INSECT HERBIVORE STOICHIOMETRY: THE EFFECT OF MACRONUTRIENT QUANTITY, RATIO, AND QUALITY (ORTHOPTERA: ACRIDAE, *Schistocerca americana*)

A Thesis

by

ANDREW WILLIAM PAYNE BOSWELL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2009

Major Subject: Entomology

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Approved by:

Chair of Committee,	Spencer Behmer
Committee Members,	Micky Eubanks
	Tony Provin
Head of Department,	Kevin Heinz

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ABSTRACT

Insect Herbivore Stoichiometry: The Effect of Macronutrient Quantity, Ratio, and Quality (ORTHOPTERA: ACRIDAE, *Schistocerca americana*). (December 2009)

Andrew William Payne Boswell, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Spencer Behmer

The field of ecological stoichiometry has been dominated by studies focusing on aquatic and benthic microinvertabrates with less attention given to herbivorous insects. These organisms rely on their food, source(s) to supply all of the building blocks (elements) they need in order to complete their life cycle. Since insect herbivores do not have the same elemental composition as the plants they use for food the question arises of how they go about building themselves. We investigated what happened when grasshoppers were fed diets with various macronutrient profiles, their total amounts, and when the protein quality varied. We discovered that under controlled conditions when using a high quality protein source that grasshoppers are able to maintain a strict level of elemental homeostasis, but that the elements directly related to manipulations made in the food seem to vary (carbon, which is associated with carbohydrates and nitrogen, associated with protein). We also discovered that when the quality of protein changes an immature grasshopper's elemental stoichiometry loses some of this strict homeostatic regulation.

DEDICATION

This thesis is dedicated to my parents, James and Nancy Boswell; to my grandparents, James and Athlyn Boswell and Horrace and Ruthie Whitehurst; to my brother, Brannon Boswell; to my sister, Emily Holiday; and to Amanda Lefler. All of whom encouraged me to never give up on my goals. To my mom and dad, thank you for always being there for me and making me understand that nothing is out of reach, until you stop trying. I will never be able to thank you enough.

To my grandparents, thank you for everything you've taught me and given me throughout my life. I would not have even been able to be in the position I am in today if it weren't for you. What you've done for me is indescribable and I will never forget you for it. I hope that one day I can be in the position you are in now and that I remember the lessons you've taught me.

To my brother, thank you for always reminding me that it is important to never give up on your dreams no matter what. Your strength and drive have been something I've always looked up to and admire. I cannot thank you enough for what you've given me over the years.

To my sister, thank you for believing in me and always having good advice. Your wisdom is truly beyond your years, and I only hope that I might possibly be able to be a mentor to your future children, and that you can offer the same to mine.

Finally, to Amanda, words cannot express the unspoken and spoken encouragement you gave me everyday.

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I would also like to thank the entire Behmer Lab. Everyone has been a pleasure to work with on many different aspects. Karl, I'll never forget getting coffee with you. Xiang-Feng, I will never forget you taking cat-naps in the side lab. Marion, the bird incident will always be remembered. Paul, your knowledge of grasshoppers and your love of entomology are something I admire. Travis and Laura, will always be thought of anytime I have to run an experiment all by myself; thanks for the help guys. I would also like to thank the department, the staff in the front office, Rebecca Hapes, the absolute best advisor at Texas A&M University, Dr. Robert Coulson for all the great life lessons, and Dr. John Jackman who passed away in the fall of 2008 but will always be remembered for teaching me how to reach students and how to tie flies. Without all of these people's help I would have never made it this far, Thank You!

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CHAPTER I

INTRODUCTION

Elements are the building blocks of all organic and inorganic chemical compounds that comprise all life on earth. During chemical reactions compounds interact and elements are often rearranged accompanied by energetic gains or losses. The study of energetics and elemental balance within chemical reactions is called stoichiometry, and the field of Ecological Stoichiometry (ES) studies the movement and balance of elements within an ecosystem (Sterner and Elser 2002). ES has taken the basic chemical and physical principles set forth in stoichiometry and focused on the movement and balance of elements relevant to organisms and ecosystems. ES studies have focused on three distinctive groups of elements: (1) the structural elements C, N, P, and S, (2) the electrochemical elements K, Na, Ca, and Mg, and (3) the catalytic elements: Fe, Cu, Zn, and Mn (Fraust da Silvia & Williams 1991). However, the structural elements, particularly C, N, and P, have received the great majority of attention in ES studies (Sterner and Elser 2002).

Carbon has been studied thoroughly because it comprises 40-50% of the dry mass of most living things (Sterner and Elser 2002). Biologically speaking carbon is found in proteins, nucleic acids, lipids, and carbohydrates. In plants it, along with other elements, is important for light absorption associated with chlorophyll and is an energetic nucleotide that has high-energy carrying capabilities. It is also the dominant component of digestible and structural carbohydrates, and is also found in a high concentration

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This thesis follows the style of *Functional Ecology*.

lipids and fatty acids. Nitrogen has significance because it is a part of all amino acids, the building blocks of proteins. Proteins have the highest N content of the biological compounds considered in ES, and be cause proteins are major a component of animals' bio-mass these proteins play a defining role in the total amount of N an animal will contain in its elemental composition. Nitrogen is necessary for growth and is essential for enzyme function. Photosynthesis is also a nitrogen-intensive process and relies on Rubisco, which can make up about half of all plant protein (Lambers et al. 1998). In animals, total animal N can comprise 8-12% of the dry mass (Sterner & Elser 2002). Phosphorus's importance lies within DNA and RNA in both plants and animals. It is also important as part of NADP and NADPH. The energy compound used by all organisms, ATP, is 18% P, most likely being the most P-rich molecule living organisms use (Sterner & Elser 2002). Phosphorous content of animals typically ranges from 1-5% of the dry mass in an organism (Sterner & Elser 2002).

Past ES studies have focused on the properties and abundance of nutrient elements in aquatic systems (Sterner & Elser 2002). The majority of these studies concentrated on *Daphnia,* a small freshwater zooplankton as the organism of interest (Elser *et al.* 2000, Elser 2001, S & E 2002, Frost *et al.* 2004, and Cross 2007). With respect to ES in terrestrial systems, most studies have focused on insects that have been caught in the field, but these studies have not been specific to an insect species nor age group (e.g. Studier 1992, Schade 2003, Bertram 2006, Knoor 1998, and Kay 2006). Where laboratory studies have been conducted, they tended to focus on the relationship between dietary phosphorus concentration in a food and the resulting carbon:nitrogen:phosphorus ratio and the possible resulting differences in growth and

feeding aspects (Sterner & Elser 2002 and Frost et al. 2004). To our knowledge, no laboratory studies have been conducted that explore a broad range of elements under controlled physiological conditions, which is important to ensure individuals are comparable and in general we know very little about elemental profiles in insects, particularly insect herbivores, which make up half of all known insect species (Bernays and Chapman 1994).

Plants are autotrophs that absorb nutrients and water from the soil and create their own energy through the process of photosynthesis. Herbivores are heterotrophs that eat plants to obtain their required nutrients, (carbohydrates, proteins, amino acids, and fats) but the stoichiometric profiles of plants and herbivores are quite different from one another (Sterner & Elser 2002). On average, nitrogen and phosphorus content of plant material is 10-20 times lower than that of herbivores (Fagan *et al.* 2002). Given the importance of N to insect growth, and its low concentration in plant material relative to insect herbivore body tissue, it is often considered to be one of the key elements limiting insect herbivore growth (Bernays and Chapman 1994). Some ES studies that have been conducted on herbivorous insects have focused on the physiological implications of phosphorus levels in food (Bertram *et al.* 2006 & 2008, Fagan 2002, Huberty & Denno 2005, Schade *et al.* 2003, and Woods *et al.* 2004 & 2006) and whether increasing or decreasing available P content in a food will change the food intake, survival, and growth of individuals.

Given the differences in elemental profile between plants and insects herbivores, a key question that arises is how do insect herbivores build themselves if they are feeding on foods that do not closely match their own body composition? In addition to the

mismatch in elemental composition, plants are highly variable in terms of their elemental composition, as a result of taxonomic affiliation (e.g. their plant family), developmental status (seedling, mature, fruiting, senescing), and local environmental conditions (soil nutrient levels, soil pH, light levels, and water availability). From an insect herbivore's perspective variation in plant protein and digestible carbohydrate content, which provide N and C, respectively, are regularly encountered. How an insect herbivore addresses this variation will have a large impact on how well they grow.

Nutrient regulation, and thus stoichiometric balance, can occur at two levels in insect herbivores: 1) behaviorally, by mixing their diets, and 2) post-ingestively, using various physiological mechanisms that control how ingested nutrients are processed. Among insect herbivores, grasshoppers show a strong ability to regulate their protein-carbohydrate intake when given the opportunity to feed from multiple diets that are nutritionally complementary (reviewed in Behmer 2009). When mixing between foods is not an option (e.g. because of predation threats or abiotic factors affecting plant nutrient content), grasshoppers can adjust their feeding so that their intake of limiting nutrients is increased, although this means that other nutrients that are not limiting will be eaten in excess of requirements. For example, grasshoppers on a diet rich in carbohydrate, but poor in protein, will greatly overeat carbohydrate to increase their protein intake. An important question to ask, is how does this compensatory behavior affect their stoichiometry? A related question is how does a macronutrient imbalance affect the flow of elements not found in protein and digestible carbohydrates (e.g. Na, Mg, Fe, Zn, just to name a few)? Different plants also have different proteins, with the key difference being the amino acid profile of the different proteins. All insects share the same requirement

for essential amino acids, but if particular proteins lack, or have low levels of key essential amino acids, this could influence growth, survival and insect herbivore elemental body composition. Another important question is how does protein quality influence insect stoichiometry?

The aim of this thesis is to explore how the macronutrient content, protein and carbohydrate are the macronutrients we will focus on, of an insect herbivore's diet influences its growth, performance and stiochiometric profile. There are two key experiments that will be conducted. In the first chapter experiments that ask how changing the protein-carbohydrate ratio or total macronutrient content of a food will affect a grasshopper's elemental composition. This question will be addressed at two levels. First, grasshoppers are given a choice of nutritionally complementary foods and allow them to freely choose between these over the course of the 6^{th} stadium. Next, a no-choice feeding regime will be used, forcing grasshoppers to ingest a single food that differs in their ratio and or amounts of protein and carbohydrate. Both of these experiments measure performance, nutrient intake, and body elemental composition. The second chapter explores how different protein quality, and thus amino acid profile, influence performance, nutrient intake, and body elemental composition over the last immature developmental stage.

By controlling dietary conditions we will limit the available nutrients and subsequently the available elements to an insect. Through the manipulation of the environment in which this experiment takes place, it can be assumed that every insect is under the same conditions and only the diet is different between individuals. This will provide specific information on the element flow from a food source into an insect, the

ultimate fate of elements ingested, and possible macronutrient bottlenecks associated with nutritionally different foods and certain elements. These experiments will allow us to determine if macronutrient ratio and total macronutrient content or protein quality will have effects on the elemental composition of grasshoppers.

CHAPTER II

DIET MACRONUTRIENT CONTENT AND ORGANISMAL STOICHIOMETRY: A CASE STUDY USING A GENERALIST GRASSHOPPER

OVERVIEW

The field of ecological stoichiometry has been dominated by studies focusing on aquatic & benthic microinvertabrates with less attention given to herbivorous insects. Studies on invertebrates suggest that a strict level of homeostasis occurs across taxonomic and age groups in regards to the elemental composition of their bodies. Although studies on insect herbivore stoichiometry have been conducted before they focus solely on the relationship between C:N:P and exclude other elements, which are essential. To date no study has investigated how macronutrient content, particularly protein and digestible carbohydrates, influence C, N, and P stoichiometry, as well as other important elements. In this study we manipulated the protein-carbohydrate ratio and amounts of artificial foods and explored the effects of food macronutrient content on the elemental body composition of 6th instar *Schistocerca americana* in a series of choice and no-choice experiments. Results showed that the amounts and concentrations of the macroelements carbon and nitrogen vary depending upon the protein:carbohydrate ratio in the diet, while strict elemental homeostasis was shown for all other elements. We discuss the results within the scope of ecological stoichiometry and use the geometric framework to explain the relationships between macronutrients and elements.

INTRODUCTION

Elemental stoichiometry addresses the balance and flow of energy and elements during chemical reactions that function in all organic and inorganic systems. Over the last decade researchers have taken these basic physical and chemical principles and applied them to higher order systems from individuals to communities to ecosystems. This new field of research, termed ecological stoichiometry (ES) focuses on the balance and flow of chemical elements in ecological interactions (Sterner & Elser 2002). Historically, ES studies have focused on the macroelements carbon, nitrogen, and phosphorous due to their structural and physiological importance, and such studies have focused mainly on aquatic and benthic invertebrates (Evans-White *et al.* 2005, Frost *et al.* 2004, Karimi & Folt 2006, and Sterner & Elser 2002). With respect to insect herbivores, ES studies have focused primarily on field collected individuals (Anderson et al. 2004 & 2005 and Studier & Sevick 1992), and the limited number of laboratory studies that have been conducted focus on the physiological implications of phosphorus levels in food (Bertram *et al.* 2006 & 2008, Fagan 2002, Huberty & Denno 2005, Schade *et al.* 2003, and Woods *et al.* 2004 & 2006). Currently we know very little about how the macronutrient content affects how insect herbivores build themselves, and how macronutrient imbalances and/or limitations affects the flow of nutrients into an insect herbivore.

The elemental composition of plants and herbivores are not equal. For example, nitrogen and phosphorus content of plant material is, on average, 10-20 times lower than that of herbivores (Fagan *et al.* 2002). However, herbivores do not simply ingest elements from plants – instead they ingest simple and complex molecules that are catabolized into useable units, which can then be reconstructed and used for metabolic

processes (Chapman 1998). Through this process of reorganizing the nutrients and elements that make up plants for the benefit of themselves, organisms have shown a remarkable ability to achieve a level of elemental homeostasis (Sterner & Elser 2002). Protein and digestible carbohydrates are two of these more complex molecules that plants provide for insect herbivores. Proteins are built from combinations of amino acids and are the primary source of nitrogen to herbivorous insects. Amino acids also provide carbon, and in the case of methionine and cystinine, sulfur. Although amino acids contain carbon atoms, the main sources of carbon for insect herbivores are carbohydrates, which can be classified as simple or complex. Simple sugars are readily utilized by insect herbivores, but the only complex carbohydrate that is readily utilized by insect herbivores is starch. Symbiotic organisms can only digest cellulose, which is where the majority of carbon in plants is found,, and these are generally not found in insect herbivores (Chapman 1998). Fats are the third major macronutrient group, but plants tend to only contain small amounts of fat, three to six percent in most cases, mostly in the form of fatty acids.

Given the variation in plant nutrient content, particularly variation in protein and digestible carbohydrates, insects are challenged with being able to successfully obtain the proper balance of nutrients (Behmer 2009). Nutrient regulation in insect herbivores is best explored using the experimental approach of the geometric framework (reviewed in Behmer 2009), which explores nutrient use by animals using multidimensional space, with each nutrient of interest represented as an axis (Raubenheimer and Simpson 1999). Within this nutrient space, the combination of nutrients that leads to optimal growth and development is described as the intake target, which is dynamic, and can change in

relation to an individual's growth, sex, and reproductive status (Raubenheimer & Simpson 2004). Integrating the GF with ES provides a powerful approach for better investigating the flow of elements and macronutrients between organisms and their food source.

In this chapter, we use the experimental approach of the GF and manipulate the protein-carbohydrate ratios and amounts of experimental test foods to explore the stoichiometry of an insect herbivore at the organismal level. In the first experiment newly molted 6th stadium *S. americana* grasshoppers are used to explore stoichiometric homeostasis at the behavioral level. Here grasshoppers are presented with a choice of two nutritionally suboptimal but complementary foods and allowed to self-select their protein-carbohydrate intake. For each grasshopper tested we analyze performance, nutrient intake and elemental body composition. In total 12 elements were analyzed, both in terms of their absolute amounts and their concentrations: C, N, P, S, K, Na, Ca, Mg, Fe, Cu, Zn, and Mn. In the second experiment grasshoppers were given a single dish of food with a fixed protein-carbohydrate ratio. The diets used range from a near optimal ratio to severely imbalanced, and also explored the effects of nutrient dilution and concentration. For each grasshopper tested we again analyze performance, nutrient intake and elemental body. This study is the first to describe the elemental body composition of an insect herbivore that has been reared under laboratory conditions and fed a chemically defined diet that varies in its protein and carbohydrate content. The results of this experiment are discussed in the context of ecological and organismal stoichiometry.

METHODS

Insects and Experimental Chambers

The polyphagous grasshopper *S. americana* occurs throughout the south and eastern United States and Mexico (Harvey 1981) and is recorded feeding on a wide range of cultivated and naturally occurring plant species (Kuitert & Connin 1952). Insects came from a culture that has been maintained on a diet consisting of seedling wheat grass tissue and wheat germ since 2006 in the Department of Entomology at Texas A&M University. They were maintained under standard laboratory conditions with a 12h:12h L:D photoperiod, and under radiant heat of 29-36 C during the light phase (supplied by 60W full spectrum incandescent bulb), and at 23-26 C during the dark phase.

Grasshoppers were removed from the culture after ecdysis to the 6th instar, sexed and weighed then placed singly into clear polystyrene arenas. 6th instar grasshoppers were used due to the amount they ingest and the mass they gain. Each arena measured 18.9 x 13.3 x 9.6 cm and contained either one food dish (experiment 1) or two food dishes (experiment 2), an aluminum wire roost for perching, and a 30-mL Solo Cup ® filled *ad libitum*, fitted with a lid that had been modified to allow the use of a cotton wick for drinking. The food dishes were modified Petri dishes designed to minimized spillage (Raubenheimer & Simpson 1990), and placed at the front of the arena while the water dishes were placed at the rear to avoid possible contamination. All test arenas were placed on heavy duty utility shelving, with shop lights containing 25 W incandescent bulbs hanging above the arenas. These lights were on a 12h:12h light:dark photoperiod. The arenas were maintained at a temperature of 29-31° C during the duration of the

experiments. Each treatment was replicated ten times, and we did not select individuals for certain treatments based on sex, instead we used the grasshoppers starting wet mass as a correction variable.

