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Impact of Carbohydrate and Protein diet in the successes survival and adult development of *Cybister Confusus*

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Abstract- Proteins and carbohydrates are two important macronutrients that provide the essential amino acids and energy and influence development, growth, and fecundity. In present study it was found that the survival from hatch to eclosion was significantly different across the seven treatments (Logistic Regression: d.f =6, χ^2= 33.91, p< 0.001). It was equally high on the diets with balanced (p21:c21) and slightly imbalanced p/c ratios (p17.5: c24.5 and p24.5: c17.5). The number of mating pairs and egg-producing pairs was highly variable due to the different survival rates across the treatments.

Kew words- Protein, Carbohydrate, *Cybister Confusus*, Adult development.

Introduction- The most diverse and prominent order of the insect, Coleoptera includes about 3.87 lakhs described species from the entire world representing about 38% of insect species of all the orders (Zhang, 2011). In all of these 12,604 species belong to aquatic beetles pertaining to at least eighteen families (Jack and Balke, 2008). In all insect *Cybister confusus* (Coleoptera: Dytiscidae) are one of the most successful group of insect, distinguished by their adaptive nature in diverse ecological and geographical range. It is a carnivorous fresh water beetle commonly found in ponds. It is a common arthropod of class insect commonly known as water beetle frequently found in the Asian countries (Sharma and Nirupma, 2012). This insect is found in area of Saharsa and Muzaffarpur district of Bihar. Rise, growth and development of this insect is a cause of concern of this water animals because this has enormous capacity to grown in the local environment and their maturation time is really a point of focus to research over this insect. Insects consume specific diets to obtain the necessary mixture of nutrients that are essential to fuel their survival, growth, and fecundity (Behmer, 2009). The fundamental reason all animals eat is to acquire nutrients that are necessary for survival, growth and reproduction. Protein and carbohydrates, which provide necessary amino acids and energy, respectively, are two important macronutrients that influence animal survival, growth and reproduction (Karasov & Martinez del Rio 2007, Simpson Raubenheimer, 2012), but for most animals there is a species-specific protein/carbohyadrate blend that results in optimal performance (Simpson et al., 2003, Lee et al., 2005, Behmer & Joern 2008, Simpson et al., 2012).

In all nutrients Proteins and carbohydrates are two important macronutrients that provide the essential amino acids and energy, respectively, that influence their development, growth, and fecundity (Karasov and del Rio 2007; Simpson and Raubenheimer 2012). Recent research has documented that some insects are capable of regulating their intake of protein and carbohydrate independently when provided the opportunity (Simpson et al. 2004, Lee et al. 2008, Dussutour and Simpson 2009, Altaye et al. 2010).

Therefore, we have to observe the impact of carbohydrate and protein in the successes survival and adult development of *Cybister Confusus*.

Material and Methods

Experimental Insects

C. confusus eggs were obtained from a *Heliothis virescens* culture. These eggs came from moths that had been reared on a corn-soy-milk (CSM)-based diet modified by the methods described in the study of Burton (1970). All experimental neonates hatched approximately at same time and within a few hours of hatching and they were transferred, using a fine-tipped paint brush. A Solo cup that contained a block of experimental food, all eggs was placed on each individual cup, and all cups were transferred to an insect growth chamber in an incubator at 30±2^{0}C, at photoperiod of 10:14 (L:D).

Experimental Diets

A total of seven CSM-based diets were used for this experiment. They all had similar combined total protein (P) and digestible carbohydrate (c) amounts (42% by dry mass) but differed in their p/c ration: (1) p10.5:c31.5 (10.5% protein and 31.5% carbohydrate), (2) pl4:c28, (3) p17.5:c24.5, (4) p21:c21, (5) p24.5: c17.5, (6) p28:c14 and (7) p31.5:c10.5. These protein/carbohydrate ratios represent values found in plants that would likely be eaten by. *H. virescens*. The inclusion of a basal amount of CSM to the diet (20% of the total dry mass of the experimental food which contributed 3.68% protein and 10.0% carbohydrates to each treatment).

The protein of the synthetic diet was a 3:1:1 mixture of casein, peptone and albumen, the digestible carbohydrate portion was entirely sucrose. Other nutrients in the synthetic diet included Wesson’s salt (1.92%), cholesterol (0.4%), linoleic acid (0.4%), ascorbic acid (0.24%) and a vitamin mix (0.16%), with the remaining portion being non-nutritive cellulose. These combined dry ingredients were suspended in a 1% agar solution, in a 1:6 dry\wet ration finally, to each treatment, formaldehyde (0.1 ml per 200 ml), methylparaben (0.4 gm per

200 ml) and a microbial inhibitor (0.5 ml per 200 ml, were added to suppress the growth of mould and other microbial agents.

