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Dietary Protein and Chlorogenic Acid Effect on Baculoviral Disease of Noctuid (Lepidoptera: Noctuidae) Larvae

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ABSTRACT Insecticidal pathogens such as baculoviruses are currently under intensive development as biorational agents for the control of lepidopteran pests. However, because the efficacy of these orally infective viruses is influenced by host diet, our ability to use baculoviruses effectively in an integrated pest management program requires understanding the influence of dietary components on the disease process. Nutritional stress caused by differences in the quality (casein or soy) or quantity (0–8%) of dietary protein altered the postinfectional course of disease caused by *Autographa californica* M nucelopolyhedrovirus in 2 species of noctuids, *Heliothis virescens* (F.) and *Trichoplusia ni* (Hübner). Lethal times of larvae infected with either the wild-type virus or a recombinant expressing a scorpion toxin (AaIT) derived from this parent virus were similarly affected by dietary protein. In general, the higher the level of dietary protein the shorter the lethal times. However, the influence of protein quality on lethal times depended on the insect species tested. The effect of chlorogenic acid on disease depended on dietary protein levels. At high protein concentrations, chlorogenic acid decreased speed of kill; whereas, at low protein levels, the phenolic had the opposite effect. The common factor among all treatments was that the faster larvae grew, the faster they died from viral infection. We suggest that insects that grow faster may support faster rates of viral replication in infected hosts. From an ecological perspective, it is possible that plants of higher protein content may increase the potential for the development of baculoviral epizootics in insect populations.

KEY WORDS *Heliothis virescens, Trichopluia ni*, dietary protein, nucleopolyhedrovirus, recombinant baculovirus, growth

PLANT QUALITY HAS a profound effect on the ability of predators and parasitoids to use their herbivorous hosts (Hogg 1986, Price 1986, Shepard and Dahlman 1988). In this regard, a variety of more mechanistic laboratory studies show that the intake of poor quality nutrients or toxins by the host insect often decreases its ability to resist attack by parasitoids or decreases the fitness of the parasitoids (Duffey and Bloem 1986, Thompson 1993, Roth et al. 1997). Moreover, an intake of surplus nutrients by the host insect can also have a negative effect on the parasitoid (House and Barlow 1961, Duffey and Bloem 1986, Thompson 1993). Likewise, differences in susceptiblity of insects to a given pathogen have been attributed to dietary stress in a number of studies (Schultz 1983, Felton and Dahlman 1984, Noguchi and Yamaguchi 1984, Shepard and Dahlman 1988, Donegan and Lighthart 1989). Dietary stress may result from a diet deficient in nutrients or from the ingestion of plant "toxins." The effect of nutrients on insect disease is poorly understood, however, and experimental results are often apparently contradictory (Benz 1987, Watanabe 1987, Duffey et al. 1995). Further, because the route of entry of baculoviruses is oral (Keddie et al. 1989), it is often assumed that factors affecting virulence must act in the insect's midgut environment within a short time. As a result of this assumption, the influence of postinfectional nutritional stress on the disease process has received little attention. During this early phase, viral replication and occlusion body production occur with a high demand for protein and DNA synthesis. Any diet-related chemical or process that restricts nutrient intake by the insect or requires significant energy expenditure to detoxify plant allelochemicals during the replicative phase of the virus may have an impact on disease progression.

Our aim was to determine how nutritional stress caused by varying the quality or quantity of dietary protein alters the postinfectional course of viral disease (survival curves) in 2 species of noctuids that are considered generalist feeders, *Heliothis virescens* (F.) and *Trichoplusia ni* (Hübner). We used 2 types of protein, soy and casein, because these proteins have

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been shown to vary in their nutritive value for noctuid larvae in a previous study (Broadway and Duffey 1986, 1988). These authors demonstrated that casein supported faster growth rates of noctuid larvae than soy. Furthermore, growth rates were strongly correlated with the arginine and lysine content of these proteins with casein containing ≈30% more of these residues than soy. In our study, we linked growth rates of noctuid larvae to speed of kill by baculoviruses. We also examined the influence of a common plant phenolic, chlorogenic acid, in artificial diets containing different levels of dietary protein on the course of viral disease. Chlorogenic acid has been strongly correlated with retarding growth or development of a number of insect species, particularly at moderate to high protein concentrations (Isman and Duffey 1982a, Isman and Duffey 1982b, Cole 1985).

Materials and Methods

To determine the influence of dietary protein quality and quantity on speed of kill by baculoviruses, we conducted bioassays with 2 different insect species using 2 different viral constructs.