Synthetic Diets

Dry, granular, chemical defined synthetic foods were made in a manner similar to Dadd (1961) and later modified by Simpson & Abisgold (1985). In total there were seven diets that varied in their ratio and/or amount of protein (p) and digestible carbohydrate (c), expressed on a dry mass basis. The first set of five diets had a total macronutrient content of 42%: (1) p7:c35, (2) p14:c28, (3) p21:c21, (4) p28:c14, and (5) p35:c7. The remaining two diets had 1:1 protein-carbohydrate ratios, but different total macronutrient content. The first one (p7:c7) was diluted relative to the first five diets, while the other (p35:c35) was concentrated relative to the first five diets. The protein component of the diets was a 3:1:1 mix of bovine casein, egg albumin, and plant peptone. The amino acid profile of this mixture closely matches that of seedling wheat (see Simpson and Abisgold 1985). The digestible carbohydrate component was a 1:1 mixture of sucrose and dextrin. The first set of five foods contained varied amounts of protein and digestible carbohydrate (henceforth carbohydrate), but the same amounts of cellulose (used as a bulk agent), Wesson's salt, vitamins, and sterols. The last two diets contained the same amounts of Wesson's salt, vitamins, and sterols as the first five diets, but contained different amounts of cellulose (82% and 26% cellulose for the p7:c7 and p35:c35 diets, respectively). The elemental profiles of each diet, plus 10-day-old seedling wheat (the food used to maintain the grasshopper culture), are shown in a table on page 17.

Experiment Protocol

Two separate experiments were performed. The first experiment, a choice-experiment, had three treatments, all with two food dishes in each arena. There were three treatments: (1) p7:c35 paired with p35:c7, (2) p7:c35 paired with p28:c14, and (3) p14:c28 paired with p35:c7. Each of the individual foods is nutritionally suboptimal, but the pairings are complementary and allow the test insects to self-select a preferred protein-carbohydrate intake target. The use of three treatments allows us to determine if protein-carbohydrate intake is an active versus random process. In the second experiment test insects were only given a single dish of food, and here there were 7 treatments: (1) p7:c7, (2) p7:c35, (3) p14:c28, (4) p21:c21, (5) p28:c14, (6) p35:c7, and (7) p35:c35. In both experiments identical protocols were followed. Foods were dished into individual dishes and then weighed to the nearest 0.01 mg after allowing the food to equilibrate to ambient room humidity levels (RH 30-40%) for about 24h. The foods were then placed into the appropriate arenas and grasshoppers were allowed to feed for 72 hrs, after which the food dishes were removed and replaced with fresh, pre-weighed dishes of the same food. The food dishes that were removed were allowed to equilibrate to room humidity (RH 30-40%) before being reweighed to determine consumption. This process was repeated every 72 hours until the grasshopper molted. Upon molting individual grasshoppers were collected and weighed (to the nearest 0.01 mg) and then frozen until needed for elemental analysis.

Postmortem Elemental and Lipid Analysis

Frozen grasshoppers were placed individually into 15 mL glass vials and transferred to a drying oven set at 40°C until they reached a constant dry mass (to the nearest 0.01 mg).

Dried grasshoppers were then pulverized to a fine powder by placing a small magnetic stir bar into the glass vial holding the dried grasshopper, capping the vial, and holding it on a vortex for approximately 1 min (Boswell et al. 2008). Powdered samples were then separated into three approximately equal aliquots (measured to the nearest 0.01 mg); one for carbon/nitrogen analysis, one for additional elemental analysis (P, K, Ca, Mg, Na, Fe, Zn, Cu, and Mn), and a third to quantify lipid mass. Each carbon/nitrogen sample was wrapped in a small sheet of tin foil, and placed individually into stainless steel crucibles. As a precautionary measure the samples were wrapped in tin foil to prevent the loss of material during removal of atmospheric air. The samples were then placed in an Elementar vario MAX CN high temperature carbon-nitrogen analyzer set at 950º C and analyzed using methods similar to those discussed by McGeehen and Naylor (1988). The second aliquot, used to measure non-N elements, was transferred to polypropylene digestion tubes. These samples were digested using trace metal grade nitric acid on a 105º C graphite block. Following digestion, samples were brought to volume and analyzed using Spectro axial CIROS inductively coupled plasma – Atomic Emission Spectrometry (Havlin & Soltanpour 1980). Finally, the third aliquot was used to obtain lipid mass. This sample was placed centrally on a piece of 7.5 cm diameter filter paper (VWR filter paper, 415) that had been previously shaped around a 15 mm diameter plastic vial. The edges of the filter paper were then folded together, and twisted to form a closed pocket around the sample. The result was a tear shaped “bag” that we could suspend in chloroform. This bag, plus the grasshopper contents, was weighed to the nearest 0.01 mg and then washed in a chloroform bath for 24-h. After this 24-h period, the chloroform was removed and replaced with fresh chloroform for an additional 24-h.

This process was repeated one final time. The sample was then dried, at 60° C to a constant mass and re-weighed. Lipid mass was then calculated as the difference between the start and end mass of the bag plus ground sample. This lipid extraction method has been previously shown to be >98% efficient relative to Soxhlet distillation (Simpson 1983) as based on Loveridge (1973).

Statistical Analysis

All statistical analyses were conducted using JMP 7.02 (SAS Institute, Inc.). The protein-carbohydrate intake points from experiment one were analyzed using MANCOVA tests, with Pillai's trace as the test statistic. Development time was analyzed using survival analysis, while ANCOVAs, with start wet mass as a covariate to correct for size differences between males and females (males were, on average, smaller), were used to analyze dry mass gain, total consumption, and body element composition, both in terms of absolute amounts, and expressed as a concentration (ppm). For both experiments, there was no difference between treatments in mean starting mass of the grasshoppers (experiment 1: ANOVA: $F_{2,26} = 0.51$, $P = 0.609$; experiment 2: ANOVA: $F_{6,54} = 1.22$, $P = 0.312$). Where significant treatment effects were observed, post-hoc treatment comparisons were made using student's t-tests.

RESULTS

Experiment 1: Choice Diet Treatments

Nutrient Intake and Performance. Figure 1.1 shows a bicoordinate plot of the amounts of protein and carbohydrate eaten over the full 6th-stadium, and during this time

grasshoppers regulated their protein-carbohydrate intake to a statistically similar point – an approximately 1:1 protein-carbohydrate ratio (MANOVA: $F_{4,50} = 0.89$, $P = 0.475$). Likewise, when development, dry mass gain, and total consumption were compared, no significant treatment effects were observed (Table 1.1).

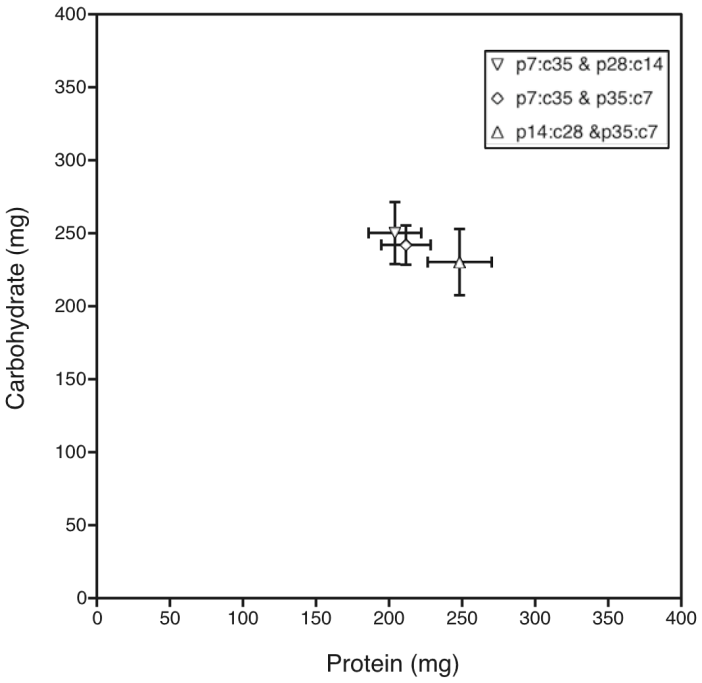


Figure 1.1 Bivariate means (±SE) for protein and carbohydrate intake for grasshoppers from the choice experiment.

Table 1.1 Elemental concentrations (expressed as a % or in ppm) present in seedling wheat and artificial diets with different protein-carbohydrate ratios (p = protein, c = carbohydrate; the numbers in each treatment represent the amount of protein and carbohydrate, respectively (expressed as a percent dry mass of the diet)).

	Element											
Diet	**C (%)**	**N (%)**	**P (%)**	**S (ppm)**	**K (ppm)**	**Na (ppm)**	**Ca (ppm)**	**Mg (ppm)**	**Zn (ppm)**	**Fe (ppm)**	**Mn (ppm)**	**Cu (ppm)**
Seedling wheat												
10-day-old	44	5.5	0.9	6276	22751	2352	2728	3241	64	93	81	12
Artificial diet												
p7:c7	41	1.2	3.4	13763	46470	57500	66062	3212	511	755	368	198
p7:c35	39	1.5	2.9	12604	41793	52177	47721	2083	227	617	368	193
p14:c28	42	2.0	3.5	20519	43517	58193	42977	2619	264	681	339	190
p21:c21	43	3.0	4.4	24755	51585	57888	53850	3453	264	800	284	147
p28:c14	42	3.9	4.6	29746	50652	63442	48508	2945	279	717	308	167
p35:c7	41	4.4	4.9	36712	55194	87339	55944	2880	518	849	468	280
p35:c35	42	3.9	4.9	33226	58688	67675	53549	3237	277	815	290	164

Element Composition and Lipid Levels

The total amount of carbon, nitrogen, phosphorus, sulfur, potassium, sodium, calcium, magnesium, iron, zinc, manganese, and copper from the grasshoppers on the three choice treatments were statistical similar (Table 1.2). Likewise, there was no difference between the treatments when individual elements were analyzed based on their tissue concentrations (Table 1.2). Finally, lipid levels on the three choice treatments were statistically similar (Table 1.2).

Table 1.2 Means (± SEM) plus test statistics for various performance measures from the choice experiment. For development time, the test statistic is χ^2, all other test statistics are F-ratios. No significant differences were found for any performance measure.

	Treatment			
Variable	p7:c35 w/ p28:c14	p7:c35 w/ p35:c7	p14:c28 w/ p35:c7	test statistic
Development (days)	13.1 (±0.8)	13.4 (±0.8)	13.4 (±0.8)	0.34
Dry mass gain (mg)	116.4 (±9.2)	124.8 (±9.3)	115.9 (±9.3)	0.55
Consumption (g)	1.08 (±0.05)	1.08 (±0.05)	1.14 (±0.05)	0.02
Body lipid (mg)	21.3 (±1.8)	19.6 (±1.8)	21.0 (±1.9)	0.25

Experiment 2: No Choice Diets

Food Consumption, Nutrient Intake and Performance. Total food consumption was significantly affected by the p:c ratio of the diet (ANCOVA: $F_{6,51} = 45.44$, $P < 0.001$; Fig. 1.2a). It was greatest on the p7:c7 and lowest on the p35:c35. On diets with 42% total macronutrient content, consumption was greatest on the p7:c35 diet, and significantly higher compared to consumption on all the other diets except the p21:c21 diet.

Consumption data, expressed as the total amounts of protein and carbohydrate eaten over the full 6th-stadium, is shown as a bi-coordinate plot in Figure 1.2b. Protein consumption was significantly affected by the p:c ratio of the diet (ANOVA: $F_{6,55} = 49.70$, $P < 0.001$), and comparison of the treatments using a student's t-test showed that protein intake was greatest on the p35:c7 diet, and lowest on the p7:c35 diet. On all the diets with total macronutrient content of 42%, protein intake was significantly different. Protein intake on the three equal ratio diets (p7:c7, p21:c21, and p35:c35) was significantly different, although the difference between the latter two diets was smaller compared to the difference between the first two diets. When the 42% total macronutrient diets were compared with the equal-ratio diets, only the p28:c14 and p35:c35 diets were statistically similar. Carbohydrate intake also differed between the treatments (ANOVA: $F_{6,55} = 43.42$, $P < 0.001$); it was greatest on the p7:c35 diet and lowest on the p35:c7 diet, and again all diets with total macronutrient content of 42% were significantly different from one another. Carbohydrate intake on the equal ratio diets was similar on the p21:c21 and c35:c35 diets, but statistically lower on the p7:c7

diet (Figure 1.2). A comparison of the 42% macronutrient diets with the equal-ratio diets showed statistically similar carbohydrate intake on the p14:c28 and p35:c35 diets, as well as on the p7:c7 and p28:c14 diets.

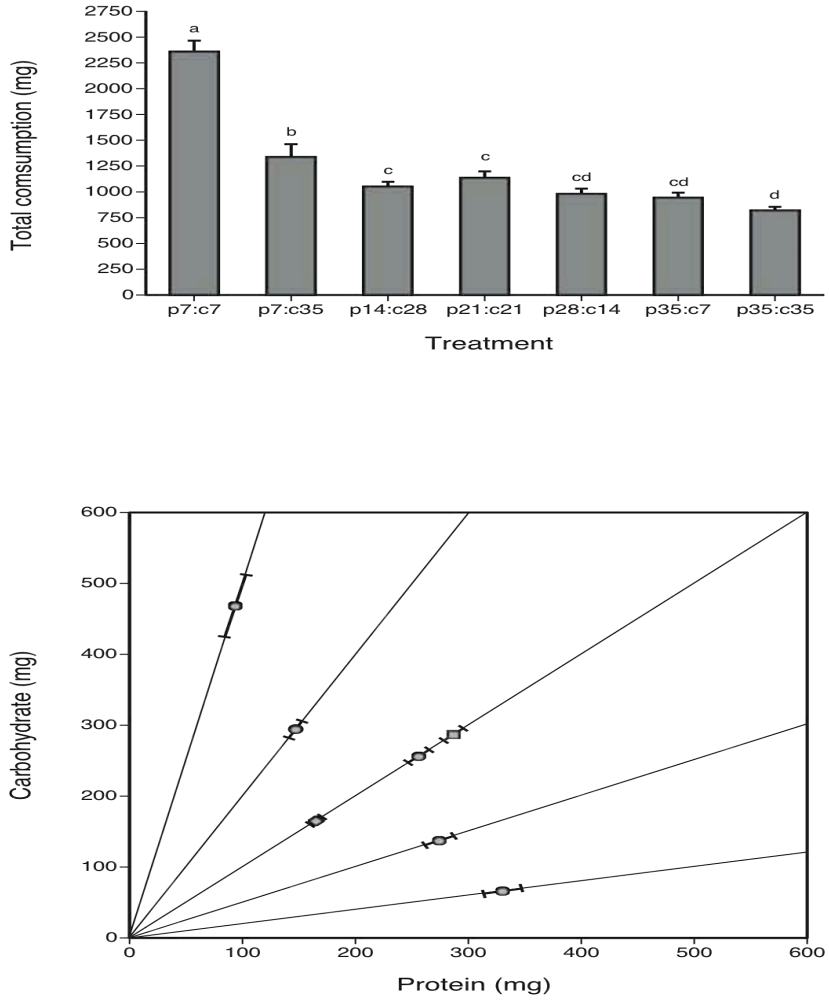


Figure 1.2. Total consumption of protein and digestible carbohydrate (means ± SE) on no-choice diet treatments; diet p7:c7 represented by an (Δ), diet p35:c35 represented by (∇), and our optimal diet p21:c21 represented by (◊).

Two measures of performance, development time and dry mass gain, were also measured. Diet p:c ratio significantly affected developmental time (1-tailed Survival Analysis: df = 6, $\chi^2 = 12.48$, $P = 0.026$; Fig. 1.3a). It was significantly longer on the p7:c35 and p35:c7 diets compared to the p7:c7, p21:c21, and p35:c35 diets, all which have a near optimal p:c ratio; development time on the other two diets (p14:c28 and p28:c14) were not significantly different compared to the three diets with equal p:c ratios. We also observed differences in dry mass gain between the treatments (ANCOVA: $F_{6,51} = 11.01$, $P < 0.001$; Fig. 1.3b). Dry mass gain was equally best on the p21:c21, p14:c28, and p35:c35 diets, and equally lowest on the p7:c7, p28:c14, and p35:c7 diets. There was no difference in dry mass gain between males and females (ANCOVA: $F_{1,51} = 0.99$, $P = 0.323$) when wet start mass was used as a covariate (ANCOVA: $F_{1,51} = 4.60$, $P = 0.037$).

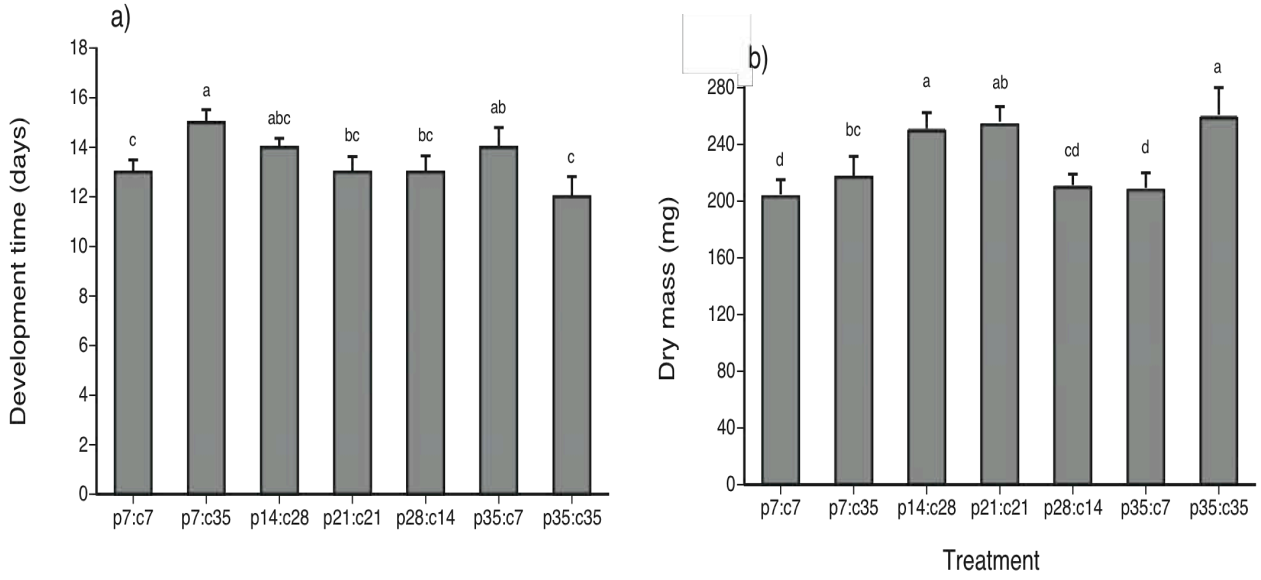


Figure 1.3. Performance measures, (a) Development time, (b) dry mass gain, and (c) total consumption (LS means ± SE).

