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Effects of protein type and concentration on development and reproduction of the German cockroach, *Blattella germanica*

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Abstract

Nymphal development and adult female reproduction were examined in the German cockroach, *Blattella germanica*, using a defined artificial diet in which the type of protein was varied. Milk proteins, including casein, supported development poorly compared to meat and plant proteins. Soybean protein supported development better than all other highly purified proteins including vitamin-free casein which is commonly used in artificial diets. Last instar females fed the soybean-based diet eclosed earlier at higher eclosion weights, developed their oocytes at a faster rate and experienced higher fecundity than females fed a vitamin-free casein-based diet. Last instar females exhibited different dose-response patterns on diets containing soybean isolate or vitamin-free casein. However, at all concentrations soybean protein was superior to casein in supporting development. The results of a food utilization study during the last instar revealed that consumption rates varied between females fed the soybean and casein based diets. However, approximate digestibility, efficiency of conversion of digested food and the efficiency of conversion of ingested food did not vary significantly between the two dietary treatments. Differential development of females fed the two diets was attributed to differences in stage-specific consumption rates and the poorer quality of casein as a source of protein for development in this species.

Introduction

The general nutritional requirements of insects are well understood and have been summarized in several recent reviews (Dadd, 1985; Reinecke, 1985). Well-defined diets as well as less defined complex diets are essential for the study of insect nutrition and the regulation of feeding; unavailability of such diets has limited nutritional research on the German cockroach, *Blattella germanica* (L.). Nutritional research on cockroaches has focused on essential nutritional requirements and optimal proportions of nutrients in the diet including the quality of various dietary constituents such as amino acids, carbohydrates, sterols, lipids and salts (House, 1949ab; Noland *et al.* 1949; Noland & Baumann, 1951; Haydak, 1953; Gordon, 1959; House, 1969). The quality of whole proteins, however has been largely ignored.

Studies on the effects of proteins on insects have shown variation in developmental and reproductive rates as well as in digestive and excretory physiology in response to changes in either the source or concentration of dietary nitrogen, or both (Noland & Baumann, 1951; Haydak, 1953; Gordon, 1959; House, 1969; Cochran *et al.*, 1979; Briegel, 1985; Hamilton & Schal, 1988; Bloem & Duffey, 1990; Hamilton *et al.*, 1990). Horie & Watanabe (1983) reported that hemolymph proteins decreased and uric acid excretion increased in *Bombyx mori* (L.) larvae fed low quality proteins such as zein and corn gluten. However, supplementation of these proteins with their limiting amino acids resulted in higher hemolymph protein concentrations and lower levels of uric acid excretion. Casein protein was found to support larval development better than soybean and corn gluten in both *Heliothis zea* (Boddie) (Bloem & Duffey, 1990) and *Spodoptera exigua* (Hubner) (Broadway & Duffey, 1986, 1988). However, optimal dietary levels of casein differed between the two species and surpluses ($>5\%$) or deficits ($<1\%$) of dietary casein were found to decrease larval weight gain in both species (Bloem & Duffey, 1990). In *Aedes aegypti* (L.) as well as in other aedine species, oogenesis and fecundity were affected by both the quantity and quality of protein ingested from host blood meal (Woke *et al.*, 1958; Lea *et al.*, 1958; Clements, 1963; Briegel, 1985, 1990). The concentration of isoleucine, which is absent in the blood of hominid primates, was determined to be a limiting factor in oogenesis in several aedine species (Briegel, 1985).

In this report we document that nymphal development and adult female reproduction of *B. germanica* are sensitive to changes in both the quantity and quality of protein in defined diets. Detailed food utilization studies were performed with two proteins to determine if differences in development were due to qualitative or quantitative differences between the proteins.

Materials and methods

Insects. German cockroaches were obtained from a stock colony raised on Purina Rat Chow (#5012) and water at 27 ± 0.5 °C under a L12:D12 photoperiod regime. Cockroaches were collected from the stock colony at various developmental stages and placed on diets containing one of fourteen proteins (see Table 1). All experiments were conducted at 27 ± 1.0 °C, 50% r.h. and a L12:D12 photoperiod regime. In all experiments, unless otherwise stated, insects were housed in 15×2 cm petri dishes with water continuously available.

Diets. All ingredients were weighed individually and mixed thoroughly in a mortar. Vitamins and corn oil were weighed separately, dissolved in a 3:1 solution of petroleum ether and methanol and added to the dry ingredients. The slurry was mixed frequently until most of the solvents had evaporated, aerated in a fume hood for 24 h and then placed in an oven at 30 °C for an additional 24 h. Diets were stored under refrigeration until used.

In order to compare proteins at the same concentration it was necessary to adjust the protein: carbohydrate ratios to control for differences in the purity of proteins (see Table 1). The quantities of all other ingredients remained constant. A complete listing of all ingredients and their proportions is in Table 2. The purities of different proteins were provided by the respective suppliers.