Insects. Eggs of *H. virescens* and *T. ni* were supplied on a weekly basis by the USDA-ARS (Stoneville, MS) and Novo Nordisk Entotech (Davis, CA), respectively. The insects received from Stoneville were from an apparently healthy colony. Both experimental and control insects were examined for symptoms of infection with viruses other than baculoviruses.

Viruses. The wild-type baculovirus *Autographa californica* multiple nucleocapsid nucleopolyhedrovirus (WT AcMNPV, C6 clone) (Ayers et al. 1994) and the recombinant virus derived from AcMNPV, termed AcAaIT, which expresses an insect-specific neurotoxin derived from the scorpion *Androctonus australis* (McCutchen et al. 1991, Stewart et al. 1991), were used for this study. AcAaIT kills noctuid larvae ≈30% faster than the wild-type virus (McCutchen et al. 1991, Stewart et al. 1991, Hoover et al. 1995). Polyhedral occlusion bodies of each virus were amplified in larvae of *H. virescens*. Occlusion bodies were then extracted, partially purified, and stored as described in Hoover et al. (1995).

Diets. Experimental diets containing different concentrations of protein were prepared according to Vanderzant et al. (1962) using either casein or soy (BioServ, Frenchtown, NJ) as the added protein source. All diets contained a background protein level of 0.6% (wet weight) as determined by the Bradford assay (Bradford 1986) and no dietary phenolics as determined by the Folins-Ciocalteau assay (Singleton and Rossi 1965). Thus, when protein concentrations are referred to in this text they indicate only the levels of casein or soy protein. Because protein concentration varied among diets, the amount of alphacel in each diet (a non-nutritive cellulose fiber, ICN Biochemicals, Cleveland, OH) was adjusted accordingly to maintain similar physical properties of all diets. Experimental diets that contained phenolics were prepared in the same manner except for the addition of 3.5 or 7.0 μmoles/g of chlorogenic acid (Sigma, St. Louis, MO) in diets containing 0.5, 2.5, or 6.0% casein only. We did not test the addition of phenolics to diets containing soy protein.

Because we did not want to confound mortality caused by inadequate nutrition with mortality caused by virus, we used a range of protein concentrations of casein and soy in the experimental diets that did not produce mortality in uninfected insects during the duration of a given bioassay (≈1 wk). Thus, for *H. virescens* we used 6 different casein concentrations (0.5, 1.0, 2.5, 4.0, 6.0, and 8.0% wet weight) and 4 different soy concentrations (1.0, 2.5, 6.0, and 8.0% wet weight). For *T. ni* we used 4 casein concentrations (1.0, 2.5, 6.0, and 8.0% wet weight) and 4 soy concentrations (2.5, 4.0, 6.0, and 8.0% wet weight). A subsample of 10 insects from each treatment group were crushed and examined under a light microscope for evidence of polyhedral occlusion bodies to verify that mortality was caused by viremia and not inadequate nutrition. If occlusion bodies were not observed, a plaque assay was performed for evidence of budded virus (O'Reilly et al. 1992). All subsampled insects showed evidence of viral infection.

Bioassays. Neonate larvae of *H. virescens* and *T. ni* were dosed using a viral preparation at a concentration of 2,000 and 1,000 occlusion bodies per microliter, respectively, with either AcAaIT or WT AcMNPV using the droplet feeding technique (Hughes et al. 1986) and transferred to their respective diets 30–45 min postingestion. We used a higher concentration of virus for droplet feeding *H. virescens* because lethal doses of AcMNPV in *H. virescens* are higher than in *T. ni* (Bonning et al. 1992, 1995). Because these concentrations deliver the approximate LD_{99} for these insect species regardless of diet quality (Hoover et al. 1998), only time to death was monitored. The effect of chlorogenic acid on speed of kill was tested on *H. virescens* dosed with WT AcMNPV only. The viral preparation used for droplet feeding contained occlusion bodies suspended in double-distilled water with 5% blue food dye (vol:vol) and 0.06% maltose (wt:vol). Controls consisted of larvae fed the same preparation without virus. Larvae were maintained at 26 ± 1°C and a photoperiod of 16:8 (L:D) h in 25-ml plastic cups containing an excess of experimental diet. Mortality was scored every 4–8 h, depending on the mortality rate until all insects had died. Each treatment consisted of 35–40 larvae. All treatments were replicated 3 times.

