspect, in the present study, the fatty acid content in artificial diet–fed coccinellids were closer to those of aphid-fed beetles than those of *E. kuehniella*–fed ones, revealing a lipid supply in the artificial diet quite suitable quantitatively but probably not qualitatively appropriate for the post-larval development of *H. axyridis*.

CONCLUSION

Based on the parameters considered, the artificial diet used in the present study is not yet optimal compared to *E. kuehniella* eggs or aphids for the complete rearing of *H. axyridis*. In this regard, aphids or, even better, a mixture of different natural prey, is probably a more representative control food than *E. kuehniella* eggs. However, the ability to use an artificial diet to rear and produce larvae, which are the usual stage released in biological control strategies, was substantiated. It must be noted that in *H. axyridis* fed on *E. kuehniella* eggs, a high variability in some biological parameters (including female fecundity and fertility) was found by Sighinolfi (2005). The present study has confirmed this variability for coccinellids produced according to the conventional procedure (e.g., nourished with *E. kuehniella* eggs). Therefore, in future studies diet suitability should also be compared with natural prey, i.e., aphids.

In any case, the artificial diet tested here may be convenient for culturing this predator (at least to integrate other foods in the production process), because it is less expensive to produce and easier to manage. Specty (2002) demonstrated that a similar artificial diet was suitable for development of *H. axyridis* from second instar larva to adult, but rearing from first instar larva was not performed in his study, nor was the diet used to feed adults. Moreover, the artificial diet was offered to coccinellids as fresh semi-liquid drops, and subsequent diet contaminations were frequently encountered. The dried artificial diet presentation used here proved to be more stable and less susceptible to contamination problems encountered with semi-liquid diets. After exposure for many weeks to experimental conditions, there was no bacterial contamination in the dried artificial diet contrary to liquid presentation. In general, accidental microbial invasions are at best neutral, and at worst harmful to rearing efforts, by either directly affecting insect health or indirectly by altering the diet composition (Grenier et al., 1994). For example, production of toxic components or microbial consumption of crucial elements for insect growth may occur (Cohen, 2004). However, the dried diet must be associated with ad libitum water supply.

Both physical and chemical components are affected by the processing history of the diet. For example, the nutrient quality and numerous other factors are affected by how the artificial diet was treated and offered, especially in terms of heat and cold treatments (Cohen, 2004). It is possible that in the dried presentation, the extra-oral digestion activity was more efficient than in the semi-liquid diets due to lower enzyme dispersion in the dried diet and, as a consequence, a higher enzyme concentration in ingested food (Cohen and Smith, 1998). This problem does not occur with natural prey or *E. kuehniella* eggs, which represent small quantities of food that are totally ingested by the predator (Specty, 2002).

According to Grenier and De Clercq (2003) the composition of key components in living organisms might be a good criterion for estimating the quality of artificially reared natural enemies. The performance of artificially reared entomophagous insects might be improved by altering diet composition. Future research will be aimed at improving the composition of the artificial diet for *H. axyridis*, to compensate for the deficiency and imbalance found in this study, especially in fatty acids, and to improve the biological parameters of larvae and adults.

The results achieved in the present work confirm that *H. axyridis* is one of the most promising species for mass production using an artificial diet. But the methodology and process described in this work could be easily applied as a guide for other coccinellids or other predaceous insects to be used in biological control strategies. The debate is open about potential risks associated with introduced exotic species compared with the advantages of not using chemical pesticides (van Lenteren et al., 2003), but that is another topic.

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