Table 1. Proteins examined in developmental and reproductive studies on *B. germanica*

	Protein type	Protein purity (%)	Supplier
Plant	Cottonseed flour	52.5	Sigma # C4898
	Soybean flour	52.0	Sigma # S9633
	Soybean concentrate	70.0	Bio-Serv # 1520
	Soybean peptone	80.6	Sigma # P1265
	Soybean isolate	90.0	Bio-Serv # 1510
	Zein from corn	92.0	Sigma # Z3625
Meat	Beef extract	78.0	Sigma # B4888
	Chicken egg albumin	82.5	Sigma # A5253
	Meat peptone	100.0	Sigma # P7750
Milk	Acid hydrolyzed casein	85.0	Sigma # C9386
	Technical casein	86.9	Sigma # C0376
	Vitamin free casein	87.0	Bio-Serv # 1100
	High nitrogen casein	88.0	Bio-Serv # 1099
	Lactalbumin	86.3	Sigma # L7252

Table 2. Composition of artificial diets

Ingredient	Percentage in diet (w/w)
Protein (type variable)[a]	22.5
White dextrin (type III)[b]	48.9
α-Cellulose[b]	20.0
Wesson's modified salt mix[c]	4.0
Cholesterol (95%)[b]	1.0
Vanderzant vitamin mix for insects[d]	0.6
Corn oil[b]	3.0
Total	100

[a] Protein:carbohydrate ratio adjusted for purity of each protein.
[b] supplied by Sigma Company.
[c] supplied by Bio-Serv.
[d] supplied by ICN.

Cooper & Schal (1992) showed differential development of *B. germanica* females fed various laboratory chows, with females developing faster on Purina Rat Chow than on two different Dog Chows. Therefore, Purina Rat Chow (Purina Mills, St. Louis, Product #5012) was selected as the control diet for this study.

Preliminary studies. Initially, all diets were compared in last instar females, with the level of dietary protein held constant at 22.5%. Newly emerging last instar females were placed in groups of twenty into petri dishes with water and one of the experimental diets. The duration of the last instar and eclosion weight were recorded for each individual. Upon eclosion females were placed into petri dishes in groups of 3 to 5 individuals to avoid deleterious effects of isolation (Gadot *et al.*, 1989). Adults were provided the same diet as the respective last instar nymphs. On day 7 females were dissected and the length of their terminal oocytes measured using an ocular micrometer in a dissecting microscope.

Nymphal development. Gravid females from the stock colony were checked daily for oothecal hatch. Newly-hatched nymphs were weighed in groups of twenty and placed into petri dishes with water and one of four experimental diets or Rat Chow. Nymphs were counted and weighed every seven days (without use of anesthetics) at which time food and water were replaced and debris removed from each dish. Development of females was assessed by comparing the weekly weights of nymphs, the duration of nymphal development and female weight at the time of eclosion.

Oocyte maturation and female reproduction. In order to examine both the utility of each protein and the effects of nymphal nutrient reserves on oocyte maturation, oocyte lengths were measured in 7-day-old adult females which had been placed on experimental diets at different developmental stages (day-0 neonates, day-0 last instars or day-0 adults).

The effects of proteins on reproduction were examined in females that had been placed on diets as last instar nymphs or as adults. Upon eclosion females were placed individually into petri dishes (10 × 2 mm) containing the respective nymphal diet and two adult males from the stock colony. Time intervals between major reproductive events (eclosion to oothecal formation, oothecal formation to hatch and hatch to formation of subsequent oothecae) were measured. The number of eggs per ootheca, number of emergent nymphs and percent hatch were also recorded. Upon hatching of each ootheca, food, water and males were replaced. This study was conducted through four reproductive cycles.

Protein concentration. The possibility that different proteins might have different dose-response patterns was investigated with 10%, 15%, 22.5%, 30%, 35% or 40% vitamin-free casein and soybean isolate. Last instar females were placed on the diets and the duration of the instar, adult weight and oocyte length in 7-day-old adults were recorded.

Consumption. Newly emerged adult females were placed individually in petri dishes containing two females with glued mouthparts, water and a pre-weighed capsule packed with diet. Consumption was monitored daily by weighing food vials on a Mettler HK 160 balance during the first four days of adulthood. Females were fed either Rat Chow or a hydrolyzed casein diet during the first three days to establish that consumption rates were the same among females within each treatment, and then switched to a new diet (Rat Chow, vitamin-free casein, hydrolyzed casein or soybean isolate). Consumption was compared during the first three days and on the fourth day.

Food utilization. Females were collected as newly emerged last instar nymphs. Groups of three females were weighed and housed in petri dishes (10 × 2 cm) containing water and either a vitamin-free casein or a soybean isolate diet. Females, feces and food vials were weighed separately on days 3, 6 and 9. Dry weights were obtained for diets, feces and females on days 0, 3, 6 and 9 after 72 hours in an oven at 65 °C. Dry mass was used to calculate the following dietetic parameters: Assimilated food (ASM) = food ingested (F)-feces produced (E), Approximate Digestibility (AD) = (ASM/F) × 100, Efficiency of Conversion of Ingested Food (ECI) = (biomass gained (G)/F) × 100 and the Efficiency of Conversion of Digested Food (ECD) = (G/ASM) × 100 (Waldbauer, 1968; Slansky & Scriber, 1984).

