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Impact of Proteins in Adult Artificial Diet of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on Biological Parameters

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ABSTRACT

Laboratory studies were carried out to determine the influence of varying amounts of different proteins in the diet of adult *Chrysoperla carnea* (Stephens). Results show that proteins play a vital role in the fecundity, fertility and all the biological parameters of F_1 for mass rearing. The tested proteins were: Casein, Protein hydrolysate, Torula yeast and Nu lure. Al these proteins were tested in laboratory at four concentrations, *i.e.,* 1, 3, 5 and 7 mg/ml in diet. Significantly higher fecundity 785.12±25.7, fertility 89.23±0.36 % of eggs and longer oviposition period 42.13±1.11 days were recorded in adults feeding on a diet containing Nu lure followed by protein hydrolysate (control diet). The F_1 progeny larvae that developed from adults consuming (Nu lure 5ml) diet had the shortest larval and pupal periods and complete all developmental stages in shortest period of time. Insects reared on different concentrations of proteins. Insects rear on different diets compared on the basis of egg to adult survival, female (%) and pupal weight (g) with low and high protein concentrations.

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INTRODUCTION

The green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is one of the most commonly and widely used natural enemy for population management of insect pests (Finney, 1948; O'Neil *et al*., 1998; Gurr *et al*., 2012). *C. carnea* is mass-produced in many European countries and commercially supplied for inundative release against many insect pests in field and glass house crops (Danne *et al*., 1996; Hoddle *et al*., 2004; Fondren *et al*., 2004). Kareim (1998) reported that *C. carnea* has been effective at controlling whitefly *Bemisia tabaci* on cotton in Pakistan. indiscriminate use of different pesticides create various problems, toxicity of different pesticides effect health, pollute environment and kills non-target organisms that are why the demand for mass production of *C. carnea* in different countries of world is increasing in the wake of adverse effects of pesticides on the environment and non-target organisms (Easterbrook *et al*., 2006). Different laboratories are trying different combinations of feed items for low cost mass production of *C. carnea* on artificial diets. Experiments on methods of mass production of *Chrysoperla* spp. started as early as 1940s. Finney (1948) studied a method developing an insectary to enhance the rearing culture and supply *Chrysoperla* spp. Finney (1950) improved methods to increase the output of eggs which had reached actual mass-production proportions. Hagen and Tassan (1965) reported the use of artificial diets to rear reproductive adults of *C. carnea*. The relationship between *C. carnea* adult diet and egg production has been studied extensively (Hagen *et al*., 1970; Tauber and Tauber, 1974; Zheng *et al*., 1993). Milevoj (1999) and Sattar and Abro (2011) studied the effect of natural and artificial diets on fecundity and fertility of *C. carnea* in laboratory to enhance the culture.

The presence of honey dew on plants appeared to influence the feeding behaviour of *Chrysoperla* (Duelli, 1987). The life history of the Genus *Chrysoperla* is complex; its larvae are entomophagous often called aphid loin, feed on more than 500 aphids during the larval period (Kift *et al*., 2005). Larvae inject enzymes through pincer-like mandibles into the body of prey which digest the internal body fluids. They feed on aphids, jassids, mealy bugs, whiteflies, scales and other soft bodied slow moving insects, when host is scarce they turn cannibal (Hagen *et al*., 1970; Hagan, 1986; Laznik *et al*., 2010). About half the species including *C. carnea* adults feed mainly on substances of plant origin: nectar, pollen and honeydew (Principi and Canard, 1984). Adults of *C. carnea* require intake of protein for high fecundity which is obtained from amino acids as a food source by feeding on honey dew (Zheng *et al*., 1993; Bozisk, 1995). Honey dew and nectar provide a supplementary food and the source of essential yeast symbionts that reside in the gut of the adults (Hagen

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and Tassan, 1972; Hagen, 1986; Villenave *et al*., 2006). Pollen is also a rich source of proteins for chrysopid adults. The total protein varies from 6-36% and usually all known protein amino acids are present in relatively large amounts except for tryptophan and phenylalanine (Ribeiro and Freitas, 2000). Kareim (1998) reported that *C. carnea* has been effective at controlling whitefly *Bemisia tabaci*, of cotton crop in Pakistan. Glycol nutrients (charbohydrates) are plant saccharides essential for adults function; they derived from plants used in adults diet (Duelli, 2001).