To verify that the influence of dietary protein on lethal times was a postinfectional effect and not the result of direct interactions between viral inoculum and dietary protein, we performed a series of cross-transfer experiments. Neonate larvae were droplet fed viral inoculum (WT AcMNPV only) as described above and immediately placed on either 1 or 6% casein diets. Two groups of 35 larvae were placed on each diet. After 24 h, one group of insects feeding on 1% was transferred to 6%, whereas the other group was transferred to fresh 1% protein. In a similar manner, one group of insects feeding on 6% was transferred to 1%,

Table 1 Influence of protein quality and quantity on lethal times of *H. virescens* infected with AcMNPV or AcAaIT

Variable	Parameter coefficient × SE	Effect on rate of mortality	z-statistic	*P* value
Virus	+1.2 ± 0.05	AcAaIT killed faster	21	<0.001
Protein quality	−0.69 ± 0.09	Rate of mortality faster on casein than soy	−7.6	<0.001
% protein	+0.14 ± 0.01	Rate of mortality " as % protein ↑	+11	<0.001
Protein quality × % protein	−0.07 ± 0.02	Rate of mortality " faster on casein than soy as % protein ↑	−3.7	<0.001

Virus (WT AcMNPV = 0, AcAaIT = 1) and protein quality (casein = 0, soy = 1) were entered as categorical variables. Model likelihood-ratio chi-square = 582, df = 4, $P < 0.001$, n = 1,612. Model: $\lambda = \exp(\beta X)$, where βX = {1.2 (virus) − 0.69 (protein quality) + 0.14 (% protein) − 0.07(protein quality × % protein)}. There were 35 larvae in each treatment and all treatments were replicated 3 times. Protein concentration of diets varied from 0.5 to 8.0% (see Figs. 1 and 2).

whereas the other group was transferred to fresh 6% protein. Larvae were maintained and time to death was monitored as described above.

Relative Growth Rates. To determine if the speed of virally induced mortality was correlated with relative growth rates of infected larvae, a separate group of 20 larvae were infected as described above and maintained for 2 d (AcAaIT-infected insects) or 3 d (WT AcMNPV-infected insects and uninfected controls) on each experimental diet. We chose these time points to measure growth rates because this was the point at which ≈50% of infected insects began to show symptoms of viral infection. After 2 or 3 d on the experimental diets, each larva was weighed to the nearest μg (Cahn 29 automatic electrobalance). Relative growth rates for each larva was calculated according to the equation in Schroeder (1986)

$$\frac{\text{(End wt.} - \text{Start wt.)}}{\text{\# Days} \times \left[\dfrac{\text{(End wt.} - \text{Start wt.)}}{\ln\left(\dfrac{\text{End wt.}}{\text{Start wt.}}\right)}\right]}$$

Twenty neonate larvae were used to obtain the mean starting weight for the equation and then discarded. Each treatment was replicated 3 times concurrently with each bioassay. To ensure that infected insects were not responding atypically to the experimental diets, relative growth rates of uninfected controls were also measured. Relative growth rates were not measured on diets containing chlorogenic acid.

Data Analysis. The Kaplan–Meier product limit estimator was used to estimate the LT_{50} values and their 95% CL for each treatment (Kalbfleisch and Prentice 1980, Collett 1994). Using Cox's proportional hazards model (a regression model) (Kalbfleisch and Prentice 1980, Collett 1994) we determined whether the survival curves were associated with dietary protein levels, protein type, viral construct, or their interactions. We evaluated each insect species separately. The same model was used to examine the influence of protein level, chlorogenic acid level, or their interaction on survival times of *H. virescens*. Because survival times were known only up to an interval of time, lethal times were estimated as the midpoints of the intervals in which they died. The hazard function is the differential function representing the rate of change in probability of dying at time (t), given survival to time (t). For Cox's model, it is given by $\lambda(t) = \lambda_o(t)\exp\{\beta X\}$, where $\lambda_o(t)$ is the baseline hazard function. The β's are unknown parameters to be estimated by fitting the model and X's are the levels for each independent variable. By definition $\exp\{\beta X\} = \exp\{\beta_o + \beta_1 X_1 + \beta_2 X_2 \ldots\}$ and is the amount by which $\lambda_o(t)$ is multiplied. The use of Cox's proportional hazards model indicates that estimated hazard functions for different covariate levels is perfectly proportional to each other over time, provided the covariates are not time-dependent. The result of this perfect proportionality is that a larger hazard function is equivalent to both a shortening of the lifespan distribution, thus leading to faster times to death, and also a higher mortality rate, thus leading to higher fractions that die at any prespecified time (t). Thus, an estimated parameter coefficient with a positive sign indicates a variable correlated with increased speed of kill; a coefficient with a negative sign indicates a variable correlated with decreased speed of kill. The best model was chosen by comparing the likelihood ratio chi-square values for each model.

Linear regression analyses of mean relative growth rates for each replicate were used to determine if relative growth rates were dependent on protein concentration (Steel and Torrie 1980). We then used Cox's proportional hazards model to evaluate whether survival curves were associated with relative growth rates.