Data analysis. All results were analyzed with SAS Institute (1985) analysis of variance (ANOVA) and Duncan's Multiple Range test ($P < 0.05$) or with Student's *t*-test ($P < 0.05$).

Results and discussion

The type of protein provided in the diet clearly affected development in *B. germanica* females. Females placed on various diets as teneral last instars exhibited significant variation in the mean duration of the last stadium (10.8 to 12.7 days), eclosion weight (57.6 to 69.5 mg) and oocyte length in 7-day-old adults (0.63–1.87 mm) depending on the type of protein provided in the diet (Fig. 1). It is important to restate that unless otherwise noted, all diets were corrected to 22.5% protein.

Although interpretation of some of these results is confounded by the wide range of protein purities (Table 1), no clear relationship was evident between the purity of proteins and development. Females fed cottonseed flour (purity of 52.5% protein) exhibited similar developmental parameters to control females fed Rat Chow, while females fed soybean flour (purity of 52% protein) exhibited significantly inferior development (Fig. 1). Similarly, of the highly purified proteins examined (purity of ≥85% protein), only females fed the soybean isolate diet experienced similar development to control females (Fig. 1).

Development was generally slow in insects fed milk proteins, including vitamin-free casein, but better on meat and plant proteins (Fig. 1). This was particularly surprising given the common use of casein as a source of protein in artificial diets for many insects, including the German cockroach. Noland & Baumann (1951) found development of *B. germanica* nymphs to be better on a casein-based diet compared to diets containing egg albumin, lactalbumin, wheat gluten, hydrolyzed casein, oxidized casein, zein, or hemoglobin. Similarly, Engelmann (1969) found that midgut protease activity was higher in *Leucophaea maderae* (F.) fed rat food or casein than in insects fed albumin, zein, hemoglobin, peptic peptone, casein hydrolysate, or protein extracted rat food. In neither study, however, was soybean protein tested.

Four proteins (soybean isolate, cottonseed flour, vitamin-free casein and hydrolyzed casein) were selected for further studies. Vitamin-free casein was selected because it is commonly used in artificial diets with cockroaches as well as other insects, while hydrolyzed casein was included to examine its constituent amino acids as a source of dietary nitrogen. Soybean isolate and cottonseed flour diets proved to be as effective as Rat Chow in all of the developmental parameters.

Nymphal development, as measured by changes in body mass, was retarded in nymphs fed vitamin-free casein or soybean isolate compared with nymphs fed either cottonseed flour or rat food (Fig. 2). Insects fed casein or soybean diets weighed significantly less as early as the third week of development, and they exhibited significant delays in eclosion, lower percentages