Mass production of *C. carnea* is required for inundative releases. This could be achieved by standardizing either the larval rearing techniques and improving the adult diet for getting the maximum fecundity (Krishnamoorthy, 1984). For mass rearing of *C. carnea* it is essential to improve the diet of adults. The objective of this study was to determine the optimum dose level of adult proteins required to maximize fecundity of *C. carnea* for mass culturing in laboratory which was used in inundative releases to suppress the pest population from the cultivated crops.

MATERIALS AND METHODS

Culture of Chrysoperla carnea

The *C. carnea* eggs were collected manually from cotton field in small size glass test tubes (5x3 mm). After hatching, healthy larvae of the same age were maintained under controlled conditions (26±2°C and 65±5% R.H. with a 14:10 L:D photoperiod). In the laboratory, selected larvae were reared on frozen eggs of *Sitotroga cerealella* (Olivier). This fictitious host, Angoumois grain moth culture was reared on treated whole wheat grains in laboratory.

To avoid cannibalism newly hatched (< 2 h) larva were shifted with the help of fine camel hair brush from hatching cover to finely perforated transparent plastic tubes (3 mm diameter, 50 mm long) provided with 0.2 g frozen *S. cerealella* eggs as diet. Every third day diet was changed until pupation. After completion of the larval period of 8-12 days these larvae pupate 7-10 days in the same transparent tubes. As larvae complete their instar, diet is offered in double quantity. After pupation, pupae were harvested gently with the help of scissors by cutting both the ends from plastic tubes and kept in glass Petri dishes (1.5 cm height and 9 cm dia.) with fresh green leaves of *Medicago sativa* to maintain humidity. The adults emerge out within 8-10 days, which were gently shifted from Petri dishes to glass cages. On daily basis an artificial diet in liquid form provided to *C. carnea* adults on sponge piece placed in small size Petri dishes and 10% sugar solution provided in cotton wicks in glass vials. After emergence of *C. carnea* adult's male and female identification takes 3-4 days. Identified adults paired and shifted in glass chimneys for this study.

Testing of proteins

Five protein sources were tested: Casein, Nu lure™ (a protein diet derived from corn, Miller Chemical & Fertilizer Corporation, USA), protein hydrolysate and Torula yeast, in different concentrations (1, 3, 5 and 7g/ml) were compared with standard diet containing sucrose (5 mg), brewer's yeast (1 mg), honey (1 mg) and water (500 ml) in the Bio-control, Mass Rearing Laboratory, Nuclear Institute of Agriculture (N.I.A.), Tando Jam, Pakistan under the controlled conditions described above.

Newly paired laboratory reared adult males and females were paired in glass chimneys (4x7 cm) covered tightly with black muslin cloth piece; diet was provided twice a day after 12 h in droplets on hard paper cards (2x3 cm) in the glass chimneys, water was provided in impregnated cotton in glass vials which were replaced daily. All chimneys were observed after 24 h until adults died. Observations were recorded on the basis of effect of different proteins and their concentration. In adults, concentration of artificial diet affects the fecundity and fertility of eggs, pre-oviposition, oviposition and post-oviposition period. Laid eggs were counted and harvested daily with the help of scissors. Collected eggs were shifted for hatching in black muslin cloth piece, kept in covered medium size glass Petri dishes. Hatched eggs were observed daily and recorded data on the period of their three instars. The fecundity and fertility of eggs of parent generation of *C. carnea* was determined from eight replications. Data was compiled and analysed.