Adult Development

Overall survival success was calculated by subtracting 35% of the pupating individuals from the starting 60 individuals for each diet. By resetting the starting population size, we were able to accurately score exclusion success. The number of enclosing adults was then divided by the revised population number, which allowed us to calculate an average total survival percentage for each diet. For individuals that successfully enclosed, development time was recorded in days from hatch to exclusion.

Upon enclosed a single male and female form same diet treatment were randomly paired and placed into an egg-laying chamber for 6 days. These cambers were composed of two components. The first was a capped 50-ml plastic centrifuge tube, standing upright, which held the mating pair. The second was I.5-ml ependorf tube, filled with a 10% sucrose solution, pushed through a hole that was drilled in the cap of the larger tube. A hole at the bottom of the 50-ml tube prevented leaked sucrose solution from accumulating. Inside each egg-laying chamber, there was a paper strip for females to lay their eggs. This strip loosely covered the inside of the larger tube in a single layer and was changed every 2 day. Adult moths were monitored daily, and when a death occurred, the date was recorded and the dead moth was death occurred, the date was recorded and then placed into separate sealed 8 oz. squat polypropylene containers to monitor offspring viability for each mating pair. Egg viability was calculated by dividing the total number of hatchlings by the number of eggs laid.

Statistical Analysis

Logistic regression was used for form the pupal to the adult stage. For total overall survival, odds ratios were used to make comparisons between treatments, Survival analyses were used for developmental time to pupation, from pupation to eclosion, and for the total time from neonate to eclosion, Finnaly, logistic regression was used to analyse: (i) the number of mating pairs and (ii) the number of egg producing pairs. ANOVA was used to compare egg production and egg viability of egg producing pairs.

Result-

Survival from hatch to eclosion was significantly different across the seven treatments (Logistic Regression: d.f =6 χ^2= 33.91, p< 0 001). It was equally high on the diets with balanced (p21:c21) and slightly imbalanced p/c ratios (p17.5: c24.5 and p24.5: c17.5), but then steadily declined as the p/c ratios of the diets became more imbalanced (Fig. 1a). Development time form hatch to eclosing was also significantly different across the treatments (Survival analysis: d.f. =6 χ^2=37.69, P<0 001; Fig. 1b). It was fastest on the p24.5:c17.5 diet and slowest on the most carbohydrate-biased diet (p10.5:c31.5). Lifetime development on the p14/c28 and p17.5:c17.5 diet. The remaining three diets, which all had protein content equal to, or > 21% developed at rates similar to the p24.5: c17.5diet.

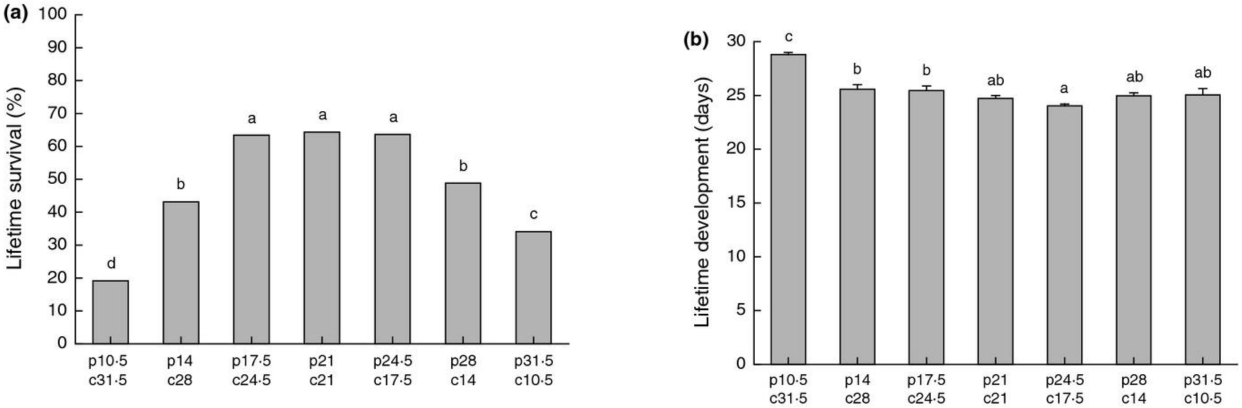

Fig- 1 (a and b)-Cumulative performance on diets with different protein/carbohydrate ratios. Fig. (a) shows survival, from hatch to eclosion, measured as a per cent. Fig- (b) shows the mean (±SEM) development time for individuals from hatch to eclosion.