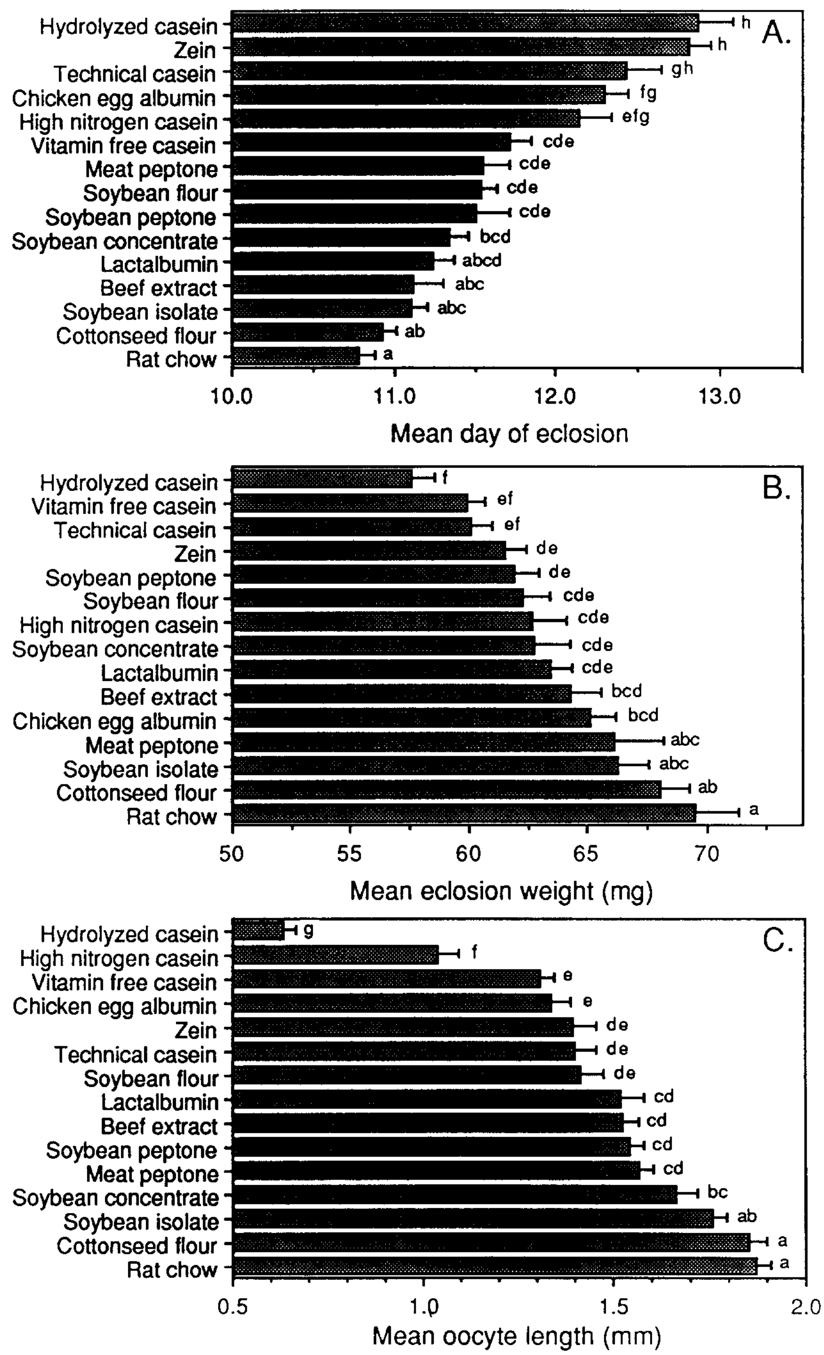

Fig. 1 Mean days of eclosion (A.), mean fresh eclosion weights (B.), and mean oocyte lengths in 7-day-old adults (C.) of female *B. germanica* fed diets containing different proteins or Rat Chow. Diets were provided to newly emerged last instar females. Bars indicate SEM. Different letters indicate significant differences among females fed diets containing different proteins ($P < 0.05$; Duncan's multiple range test).

of emergence and lower eclosion weights (Table 3). Although nymphal development on the cottonseed flour-based diet was indistinguishable from that on Rat Chow, the high percentage of unknown constituents (47.5%) in this protein source limits its utility in defined diets.

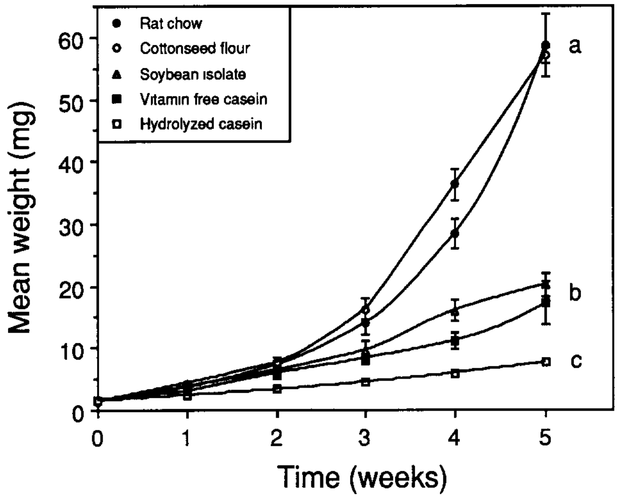

Fig. 2. Mean weekly fresh body weights ($\pm$ SEM) throughout the first five weeks of nymphal development. Neonate nymphs were fed Rat Chow or one of four diets containing 22.5% of different proteins. Different letters indicate significant differences between treatments during weeks 3, 4 and 5 ($P<0.05$; Duncan's multiple range test).

Noland *et al.* (1949) reported that nymphs raised on an artificial diet containing 30% casein developed faster than on the best crude control diet (dog biscuits). In our laboratory, however, nymphs provided the standard 22.5% casein diet or a 30% casein diet developed at similar rates (Cooper, unpubl.). It is possible that impurities in both diets may have contributed to the disparate results. Growth factors, usually present in diets as trace nutrients, have been implicated as limiting factors for proper development of insects (see review: Dadd, 1985). Often these necessary factors are absent in highly purified diets, complicating the development of these diets and thus the study of nutrition. Casein often lacks or is deficient in some amino acids such as cystine, glutamic acid and glycine (see review: Reinecke, 1985).

Supplementation of a casein diet with L-cystine and glycine was necessary for normal development in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Vanderzant & Reiser, 1956). With the exception of the study by Noland *et al.* (1949), our results are in accordance with previous studies on *B. germanica*, demonstrating that the defined diets were inferior to complex diets for cockroach nymphal development (House, 1949b; Haydak, 1953; Forgash, 1958; Gordon, 1959).