Performance of F_1 Chrysoperla carnea

Eggs were allowed to develop in black muslin cloth piece and the duration of the incubation period was recorded. There were 100 eggs per replicate. Neonate (< 2 h old) larvae of *C. carnea* were placed individually inside finely perforated transparent plastic tubes (3mm diameter, 50 mm length). Weighed frozen eggs (0.2 g) of *S. cerealella* were used as food and introduced in each tube with the help of fine camel hair brush and then both ends of the tube sealed with impulse sealer. Food was changed after every third day till pupation. The larvae were examined each day to determine survival and larval stadia. Pupae were harvested gently without any physical handling by cutting both ends of tube with the help of scissors and placed in covered glass Petri dishes. Fresh *Medicago sativa* leaves were provided in Petri dishes to maintain moisture inside the Petri dishes. After six to eight days, these Petri dishes were placed in glass cages for adult emergence. Pupal weight was recorded two days after pupation using an

electronic balance (Ohaus®, Galaxy 160). Sex was determined within 2-3 days of adult eclosion. Life history parameters measured were: incubation period, larval/ pupal period, pupation, emergence, male/female ratio and longevity of adults. Larval and pupal period, development time, egg to adult survival, percent female and pupal weight was determined from 25 insects per treatment replicated eight times.

Data were subjected to analysis of variance and all the treatment means of above parameters were compared using Tukey HSD test with the help of MASTATC computer software.

RESULTS

Influence of proteins on adults biology

Biology of *Chrysoperla carnea* adults had significant effect when reared on different concentrations of proteins. Results in Table I showed that in Casein diet, among low and high concentrations maximum fecundity (662.40±22.54) and highest percent fertility (77.21±2.81) was recorded in 3.0 mg diet. Whereas in protein hydrolysate, maximum oviposition days (41.40±0.82 and 35.90±1.48) were recorded in 5.0 and 3.0 mg diets, respectively. Similarly, maximum percent fertility (65.25±5.13) was recorded in Torula yeast (1.0 mg) diet, whereas highest oviposition (30.38±0.75 and 30.0±0.77) days were recorded in 5.0 and 3.0 mg, respectively. In Nu lure diet maximum number of eggs obtained were 785.12±25.75 (F= 184.95; DF=15, 112; P <0.001) and highest percent fertility of eggs achieved was 89.23±0.36 (F= 25.43; DF= 15, 112; P <0.001), whereas maximum oviposition days (42.13 ± 1.11) was recorded in diet containing 5.0 ml. In Table I minimum pre-oviposition 3.25±0.16 and maximum post-oviposition 10.13 ± 0.71 days were recorded in Nu lure and Torula yeast diets with the concentrations 7.0 ml and 1.0 mg, respectively.

Performance of F_1

Table II shows the performance of F_1 progeny reared on artificial diet with various low and high concentrations of different proteins. There was significant difference in incubation period (F= 21.47; DF= 15, 112; P <0.001), shortest incubation period (4.36±0.01 days) was recorded in Nu lure (7.0 ml) diet and longest incubation period (5.00±0.00 days) were recorded in Torula yeast (1.0 mg) diet. Results of Table II showed that long and short larval periods were significantly different (F= 14.48; DF= 15, 112; P <0.001). Shortest larval periods 13.13±0.12, 12.88±0.12, 13.63±0.26 and 12.38±0.18 days were recorded in Casein (3.0 mg), protein hydrolysate (5.0 mg), Torula yeast (7.0 mg) and Nu lure (5.0 ml).

Table I.- Effect of different proteins and their concentrations in adult artificial diet on biological parameters of *C. carnea* under laboratory conditions.