Results

Influence of Dietary Protein Quality and Quantity on Survival Curves. The models depicting the influence of protein identity and concentration on the survival curves were not the same for *H. virescens* and *T. ni* because of important differences produced by interactions between protein quality and percent protein or protein quality and viral construct. As expected for both insect species, lethal times were faster when insects were infected with AcAaIT than WT AcMNPV (Tables 1 and 2). Furthermore, increasing the concentration of dietary protein generally increased the speed of kill for both insect species (Tables 1–3). However, casein and soy produced opposite effects on lethal times of the 2 insect species. The interaction between protein quality and protein level had a negative sign for *H. virescens* and a positive sign for *T. ni*.

Table 2. Influence of protein quality and quantity on lethal times of *T. ni* infected with AcMNPV or AcAaIT

Variable	Parameter coefficient × SE	Effect on rate of mortality	z-statistic	*P* value
Virus	+1.2 ± 0.08	AcAaIT killed faster	+15	<0.001
Protein quality	−0.10 ± 0.14	See interaction terms	−0.75	0.460
% protein	+0.02 ± 0.01	See interaction terms	+1.3	0.180
Protein quality × % protein	+0.08 ± 0.01	Rate of mortality ↑ faster on soy than on casein as protein ↑	+3.4	<0.001
Protein quality × virus	+0.68 ± 0.11	Rate of mortality ↑ faster on soy for insects infected with AcAaIT	+6.1	

Virus (WT AcMNPV = 0, AcAaIT = 1) and protein quality (casein = 0, soy = 1) were entered as categorical variables. Model likelihood-ratio chi-square = 750, df = 5, $P < 0.001$, n = 1,328. Model: $\lambda = \exp(\beta X)$, where βX = {1.2 (virus) − 0.10 (protein quality) + 0.02 (% protein) + 0.08(protein quality × protein) + 0.68 (protein quality × virus)}. There were 35 larvae in each treatment and all treatments were replicated 3 times. Protein concentration of diets varied from 1.0 to 8.0% (see Figs. 3 and 4).

Thus, for *H. virescens*, increasing the level of dietary casein increased the speed of kill to a greater extent than increasing the soy concentration (Table 1). Furthermore, these effects were equivalent for both viral constructs because the interaction between protein quality and viral construct was not significant (unpublished data). In contrast, both interaction terms we evaluated carried a positive sign for *T. ni* (Table 2). The estimates for these variables show that although increasing dietary protein level increased speed of kill for *T. ni* on casein diets for insects infected with the wild-type virus, insects infected with the recombinant virus or insects that fed on soy diets died even faster as protein level increased. Speed of kill increased even more for insects infected with AcAaIT that fed on soy (both interaction terms took on positive values further increasing the hazard of dying).

Table 3. LT_{50} values for *H. virescens* and *T. ni* infected with WT AcMNPV or AcAaIT and fed on casein or soy diets containing different protein concentrations

% protein	WT AcMNPV LT_{50}, h[a]	AcAaIT LT_{50} (h)	95% CL	
0.5	125		88.0–104	76
1.0	130		103–114	75
2.5	113		80.0–90.2	78
4.0	111		80.8–93.0	72
6.0	101		80.0–88.0	72
8.0	119		73.5–79.8	81
1.0	112		83.0–97.0	77
2.5	111		76.0–83.0	77
6.0	104		72.0–76.0	81
8.0	103		68.0–77.0	89
	95.5		71.5–76.5	83
	104		72.0–81.0	79
6	97		71.5–77.5	77
8	102		71.5–78.5	77
2.5	105		67.0–77.0	104
4	98.5		63.0–67.0	102
	97.0		63.0–67.0	93
8	93.0		63.0–67.0	83

LT_{50} values and 95% confidence limits (CL) represent the median of 3 replicates using pooled data calculated by the Kaplan Meier product limit estimator (see *Materials and Methods*). Because we were using a viral dosage designed to deliver an approximate LD_{99}, 99–100% of the insects died in every treatment and every replicate. All insects had died by 8 d postinoculation.

In an experiment designed to verify whether the influence of dietary protein on lethal times was a postinfection effect or a result of interactions between dietary protein and the viral inoculum, we found that if we placed an insect on a high protein diet (6%) immediately after droplet feeding viral inoculum and then transferred these insects to a low protein diet (1%) 24 h later (or vise versa), lethal times were influenced only by what the insects consumed after the 24-h transfer. In other words, the protein level they received immediately after dosing did not influence lethal times. For example, insects droplet fed viral inoculum and placed immediately on 1% protein had an LT_{50} of 111 hpi (95% CL, 107–114), whereas insects dosed and placed immediately on 6% protein followed by transfer to 1% had an LT_{50} of 109 hpi (105–114). In a similar manner, insects droplet fed virus and then placed on 6% protein had an LT_{50} of 98.1 hpi (95.7–101), whereas insects dosed and placed immediately on 1% followed by transfer to 6% had an LT_{50} of 98.8 hpi (89.2–102). Furthermore, at the viral dosage used in this study, dietary protein only influenced speed of kill because percentage mortality was 99–100% regardless of protein level or type.