Element Composition and Lipid Levels

The effects of diets with different p:c ratios and/or amounts on twelve total elements were analyzed (C, N, P, S, K, Na, Ca, Mg, Zn, Fe, Mn, and Cu), both in terms of their absolute amounts, and their proportions (expressed either as a % or ppm). Of these twelve elements, the effects of diet p:c ratio were only observed for C, N, K and Cu (Table 1.3).

With respect to absolute amounts, we observed effects of diet on C, N, and K (Table 1.3). Body C amount was highest on the p35:c35 diet, but C amounts from grasshoppers on this diet did not differ significantly compared to those on the p7:c35, p14:c28 and p21:c21 diets (Figure 1.4a). Body C amount was lowest on the p7:c7 diet, although C amounts on the p28:c14 and p35:c7 diets were equally low. Body N content, expressed in absolute amounts, was highest on the p35:c35 diet, but N amounts on the p14:c28 and p21:c21 diets were statistically similar. Body N levels were equally low on the p7:c7, p7:c35, and p35:c7 diets. Lastly, body K levels were highest on the p21:c21 diet, although K levels on the p14:c28, p28:c14, and p35:c35 diets were statistically similar. Body levels of K were lowest on the p7:c35 diet, but were not statistically different from p7:c7, p28:c14, and p35:c7. Structural elemental amounts and concentrations can be seen in figure 1.4.

Table 1.3 Mean (±SEM) total amount (mg or μg) and concentration (ppm) of each element in newly molted adult grasshoppers from the different treatments from the choice experiment. All comparisons were made using ANOVA – no significant differences were found for any element.

Element	expressed as	Treatment: p7:c35 w/ p28:c14	Treatment: p7:c35 w/ p35:c7	Treatment: p14:c28 w/ p35:c7	F-ratio
C	mg	108.6 (±6.5)	113.7 (±6.5)	117.6 (±6.5)	0.36
	%	48.3 (±1.4)	48.5 (±1.4)	50.5 (±1.4)	1.17
N	mg	25.3 (±1.6)	26.6 (±1.6)	28.9 (±1.6)	0.79
	%	11.1 (±0.4)	11.3 (±0.4)	12.5 (±0.4)	2.60
P	mg	1.6 (±0.12)	1.6 (±0.12)	1.7 (±0.12)	0.29
	%	0.7 (±0.05)	0.7 (±0.05)	0.7 (±0.05)	0.43
S	μg	806 (±76)	893 (±76)	873 (±77)	0.54
	ppm	3598 (±319)	3856 (±320)	3829 (±323)	0.45
K	μg	1706 (±71)	1593 (±71)	1785 (±72)	0.86
	ppm	7557 (±271)	6865 (±272)	7754 (±275)	2.90
Na	μg	749 (±19)	806 (±119)	785 (±120)	0.13
	ppm	3449 (±582)	3499 (±583)	3510 (±589)	0.07
Ca	μg	300 (±38)	344 (±38)	309 (±38)	0.48
	ppm	1366 (±175)	1503 (±176)	1360 (±177)	0.20
Mg	μg	148 (±15)	159 (±15)	163 (±15)	0.40
	ppm	663 (±70)	687 (±70)	714 (±71)	0.36
Zn	μg	30 (±4)	27 (±4)	31 (±4)	0.25
	ppm	138 (±16)	117 (±16)	137 (±16)	0.74
Fe	μg	14 (±2)	18 (±2)	15 (±2)	1.79
	ppm	62 (±8)	79 (±8)	63 (±8)	1.40
Mn	μg	1.8 (±0.3)	2.0 (±0.3)	1.8 (±0.3)	0.21
	ppm	8.4 (±1.4)	8.8 (±1.4)	7.8 (±1.4)	0.04
Cu	μg	3.5 (±0.7)	4.2 (±0.7)	3.9 (±0.7)	0.29
	ppm	16.6 (±3.5)	18.8 (±3.5)	17.8 (±3.6)	0.16

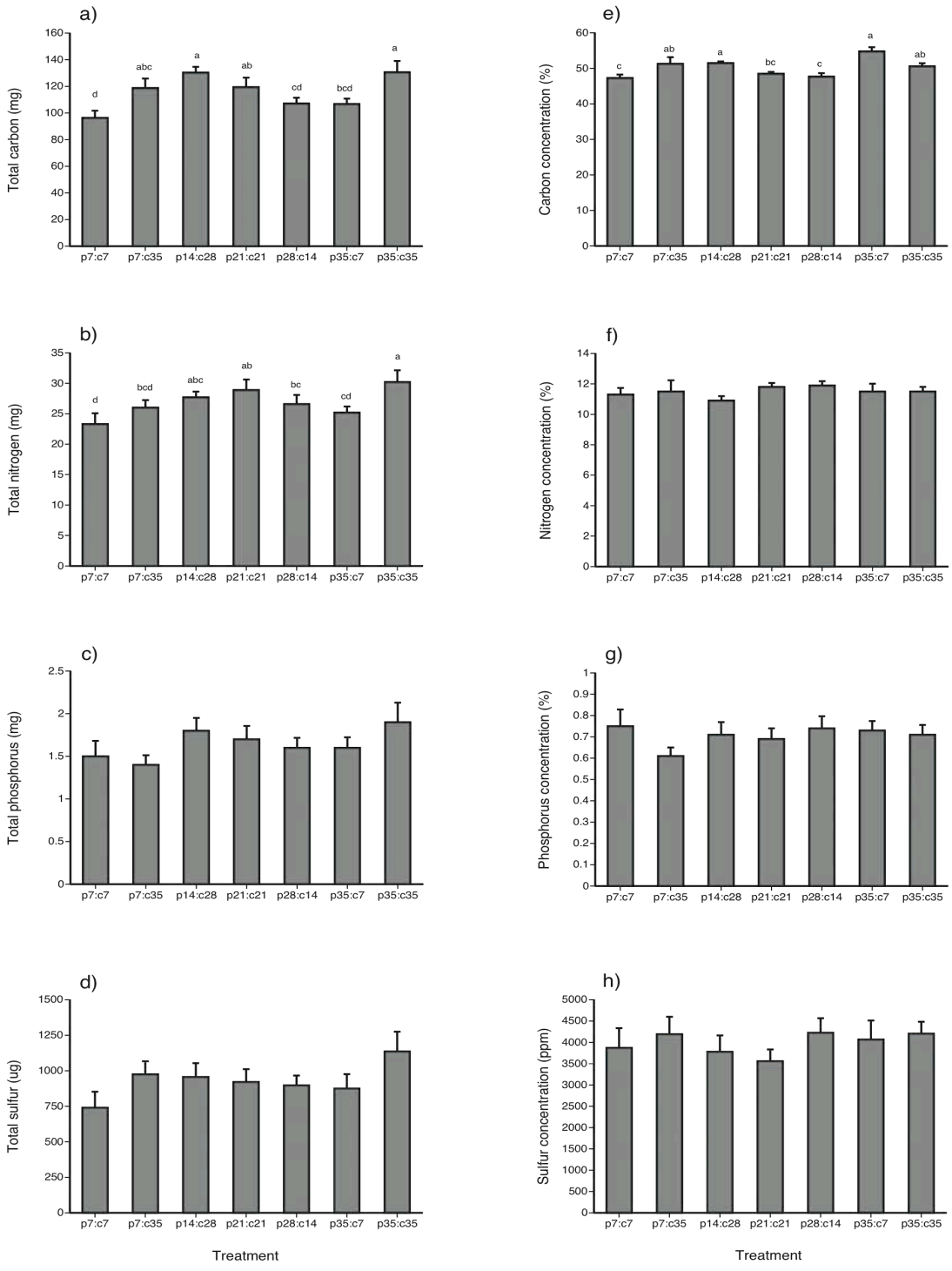


Figure 1.4. The left column contains total amounts (mg or μg) of each structural element present at end the experiment (LS means ± SE). The right column shows the concentration of the structural elements (% or ppm) (LS means + SE).

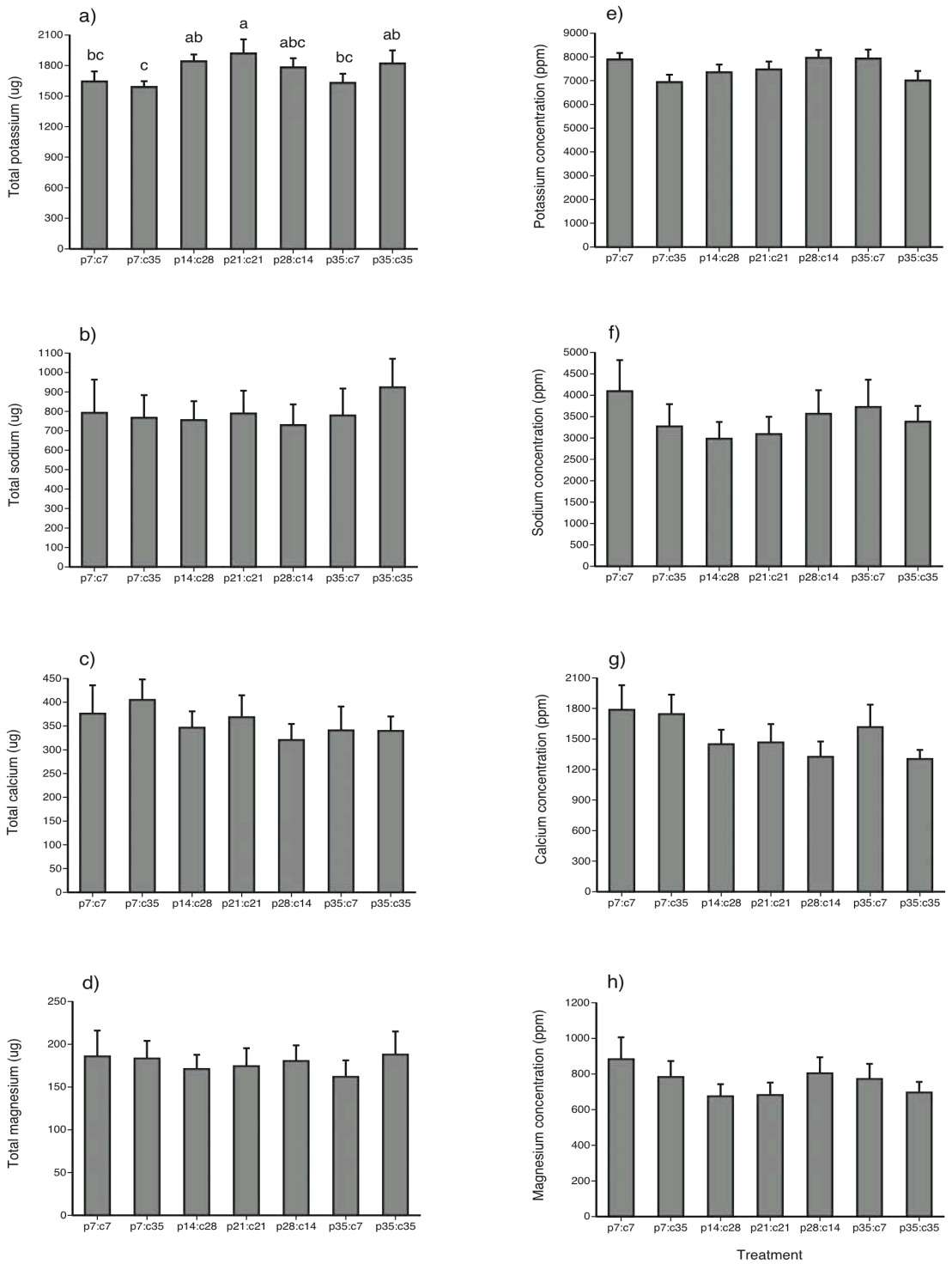


Figure 1.5. The left column contains total amounts (μg) of each electro-chemical element present at end the experiment (LS means ± SE). The right column shows the concentration of the electro-chemical elements (ppm) (LS means + SE).

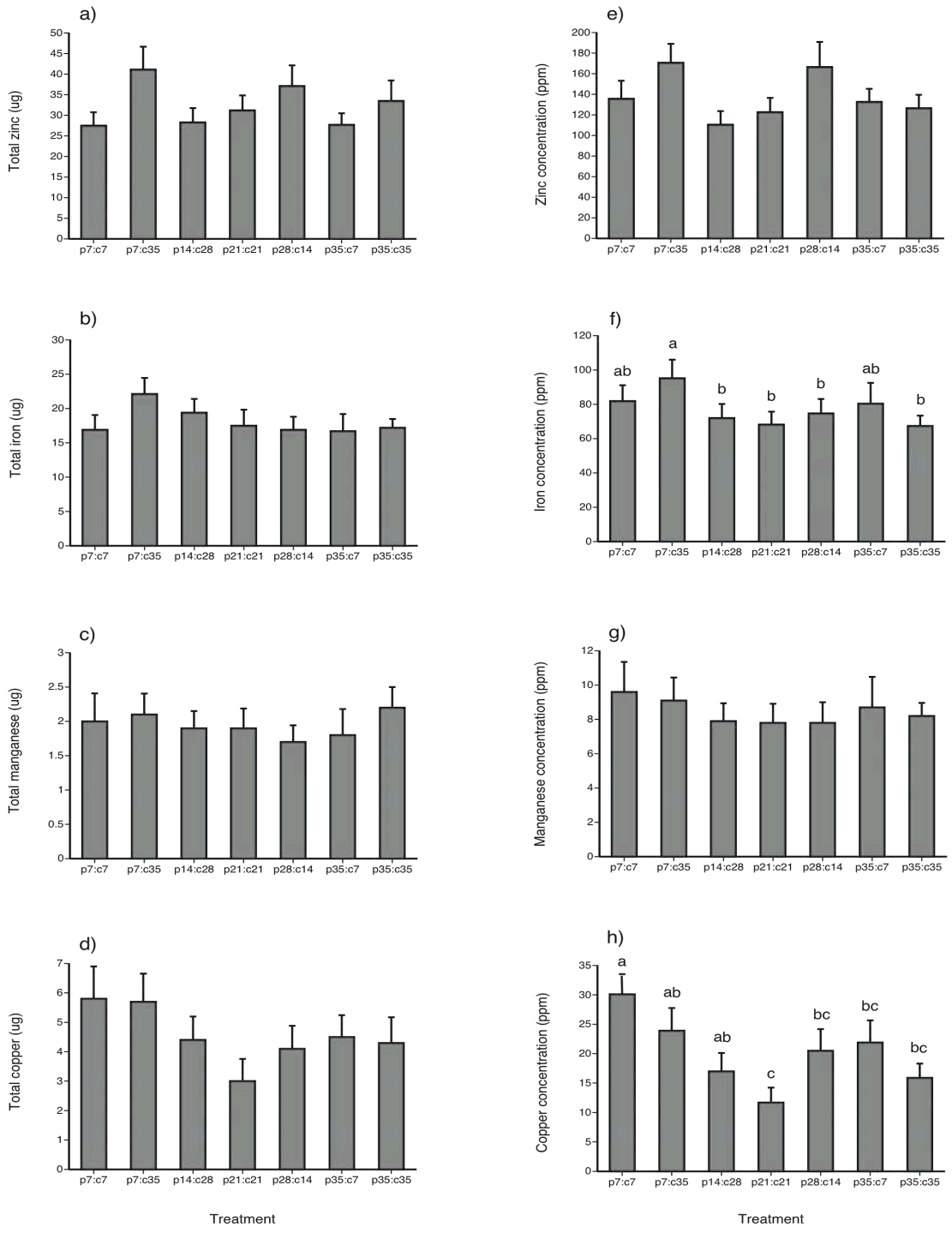


Figure 1.6. The left column contains total amounts (μg) of each catalytic element that was present at end the experiment (LS means ±SE). The right column shows the concentration of the catalytic elements (ppm) (LS means ± SE).

All other elemental amounts and concentrations can be found in figures 1.5 and 1.6.

In terms of elements expressed as a proportion of the body, only C and Cu were significantly affected by the p:c ratio of the diet (Table 1.3). As a percentage of body mass, C was equally high on the p7:c35, p14:c28, p35:c7, and p35:c35 diets. It was lowest on the p7:c7, p21:c21 and p28:c14 diets. Body Cu concentration, expressed as ppm, was highest on the p7:c7, p7:c35, and p35:c7 diets. It was lowest on the p21:c21 diet, but Cu concentrations on the p14:c28, p28:c14, p35:c35 diets were not different compared to the p21:c21 diet.

Finally, lipid content was also significantly affected by diet p:c ratio, both in terms of absolute amounts (ANCOVA: $F_{6,55} = 6.61$, $P < 0.0001$) and as a percent of the total body dry mass (ANCOVA: $F_{6,55} = 4.05$, $P = 0.002$). Whether measured as total mass, or as a percent of the total body mass, lipid amounts were equally high on diets that had at least 21% digestible carbohydrate, and equally low on diets with less than 21% digestible carbohydrate (Figure 1.7).