Protein	Conc. mg/ml diet	Fecundity (no. of eggs)	Fertility (%)	Pre-Oviposition (days)	Oviposition (days)	Post-Oviposition (days)
Casein (ml)	1.0	331.5±19.47 fg	62.20±2.52 de	4.90±0.12 a	25.60±0.91 g	5.40±0.49 defg
	3.0	662.40 22.54 bc	77.21±2.18 bc	4.50±0.19abc	38.20±0.16 bc	4.9±0.69 efg
	5.0	271.80±13.47 h	47.90±2.29 gh	4.25±0.18 abc	32.0±1.13 ef	4.40±0.33 fg
	7.0	169.50±20.18 e	45.06±2.47 h	4.75±0.16 ab	31.63±1.03 ef	4.20±0.25g
Protein hydrolysate (mg)	1.0	254.50±10.33 h	74.063±2.34 c	4.625±0.18 abc	25.63±0.59 g	9.00±0.27 a
	3.0	565.2±24.00 d	81.21±1.2 abc	4.40±0.18 abc	35.90±1.48 cd	7.63±0.53 b
	5.0	684.00±6.14 a	80.59±1.80 abc	3.40±0.18 d	41.40±0.82 ab	4.65±0.26 efg
	7.0	356.30±15.80 ef	57.33±1.77	3.50±0.19 d	31.13±1.06 ab	4.50±0.33efg
Torula yeast (ml)	1.0	110.12±7.16 f	65.25±5.13 d	4.40±0.18abc	17.25±1.08 h	10.13±0.72 a
	3.0	167.00±9.97 i	54.87±1.75efg	4.25±0.29 c	30.0±0.78 f	7.65±0.49 b
	5.0	88.630±4.95 j	53.79±4.74 efgh	3.40±0.18 d	30.38±0.75 f	6.63±0.42 bcd
	7.0	137.80±13.04ij	47.34±4.59 gh	3.40±0.18 d	24.00±1.04 g	7.13±0.35 bc
Nu lure (ml)	1.0	302.62±20.79 gh	65.71±4.11 d	4.40±0.18 abc	29.50±1.07 f	5.80±0.37 def
	3.0	628.62±22.67 c	83.36±0.90a	4.25±0.16 bc	34.25±0.99 de	5.90±0.55 cde
	5.0	785.12±25.75 a	89.23±0.36 a	3.25±0.16 d	42.13±1.11 a	4.63±0.32 efg
	7.0	396.12±5.51e	50.01±0.79 fgh	3.25±0.16 d	36.63±2.03 cd	5.25±0.33defg
LSD-=0.050	-	46.79	8.093	0.5195	3.097	1.246
F-value	-	184.950	25.428	10.380	39.804	15.789
P-value	-	0.001	0.001	0.001	0.001	0.001

Figures followed by same letter in a column are not significantly different from each other at 5% DMRT.

Table II.- Effect of different proteins and their concentrations in adult artificial diet on biological parameters of *C. carnea* under laboratory conditions.

Protein	Conc. mg/ml diet	Incubation period (days)	Larval period (days)	Pupal period (days)	Developmental period (days)
Casein (ml)	1.0	4.73±0.07 cd	14.38±0.18 bc	10.25±0.31 c	29.49
	3.0	4.57±0.08 fg	13.13±0.12 fg	9.50±0.19 d	27.24
	5.0	4.58±0.06 ef	13.25±0.16 efg	9.12±0.12 de	26.99
	7.0	4.51±0.04 fghi	13.38±0.18 efg	9.25±0.17 de	27.37
Protein hydrolysate (mg)	1.0	4.64±0.35 de	14.25±0.31 bcd	9.37±0.18 de	28.37
	3.0	4.58±0.02 ef	13.75±0.16 cdef	9.25±0.16 de	27.64
	5.0	4.55±0.02 efgh	12.88±0.12 gh	8.87±0.23 e	28.34
	7.0	4.45± 0.02 ghij	13.13±0.12 fg	9.00±0.19 de	27.83
Torula yeast (ml)	1.0	5.00±0.00 a	15.25±0.16 a	11.13±0.29 a	31.37
	3.0	4.87±0.04 b	14.63±0.81 b	11.00±0.19 a	30.49
	5.0	4.82±0.04 bc	13.88±0.29 cde	10.88±0.23 ab	30.28
	7.0	4.76±0.04 bc	13.63±0.26 def	10.38±0.23 bc	30.11
Nu lure (ml)	1.0	4.47±0.007 fghij	14.25±0.25 bcd	9.00±0.00 de	27.87
	3.0	4.43±0.01 hij	13.13±0.22 fg	9.00±0.00 de	26.00
	5.0	4.39±0.01 ij	12.38±0.18 h	8.75±0.16 e	25.78
	7.0	4.36±0.02 j	12.75±0.16 gh	8.87±0.12 e	26.53
LSD =0.050	-	0.1130	0.5674	0.5307	-
F-value	-	21.471	14.476	19.188	-
P-value	-	0.001	0.001	0.001	-

Figures followed by same letter in a column are not significantly different from each other at 5% DMRT.