Influence of Dietary Protein Quality and Quantity on Relative Growth Rates. In an attempt to explain these differences in the course of viral disease of *H. virescens* and *T. ni* on soy versus casein diets, we measured relative growth rates of insects in the above treatments. For both insect species, relative growth rates increased with increasing dietary protein level regardless of the viral construct used to infect the insects or the identity of the protein used in the diet (Figs. 1–4). Above 6.0% protein, however, growth rates plateaued or even began to decline (Figs. 1–3), with 1 exception. The exception was that relative growth rates of *T. ni* infected with AcAaIT fed on soy diets increased linearily with increasing soy concentration without reaching a plateau (Fig. 4). More importantly, speed of kill varied directly with growth rates for both insect species regardless of the protein quality. The faster the insects grew, the faster they died from viral infection regardless of the treatment

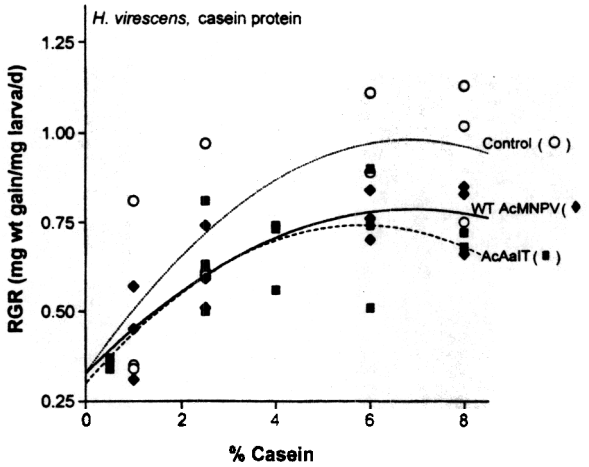

Fig. 1. Influence of casein protein concentration on relative growth rates (RGR) of neonate *H. virescens*. Insects were droplet fed 1 of 2 viruses (WT AcNPV or the recombinant virus AcAaIT) or water (control). Datum points are the mean RGR of 20 insects with each treatment replicated 3 times. All growth rates varied with protein concentration as a 2nd-order polynomial. WT AcMNPV RGR = 1/[3.6 − 0.83 (% protein) + 0.07 (% protein)^2], F = 9.0; df = 2, 14; P = 0.003, adjusted R^2 = 0.50; AcAaIT RGR = 1/[3.1 − 0.64 (% protein) + 0.06 (% protein)^2], F = 21; df = 2, 14; P = 0.001, adjusted R^2 = 0.75; control RGR = 1/[2.1 − 0.44 (% protein) + 0.05 (% protein)^2], F = 51; df = 2, 14; P < 0.001, adjusted R^2 = 0.86. Standard errors were ≤ 0.04 mg/mg/d.

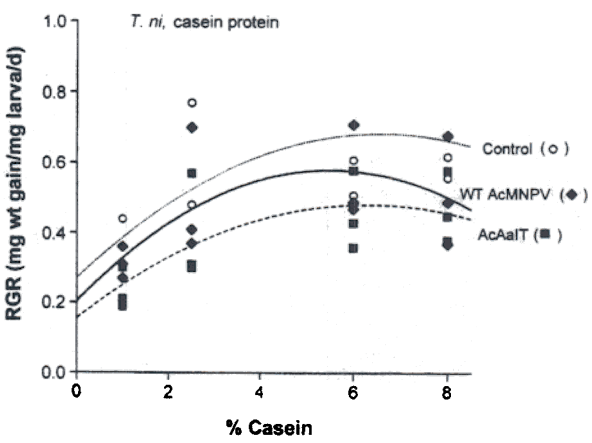

Fig. 3. Influence of casein protein concentration on relative growth rates (RGR) of neonate *T. ni*. Insects were droplet fed 1 of 2 viruses (WT AcNPV or the recombinant virus AcAaIT) or water (control). Datum points are the mean RGR or 20 insects with each treatment replicated 3 times. All growth rates varied with protein concentration as a 2nd-order polynomial. WT AcMNPV RGR = 1/[3.9 − 0.77 (% protein) + 0.07 (% protein)^2], F = 5.6, df = 2, 9; P = 0.026, adjusted R^2 = 0.46; AcAaIT RGR = 1/[5.3 − 1.1 (% protein) + 0.09 (% protein)^2], F = 7.8; df = 2, 9; P = 0.011; adjusted R^2 = 0.55; control RGR = 1/[3.2 − 0.54 (% protein) + 0.04 (% protein)^2], F = 6.9; df = 2, 9; P = 0.015, adjusted R^2 = 0.52. Standard errors were ≤ 0.04 mg/mg/d.