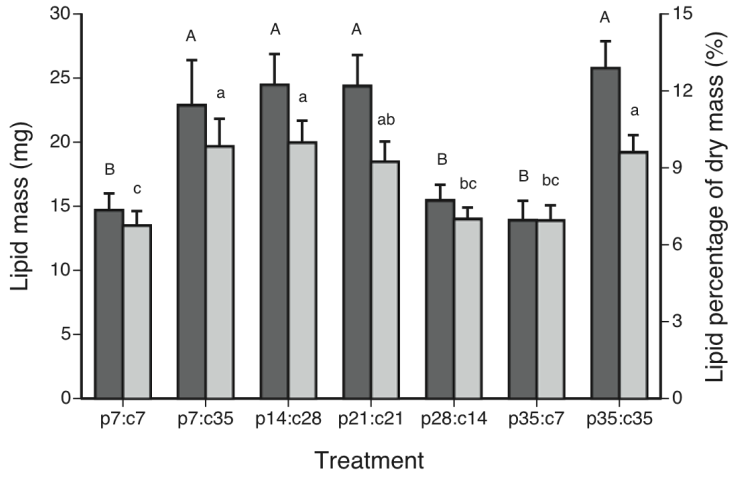


Figure 1.7 This figure shows the total lipid mass and the lipid percentage of dry mass (LS means ± SE).

DISCUSSION

This is the first study to provide a detailed account of the elemental body composition for an insect herbivore when raised on chemically defined foods that differ in their macronutrient content. Our results indicate that 6th instar *S. americana* grasshoppers allowed to self-select their diet regulate their protein-carbohydrate intake tightly and in doing so achieve similar measures of performance (survival, development time, mass gain) and maintain tight elemental homeostasis. When 6th instar *S. americana* grasshopper are confined to single diets with a range of different p:c ratios and total amounts of macronutrients, and not allowed to self-select their nutrient intake, performance differed across treatments, but interestingly grasshoppers generally practiced strict elemental homeostasis (in terms of elemental concentration) for all elements, excluding carbon and two metals – iron and cooper. Another key finding from this study was the result that digestible carbohydrate content was more limiting, in terms of growth, than was nitrogen content.

Insect herbivores, when given the opportunity, actively regulate their ingestion of foods to obtain precise levels of nutrients (Raubenheimer & Simpson 1993, Bernays *et al.* 1994, Behmer & Joern 2008, Lee 2007). In this study we found that *S. americana* nymphs also regulate their protein-carbohydrate intake to a slightly less than a 1:1 protein-carbohydrate ratio when they are allowed to self-select from nutritionally suboptimal, but complementary foods. As a result of ingesting similar total amounts and proportions of protein and digestible carbohydrate across the three treatments in the choice experiment, grasshoppers grew equally well, and had similar body elemental composition. Past ecological stoichiometry studies have shown that field collected

insects tend to practice strict elemental homeostasis (Bertram *et al.* 2006 and 2008, Fagan *et al.* 2002, Huberty & Denno 2006, Schade *et al.* 2003, Studier & Sevick 1992, and Woods *et al.* 2004). If insect herbivores have access to multiple plants in the field, and can freely move between these plants to regulate their nutrient intake, tight stoichiometric regulation in field caught insect herbivores may not be particularly surprising.

The true extent of strict elemental homeostasis practiced by insect herbivores can best be observed by restricting individuals to a range of foods with fixed or narrow nutrient content. In the field this might happen as a result of abiotic conditions limiting the total amount of plant material available, or as a result of a lack of nutritionally complementary plants in the insects habitat. It might also occur because potential predators limit the range of food options (Schmitz 2004, 2006, 2007, & 2008, Danner & Joern 2003). A good starting point for exploring food nutrient effects on performance and body elemental composition is to examine foods with optimal protein-carbohydrate ratios (in this case 1:1), but which have different absolute concentrations of macronutrient (e.g. the p7:c7, p21:c21, and p35:c35 diets). Grasshoppers on the p21:c21 and p35:35 diets ate different absolute amounts of food, but ingested relatively similar amounts of protein and digestible carbohydrate. As a result, grasshoppers on these two treatments had similar growth rates, elemental profiles and lipid content. In contrast, grasshoppers on the p7:c7 diet (which was heavily diluted compared to the p21:c21 and p35:c35 diets) attempted to compensate for nutrient dilution by eating more total food. Despite eating nearly 2x and 2.5x as much food as grasshoppers on the p21:c21 and p35:c35 diets, respectively, they could not match these latter two treatments in terms of protein and carbohydrate intake. Interestingly these grasshoppers developed at an

equivalent rate to the other two treatments, although they were significantly smaller. In nature, however, temperature constraints (Behmer 2009) and risks of predation during feeding (Benrey & Denno 1997) would likely result in extended development for grasshoppers on nutritionally dilute diets.

But what happened in terms of elemental profiles as nutrients become diluted? Grasshoppers on the p7:c7 diets had lower absolute amounts of C and N relative to the p21:c21 and p35:c35 treatments, and K levels on the p7:c7 were significantly lower compared to p21:c21 diet, but not the p35:35 diet. In terms of elemental concentration (the true indication of stoichiometry), the only element that differed among these three treatments was copper, which was highest in the p7:c7 diet. Interestingly the grasshoppers on the p7:c7 diet ate significantly more food than did grasshoppers on the other two diets – which suggests that cooper uptake is likely a passive process, and that the body concentrations are a function of the total amount of cooper ingested.

Another challenge that insect herbivores may encounter is variation in the ratio of potentially limiting macronutrients – especially protein and digestible carbohydrates (Bernays and Chapman 1994). In terms of overall performance, insects on the p21:c21 diets did best relative to the other diets with 42% total macronutrient content (i.e. p7:c35, p14:c28, p21:c21, p28:c14, and p35:c7); this is also the p:c ratio nymphs selected when given a choice of foods (see Figure 1.2).

Insects that put on the most mass also generally had the highest absolute element amounts, but interestingly grasshoppers on diets with very different p:c ratios (from highly carbohydrate-biased to highly protein-biased) did not differ in terms of elemental stoichiometry, with the two exceptions of carbon and cooper. Variation in carbon, however, was within the range seen for *S. americana* nymphs reared on 10-day-old seedling wheat (Boswell et al. 2008), and therefore this variation is likely of little biological significance. In terms of cooper body concentrations, the concave shaped response is likely associated with the finding that cooper levels in the diet were lowest on the p21:c21 treatment (see Table 1.4), and highest on the two most nutritionally imbalanced diets (e.g. p7:c35 and p35:c7). Again, this result suggests cooper uptake is a passive process, and there is little post-ingestive regulation of cooper once it is absorbed. Overall, the just discussed results indicate that the macronutrient profile of a food (the protein to carbohydrate ratio) has the greatest impact on the dry mass gain of individuals, and little influence terms of stoichiometry.

Table 1.4 Test statistic expressed as the F-ratio for the total amount (mg or μg) and concentration (% or ppm) of each element in newly molted adult grasshoppers from the different treatments from the no-choice experiment. All comparisons were made using ANCOVA –significant differences are noted in bold.

Element	expressed as	F-ratio
C	mg	**9.30**
	%	**3.41**
N	mg	**4.18**
	%	0.72
P	mg	1.71
	%	0.96
S	μg	1.70
	ppm	0.58
K	μg	**2.76**
	ppm	1.59
Na	μg	0.27
	ppm	0.65
Ca	μg	0.51
	ppm	1.45
Mg	μg	0.19
	ppm	0.97
Zn	μg	1.72
	ppm	2.09
Fe	μg	1.58
	ppm	**2.36**
Mn	μg	0.29
	ppm	0.35
Cu	μg	1.31
	ppm	**3.40**

So how did changing food macronutrient profile influence grasshopper growth? Typically protein (or more simply nitrogen) is considered the most limiting nutrient for insect herbivores (White 1993 & Joern & Behmer 1997), but results from this study suggest that energy limitations, particularly the amount of digestible carbohydrate (simple sugars and starch) in the food, is equally, if not more important as a limiting growth factor. This is best seen by making comparison among 4 key diets: p7:c7, p7:c35, p35:c7, and p35:c35. For example, when protein is held constant, but carbohydrate is varied (p7:c7 is compared with p7:c35, and p35:c7 is compared with p35:c35), growth is always better on the diet with more carbohydrate; and this difference holds after adjusting for differences in fat content for grasshoppers on carbohydrate-rich diets. More interesting, though, are comparisons where protein content is varied, but carbohydrate is kept constant (p7:c7 is compared with p35:7, and p7:c35 is compared with p35:c35). If nitrogen was the key limiting-factor, growth of grasshoppers on the p35:c7 diets should have been significantly higher compared to growth on the p7:c7 diets. Not surprisingly, where protein is readily available and coupled with a large supply of energy (e.g. the p35:c35 diet compared to the p7:c35 diet), growth is high. However, the real value of energy to the process of growth is seen when growth on the p7:c35 and p35:c7 diets are compared. For the latter diet energy is limiting, so nitrogen that would go to growth is not used. In contrast, when energy is in sufficient supply (e.g. p7:c35), ingested nitrogen (in the form of protein) can be used with great efficiency (see Zanotto et al. 1993). The result is that growth is superior on the carbohydrate-rich diet compared to the protein-rich diet. The trade-off here, though, is that development on the protein-rich diet is faster.

Lastly, some comments should be made about the elemental content of the artificial diets used in this study. First, compared to 10-day-old seedling wheat, which is used to maintain the grasshoppers culture that provided experimental insects for this study, our artificial diets contained elevated levels of all elements except for carbon, nitrogen, and magnesium (see Table 1.4); and in some instances these levels were quite extreme (e.g. sodium and calcium were more than an order of magnitude higher). It did not seem as though these elevated levels negatively affected insect growth and development, but it is clear that if the forms in which these elements occurred in the diet were accessible, most elements in our diets would never have been limiting. As a result, the experimental insects in this study likely were faced with having to regulate elements, especially those not related to protein and carbohydrate, by excreting those that exceeded requirements. Future analysis of frass from these experimental animals is needed to understand the extent to which this excretion was a mechanism used to keep concentrations at a particular threshold level. Interestingly phosphorus and sulfur concentrations in grasshoppers in this study were lower compared to grasshoppers reared on seedling wheat (Boswell et al. 2008). Given the lower levels of phosphorus and sulfur in the wheat, it may be that the phosphorous and sulfur in the diet was not completely accessible. Both phosphorous and sulfur levels were positively correlated with casein levels in the diet, and analysis of casein shows that it and is heavily phosphorylated and rich in sulfur (Reynolds *et al.* 1999) – whether grasshoppers can utilize the phosphorus and sulfur associated with casein, though, is unknown.

A second point relating to our diets concerns the relationship between carbon content and available energy (in the case of our grasshoppers the digestible carbohydrates

sugar and starch). The transfer of carbon across trophic systems is a fundamental issue in ecological stoichiometry (Sterner & Elser 2002), but it is critical to recognize that much of the carbon found in plants (cellulose) is inaccessible to insect herbivores. Our diets differed radically in their digestible carbohydrate content, and in two cases their cellulose content. However, a simple carbon analysis of our diets shows that they all contain similar concentrations of carbon (ranging from 39-43%). The clear effect of concentrations of digestible carbohydrates on growth and lipid levels in our grasshoppers argues that stoichiometric studies that focus on terrestrial systems, and particularly those studying players that cannot metabolize non-soluble carbohydrates, need to turn their attention to quantifying biomolecules that are relevant for the heterotrophs being investigated.

This study has shown that macronutrient bottlenecks have their biggest impact on determining insect growth, but little impact on elemental homeostasis. More research needs to be conducted where elements other than N and C are manipulated to better understand the relationship between a food source, its elemental composition, and the insect herbivore's elemental composition. A necessary next step is to manipulate elemental levels in a controlled fashion in plants, and explore the effects of these manipulations on elemental regulation in insect herbivores.

CHAPTER III

THE EFFECT OF PROTEIN TYPE ON ELEMENTAL HOMEOSTASIS IN A GENERALIST GRASSHOPPER

OVERVIEW

In nature organisms have the ability to select the foods that comprise its diet, unless it is a specialist one a single food source or there is a confounding factor resulting in only one food source being available. Interestingly most organisms' elemental compositions are not representative of their food source(s). We now ask the question of what happens to the elemental composition of an insect herbivore when the protein source and subsequently the amino acid profiles food is changed. In this study, we alter the protein quality by using three difference protein sources and the protein-carbohydrate ratio in a series of synthetically made foods. We see that the protein quality of an insect herbivore's diet has a significant affect on the elemental composition of our grasshoppers. This protein quality also plays a significant role in the total consumption, nutrient consumption, survival, and growth of individuals. We also found that the protein to carbohydrate ratio has the ability to significantly affect the elemental composition of grasshoppers when protein quality is varied. We have shown that for an insect herbivore the quality of protein on which it feeds, has the ability to alter the elemental levels at that are found in the body. Protein type in certain quantities also plays a significant role in immature insect mortality. We believe it is the differences in amino acids, and the specific elemental differences in each protein as well as the ratio of available protein to

carbohydrate that lead to multiple different outcomes depending on amino acid availability in the protein source.

INTRODUCTION

Physiological processes require insects to ingest proper amounts of a mixture of nutrients, including amino acids, carbohydrates, sterols, phospholipids, fatty acids, vitamins, minerals, trace elements, and water to meet optimal levels of growth and fitness (Behmer 2009, Behmer & Joren 2008, Bernays *et al.* 1994, Chapman 1998, & Schoonhoven *et al.* 2005). Insect herbivores therefore have to rely on plants, relatively nutrient poor foods, to obtain this suite of nutrients. The elemental composition of plants and herbivores are not equal, and on average, nitrogen and phosphorus content of plant material is 10-20 times lower than that of herbivores (Fagan *et al.* 2002). An animal does not simply ingest elements from the plant, but instead they ingest simple and complex molecules that are catabolized into useable units, which can then be reconstructed and used for metabolic processes (Raubenheimer and Simpson 2004). Through the process of reorganizing nutrients, organisms have the ability to practice elemental homeostasis (Sterner & Elser 2002). Plants are not nutritionally equally though, and each plant species has its own unique chemistry (Schoonhoven *et al.* 2005).

Two of these more complex nutritional molecules that plants provide for insect herbivores are the macronutrients proteins and carbohydrates. Carbohydrates come in different forms, but generally share the same elemental formula [$(CH_2O)_n$]. However, only soluble carbohydrates (e.g. simple sugars and starch) are available to insect herbivores. In contrast, complex carbohydrates like cellulose lignin, and hemicellulose,

which make up a large proportion of the total carbohydrate profile (often greater than 50-60%), provide no nutritional value of insect herbivores.

Another chemical difference between plants relates to their protein content, both in terms of amounts and quality. Proteins are built from combinations of amino acids, and are the primary source of nitrogen to herbivorous insects. However, the proteins found in different plant species, especially those in different plant families, can differ in terms of their amino acid profile. All insect herbivores require ten essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methoionine, phenylalanine, threonine, tryptophan, and valine; and while not required the ten non-essential amino acids can be either synthesized from the essential amino acids or ingested with food: alanine, aspartic acid, cystenine/cystine, glutamic acid, proline, glycine, serine, and tyrosine (Chapman 1998). Different proteins, with their different amino acid profiles, are known to differentially influence caterpillar growth and development (Broadway and Duffey 1988) but currently we know little about how protein quality influences grasshopper growth, and nothing about how protein quality influences elemental homeostasis in any insect herbivores.

Boswell *et al.* (2009) recently used artificial foods with different nutritional profiles to explore elemental homeostatic ability in a generalist insect herbivore. Here organismal stoichiometry was studied using the experimental design of the geometric framework (GF) (Raubenheimer and Simpson 1999, 2004), and results showed strong elemental homeostasis across a range of diets that differed in their absolute amounts and/or ratios of protein and carbohydrate. Stoichiometric studies have mainly focused on the macroelements carbon, nitrogen, and phosphorous due to their structural and physiological importance on aquatic and benthic invertebrates (Evans-White *et al.* 2005,

Frost *et al.* 2004, Karimi & Folt 2006, and Sterner & Elser 2002), but the Boswell et al. (2009) study included analysis of 9 additional elements.

In the current paper, the effects of protein quality and quantity on stoichiometry of an insect herbivore are explored at the organismal level. This study is the first that describes the relationship between a protein's amino acid content and the elemental body composition of an insect herbivore reared in a laboratory and fed a chemically defined diet. In total of 12 different elements are quantified: C, N, P, S, K, Na, Ca, Mg, Fe, Cu, Zn, and Mn. Generally these elements are split into three broad categories (Fraust da Silvia & Williams 1991): 1) the structural elements, which include C, N, P, and S, 2) the electrochemical elements, which include K, Na, Ca, and Mg, and 3) the catalytic elements, which include Fe, Cu, Zn, and Mn. It is a gross generalization to classify these elements within these groups – it is done only as a way to simplify the results and to view them in a classical way. We discuss the results of this experiment in the context of ecological and organismal stoichiometry.

METHODS

Insects and Experimental Chambers

The polyphagous grasshopper *S. americana* occurs throughout the south and eastern United States and Mexico (Harvey 1981) and is recorded feeding on a wide range of cultivated and naturally occurring plant species (Kuitert & Connin 1952). Insects came from a culture that has been maintained on a diet consisting of seedling wheat and wheat germ since 2006 in the Department of Entomology at Texas A&M University. They

were maintained under standard laboratory conditions with a 12h:12h L:D photoperiod, and under radiant heat of 29-36 C during the light phase (supplied by 60W full spectrum incandescent bulb), and at 23-26 C during the dark phase.

Grasshoppers were removed from the culture after ecdysis to the 6th instar, sexed and weighed then placed singly into clear polystyrene arenas. Each arena measured 18.9 x 13.3 x 9.6 cm and contained one food, an aluminum wire roost for perching, and a 30-mL Solo Cup ® filled *ad libitum*, fitted with a lid that had been modified to allow the use of a cotton wick for drinking. The food dish was modified Petri dishes designed to minimized spillage (Raubenheimer & Simpson 1990), and placed at the front of the arena while the water dish was placed a the rear to avoid possible contamination. All experiments were conducted at a constant temperature of 29-31 °C and a 12:12h light:dark photoperiod. Approximately the same number of males and females were placed on each diet, and treatment was replicated ten times.