The number of mating pairs and egg-producing pairs was highly variable due to the different survival rates across the treatments. Both the number of mating pairs (Logistic Regression: d.f = χ^2= 31.38, P<0.001) and the number of egg producing pairs (Logistic Regression: d.f=6 χ^2=22.14, P = 0.001) were statistically different across treatments. When egg production was analyses, no significant treatment effect was observed (ANOVA: $F_{5,\ 30}$=1.70, P=0.165). All mating pairs that produced eggs generated viable offspring, but egg viability was significantly affected by the p/c ration of a diet (ANOVA: $F_{5,\ 30}$ = 4.08, P= 0.006).

Discussion

For capital-breeding insects, larval food quality impacts both larval growth and adult reproduction (Boggs 1997; Casas et. al 2005; wesels 2000). However, it is becoming well established that suboptimal nutrition during development can significantly affect physiological processes later in life (Desai & Hales 1997; Metcalfe & Monaghan 2001; Criscuolo et al. 2008). Our pupal eclosion results (especially survival) provide another example of this phenomenon, with one notable difference – females, compared with males, appear to be more resilient to deviations from an optimal p/c ration. In the studies by Telang et al. (2001) and Lee (2010), it has shown that male *Cybister confususs* prefer diets with a balanced p/c ration while female *C. confusus* self-select a slightly protein biased diet. Our eclosion results suggest that balanced diets are functionally best for males. But why are male pupae, compared with females, so sensitive to protein/carbohydrate imbalances?

Where dietary protein and carbohydrates are imbalanced relative to requirements, animals (including caterpillars will eat greater amounts of food to increase their intake of nutrients that are in deficit (Behmer 2009; Simpson & Raubenheimer 2012). Thus, *Cybister confusus* on foods with high p/c ratios (e.g. p28:c14 and p31.5: c10.5) will ingest large amount of protein to acquire carbohydrates that are in deficit [as shown in *H. virescens*. (Lee et al., 2006)]. This compensatory behavior can, however, come at a cost. For example , ants self–select carbohydrate-biased diets, but when they are maintained on protein biased diets, they suffer high mortality rates (Dussutour & Simpson 2009, 2012, Cook et al. 2010); it was suggested that for worker ants, protein is toxic when ingested in excess of requirements (although the toxicology of this phenomenon is poorly understood). Perhaps too much dietary protein is also detrimental of male caterpillars? Lee (2010) has shown that female *C. confusus* on high protein diets, compared with males, are more efficient at utilizing ingested protein for growth. Additionally, females, in contrast to males, sequester metabolized protein (absorbed across the gut lumen, and into the haemolymph, in the form of amino acids) using storage proteins (Telang et al. 2001). This serves the following two purposes: First, metabolic cost are reduced because less nitrogenous waste is produced, processed and excreted, second, during the adult stage, females can mobilize amino acids from storage proteins to produce vitally, the primary protein found in egg yolk (Chapman, Simpson & Douglas 2013).

C. confusus, including *H. virescens*, also over eat foods that are carbohydrate- biased, in an attempt to redress protein deficits (Behmer 2009). In Most insects, sugar ingested in excess of requirement is converted to lipids and stored (Warbrick-smith) et al. 2006). Thus as a consequence of overeating carbohydrate biased foods to meet protein needs, lipid levels on our two most carbohydrate- biased diets (p10.5:c31.5 and p14:c28) were very high. High lipid content in females is not necessarily a bad thing, because lipids are an important egg component, with triacylglycerol comprising about 40% of a terminal oocyte (Chapaman 2013). In contrast, males do not have a great need for excess lipids (With the potential exception of migration). It is also the case that male *C. confusus*, compared with females. Have reduced carbohydrate utilization efficiency (Lee 2010). Our low eclosion success for males on the two most carbohydrate-biased diets suggests that there is a tangible metabolic cost associated with males when they consume excess carbohydrates.

To our knowledge, this is the first study to quantify the effects of food p/c ration on reproductive output in a Coleopteran, the reason for the paucity of data relates to the failure of earlier nutritional defined artificial diets (specifically ones with modified protein carbohydrate con-tent) to adequately support larval growth through to eclosion, so that reproductive ability of individuals could be measured.

Population sizes were calculated using survival to eclosing, egg production, and egg viability for each treatment. It is well established that food nutrient quality (especially food p/c ration) significantly affects insect herbivores, but our study demonstrates how a more complete understanding can be gained by tracking food p/c ration effects through multiple life stages. Our study also highlights the importance of separately tracking dietary effects on males and females.

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