(Tables 4 and 5). Furthermore, the interaction term in the models indicates that as relative growth rates increased, speed of kill increased at a faster rate for insects infected with the recombinant than for the wild-type virus for both insect species (Tables 4 and 5).

A group of uninfected insects of each species were allowed to develop on the experimental diets for up to

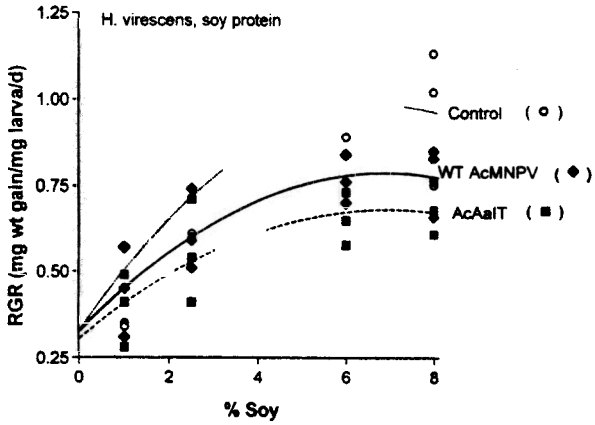

Fig. 2. Influence of soy protein concentration on relative growth rates (RGR) of neonate *H. virescens*. Insects were droplet fed 1 of 2 viruses (WT AcNPV or the recombinant virus AcAaIT) or water (control). Datum points are the mean RGR of 20 insects with each treatment replicated 3 times. All growth rates varied with protein concentration as a 2nd-order polynomial. WT AcMNPV RGR = 1/[28 − 0.51 (% protein) + 0.04 (% protein)^2], F = 6.9; df = 2, 9; P = 0.015, adjusted R^2 = 0.52; AcAaIT RGR = 1/[3.1 − 0.52 (% protein) + 0.04 (% protein)^2], F = 5.8; df = 2, 9; P = 0.024, adjusted R^2 = 0.47; control RGR = 1/[2.8 − 0.63 (% protein) + 0.05 (% protein)^2], F = 6.0, df = 2, 9; P < 0.022, adjusted R^2 = 0.48. Standard errors were ≤ 0.04 mg/mg/d.

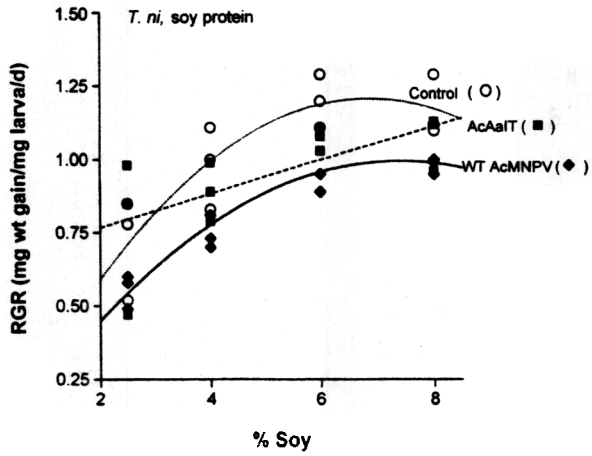

Fig. 4. Influence of casein protein concentration on relative growth rates (RGR) of neonate *T. ni*. Insects were droplet fed 1 of 2 viruses (WT AcNPV or the recombinant virus AcAaIT) or water (control). Datum points are the mean RGR of 20 insects with each treatment replicated 3 times. All growth rates varied with protein concentration as a 2nd-order polynomial. WT AcMNPV RGR = 1/[3.0 − 0.57 (% protein) + 0.04 (% protein)^2], F = 43, df = 2, 9; P = 0.001; adjusted R^2 = 0.89; AcAaIT RGR = 1/(1.1 (% protein)^2], F = 3.7; df = 1, 10; P = 0.085; adjusted R^2 = 0.27; control RGR = 1/[8.5 − 0.50 (% protein) + 0.04 (% protein)^2], F = 8.5; df = 2, 9; P = 0.009; adjusted R^2 = 0.58. Standard errors were ≤ 0.04 mg/mg/d.