Oocyte maturation in adult females was dependent on both the type of protein in the diet (Fig. 1) and the developmental stage during which females were provided the diet. Females fed diets containing soybean or casein proteins since the first instar failed to mature their oocytes beyond the state of newly eclosed females (Fig. 3A). However, when these diets were provided to last instar nymphs, oocyte maturation was not inhibited (Fig. 3B). As shown in a previous experiment (Fig. 1), provision of vitamin-free casein during the last instar resulted in females with significantly smaller oocytes than in females fed soybean protein, cottonseed flour or Rat Chow. Except for hydrolyzed casein, the type of protein had no effect on oocyte maturation when

Table 3. Nymphal development in female *B. gemanica* fed diets with different proteins

Diet[1]	N	Mean day of eclosion[2]	% emergence	Mean adult weight (mg)[2]
Rat food	100	39.0 ± 0.1a	89	64.0 ± 1.2a
Cottonseed flour	100	42.3 ± 0.3b	83	63.0 ± 0.9a
Soybean isolate	100	55.2 ± 0.7c	30	55.0 ± 1.9b
Vitamin free casein	100	58.0 ± 0.5c	9	54.0 ± 2.6b
Hydrolyzed casein	100	–	0	–

[1] All diets contained 22.5% protein. Females were placed on diets as newly hatched first instar nymphs.
[2] The same letters within columns indicate no significant differences (Duncan's multiple range test; $P>0.05$). – indicates failure of all females to reach adulthood.

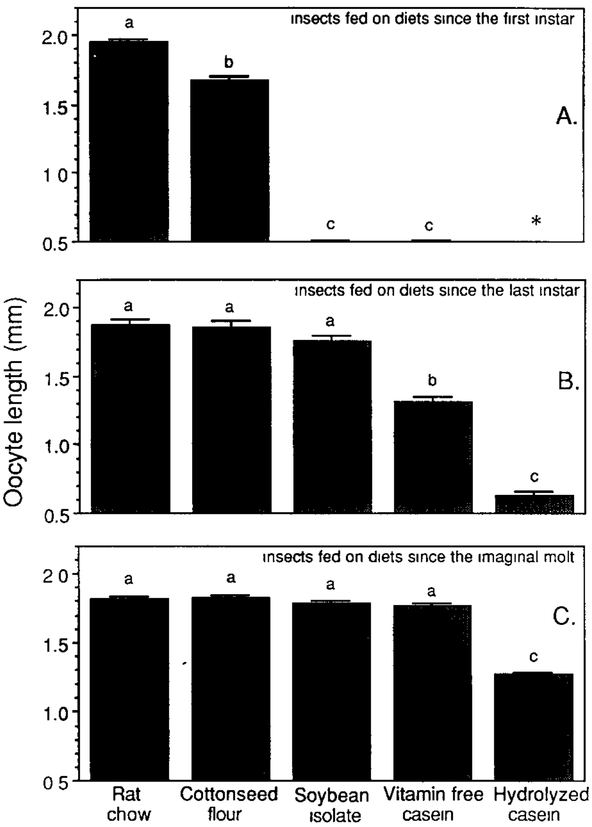

Fig. 3. Oocyte lengths in 7-day-old adult females which had been fed Rat Chow or one of four diets varying in protein type since the first instar (A.), since the last instar (B.) or as newly emerged adults (C.). Bars indicate SEM. Different letters indicate significant differences among treatments ($P<0.05$; Duncan's multiple range test). An * indicates failure of all females to eclose

females were provided the diets as adults only (Fig. 3C).

The first four reproductive cycles were studied in females that were placed on these diets as last instar nymphs. The effects of protein type on female fecundity were similar to those on oocyte maturation. Females fed a cottonseed flour-based diet reproduced normally throughout all four reproductive cycles compared with females fed Rat Chow (Tables 4, 5). Females fed the soybean protein diet reproduced normally through three reproductive cycles before significant delays in reproduction and reductions in fecundity were realized. However, when vitamin-free casein was provided during the last instar, females experienced delayed reproduction and produced smaller oothecae that contained significantly fewer eggs in the first gonotrophic cycle. These effects were delayed to the third reproductive cycle when the casein diet was provided during the adult stage only (Tables 4, 5). When fed diets as last instar nymphs, total fecundity during the first four reproductive cycles was reduced by 35% in females fed vitamin-free casein and by 83% in females fed hydrolyzed casein, compared with control females. Furthermore, only 50% of the females that were fed vitamin-free casein produced a third ootheca and only 8 of 20 females produced a fourth egg case (Table 4). However, the percent hatch and incubation period of all oothecae were similar among all treatments throughout all four reproductive cycles (Cooper, unpubl.).

