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EFFECTS OF ADULT DIET ON LONGEVITY AND REPRODUCTIVE PERFORMANCE OF *COCCINELLA SEPTEMPUNCTATA* AND *CHRYSOPERLA CARNEA* UNDER CONTROLLED CONDITIONS

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Abstract: Adult nutrition is a key factor influencing reproductive performance and survival of predatory insects used in biological control programs. This study evaluated the effects of different adult diets on longevity and reproductive parameters of *Coccinella septempunctata* and *Chrysoperla carnea* under controlled laboratory conditions. Longevity, pre-oviposition period, total and daily fecundity and egg viability were recorded over a six-week period. In *C. septempunctata*, the natural aphid-based diet resulted in significantly higher longevity, fecundity and egg viability compared to all artificial diets. Among artificial treatments, the mixed protein-carbohydrate diet produced intermediate reproductive performance. In *Ch. carnea*, carbohydrate-based diets enhanced adult longevity, whereas reproductive output and egg viability were maximized under the mixed diet. Protein-only diets supported egg production but reduced survival. The results highlight species-specific nutritional requirements reflecting different trophic strategies. While natural prey remains essential for optimal reproduction in *C. septempunctata*, balanced artificial diets can represent a practical alternative for mass rearing. For *Ch. carnea*, mixed protein-carbohydrate diets are recommended to optimize both survival and reproduction.

Key words: *Coccinella septempunctata*, *Chrysoperla carnea, mass rearing program, biological control*

INTRODUCTION

Biological control of insect pests represents an essential component of integrated pest management (IPM) strategies, contributing to the reduction of chemical pesticide use and to the sustainability of agroecosystems. Among the most effective and widely utilized natural enemies are the aphidophagous predators *Coccinella septempunctata* Linnaeus 1758 (Coleoptera:Coccinellidae) and *Chrysoperla carnea* (Stephens 1836) (Neuroptera: Chrysopidae), both recognized for their high predation capacity on aphids and other soft-bodied pests affecting agricultural crops worldwide (van Lenteren, 2012; Obrycki & Kring, 1998; McEwen et al., 2001).

The mass rearing of aphidophagous predators under controlled conditions has gained increasing importance over recent decades, both at global level and in Europe, as a response to the growing demand for environmentally friendly pest control solutions. The rearing capacity to a large variety of natural enemies has improved over the past decades thanks to developments in insect rearing technologies, which have included the development of artificial diets (Hangay et al., 2008). Commercial and semi-commercial rearing systems for coccinellids and chrysopids have been developed in numerous countries, focusing on optimizing survival, reproduction and quality of released individuals (van Lenteren et al., 2018). In Romania, interest in the controlled rearing of beneficial insects has intensified in the

context of sustainable agriculture and organic farming, with research institutions and experimental laboratories exploring feasible rearing protocols adapted to local agroecological conditions. However, large-scale application is still limited, and further research is required to refine rearing techniques, particularly regarding adult nutrition and reproductive performance (Georgescu et al., 2023).

One of the most critical factors influencing the success of controlled breeding programs for predatory insects is adult diet. Adequate nutrition plays a fundamental role in determining key life-history traits such as adult longevity, pre-oviposition period, fecundity, egg viability and overall population growth rate, which ultimately affect the effectiveness of these species as biological control agents (Dixon, 2000). While larvae of both *C. septempunctata* and *Ch. carnea* are primarily carnivorous, adult feeding habits are more diverse and flexible, making diet formulation a decisive aspect of rearing optimization. Recent studies have demonstrated that supplementing adult diets with specific nutritional components, such as pollen, free amino acids, protein-rich sources (e.g. egg yolk, yeast or whey protein concentrates), and carbohydrates (e.g. honey, sucrose or glucose solutions), can significantly enhance reproductive output, longevity, and egg hatching rates in both coccinellids (Lundgren, 2009; Hodek et al.,2012) and chrysopids (Canard, 2001).

Despite these advances, important knowledge gaps persist. In particular, the interactions between macronutrients (proteins and carbohydrates) and micronutrients (lipids, vitamins, minerals, antioxidants) and their combined effects on reproductive performance remain insufficiently understood. Moreover, comparative experimental data obtained under standardized and controlled conditions are still scarce. There is a clear need for studies that simultaneously evaluate comparable biological parameters, such as adult longevity, pre-oviposition period, total fecundity and egg viability, in response to different adult diets (Grenier & De Clercq, 2003; Cohen, 2004). Such information is essential for developing efficient and species-specific rearing protocols.

From a practical perspective, optimizing adult diet composition is crucial not only for maximizing egg production or adult survival, but also for producing high-quality predator populations with enhanced biological control potential (Riddick, 2009).

The aim of the present study was to evaluate the influence of different adult diets on reproductive parameters of *Coccinella septempunctata* and *Chrysoperla carnea* reared under controlled conditions. Specifically, the study compares the effects of natural diets (aphids and/or pollen) with those of artificial diets and selected nutritional supplements, with the objective of identifying optimal feeding strategies and proposing recommendations for improving predator rearing protocols under experimental conditions.