Table III.- Effect of different proteins and their concentrations in adult artificial diet on biological parameters of F1 progeny of *C. carnea* under laboratory conditions.

Protein	Conc. mg/ml diet	Egg to adult survival (%)	Female (%)	Pupal weight (mg)
Casein (ml)	1.0	19.76±1.44 f	54.23±2.71	4.88±0.12 h
	3.0	39.70±3.3 d	57.52±1.62	5.88±0.12 def
	5.0	7.76 ± 0.37 h	55.69±3.48	6.13±0.12 d
	7.0	6.66±1.01 h	52.44±6.35	6.13±0.12 d
Protein hydrolysate (mg)	1.0	28.38±1.81 e	58.78±2.48	5.50±0.19defg
	3.0	46.82±1.55 c	59.52±2.43	6.0±0.00 de
	5.0	55.78±2.33 b	56.78±1.138	7.63±0.18 ab
	7.0	21.16±1.78 f	60.47±3.57	7.38±0.26 bc
Torula yeast (ml)	1.0	20.97±1.79 f	54.82±2.38	5.25±0.16 gh
	3.0	8.38±0.82 gh	54.82±2.38	5.25±0.16gh
	5.0	8.38±0.82 gh	54.85±4.15	5.50±0.19defg
	7.0	15.44±1.38 fg	56.62±3.53	5.63±0.18 defg
Nu lure (ml)	1.0	35.97±3.76 d	60.39±1.83	5.37±0.18 fgh
	3.0	54.54±3.19 b	58.28±1.90	7.00.27 c
	5.0	67.4±1.76 a	63.16±0.97	8.0±0.00 a
	7.0	29.275±2.15 e	58.11 ± 1.03	7.00 ± 0.27 c
LSD-=0.050	-	5.705	NS	0.4953
F-value	-	87.815	NS	27.600
P-value	-	0.001	NS	0.001

Figures followed by same letter in a column are not significantly different from each other at 5% DMRT.

Whereas, the longest larval periods of 14.38±0.18, 14.25±0.31, 15.25±0.16 and 14.25±0.25 days were recorded when larvae were fed on Casein (1.0 mg), protein hydrolysate (1.0 mg), Torula yeast (1.0 mg) and Nu lure (1.0 ml), respectively. Shortest pupal period was significantly different (F= 19.19; DF= 15, 112; P <0.001) in casein diet (9.12±0.12 days), in protein hydrolysate (8.87±0.26 days), in Torula yeast (10.38±0.22 days) and in Nu lure (8.75±0.16 days) when larvae were fed on 5.0 mg, 5.0 mg, 7.0 mg and 5.0 ml of diet, respectively. Total developmental period are shown in Table II, minimum period was recorded in casein (5.0 mg) diet (26.99 days), in protein hydrolysate (3.0 mg) diet (27.64 days), in Torula yeast (7.0 mg) diet (30.11 days) and in Nu lure (5.0 ml) diet (25.78 days), respectively.

Table III shows adults survival from egg to adult of F_1 progeny of *C. carnea* (F= 87.81; DF=15, 112; P <0.001). Maximum percent survival was recorded in all proteins with same concentration (5.0mg/ml) *i.e.,* in casein diet maximum percent survival was 77.59±0.36, in protein hydrolysate diet 55.78±2.33, in Torula yeast diet 83.8±0.81 days and in Nu lure diet 68.41±1.76 days. The percent female emergence was however, not significant between diets (F= 0.94; DF= 15, 112; P >0.05). Maximum percent emergence of females were recorded in Nu lure (5.0 ml) diet (63.16±0.97) followed by protein hydrolysate (5.0 mg) diet (60.46±3.57). It was recorded that the differences in pupal weight due to feeding on different concentrations of various proteins were significant (F= 27.60; DF= 15, 112; P <0.001). Highest pupal weight (8.0±0.00) was recorded in Nu lure (5.0 ml) diet whereas lowest pupal weight (4.87±0.12) was recorded in casein (1.0 mg) diet.