In a closely related cockroach, *Supella longipalpa* (F.), females exhibited delays in reproduction and reduced fecundity on the fourth reproductive cycle when provided a low protein diet in the adult stage only (Hamilton *et al.*, 1990). These delays were shifted to the first gonotrophic cycle when the protein deficient diet was provided in both the last instar and the adult stage. It was suggested that nutrient reserves which were accumulated during nymphal development might have been mobilized and used to maintain normal reproduction in adult females fed a low protein diet. The results of our study indicate that the type of protein present in the diet affects oocyte maturation and reproduction in adult female German cockroaches and that nymphal reserves play a similar role as demonstrated in *S. longipalpa*.

Results from these experiments suggest that for *B. germanica* soybean isolate is a better protein source than vitamin-free casein. There are several possibilities for the observed differences between casein and soybean proteins: Females may feed differentially on the two diets, the utilization of the two proteins by females may vary, the required dietary levels of soybean and casein proteins may differ, or differential development may be related to differences in the quality of the two proteins. Experiments were conducted to examine each of these possibilities.

High concentrations of protein have been

Table 4. Mean time intervals (days ± SEM) between reproductive events during the first four reproductive cycles of female *B. germanica* fed diets containing different proteins

Diet[1]	A-EC1[2]	EC1-EC2[2]	EC2-EC3[2]	EC3-EC4[2]
A. On diet as last instar nymphs and adults				
Rat Chow	10.2 ± 0.1 a (20)	5.3 ± 0.5 a (20)	6.9 ± 0.9 a (17)	6.7 ± 0.2 a (17)
Cottonseed flour	8.9 ± 0.4 a (20)	6.3 ± 0.5 a (20)	5.9 ± 0.1 a (18)	7.5 ± 0.7 ab (18)
Soybean isolate	9.4 ± 0.4 a (20)	6.2 ± 0.4 a (20)	6.8 ± 0.8 a (18)	9.7 ± 0.6 b (15)
Vitamin free casein	12.1 ± 0.6 b (18)	8.2 ± 0.6 b (16)	10.8 ± 0.6 b (10)	11.0 ± 0.1 c (8)
Hydrolyzed casein	14 9 ± 0.7 c (16)	11.0 ± 0.8 c (10)	–	–
B. On diet only as adults				
Rat Chow	10.2 ± 0.1 a (20)	5.3 ± 0.5 a (20)	6.9 ± 0.9 a (17)	6.7 ± 0.2 a (17)
Cottonseed flour	9.2 ± 0.5 a (20)	5.9 ± 0.4 a (20)	6.8 ± 0.3 a (20)	6.9 ± 0.6 a (20)
Soybean isolate	9.5 ± 0.3 a (20)	6.0 ± 0.4 a (20)	6.5 ± 0.3 a (20)	7.1 ± 0.4 a* (18)
Vitamin free casein	9.5 ± 0.4 a* (20)	7.9 ± 0.4 b (19)	10.9 ± 0.6 b (18)	11.2 ± 0.6 b (15)
Hydrolyzed casein	11.9 ± 0.6 b* (20)	11.2 ± 0.9 c (12)	–	–

[1] All diets contained 22.5% protein. Females were maintained on Rat Chow and placed on artificial diets beginning either as newly emerged last instar nymphs or as newly emerged adults.
[2] A = adult emergence, EC1 = oviposition of first ootheca, EC2 = oviposition of second ootheca, etc. The same letters within columns indicate no significant differences (Duncan's multiple range test; $P > 0.05$). – indicates failure of all females to produce oothecae. An * indicates significant differences between the time taken to produce an ootheca by females fed an artificial diet as an adult only, as compared to females fed the same diet as both a last instar nymph and an adult (Student's *t*-test; $P < 0.05$) N is indicated in parentheses.

shown to delay reproduction, reduce fecundity and decrease longevity in a number of cockroach species, including *B. germanica* (Noland & Baumann, 1951; Haydak, 1953; Mullins & Cochran, 1973; Hamilton & Schal, 1988, Hamilton *et al.*, 1990). Disparate optimal levels of dietary casein have been reported for the German cockroach ranging from 11% (Haydak, 1953) to 40% (Noland & Baumann, 1951), but similar studies have not been conducted with any other proteins. Recently, Bloem & Duffey (1990) reported that the optimal levels of dietary protein for larval *H. zea*

Table 5. Mean number of eggs per ootheca (± SEM) during the first four reproductive cycles for *B. germanica* fed diets containing different proteins