MATERIALS AND METHODS

Source of biological material and laboratory environmental conditions

Adults of *C. septempunctata* and *Ch. carnea* were obtained from laboratory colonies maintained at the Research and Development Institute for Plant Protection (R.D.I.P.P.), Bucharest. The colonies were continuously reared on aphids (*Aphis fabae* Scopoli, 1763) for both larvae and adults of *C. septempunctata*, while larvae of *Ch. carnea* were reared on aphids and adults were maintained on a standard diet consisting of sucrose solution and pollen. For the experiment, pairs of the same age (2 days old), originating from the sixth laboratory generation, were selected.

Experiments were conducted in a climate-controlled chamber under the following conditions: temperature 25 ± 1°C, relative humidity 65 ± 5% and a photoperiod of 16:8 h (light:dark). Each adult pair was placed in transparent plastic containers (9 × 6 cm), lined with filter paper at the bottom. The containers were covered with a fine mesh lid to ensure adequate ventilation.

Experimental diets and design

The dietary treatments were selected based on their frequent use in laboratory rearing or mass production of predatory insects.

A. Diets for *C. septempunctata*

Four adult diets were tested: Treatment I. Protein-based artificial diet (PD) Composition: whey protein hydrolysate + casein (20g), brewer's yeast (10g), multifloral honey (30g), distilled water (40ml), vitamin mix (0.2g); the mixture was homogenized and stored at 4°C; administration of 0.2 ml /adult/day.

Treatment II. Carbohydrate-based diet (CD**)** represented by 30% honey-water solution and dried pollen (5 mg /adult/day).

Treatment III. Mixed protein + carbohydrate diet (MD) respectively 1:1 mixture (v/v) of PD and CD; administration of 0.3 ml of diet mixture and 5 mg dried pollen /adult/day.

Treatment IV. Natural prey diet (NF) represented by larvae of *A. fabae* reared on broad bean plants (*Vicia faba*); administration of 40-60 aphids per adult per day.

B. Diets for *Ch. carnea*

Because adults of *Ch. carnea* are non-predatory and mainly consume carbohydrate-rich food sources, the natural aphid-based diet was excluded. Three artificial diets (PD, CD, MD) identical to those described above were tested. All diets were offered on small Parafilm® discs and replaced every 24-48 h to prevent fungal contamination.

A completely randomized design was used, with four dietary treatments and 25 adult pairs (replicates) per treatment for each species, totalling 400 individuals (200 *C. septempunctata* and 200 *Ch. carnea*). The experiments lasted five weeks, during which observations were recorded daily. Parameters assessed were: female longevity (days), pre-oviposition period (days), total fecundity (number of eggs per female), daily fecundity (eggs per female per day) and egg viability (% hatch rate). Egg hatch rate was calculated as the percentage of larvae emerging from the total number of eggs laid. All containers were cleaned every 48 h and biological residues were removed to prevent microbial contamination. Insects were handled using soft brushes to minimize mechanical stress.

Statistical analysis

Biological and reproductive parameters were analyzed using one-way ANOVA, with diet as a fixed factor. Differences were considered statistically significant at $p < 0.05$

RESULTS AND DISCUSSIONS

Effect of adult diet on biological parameters of *C. septempunctata*

The results summarized in Table 1 clearly indicate that the natural aphid-based diet produced the highest values for most biological parameters. Females fed on *A. fabae* exhibited maximum longevity (38.5 days), the shortest pre-oviposition period (6.2 days), the highest

total fecundity (243.5 eggs per female) and daily fecundity (8.7 eggs per day) and the greatest egg viability (92.3%).

Table 1. The effect of the diet offered to adults on the biological parameters of *Coccinella septempunctata* under laboratory conditions

Treatment no.	Diet	Female longevity (days)	Pre-oviposition period (days)	Eggs/female (total number)	Eggs/female/ day (average number)	Hatching (%)
I.	Artificial protein diet (PD)	24.5 d	7.3 b	71.4 c	4.7 c	54.3 c
II.	Carbohydrate-based diet (CD)	32.3 b	8.1 a	59.4 d	2.8 d	28.9 d
III.	Mixed protein + carbohydrate diet (MD)	26.3 c	6.8 c	91.8 b	5.4 b	65.2 b
IV.	Natural food (*A. fabae*) (NF)	38.5 a	6.2 d	243.5 a	8.7 a	92.3 a

Means followed by different letters differ statistically significantly (Tukey test, $p \leq 0.05$).

Results profile in our laboratory experiments are in concordance with those reported for coccinellids, which show superior reproductive performances when fed on natural prey rich in essential amino acids, sterols and lipids necessary for insects for vitellogenin synthesis and oogenesis (Hodek & Evans, 2012; Dixon, 2000; Obrycki & Kring, 1998).