Synthetic Foods

Dry, granular, chemical defined synthetic foods were made in a manner similar to Dadd (1961) and later modified by Simpson & Abisgold (1985). In total there were 18 diets that varied in the type of protein used and the ratio and/or amount of protein (p) and digestible carbohydrate (c), expressed on a dry mass basis. In terms of the macronutrient content, there were six combinations: (1) p7:c35, (2) p14:c14, (3) p14:28, (4) p21:c21, (5) p28:c14, and (6) p35:c7. In terms of protein content, three different protein sources were used: (1) casein plus albumin (mixed in a 3:1 ratio), (2) soy protein, and (3) wheat protein. The casein and albumin, which are the typical protein sources used in a broad range of grasshopper nutritional studies (e.g. Behmer 2001, 2003), were obtained from

Sigma (C5890 and A5253, respectively). The soy protein was Peptone Hy-Soy® T, enzymatic hydrolysate, also obtained from Sigma (P6463). Finally, the wheat protein was Wheat Protein Isolate 8000, obtained from LifeSource Foods, LLC (Louisville, KY). The digestible carbohydrate component was a 1:1 mixture of sucrose and dextrin. All experimental foods except the p14:c14 diet contained identical total macronutrient content, the same amounts of cellulose (used as a bulk agent), Wesson's salt, vitamins, linolenic acid and sterols. The p14:c14 diet contained only 28% total macronutrient content, but compared to the other diets had similar absolute amounts of Wesson's salt, vitamins, and sterols. The bulk difference in the p14:c14 diet was made up using cellulose (68% of the total).

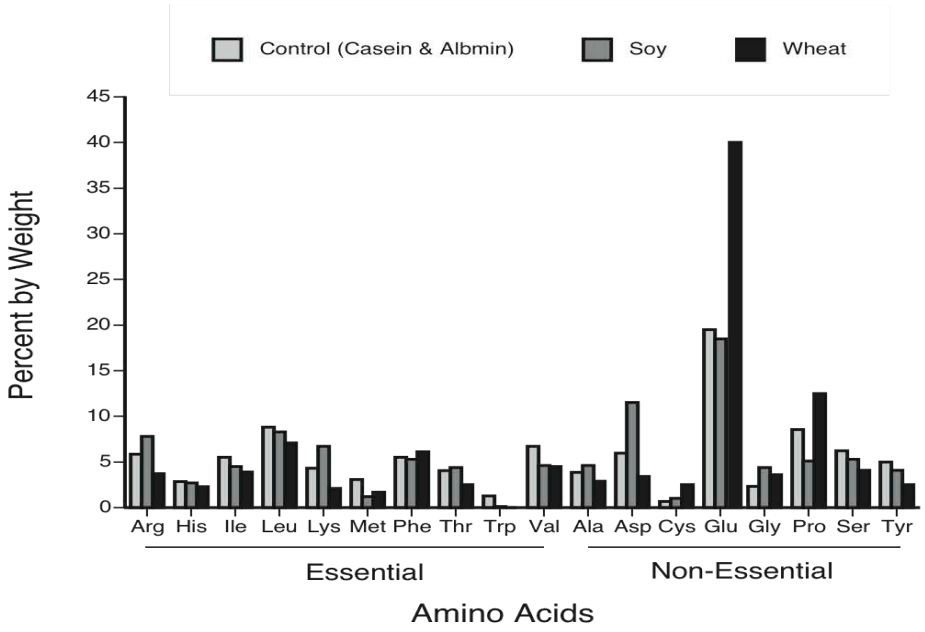


Figure 2.1. The three protein sources we used in our diets are shown with their respective amounts of amino acids present in each. NOTE: These amounts of amino acids are averages and do not represent the exact amino acid content of our protein sources.

Two key differences existed between the different protein sources. First, as summarized in Figure 2.1, the different protein sources had different amino acid profiles [information on casein and albumin came from the following website (http://www.scientificpsychic.com/fitness/aminoacids1.html), information for soy protein was gathered from Sigma, Erasmus *et al.* (1994), and the following website (http://www.scientificpsychic.com/fitness/aminoacids1.html), while information for wheat protein was collected from Anjum *et al.* (2004) and Lasztity *et al.* (1985)]. The main differences to note are that the casein and albumin mixture, and the soy protein, relative to the wheat protein, have higher proportions of the following essential amino acids: Arginine (Arg), Lysine (Lys), and Tryptophan (Trp). With respect to Theronine (Thr), it is found in the casein plus albumin mixture, but is only at trace levels in the both the soy and wheat protein. On average, the casein plus albumin mixture has a higher percentage of Methonine (Met) compared to the soy and wheat protein. With respect to non-essential amino acids, the most notable difference is that wheat has a much higher percentage of Glutamic acid (Glu), and somewhat higher percentage of proline (Pro), compared to the other two protein sources. Cysteine (Cys) concentrations were highest in wheat, intermediate in soy, and lowest on the protein plus albumin mixture. Finally, Aspartic acid (Asp) was highest in soy, followed by casein plus albumin mixture, and then the wheat. The percentages of all other amino acids were fairly similar. The second key difference between the proteins was their total nitrogen content, despite each protein source coming from a pure source (> 99%). The casein and albumin mixture, as well as the wheat protein, were approximately 14% total nitrogen, but the soy protein contained only 8% total nitrogen.

Experimental Protocol

Two separate no-choice experiments were preformed. The first experiment had three p:c ratios (p7:c35, p21:c21, and p35:c7) and three protein sources (casein plus albumin, soy, and wheat). The p:c ratios used represented a near optimal mixture (p21:c21), and two highly imbalanced mixtures (p7:c35 and p35:c7) on either side of the optimal mixture. Foods were placed into individual dishes and then weighed to the nearest 0.01 mg after allowing the food to equilibrate to ambient room humidity levels (RH 30-40%) for about 24hrs. The foods were then placed into the appropriate arenas and grasshoppers were allowed to feed for 72 hrs, after which the food dishes were removed and replaced with fresh, pre-weighed dishes of the same food. The food dishes that were removed were allowed to equilibrate to room humidity (RH 30-40%) before being reweighed to determine consumption. This process was repeated every 72 hours until the grasshopper molted. Upon molting individual grasshoppers were collected and weighed (to the nearest 0.01 mg) and then frozen until needed for elemental analysis.

The second experiment was identical to the first expect that protein type was only examined across a single p:c ratio (p14:c14). This treatment had a near optimal protein-carbohydrate ratio, but was slightly diluted compared to the earlier p21:c21 diet. We choose to use this protein to carbohydrate ratio after seeing that protein ratios of p7:c35 lead to significant death when using the wheat and soy protein.

Postmortem Elemental Analysis

Frozen grasshoppers were placed individually into 15 mL glass vials and transferred to a drying oven set at 40°C until they reached a constant dry mass (to the nearest 0.01 mg). Dried grasshoppers were then pulverized to a fine powder by placing a small magnetic

stir bar into the glass vial holding the dried grasshopper, capping the vial, and holding it on a vortex for approximately 1 min (Boswell et al. 2008). Powdered samples were then separated into two approximately equal aliquots (measured to the nearest 0.01 mg); one for carbon-nitrogen analysis, one for additional elemental analysis (P, K, Ca, Mg, Na, Fe, Zn, Cu, and Mn), and a third to quantify lipid mass. Each nitrogen sample was wrapped in a small sheet of tin foil, and placed individually into stainless steel crucibles. As a precautionary measure the samples were wrapped in tin foil to prevent the loss of material during removal of atmospheric air. The samples were then placed in an Elementar vario MAX CN high temperature carbon-nitrogen analyzer set at 950º C and analyzed using methods similar to those discussed by McGeehen and Naylor (1988). The second aliquot, used to measure non-N elements, was transferred to polypropylene digestion tubes. These samples were digested using trace metal grade nitric acid on a 105º C graphite block. Following digestion, samples were brought to volume and analyzed using Spectro axial CIROS inductively coupled plasma – Atomic Emission Spectrometry (Havlin & Soltanpour 1980).

Statistical Analysis

All statistical analyses were conducted using JMP 7.02 (SAS Institute, Inc. Development time was analyzed using survival analysis, while ANCOVAs, with start wet mass as a covariate to correct for size differences between males and females (males are, on average, smaller), was used to analyze dry mass gain, total consumption, and body element composition, both in terms of absolute amounts, and as a concentration. Where significant treatment effects were observed, post-hoc treatment comparisons were made using student's t-tests.

RESULTS

Experiment 1

It is first worth noting that none of the grasshoppers on the p7:c35 diet with wheat protein completed development to the adult stage. Also worth noting is that only three of ten grasshoppers on the soy p7:c35 treatment completed development. On all other treatments, survival was at or near 100%. As a result of the high mortality on two of the p7:c35 treatments, analyses were only compared with ratios, using protein type as the key main effect of interest.

Total Consumption, Nutrient Intake, and Performance

Figure 2.2 shows total consumption for each protein type and each p:c ratio. On the p21:c21 diets, protein type did not significantly effect consumption, but when the p:c ratio was imbalanced significant differences were observed (Table 2.1). On the p35:c7 diets consumption was highest on the soy protein and equally low on the casein plus albumin and wheat treatments. On the p7:c35 diets consumption was significantly higher on the soy treatment. The consumption data is also shown as protein-carbohydrate intake, and as nitrogen-carbohydrate intake (Figure 2.2a & 2.2b). The protein-carbohydrate intake data is similar, in terms of patterns of significance, as the consumption data (Table 2.1). When the nitrogen-carbohydrate intake data was analyzed, however, significant differences were observed for each p:c ratio grouping (Table 2.1).

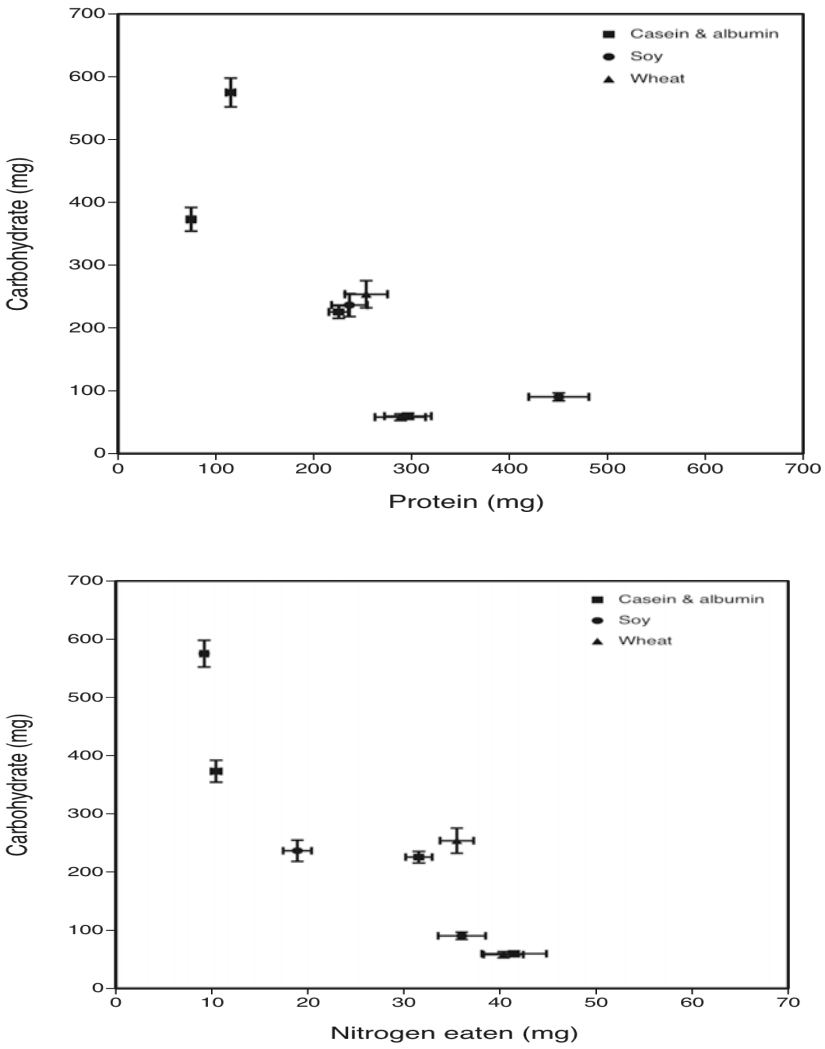


Figure 2.2. (a) Consumption of protein and digestible carbohydrate (means ± SE) and the (b) amount of nitrogen eaten compared to the amount of carbohydrate eaten in experiment 1 diet.

Table 2.1 Elemental concentrations (expressed as a % or in ppm) present in seedling wheat and the three artificial diets.

	Element											
Diet	**C (%)**	**N (%)**	**P (%)**	**S (ppm)**	**K (ppm)**	**Na (ppm)**	**Ca (ppm)**	**Mg (ppm)**	**Zn (ppm)**	**Fe (ppm)**	**Mn (ppm)**	**Cu (ppm)**
Seedling wheat												
10-day-old	44	5.5	0.9	6276	22751	2352	2728	3241	64	93	81	12
Casein plus albumin												
p7:c35	41	1.1	3.4	12232	44348	39529	54172	4240	167	718	326	137
p21:c21	42	3.3	4.2	23125	41814	60093	55421	3381	332	704	415	230
p35:c7	43	4.5	4.8	34059	52189	62858	57838	3881	298	1024	336	172
p14:c14	43	1.9	4.1	15536	88289	60974	60399	9768	333	680	426	192
Soy protein												
p7:c35	41	0.8	3.7	13527	57077	44220	50334	3660	379	764	295	158
p21:c21	41	1.7	5.6	17542	93709	53802	51086	5529	804	807	391	200
p35:c7	41	2.8	7.5	12232	130242	53335	53224	7771	1176	1014	374	177
p14:c14	42	1.4	2.9	20778	38276	31262	46392	3668	187	711	242	105
Wheat protein												
p7:c35	42	1.2	4.8	10251	78179	34227	46872	5498	512	1401	218	198
p21:c21	39	3.0	4.5	10036	76142	38670	50455	5151	483	1538	264	193
p35:c7	43	5.1	7.3	16064	123213	55112	57054	7586	959	1704	389	190
p14:c14	42	2.2	2.8	17452	38537	45813	50705	3469	272	1306	338	175

When the nitrogen-carbohydrate intake data was analyzed, however, significant differences were observed for each p:c ratio grouping (Table 2.1). On the p21:c21 treatments, the casein plus albumin and wheat values were similar, but the soy nitrogen-carbohydrate intake was different, mostly as a result of decreased nitrogen intake. On the p35:c7 treatments, the nitrogen-carbohydrate values were closely grouped, but the soy treatment differed from the casein plus albumin and the wheat treatment, but the latter two treatments did not differ from one another. On the p7:c35 treatments there was a significant difference between the nitrogen-carbohydrate intake on the two protein sources. Two measures of performance, development time and dry mass gain, were measured. Protein type had a significant affect on the survival of grasshoppers in experiment. On the p21:c21 treatments development was equal fast on the casein plus albumin and soy diets, and longest on the wheat protein (Figure 2.3b; Table 2.2). On the p7:c35 diets, development on the soy treatments was significantly longer compared to the casein plus albumin diets (Figure 2.3b; Table 2.2).

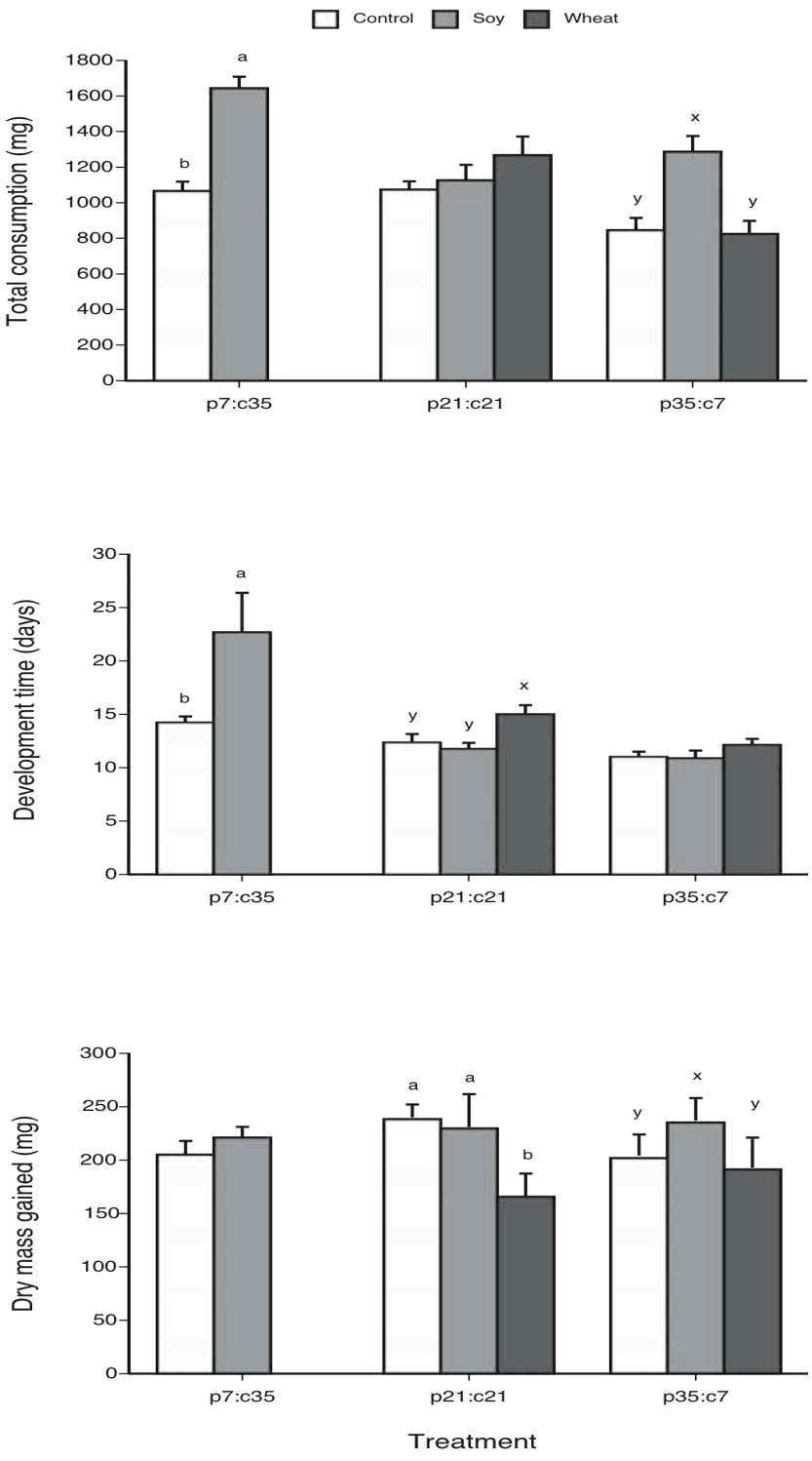


Figure 2.3. (a) Total consumption of food for grasshoppers in experiment 1, (b) development time, and (c) dry mass gain (LS means ± SE).