DISCUSSION

In the present study an almost clear dose-response relationship was evident which showed the importance of proteins and indicated a proper dose of protein in the adult diet for reproductive biology of *C. carnea* which enhance the efficiency of predator. Tulisalo and Korpela (1973) reared *C. carnea* adults with a mixture of protein hydrolysate (yeast), sugar and water (5: 6: 10) spread as a moist paste on the walls of the cage; adults also had access to water on cotton-wool. Females laid an average of 700 eggs each, having 30-40% fertility. Results of present study indicated that concentrations of various proteins were to determine the optimum dose for maximum egg production and healthy larvae. Vanderzant (1973) made improvements in adult diet of *C. carnea*, prepared a diet containing soy and casein hydrolysate and found satisfactory result till 8 generations. Hassan (1974) tried sucrose as substitute for fructose and protein hydrolysate was replaced with 18 free amino acids including 10 essential ones which were found indispensable for growth of *C. carnea* adults in laboratory.

Hassan (1975) further described the mass-rearing of *C. carnea*, with an artificial diet containing brewer's yeast, honey and water. Principi and Canrad (1984) recorded that the inclusion of yeast was required for egg production, but few eggs were produced on yeast solution alone. For maximum egg production, food containing yeast and sugar must be offered on more than one occasion, compared with the insects given a diet comprising yeast, protein hydrolysate, sugar and water in the ratios 4:7:10, small but significant reductions in egg production rate were noted when the amount of either yeast, or both the yeast and the sugar was halved. Duelli (1987) reported that adults of *C. carnea* were attracted to pollen but not consumed as they needed, so prepared an artificial diet of *C. carnea* adults with honeydew and nectar, as a supplementary diet and a good source of yeast that increase number of eggs laid but fertility is not more than 40 percent.

Neuropterans life cycle is complex; larvae are voracious feeder of soft bodied pests while adults feed on honey dew and nectar (Bozisk, 1995). An optimum dose of different proteins play vital role in colony maintenance and effect on female emergence and increase oviposition days. McEwen and Kidd (1995) investigated the role of artificial foods comprising yeast products such as sugar and water, which were sprayed in the field to increase numbers of naturally occurring lacewing populations. McEwen *et al*. (1996) studied the influence of an artificial food supplement on larval and adult performance of *C. carnea*. The adult diet comprised of yeast, protein hydrolysate, sugar and water in the ratio of 4:7:10.

Duelli (2001) reported that yeast is important ingredient in adults food of *C. carnea*, they consume honey dew, pollen and nectar because saccharides are basic need in adults diet. Tesfaye *et al*. (2002) examined the effects of different combinations of 50 percent honey solution, castor pollens and yeast on the longevity, fecundity, reproductive age and other reproductive attributes of *C. carnea*. The highest number of eggs/female (245.2) was laid when adults were supplemented with baker's yeast granules+50% honey and castor pollen+50% honey. Sattar *et al*. (2007) used an artificial adult diet based on casein and yeast, released *C. carnea* for controlling several insect pests of cotton and maize. The best productive age of female was observed to reach up to 8, 9, 8 and 4 weeks when fed with baker's yeast granules+50% honey, castor pollen+50% honey and 50% honey, respectively.

Quantity of protein plays vital role in biological parameters and there was a close association between optimum dose of nutritious diet in mass rearing of *C. carnea* for highest fecundity and fertility of eggs (Sattar

and Abro, 2011). In laboratory experiment, results revealed that the insects receiving diets containing both sugar and yeast lived significantly longer than those receiving only sugar solution ($P < 0.05$). Sattar *et al*. (2011) reported that feeding of *C. carnea* larvae on different diets showed significant effect on all biological parameters such as on larval survival and larval period and enhance the predatory efficiency.

CONCLUSIONS

In conclusion of this study, the adults best nutritious diet was based on Nu lure and protein hydrolysate, which significantly influenced on adults both male and female, enhance oviposition period, fecundity, larval survival and egg to adult survival while having no significant effects on pre and post-oviposition period.

Statement of conflict of interest

Authors have declared no conflict of interest.

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