Table 4. Influence of relative growth rate on lethal times of *H. virescens* infected with AcMNPV or AcAaIT

Variable	Parameter coefficient	Effect on rate of mortality	z-statistic	P value
Virus		AcAaIT killed faster		
Mean RGR		Rate of mortality ↑ as RGR ↑		
Virus × Mean RGR		Mortality rate ↑ faster for insects infected with AcAaIT as RGR ↑		

Virus (WT AcMNPV = 0, AcAaIT = 1) was entered as a categorical variable. Model likelihood-ratio chi-square = 459, df = 3, $P < 0.001$, n = 1,537. Model: $\lambda = \exp(\beta X)$, where βX = {0.53 (virus) + 1.0 (mean RGR) − 1.0 (virus × mean RGR)}. There were 20 larvae in each treatment and all treatments were replicated 3 times. Protein concentration of diets varied from 0.5 to 8.0% (see Figs. 1 and 2).

2 wk to determine which test diets would eventually allow insects to pupate. Larvae of *H. virescens* pupated at all soy and casein levels except 1.0 and 0.5%, respectively. *T. ni* proved to be more sensitive to dietary protein quantity and quality than was *H. virescens*. Below 1.0% casein or 2.5% soy, larvae did not survive more than a few days and those that did survive failed to pupate. As a general trend, *T. ni* grew faster on soy diets than casein at the same protein concentrations (compare relative growth rates of contols in Figs. 3 and 4).

Influence of Phenolics in Diets of Different Protein Concentrations on Survival Curves of *H. Virescens* Infected with WT AcMNPV. Speed of kill of *H. virescens* was influenced by a negative interaction between dietary casein level and chlorogenic acid concentration (Table 6); the higher the phenolic content of the diet the longer it took infected insects to die. In contrast, diets containing a low level of protein (0.5%) had the opposite effect on lethal times. Lethal times of insects that fed on diets containing 0.5% protein decreased as the phenolic content increased.

Discussion

The effect of dietary protein quality and quantity on the course of viral disease (survival curves) in *H. virescens* and *T. ni* appeared to depend strongly on the physiological status of the host insects as evidenced by the direct relationship between speed of kill and larval growth rates. These findings concur with a previous study that found a positive correlation between growth rates and speed of kill of infected insects that fed on diets containing different concentrations of the antibiotic streptomycin or diets of different ages (Hoover et al. 1996). In the current study, it is also likely that the interaction between chlorogenic acid and protein level in influencing viral disease was caused by variation in larval growth rates. The growth-reducing properties of chlorogenic acid (and rutin) to lepidopteran larvae have been shown to be highly dependent on the identity and quantity of dietary protein (Elliger et al. 1980, 1981; Isman and Duffey 1982b). For example, growth reduction of noctuid larvae as a result of ingestion of chlorogenic acid was correlated with increasing protein quality of artificial diets (Duffey and Bloem 1986, Bloem and Duffey 1989a, Felton et al. 1992). Further, for a given protein type, the higher the level, the more toxic the phenolic.

The differences observed between *H. virescens* and *T. ni* in sensitivity to protein identity and level concurs with previous studies involving 2 other polyphagous noctuids, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Broadway and Duffey 1986, Bloem and Duffey 1989a). High and low levels of dietary protein significantly decreased larval weight gain in both of these species (Broadway and Duffey 1988, Bloem and Duffey 1989a). In a similar manner to the response of *H. virescens* to protein levels reported herein, *H. zea* was able to develop over a broad range of protein levels, although development was much slower outside the optimum level of 1.2%. In contrast, maximal development of *S. exigua* occurred over a broad range of casein concentrations (1.2–4.8%); larvae did not develop at all on low casein diets (Broadway and Duffey 1986). In our study, we found that *T. ni* shared a similar sensitivity to *S. exigua* in response to dietary protein in that *T. ni* did not develop below 1.0% casein or 2.5% soy. In agreement with previous studies (Broadway and Duffey 1988, Bloem and Duffey 1989a), we also found that protein may be toxic at high levels to noctuid larvae. Larval growth rates (Figs. 1–4) and lethal times (Table 3) levelled off and in some cases were slower at 8% protein. We suggest that these responses would slow even further above 8% protein.

Table 5. Influence of relative growth rate on lethal times of *T. ni* infected with AcMNPV or AcAaIT

Variable	Parameter coefficient ± SE	Effect on rate of mortality	z-statistic	P value
Virus	+1.2 ± 0.15	AcAaIT killed faster	+7.8	<0.001
Mean RGR	+1.1 ± 0.18	Rate of mortality ↑ as RGR ↑	+6.2	<0.001
Virus × Mean RGR	+0.78 ± 0.22	Mortality rate ↑ faster for insects infected with AcAaIT as RGR ↑	+3.6	<0.001

Virus (WT AcMNPV = 0, AcAaIT = 1) was entered as a categorical variable. Model likelihood-ratio chi-square = 782, df = 3, $P < 0.001$, n = 1,328. Model: $\lambda = \exp(\beta X)$, where βX = {1.2 (virus) + 1.1 (mean RGR) − 0.78 (virus × mean RGR)}. There were 20 larvae in each treatment and all treatments were replicated 3 times. Protein concentration of diets varied from 1.0 to 8.0% (see Figs. 3 and 4).

