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Uptake of dietary micronutrients from artificial diets by larval *Heliothis virescens*

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Abstract

Micronutrient assimilation from artificial diet by larvae of *Heliothis virescens* during selenium (Se) supplementation was studied. The metal content of pupae and plugs of the artificial diet on which they had developed from hatching was analyzed by inductively coupled plasma-mass spectrometry. Levels of the metals Cr, Co, Fe, Mg, Mn, Ni, Se, Na, and Zn were not bioaccumulated from the diet regardless of the amount of Se added to the diet. Only pupal Cu and Mo bioaccumulation were found to be altered significantly by dietary Se supplementation. Larvae fed Zn, which was found in higher levels in pupae than diet, had a deleterious response to increasing levels of dietary Zn. Larvae fed Cr, found in higher levels in diet than in pupae, were not adversely affected when increasing levels of Cr were added to the diet. Based on this analysis, metals were identified that might well impact the fitness of a given colony of insects in relation to their diet.

Keywords: Chromium; Copper; Molybdenum; Selenium; Zinc

1. Introduction

Micronutrients are metals or metal-containing inorganic compounds utilized as enzymatic cofactors or as intracellular messengers. Dietary micronutrient intake can affect the growth, development and immunocompetence of vertebrates (Wellinghausen et al., 1997; Beck et al., 2004). The essentiality of these elements in insect nutrition is well established (Dadd, 1985); however, the optimal micronutrient requirements of insects are largely unknown. We recently demonstrated that dietary supplementation with selenium (Se) (in the form of selenite) boosts the immunocompetence of the larval lepidopterans, the cabbage looper (*Trichoplusia ni*) (Popham et al., 2005), and the tobacco budworm (*Heliothis virescens*) (Shelby and Popham, 2006), elevating larval resistance to *per os* challenge with a fatal baculovirus infection. While the mechanism of Se-dependent baculoviral resistance is as yet unknown, the observation opens several new lines of investigation: (i) the optimal dietary concentration range and formulation of Se; (ii) dietary Se assimilation, metabolism and excretion; (iii) Se-dependent biochemical or physiological processes in larval or adult insects; (iv) micronutrients required for optimal, or maximal fitness; and (v) interactions of other nutrients with dietary Se.

An immunostimulatory effect was observed when diet was supplemented with 5–25 parts per million (ppm) Se (Popham et al., 2005; Shelby and Popham, 2006). Higher levels of Se appeared to exceed the tolerable upper level, with slowed or disrupted larval development. Thus, for the species studied we reported an upper and lower boundary for Se concentration between beneficial supplementation and toxicity. These effects on larval immunocompetence indicate that the artificial diets commonly used to rear insects in the laboratory, which are sufficient to support growth, development and reproduction (measures of fitness), may have specific micronutrient deficiencies which could affect other types of bioassays. For example, interactions with the highly complex mix of phytochemicals present in foliar tissues, and largely absent from artificial diets, may alter the response of feeding larvae to Bt toxins

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(Tabashnik et al., 2003). Understanding of the influence of a single inorganic element on insect biology is clearly far more complex than a simple issue of dietary requirements.

Because of the importance of other metal micronutrients besides Se to vertebrate immunity (Wellinghausen et al., 1997; Wellinghausen and Rink, 1998; Erickson et al., 2000), and the stimulatory action of Zn on *Manduca sexta* hemocytes (Willot and Tran, 2002), and of Fe on *Galleria mellonella* immunity (Dunphy et al., 2002), we have evaluated the hypothesis that the dietary levels of additional micronutrients may impact the fitness or immunocompetence of insects reared on artificial diets. Assimilation and bioaccumulation of metals directly from the diet into tissues of the insect was studied.

In vertebrates, absorption and utilization of iodine, Zn, Fe, Cr, and vitamin A are supported by adequate dietary Se (Lyons et al., 2004). The observed immunomodulatory effects of Se supplementation could be caused by an antagonistic interaction with other vital micronutrients such as Zn, Cr, etc., or by a positive interaction with dietary toxins such as Hg (Jensen et al., 2006). Therefore, we investigated the hypothesis that dietary Se supplementation may affect the uptake or assimilation of other micronutrients from artificial diets commonly used to rear insects in the laboratory.