Diet[1]	EC1	EC2	EC3	EC4	Total
A. On diet as last instar nymphs and adults					
Rat chow	42.5 ± 1.8 a	43.5 ± 0.85 a	39.8 ± 1.3 a	41.5 ± 1.2 a	167.3 ± 1.5 a
Cottonseed flour	44.3 ± 1.1 a	42.4 ± 1.17 a	42.7 ± 0.8 a	39.8 ± 1.3 a	169.2 ± 1.2 a
Soybean isolate	43.4 ± 1.8 a	42.9 ± 0.89 a	39.0 ± 1.4 a	32.2 ± 1.3 b	157.5 ± 1.7 b
Vitamin free casein	34.0 ± 1.8 b	30.2 ± 1.94 b	26.0 ± 1.5 b	25.0 ± 0.5 c	115.2 ± 1.8 c
Hydroloyzed casein	26.0 ± 1.3 c	22.5 ± 0.96 c	–	–	48.5 ± 1.2 d
B. On diet only as adults					
Rat chow	42.5 ± 1.8 a	43.5 ± 0.9 a	39.8 ± 1.3 a	41.5 ± 1.2 a	167.3 ± 1.5 a
Cottonseed flour	44.1 ± 1.4 a	43.0 ± 1.23 a	41.6 ± 0.1 a	40.1 ± 1.3 a	168.8 ± 1.2 a
Soybean isolate	43.8 ± 1.3 a	43.9 ± 1.10 a	43.1 ± 1.2 a	39.9 ± 1.0 a	170.7 ± 1.1 a*
Vitamin free casein	42.3 ± 1.6 a*	33.9 ± 1.53 b	30.1 ± 1.2 b	27.6 ± 0.9 b	133.9 ± 1.4 b*
Hydroloyzed casein	28.2 ± 1.1 b	23.4 ± 0.75 c	–	–	51.6 ± 0.9 c

[1] See Table 4 for details. N as in Table 4

and *S. exigua* were lower for casein (2–3%) than for soybean protein (5–6%). Our results indicate that a similar phenomenon might occur in female *B. germanica*.

When fed different diets as last instar nymphs, eclosion times, eclosion weights and oocyte development varied significantly depending on both the type and level of protein in the diet (Fig. 4). In females fed the casein diet oocyte development was significantly advanced at low levels of protein but severely retarded at levels above 22.5%. In contrast, development of oocytes was maximal over a broad range between 22.5% and 35% protein in females fed the soybean based diet. Thus, casein and soybean proteins have different dose-response effects on development and reproduction in *B. germanica*. Although the optimal level of dietary protein varies depending on whether soybean or vitamin-free casein is present in the diet, development of females was better on optimal levels of soybean compared to casein. This indicates that the poor development of females fed the vitamin-free casein diet is due to factors other than protein concentration.

Differences in nymphal and eclosion weights suggested that females might not be consuming equal amounts of the soybean and casein diets. To address this possibility females were fed either Rat Chow or a hydrolyzed casein diet for the first three days after the imaginal molt and on day 4 were switched to different diets. Daily consumption was similar during the first three days in females fed the same diet, but differed significantly depending on whether females were provided Rat

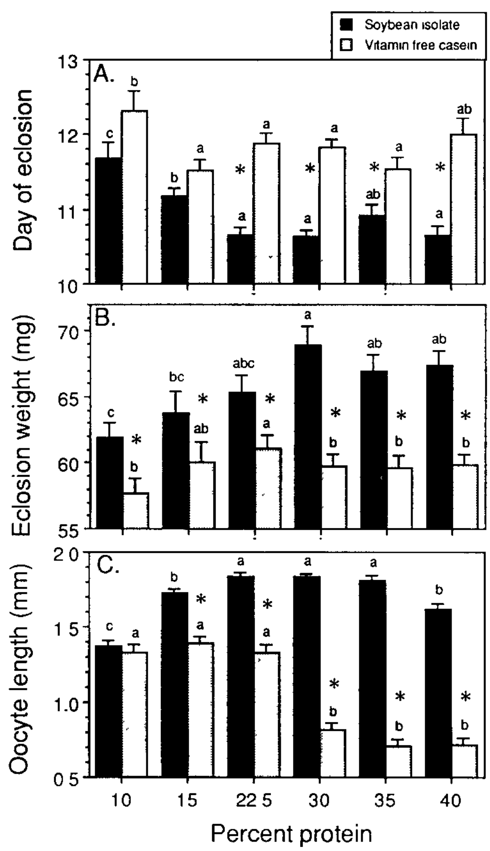

Fig. 4. Mean days of eclosion (A.), mean fresh eclosion weights (B.), and mean oocyte lengths in 7-day-old adult females (C.). Last instar females were fed either soybean isolate or vitamin-free casein based diets varying in the concentration of protein Bars indicate SEM. Different letters indicate significant differences among females fed different concentrations of the same protein ($P<0.05$; Duncan's multiple range test). An * indicates significant differences between females fed different diets of the same protein concentration ($P<0.05$; t-test).