Among the artificial diets tested, MD diet produced intermediate reproductive performance, significantly higher than that recorded for PD or CD diets. Although female fecundity under MD treatment (91.8 eggs/female) remained markedly lower than under natural prey conditions (243.5 eggs/female), it exceeded the value observed for the other artificial diets, suggesting that the combined nutrient supply partially satisfies the physiological requirements of adults. This result suggests that the combined supply of proteins and carbohydrates partially fulfils the nutritional requirements of adult *C. septempunctata*, although it does not fully replace the complexity of natural prey. Similar findings were reported by Grenier & De Clercq (2003) who emphasized that artificial diets for predatory beetles must provide balanced macronutrients to sustain reproduction

The PD supported moderate fecundity (71.4 eggs/female) but resulted in reduced longevity (24.5 days) and egg viability (54.3%), while the CD treatment produced the poorest reproductive outcomes: 59.4 eggs per female and 28.9 % egg viability. Diets lacking sterols, complex lipids, and micronutrients essential for egg development are known to impair reproductive success in coccinellids (Hodek et al., 2012; Lundgren, 2009). Statistical analysis confirmed a significant effect of diet on all parameters evaluated ($p < 0.05$), highlighting the critical role of adult nutrition in regulating reproductive processes in *C. septempunctata*.

Effect of adult diet on biological parameters of *Ch. carnea*

Consistent with previous studies, adults of *Ch. carnea* are primarily adapted to consume carbohydrate-rich resources such as nectar, pollen and honeydew (McEwen et al., 2001; Duelli, 2001). Accordingly, the present analysis focused on artificial diets differing in protein and carbohydrate composition. The highest longevity was recorded for adults fed the carbohydrate-based diet (19.2 days) followed by the mixed diet (15.1 days) and the protein-based diet (14.3 days) (Table 2). This pattern reflects the high energetic demands of adult lacewings and the essential role of carbohydrates in sustaining survival and flight activity. Similar results were reported by Pappas et al. (2011), who demonstrated that carbohydrate intake is a key determinant of adult survival in chrysopids.

Table 2. The effect of the diet offered to adults on the biological parameters of *Chrysopa carnea* under laboratory conditions

Treatment no.	Diet	Female longevity (days)	Pre-oviposition period (days)	Eggs/female (total number)	Eggs/female /day (average number)	Hatching (%)
I.	Artificial protein diet (PD)	14.3 c	3.4 b	83.6 b	8.9 b	81.6 b
II.	Carbohydrate-based diet (CD)	19.2 a	4.2 a	64.8 c	5.4 c	71.2 c
III.	Mixed protein + carbohydrate diet (MD)	15.1 b	3.0 c	94.8 a	9.2 a	87.3 a

Means followed by different letters differ statistically significantly (Tukey test, $p \leq 0.05$).

In contrast, reproductive performance was maximized under the MD treatment, which resulted in the shortest pre-oviposition period, the highest fecundity, and the greatest egg viability. The pre-oviposition period ranged from 3.0 to 4.2 days, with the shortest duration observed under the MD. Maximum fecundity was obtained with the MD (94.8 eggs/female), followed by the PD (83.6 eggs), while the CD resulted in significantly lower egg production (64.8 eggs). Daily fecundity followed the same trend. Egg viability was also highest under the MD (87.3%) indicating that balanced nutrient intake supports optimal allocation of resources to egg quality. Protein intake is essential for vitellogenin synthesis and egg maturation, while carbohydrates supply the energy required to sustain oviposition activity (Grenier & De Clercq, 2003; Canard, 2005). Diets consisting exclusively of carbohydrates delayed oviposition and reduced fecundity, whereas protein-only diets supported egg production but compromised adult longevity. These findings confirm that a balanced protein-carbohydrate diet is required to optimize both survival and reproductive output in adult *Ch. carnea*.

Interspecific comparison of dietary responses

The contrasting responses observed between the two species reflect their distinct trophic strategies. *C. septempunctata* is a strictly predatory species throughout all life stages and depends heavily on animal-derived nutrients, making artificial diets less effective than natural prey, as other studies have reported (Dixon, 2000; Hodek et al., 2012). In contrast, *Ch. carnea* adults possess a physiology adapted to carbohydrate-rich diets, allowing them to utilize artificial diets more efficiently, provided that sufficient protein is available to support reproduction a fact also described by McEwen et al. (2001).

These findings are particularly relevant for mass-rearing programs, where diet optimization is considered a key limiting factor for large-scale application of biological control agents (van Lenteren, 2012).

CONCLUSIONS

The present study confirms fundamental differences between *Coccinella septempunctata* and *Chrysopa carnea* related to their trophic strategies and nutritional requirements. Protein intake plays a major role in reproductive performance in both species, with higher protein availability being positively correlated with fecundity. Carbohydrate-based diets significantly increase adult longevity but do not adequately support oviposition when provided alone. Pollen proved to be a key dietary component, particularly for *Ch. carnea*, supplying amino acids, lipids, and sugars essential for reproduction. In *C.*

septempunctata, pollen supplementation improved survival and fecundity, although performance remained inferior to that obtained with natural prey.

Although natural prey yielded the best overall results for *C. septempunctata*, the mixed protein - carbohydrate diet represents a viable alternative for laboratory rearing during the period when aphids are unavailable. In *Ch. carnea*, the mixed diet optimized fecundity, egg viability, and pre-oviposition period while maintaining adequate longevity.

Further studies are needed to evaluate the long-term effects of these diets across multiple laboratory generations.

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