Table 2.2 Test statistics for protein-carbohydrate consumption, nitrogen-carbohydrate consumption, total consumption, development, and dry mass gained. The test statistic is an F-ratio for all variables except development time, which is χ^2. Where significant differences ($P < 0.05$) were detected they are noted in bold.

	Variable of interest				
Diet	**Protein-carbohydrate consumption (mg)**	**N-carbohydrate consumption (mg)**	**Total consumption (mg)**	**Development time (days)**	**Dry mass gain (mg)**
p7:c35	**3.54**	**12.05**	**30.45**	**13.60**	1.55
p21:c21	0.37	**11.53**	1.92	**10.01**	**8.63**
p35:c7	**4.11**	**9.20**	**12.85**	3.14	**3.74**
p14:c14	**2.73**	**10.42**	**7.97**	**28.10**	**33.31**

No difference in development time was observed for the three protein sources when the p:c ratio was p35:c7 (Table 2.2). The protein type also significantly affected dry mass gain. On the p21:c21 diets grasshoppers gained equally high amounts of dry mass on the casein plus albumin mixture and the soy protein, and the lowest amounts on the wheat protein (Figure 2.3c; Table 2.2). Significant differences in dry mass gain were also observed on the p35:c7 treatments, with mass gain being highest on the soy protein, and equally low on the casein with albumin and wheat diets (Figure 2.3c; Table 2.1). No significant differences in dry mass were observed on the p7:c35 treatments.

Elemental Composition

A total of 12 elements were analyzed both in terms of their absolute amounts, and their concentrations (either as a % or as ppm): carbon, nitrogen, phosphorus, sulfur, potassium, sodium, calcium, magnesium, iron, zinc, manganese, and copper.

Figure 2.4 and table 2.3 shows that the total amount and concentration of carbon, nitrogen, phosphorous, and sulfur for each p:c ratio and protein type. We only observed differences for phosphorous, and only for total amounts.

Table 2.3 ANCOVA test statistics (expressed as the F-ratio) for the total amount (mg or μg) and concentration (% or ppm) of each element in newly molted adult grasshoppers from the different treatments from the no-choice experiment. Where significant differences ($P < 0.05$) were detected they are noted in bold.

Element	expressed as	p7:c35	p21:c21	p35:c7
C	mg	4.29	2.12	2.41
	%	1.78	2.72	**0.16**
N	mg	0.02	0.93	1.89
	%	0.08	1.53	1.22
P	mg	1.71	**14.1**	**6.02**
	%	4.21	2.06	2.51
S	μg	0.32	2.90	0.95
	ppm	0.01	0.62	1.21
K	μg	0.38	**14.8**	**7.35**
	ppm	3.38	0.89	0.75
Na	μg	1.41	**3.82**	1.18
	ppm	0.22	**4.58**	0.97
Ca	μg	4.68	2.95	1.72
	ppm	3.19	0.31	0.88
Mg	μg	3.34	**4.71**	**8.33**
	ppm	0.95	0.47	1.93
Zn	μg	3.73	**4.07**	**4.39**
	ppm	1.24	**4.28**	0.93
Fe	μg	0.04	**4.63**	**11.04**
	ppm	0.26	**7.36**	**13.97**
Mn	μg	0.08	0.45	0.12
	ppm	0.01	2.17	1.09
Cu	μg	2.26	1.94	3.01
	ppm	1.26	**9.87**	2.19

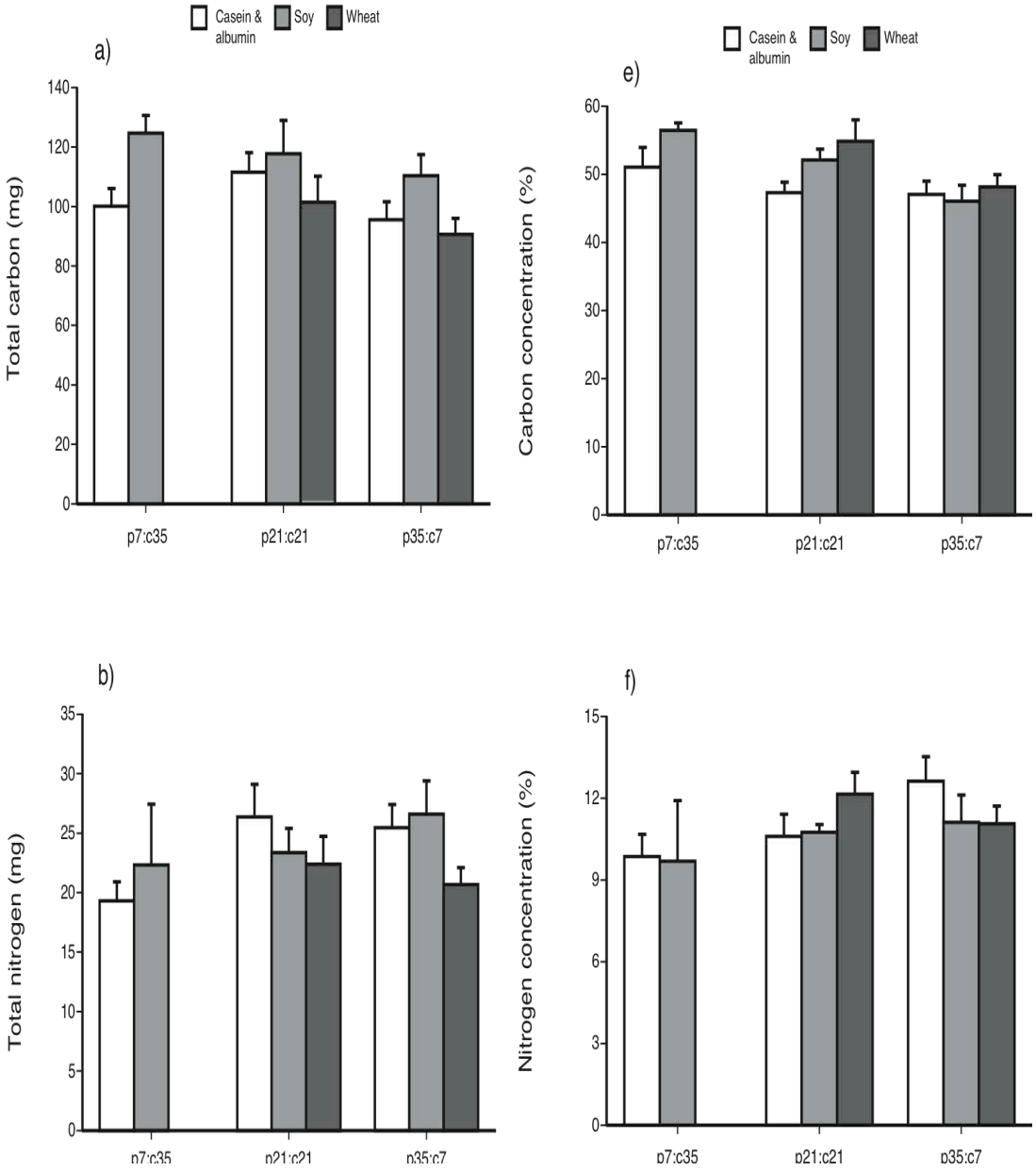


Figure 2.4. The left column contains total amounts (mg or μg) of each structural element present at end of experiment 1 (LS means ± SE). The right column shows the concentration of the structural elements (% or ppm) (LS means + SE).

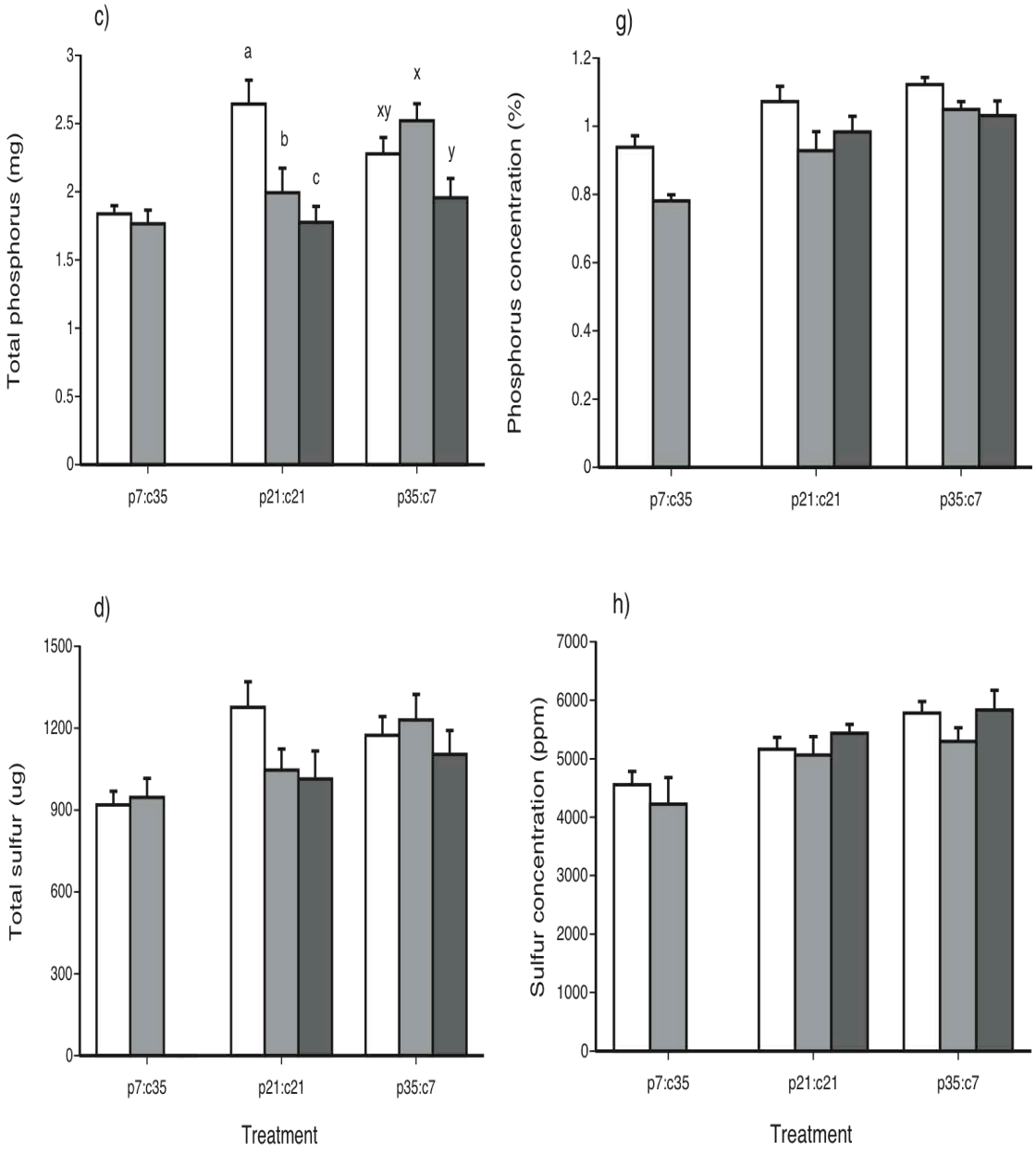


Figure 2.4 continued.

On the p21:c21 diets, grasshoppers on the casein & albumin protein source had the highest amounts of phosphorus, those on soy had an intermediate level, and those on wheat protein showed the lowest levels (Figure 2.4c). On the p35:c7 diets phosphorous amounts were highest on the soy diet, lowest on the wheat, and intermediate on the casein plus albumin treatment (Figure 2.4c).

Figure 2.5 shows that the total amount and concentration of elements that are typically classified as electro-chemical elements (K, Na, Ca, and Mg). Significant differences in total amounts were observed for K, Na, and Mg, and in concentration for Na. There was no significant effect of p:c ratio or protein for Ca. The total amount of potassium on the p21:c21 diets was significantly higher for grasshoppers on the casein plus albumin and the soy protein source, and lowest on the wheat source. On the p35:c7 diets, grasshoppers again had higher K amounts on the casein plus albumin mixture and the soy diet compared to grasshoppers on the wheat protein source. The total amount and concentration of sodium was only significantly affected on diets with a p21:c21 ratio.

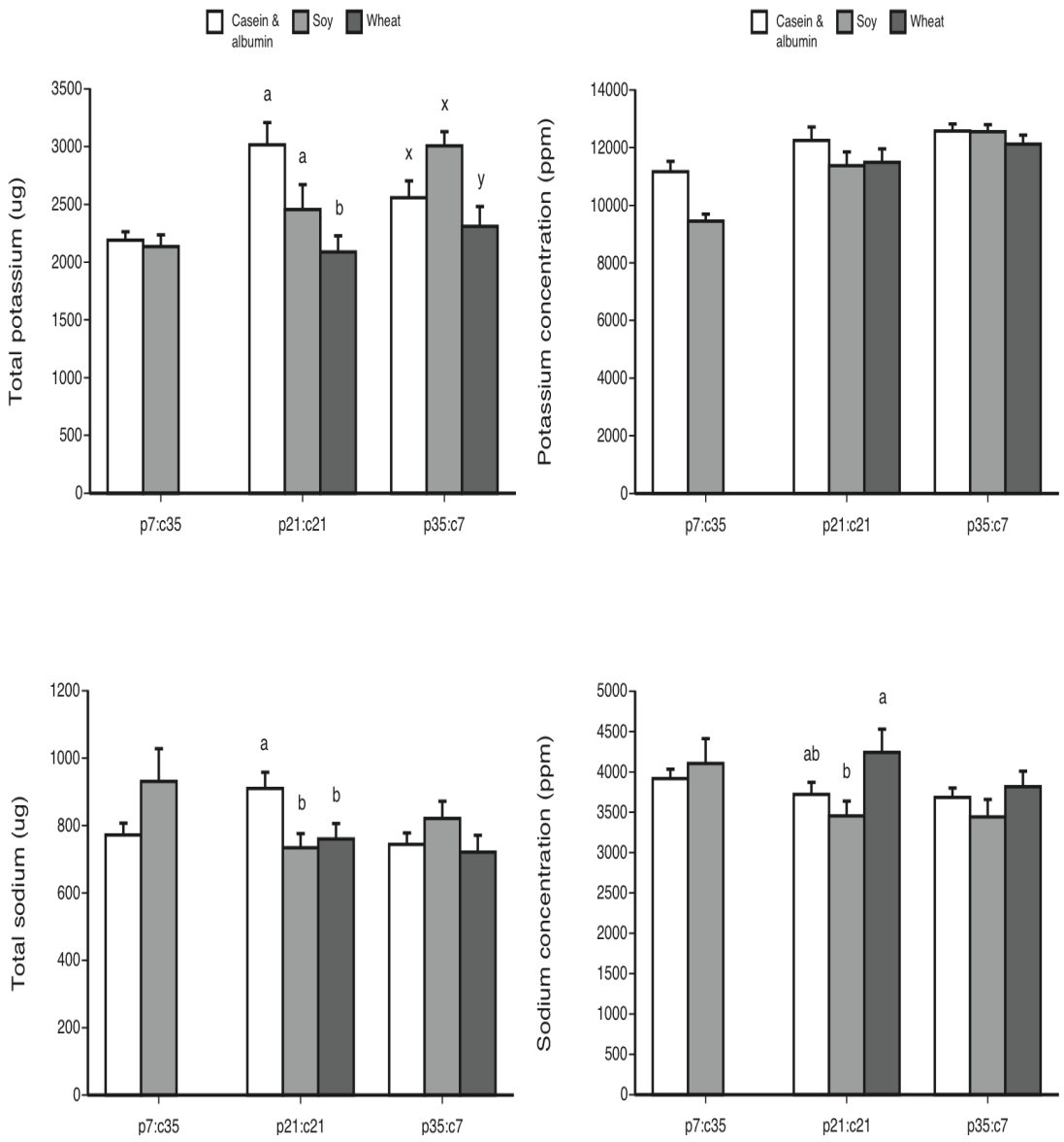


Figure 2.5. The left column contains total amounts (μg) of each electro-chemical element present at end of experiment 1 (LS means ± SE). The right column shows the concentration of the electro-chemical elements (ppm) (LS means + SE).