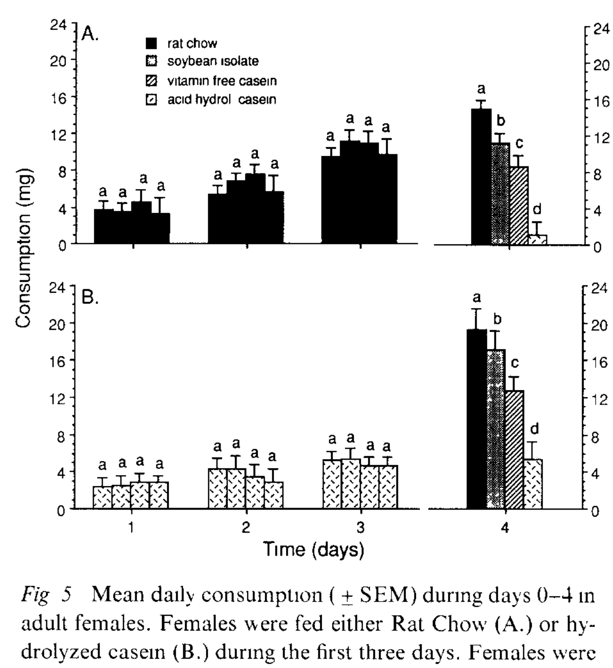

Fig 5 Mean daily consumption ($\pm$ SEM) during days 0–4 in adult females. Females were fed either Rat Chow (A.) or hydrolyzed casein (B.) during the first three days. Females were then switched to 22.5% protein diets varying in protein type or maintained on the same diet as during days 0–3. Different letters for each day indicate significant differences in consumption among females fed different diets ($P<0.05$; Duncan's multiple range test).

Chow or hydrolyzed casein (Fig. 5). Daily consumption of Rat Chow increased from approximately 4 to 10 mg during the first three days but remained at a relatively constant level (approximately 3–5 mg) when hydrolyzed casein was provided. When switched to new diets on day 4, food intake changed depending on the type of protein in the diet. Consumption on day 4 was greatest in females fed Rat Chow, followed by soybean isolate, vitamin-free casein and finally, hydrolyzed casein. Interestingly, females switched from the hydrolyzed casein diet consumed greater quantities of each of the diets on day 4 than females switched from Rat Chow (Fig. 5A).

The results on consumption rates in last instar females between days 0–9 provide additional support for differential intake of casein and soybean proteins in *B. germanica*. Females consumed similar quantities of each diet by the end of the nine day interval; however, consumption rates within this interval varied significantly between treatments (Table 6). Those females that were fed soybean protein consumed greater quantities during the first three days of the last instar than females fed vitamin-free casein. Consumption rates were similar for both treatments during days 3–6 but in females fed vitamin-free casein food intake on days 6–9 was twice that in females fed soybean isolate (Table 6). These results demonstrate that, like adult female *B. germanica* (Cochran, 1983; Hamilton & Schal, 1988), last instar females exhibit stage-specific feeding patterns. When fed a soybean protein diet they feed more early in the instar and less later, toward the molt (Table 6). Nymphs provided with a casein diet in the last instar are developmentally delayed and between days 6 and 9 still exhibit relatively high intake rates.

No significant differences were apparent in the approximate digestibility (AD), conversion efficiencies of digested food (ECD) or conversion efficiencies of ingested food (ECI) in last instar nymphs fed either soybean or casein based diets (Table 6). Thus, delayed development, lower eclosion weights and reduced fitness in females fed vitamin-free casein may be due to lower con-

Table 6. Utilization of two diets by last instar female *B. germanica*

	Weight gain[1] (mg)	Consumption (mg)	AD	ECI	ECD
Days 0–3					
Soybean isolate	34.0 ± 1.7	62.5 ± 2.3	98%	54%	54%
	*	*			
Casein, vitamin-free	27.9 ± 1.4	54.0 ± 1.9	98%	53%	54%
Days 3–6					
Soybean isolate	23.8 ± 1.0	42.0 ± 1.5	98%	54%	55%
Casein, vitamin-free	23.5 ± 0.8	43.5 ± 1.3	97%	52%	54%
Days 6–9					
Soybean isolate	2.6 ± 0.1	4.9 ± 0.1	98%	53%	55%
	*	*			
Casein, vitamin-free	5.8 ± 0.2	9.4 ± 1.0	98%	58%	60%
Days 0–9					
Soybean isolate	60.4 ± 1.9	109.4 ± 2.1	98%	53%	55%
Casein, vitamin-free	57.2 ± 1.6	106.9 ± 1.8	98%	55%	56%

[1] Weight gain, consumption and conversion efficiencies are based on dry weights of at least 20 samples. Females were fed 22.5% protein diets as newly emerged last instar nymphs. Significant differences between diets within the same time period are indicated by * (Student's *t*-test; $P < 0.05$). See text for formulas for dietetic parameters.

sumption rates early in the last instar and to the lower quality of casein protein relative to soybean protein.

As the relationships between nutrition and complex physiological processes in cockroaches become more clearly understood, defined diets with which these relationships can be studied become increasingly important. Recently, Hamilton & Schal (1990) showed that the type of cellulose used in the diet affects consumption, digestibility and reproduction in *S. longipalpa*. Our study indicates that the type, source and concentration of protein strongly affects development in the German cockroach. We have developed a defined diet containing 22.5% soybean isolate which is suitable for use in short term nutritional studies with *B. germanica*. However, continued research is necessary to elucidate limiting factors in this diet which retard nymphal development.

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