Table 6. Influence of casein and chlorogenic acid concentrations on lethal times of *H. virescens* infected with wild-type AcMNPV

Variable	Parameter coefficient ± SE	z-statistic	*P* value
% casein	+0.08 ± 0.04	+1.9	0.031
Chlorogenic acid (CHA)	+0.07 ± 0.03	+2.0	0.025
Casein × CHA	−0.03 ± 0.01	−2.9	0.002

Model chi-square = 9.8, df = 3, $P = 0.021$, $n = 284$. Model: $\lambda = \exp(\beta X)$, where βX = {0.08 (% casein) + 0.07 (chlorogenic acid concentration) − 0.03 (casein × chlorogenic acid concentration)}. There were 35 larvae in each treatment and all treatments were replicated twice. Diets contained all combinations of 0.5, 2.5 or 6.0% casein and 0, 3.5 or 7.0 μmoles/g of chlorogenic acid. The interaction term signifies that at low protein levels (0.5%), increasing CHA concentration increased speed of kill. At moderate to high protein levels (2.5 or 6.0% protein), increasing CHA concentration decreased speed of kill.

On the surface, the ways in which nutrients influence disease often appear contradictory (reviewed by Duffey et al. 1995). However, what may appear to be contradictory results can be explained if we consider that nutritional imbalance may alter the course of the disease separate from the protein altering infectivity. In what may appear to be contradictory to our results, several studies on the influence of protein on viral disease have concluded that high dietary protein levels decrease larval susceptibility to viral disease (David et al. 1972, David and Taylor 1977, Watanabe and Imansihi 1980, Keating et al. 1989). For example, when the level of casein in the diet of *Pieris brassicae* (Lepidoptera: Pieridae) was reduced, the larvae were more likely to die from a granulovirus infection (David et al. 1972, David and Taylor 1977). Similar results were found for *Bombyx mori* (L.) (Lepidoptera: Bombycidae) (Watanabe and Imansihi 1980) and the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) (Keating et al. 1989). In all of these studies, viral inoculum was acquired simultaneously with the ingestion of the experimental diets. From a physiological standpoint, it has been suggested that large amounts of dietary protein may diminish larval susceptibility to viral disease by altering the rate of virion inactivation by midgut proteases (Pritchett et al. 1984, Keating et al. 1989). In contrast, once a lethal viral dose has been acquired by the insect, it is possible that our findings that higher protein levels shortened lethal times occurred by a different physiological mechanism.

We suggest that protein may influence the speed of virally induced mortality by a physiological mechanism whereby low protein levels retard insect growth, slowing the rate of viral replication in the host insect. Insects that grow more slowly may produce fewer viral progeny during systemic infection and thus they die more slowly. There is considerable evidence that smaller insects produce fewer viral progeny. For example, larvae of *T. ni* infected with recombinant viruses (AcAaIT or AcJHE.KK) derived from the parent AcMNPV weighed less, produced fewer polyhedra, and possessed lower viral titers than larvae infected with the wild-type virus (Kunimi et al. 1996). Other studies have shown that stress in the form of starvation or reduced food intake can have a detrimental effect on viral development and replication in lepidopteran larvae (Steinhaus and Dineen 1960, see Benz 1987). A similar mechanism may be responsible for the influence of nutritional factors on the development of parasitoids or predators that use the herbivore. The diet of *H. zea* affected the growth and development of one of its principle endoparasitoids, *Hyposoter exiguae* (Viereck) (Hymenoptera: Ichneumonidae) (Bloem and Duffey 1989a, b). Diets that retarded the development of the host to the greatest extent had the greatest impact on the ability of the parasitoid to develop.

Whether our findings on the influence of growth rates mediated by dietary protein identity and levels on lethal times of host insects infected with baculoviruses has relevance to the field requires further exploration. The influence of protein quality and quantity on the growth and development of noctuid larvae is strongly influenced by interactions with other plant factors such as oxidative enzymes (phenolases) (Felton et al. 1992) and tannins (Feeny 1968). Further studies are needed to assess how protein in the context of other phytochemicals in plants may influence the acquisition of a lethal dose and the subsequent course of viral disease. If these results do hold in the field, plants of higher protein content may favor the development of epizootics. Epizootics are favored by short incubation periods and high rates of replication during the infective stage of the pathogen (May 1986). Further studies are also needed to determine the mechanism whereby enhanced larval growth rates increased speed of kill by baculoviruses.

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