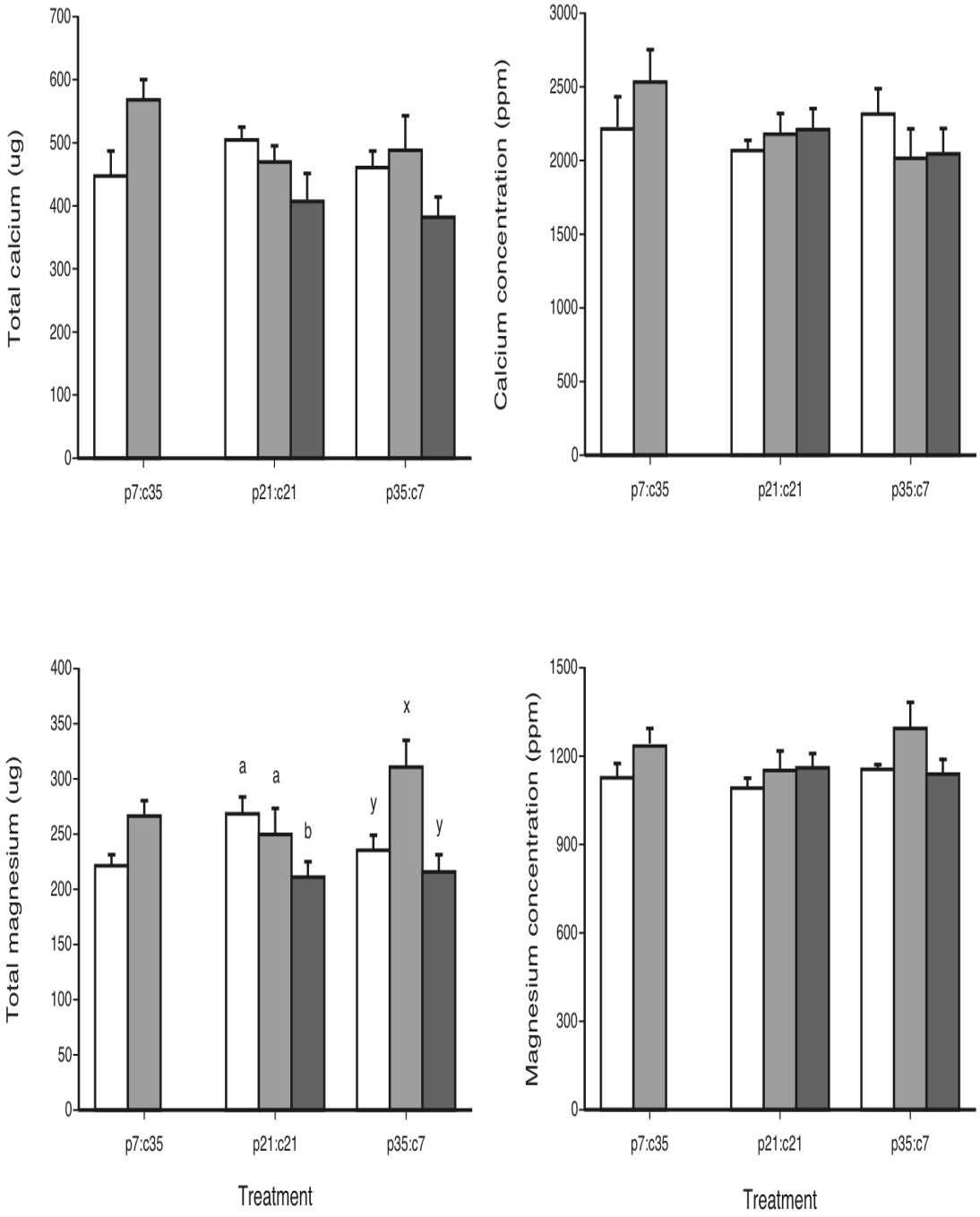


Figure 2.5 continued.

The highest Na amounts were for grasshoppers on the casein plus albumin diets, and equally low on the soy and wheat diets. In terms of concentration, grasshoppers on the soy p21:c21 had the lowest Na concentration, while those on the wheat had the highest. Grasshoppers on the casein plus albumin mixture had an intermediate concentration of Na. The total amount of magnesium differed for across the p21:c21 and p35:c7 ratios. Total amounts of Mg were equally high the casein plus albumin mixture and the soy protein and lowest on the wheat protein. On the p35:c7 diets, grasshoppers on the soy protein had higher amounts of Mg compared to the other two protein sources.

The catalytic elements (Zn, Fe, Mn, and Cu) are shown in Figure 2.6. There are also no differences, either in total amount or concentration was observed on diets with a p7:c35 ratio. A number of differences were observed on the p21:c21 and p35:c7 diets. The protein type significantly affected the total amounts and concentrations of zinc and iron. Zinc amounts on the p21:c21 diets were highest in the casein plus albumin mixture, lowest on soy protein, and intermediate on the wheat diets. On the p35:c7 diets zinc amounts were highest on grasshoppers fed soy protein, lowest on those given wheat, and intermediate on those given the casein plus albumin mixture. Zn concentrations on the p21:c21 diet were equally high on the casein plus albumin and wheat diets, and lowest on the soy protein. Total iron amounts were highest on the soy protein for both p21:c21 and p35:c7. On the p21:c21 Fe levels on the casein plus albumin and wheat diets were equally low, but on the p35:c7 diets Iron amounts on the casein plus albumin were significantly lower compared to the wheat diet. In terms of Fe concentration, on the p21:c21 diet it was highest on the soy diet, and equally low on the other two diets. On the p35:c7 diets, it was lowest on the casein and albumin diet, and equally high on the other

two diets. The concentration of copper was only different on the p21:c21 diets. It was highest when grasshoppers were fed the wheat protein and equally low on the casein plus albumin and soy diets.

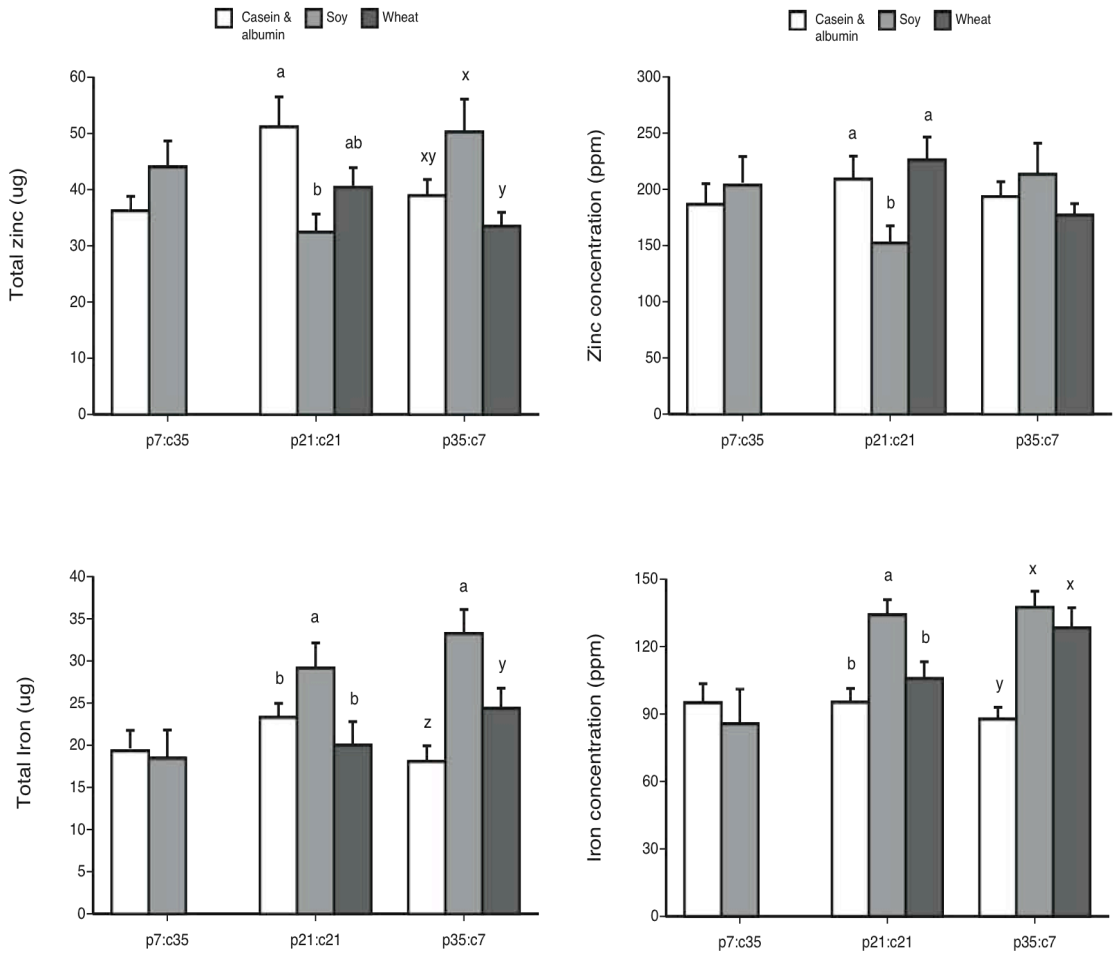


Figure 2.6. The left column contains total amounts (μg) of each catalytic element present at end of experiment 1 (LS means ± SE). The right column shows the concentration of the electro-chemical elements (ppm) (LS means + SE).

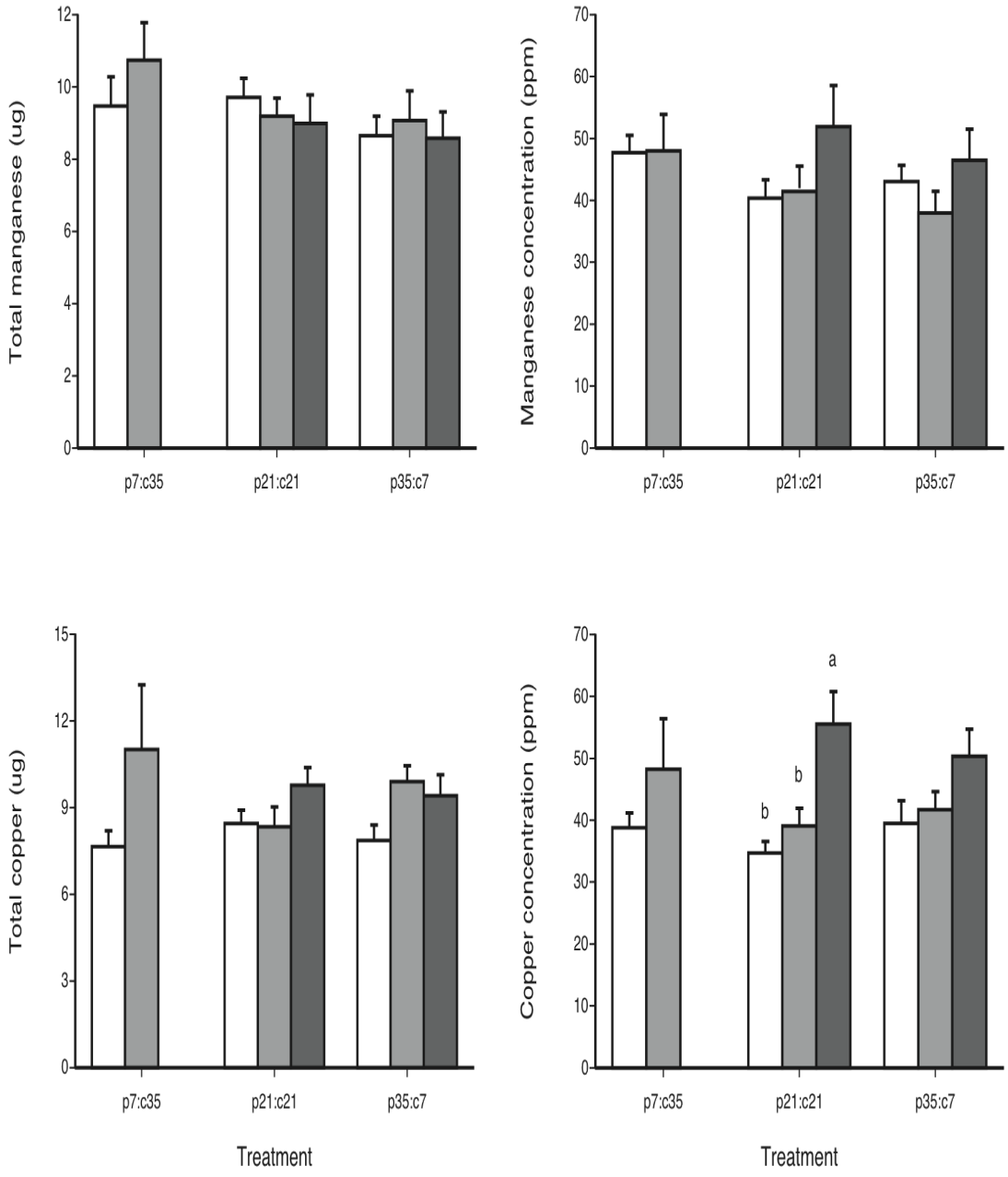


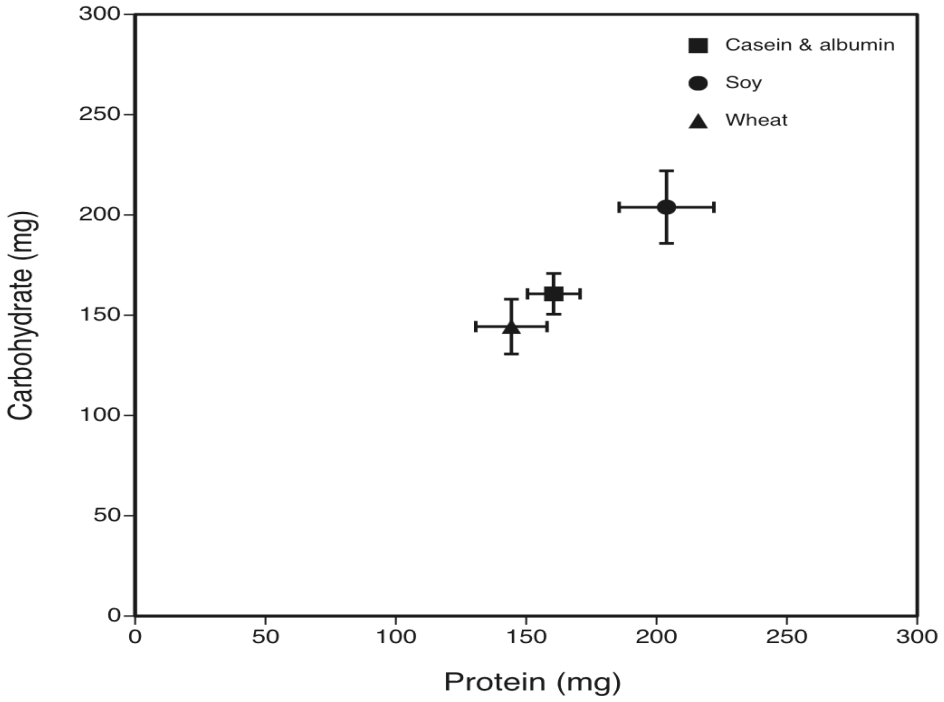
Figure 2.6 continued.

Experiment 2

In contrast to the previous experiment, there was good survival across the three treatments used for this experiment.

Total Consumption, Nutrient Intake and Performance

Consumption expressed in terms of protein-carbohydrate eaten is shown in Figure 2.7a. Figure 2.8a shows total food consumption, which was significantly affected by the protein source (Table 2.2). Consumption was highest on the soy protein diet and equally low on the casein plus albumin and wheat treatments. The statistical pattern followed that of consumption (Table 2.2), with protein and carbohydrate intake being highest on the soy treatment, and statistically similar on the other two treatments. In terms of nitrogen-carbohydrate intake, protein type had a significant effect (Table 2.2). Carbohydrate intake was greatest on the soy treatment, but nitrogen intake was higher on the other two treatments (Figure 2.7b).



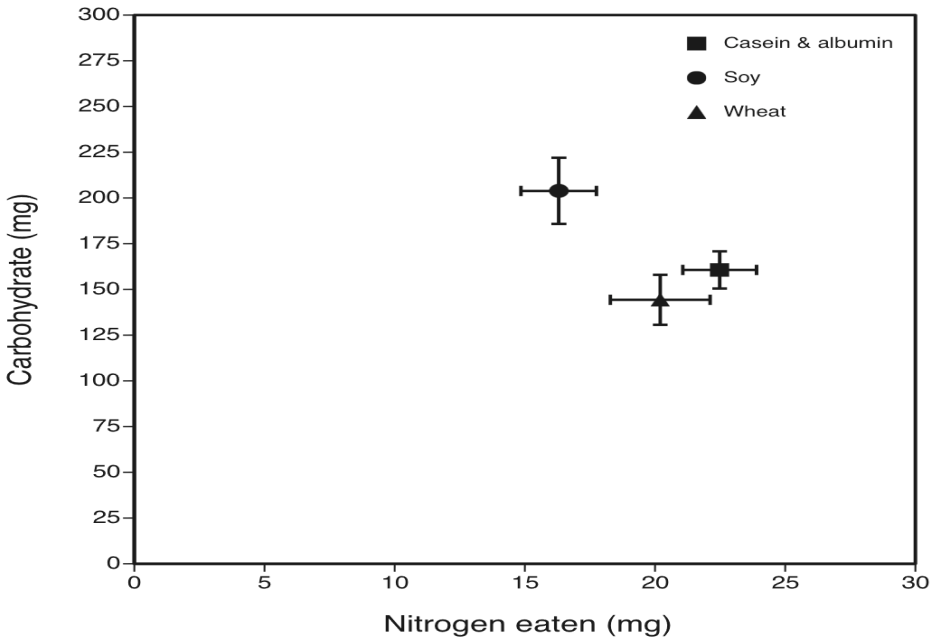


Figure 2.7 (a) Consumption of protein and digestible carbohydrate (LS means ± SE) and the (b) amount of nitrogen eaten compared to the amount of carbohydrate eaten (LS means ± SE) in experiment 2.