2. Materials and methods

2.1. Insects

H. virescens eggs were received from the North Carolina State University Dept. of Entomology Insectary. Their insectary colony was established from field insects collected in July of 2002. Larvae were reared individually on an artificial wheat germ-based diet (Catalog # F9781B, BioServe, Frenchtown, NJ) containing Wesson's Salt Mixture minerals with no added Se (Popham et al., 2005; Shelby and Popham, 2006). Larvae were reared under a photoperiod of 14 h:10 h (L:D) at 55% relative humidity at 28 °C.

2.2. Supplemented diets

Se-supplement diets were prepared with Se added to the stock culture medium in the form of Na_2SeO_3 (Sigma Chemical, St. Louis, MO, USA) at 5, 10, 25, and 50 ppm. Cr-supplement diets were prepared with added Cr in the form of $Cr(histidine)_3$ or $Cr(picolinate)_3$ at 0.5, 3, and 10 ppm. Zn-supplement diets were poured with Zn added in the form of $ZnSO_4$ or $ZnCl_2$ (Sigma Chemical, St. Louis, MO, USA) at 20, 40, 60, 80, 100, and 200 ppm. Diet without added dietary Se, Cr, or Zn was considered to be basal Se, Cr, and Zn, respectively. Larvae were monitored daily for death, pupation, and emergence. The presence of developmental irregularities including larval/pupal or pupal/adult intermediates was recorded during each study.

2.3. ICP-MS analysis of metal content

Individual pupae or small core samples of diet were placed in pre-tared vials, weighed, and oven dried at 65 °C for two or more days until no further loss of water mass was noted. Dry mass was calculated from these final dried samples. Pupae were chosen for analysis because larvae completely void midgut contents prior to pupation, minimizing contributions of diet contents within the digestive system. Three male and three female pupae from larvae reared on each different level of Se were analyzed in each group. Metal determinations were performed by the University of Missouri Research Reactor Analytical Services by inductively coupled plasma-mass spectrometry (ICP-MS) (Beauchemin, 2004). Samples were subjected to microwave digestion in nitric acid. The microwave digestion system was a CEM brand MDS-2000 with Advanced Composite Vessels (CEM Corporation; Matthews, NC, USA). During the microwave program, the setpoint pressure for the vessels was stepped up from 40 psi (hold 5 min), to 60 psi (hold 5 min), to 80 psi (hold 5 min), and to 100 psi (hold 10 min). Digestates (approximately 12 ml) were analyzed undiluted and with gravimetric dilutions (200 × and 1000 × for most samples). Na, Mg, Mn, Fe, and Zn were quantitated from dilutions. All others were quantitated from digestates. The elements scandium and yttrium were included as internal standards in all digestates, dilutions and calibration standards. The ICP-MS used for all of the elements was a VG Elemental Axiom High-Resolution ICP-MS (Thermo Electron Corp., VG Elemental, Waltham, MA, USA). Metal concentrations were expressed as μg/g dry mass (ppm). The sum of all determined isotopes of each metal are presented. Two independent replicates were performed. Statistical comparisons were made with the Holm–Sidak pairwise multiple comparison method when a significant ANOVA value was found ($p<0.05$) (SigmaStat, Systat Software Inc., Point Richmond, CA, USA).

2.4. Plasma in vitro virucidal activity assay

The *Helicoverpa zea* cell line, *Hz*AM-1, was maintained as monolayers at 28 °C in Excel 401 medium (JRH Biosciences, Lenexa, KS, USA) supplemented with 10% fetal bovine serum (Intergen Co., Purchase, NY, USA). Virucidal activity in larval *H. virescens* plasma was quantified by endpoint dilution assay as detailed (Popham et al., 2004). In brief, plasma dilutions were combined with *Helicoverpa zea* single nucleopolyhedrovirus (*Hz*SNPV) at a ratio of 3:1 (v/v) and allowed to incubate at 20 °C for 1 h. PBS was used as a control in the absence of plasma. Viral titers of these incubations were determined by endpoint dilution assay. *Hz*AM-1 cells were seeded at 5×10^4 cells/ml in P96 well plates (BD Falcon) and allowed to attach for 1 h. The cells were infected with dilutions of virus/plasma or virus/PBS at dilutions of 10^{-1}–10^{-6} and plates were incubated for 1 week at 28 °C. The plate wells were then