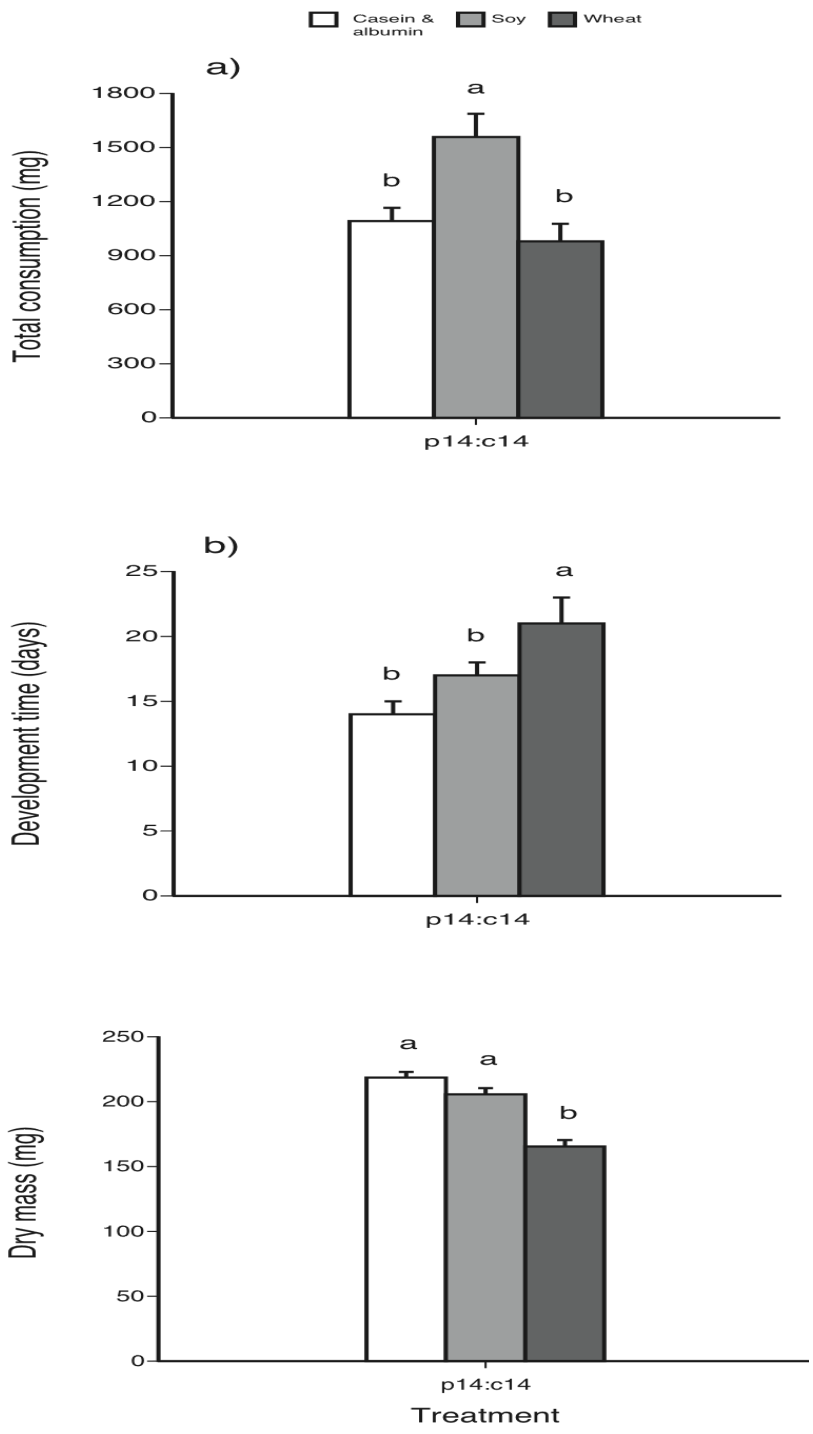


Figure 2.8 (a) Total consumption of food (LS means ± SE) for grasshoppers in experiment 2, (b) development time (LS means ± SE), and (c) dry mass gain (LS means ± SE).

Two measures of performance, development time and dry mass gain, were again measured. Protein type had a significant affect on grasshopper development (Figure 2.8b, Table 2.2). Individuals on the wheat protein took the longest to develop, while those on the other two diets developed equally fast. The total dry mass gained was also significantly affected by protein type. The casein plus albumin and soy diets had equally high amounts of dry mass gain while grasshoppers on the wheat protein gained significantly lower amounts (Figure 2.8c, Table 2.2).

Elemental Composition

Again, 12 elements were analyzed both in terms of their absolute amounts, and their concentrations (either as a % or as ppm): carbon, nitrogen, phosphorus, sulfur, potassium, sodium, calcium, magnesium, iron, zinc, manganese, and copper. Figure 2.9 and table 2.4 shows that within the structural elements (C, N, P, and S) the total amounts of all are significantly affected by the protein source. The concentration of carbon, nitrogen, and phosphorus is also significantly affected by the protein source as well. The highest amounts of carbon are seen in grasshoppers fed the casein plus albumin and soy proteins, with significantly lower amounts on the wheat protein. The concentration of carbon is equally high in both the soy and wheat protein, but lowest in the casein plus albumin protein source. The highest total amounts of nitrogen were seen on the casein plus albumin diets, with intermediate levels on the soy diets, and the lowest levels on the wheat diets. Nitrogen concentration was highest in grasshoppers fed the casein plus albumin protein source and equally low on soy and wheat protein sources. The total amount of phosphorus was equally high on both the casein plus albumin and soy protein, with significantly lower amounts found in individuals fed wheat protein.

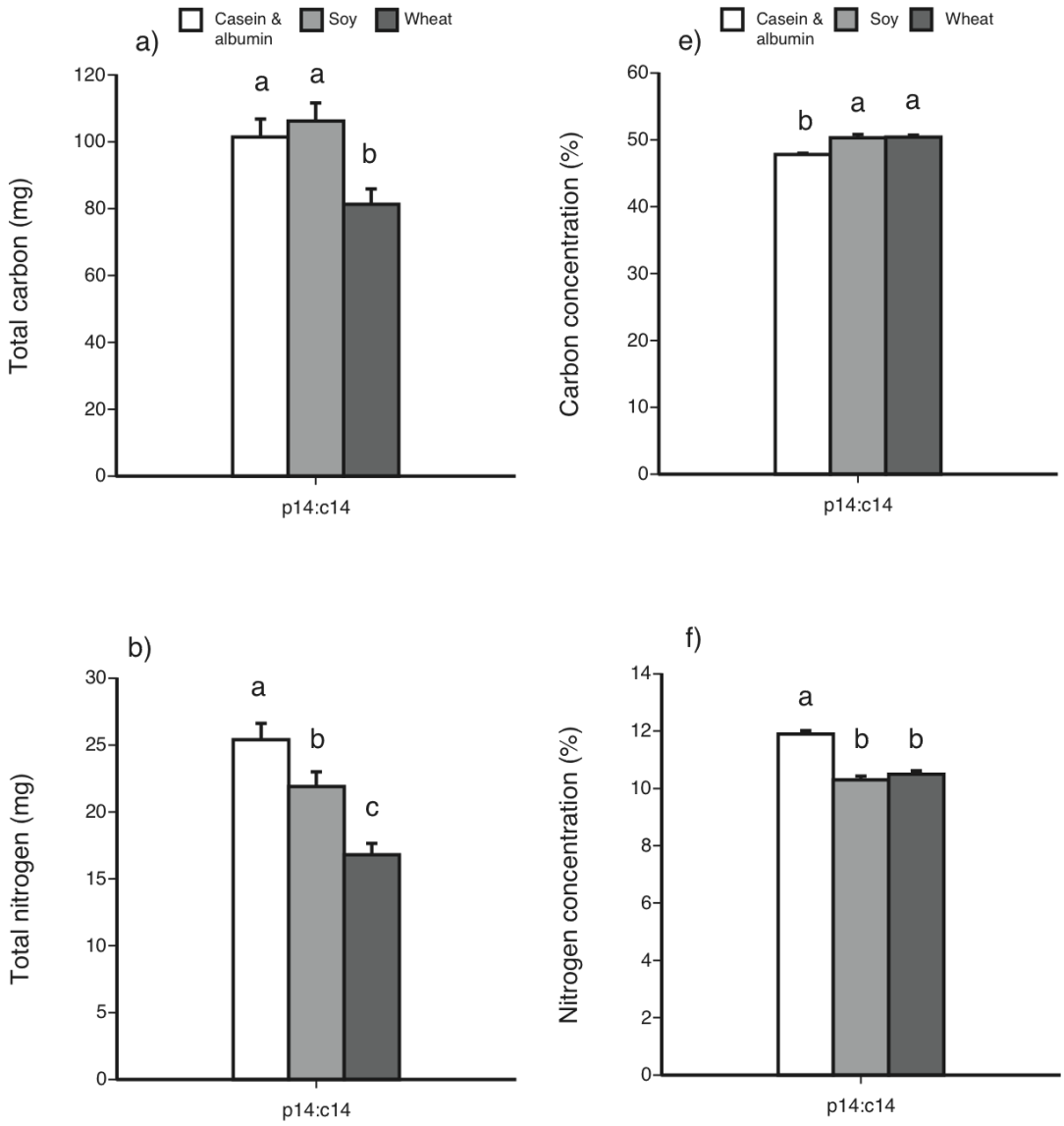


Figure 2.9 The left column contains total amounts (mg or μg) of each structural element present at end of experiment 2 (LS means ± SE). The right column shows the concentration of the structural elements (% or ppm) (LS means + SE).

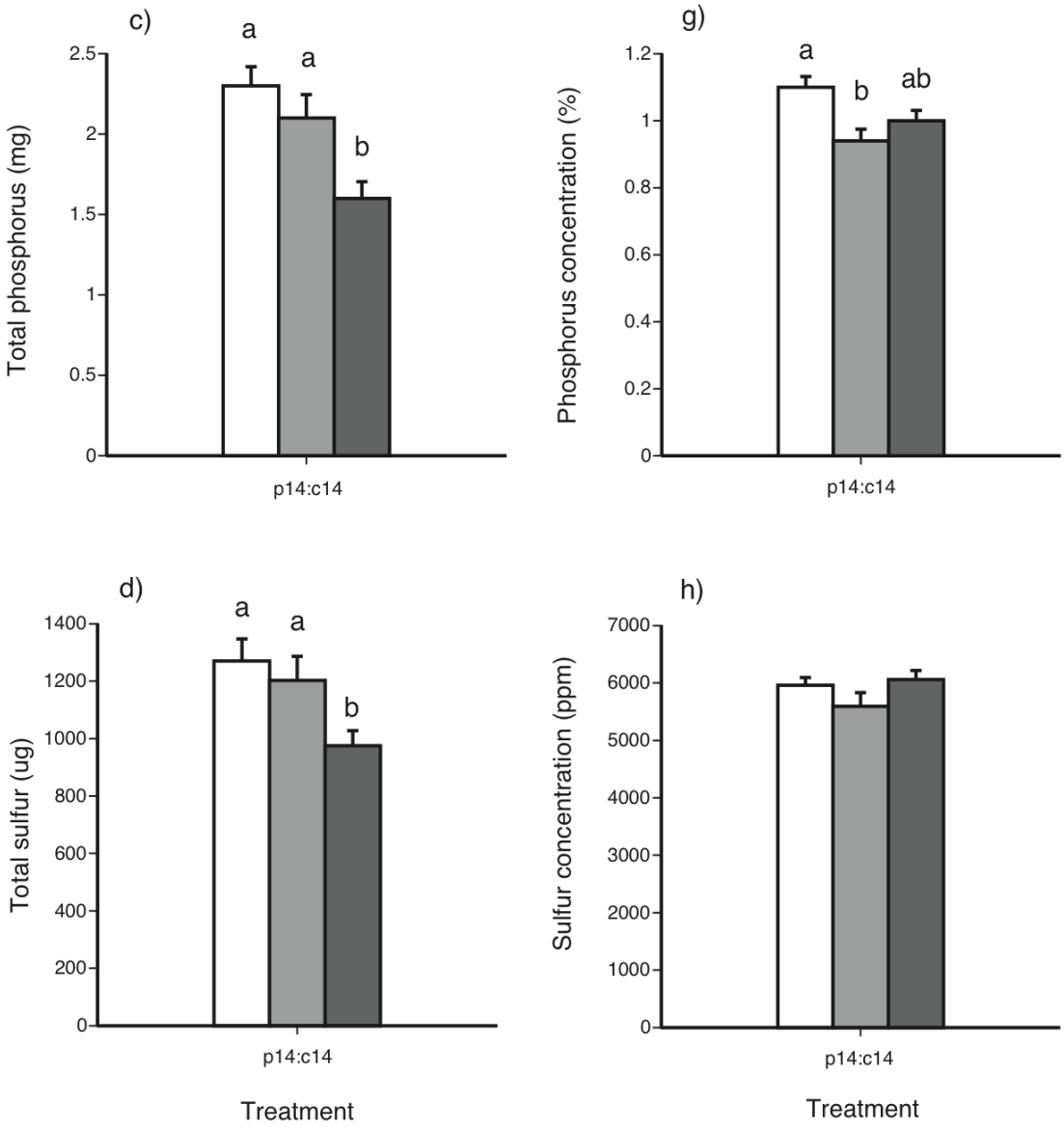


Figure 2.9 continued.

Table 2.4 ANCOVA test statistic (expressed as the F-ratio) for the total amount (mg or μg) and concentration (% or ppm) of each element in newly molted adult grasshoppers from the different treatments from the no-choice experiment. Where significant differences ($P < 0.05$) were detected they are noted in bold.

Element	expressed as	p14:c14
C	mg	**22.7**
	%	**17.9**
N	mg	**68.1**
	%	**58.8**
P	mg	**16.4**
	%	**3.64**
S	μg	**10.8**
	ppm	1.44
K	μg	**15.7**
	ppm	0.64
Na	μg	1.89
	ppm	**4.14**
Ca	μg	**4.92**
	ppm	2.44
Mg	μg	**12.4**
	ppm	0.17
Zn	μg	2.28
	ppm	0.57
Fe	μg	**11.3**
	ppm	3.18
Mn	μg	2.49
	ppm	3.35
Cu	μg	0.37
	ppm	**4.26**

The highest phosphorus concentration was observed in grasshoppers fed the casein plus albumin diets, intermediate on the wheat protein, and lowest on the soy protein. Equally high total amounts of sulfur were observed in grasshoppers fed the casein plus albumin and the soy protein, with the lowest amounts seen in individuals fed the wheat protein.

Figure 2.10 illustrates the electro-chemical elements. Only the total amounts of potassium, calcium, and magnesium were significantly affected by protein source, with sodium showed significant affects in concentration. The casein plus albumin and soy protein were equally high in regards to total potassium levels, and individuals on wheat protein diets had the lowest amounts. The concentration of sodium is highest in grasshoppers fed wheat diets, intermediate on the casein plus albumin diets, and lowest in grasshoppers fed soy protein. The total amount of calcium showed the highest levels on the casein plus albumin diets and equally low amounts on the soy and wheat protein. Lastly, the total amount of magnesium was equally high in the casein plus albumin and the soy protein; the lowest levels were seen in grasshoppers fed the wheat protein.

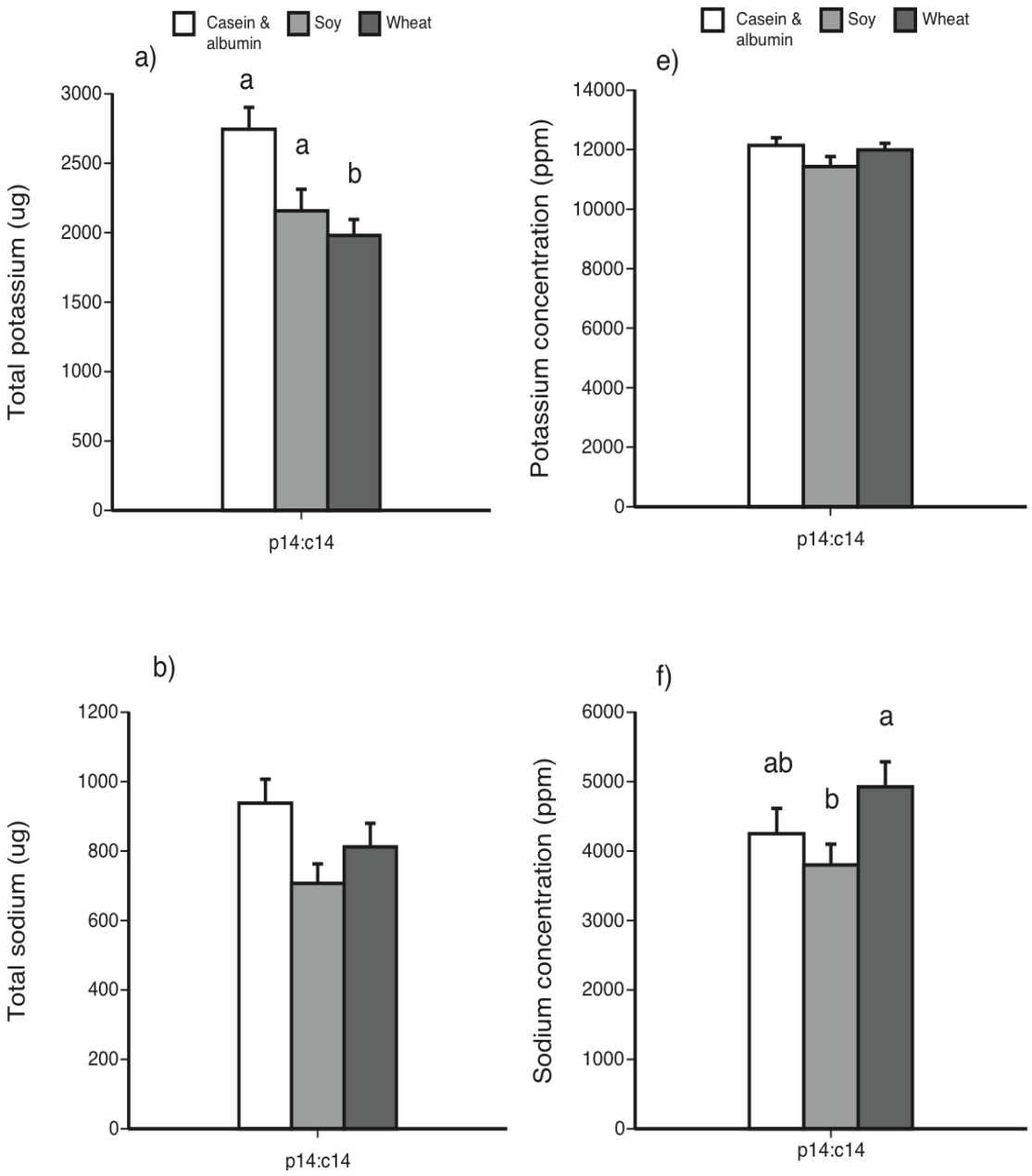


Figure 2.10. The left column contains total amounts (μg) of each electro-chemical element present at end of experiment 2 (LS means ± SE). The right column shows the concentration of the electro-chemical elements ppm) (LS means + SE).

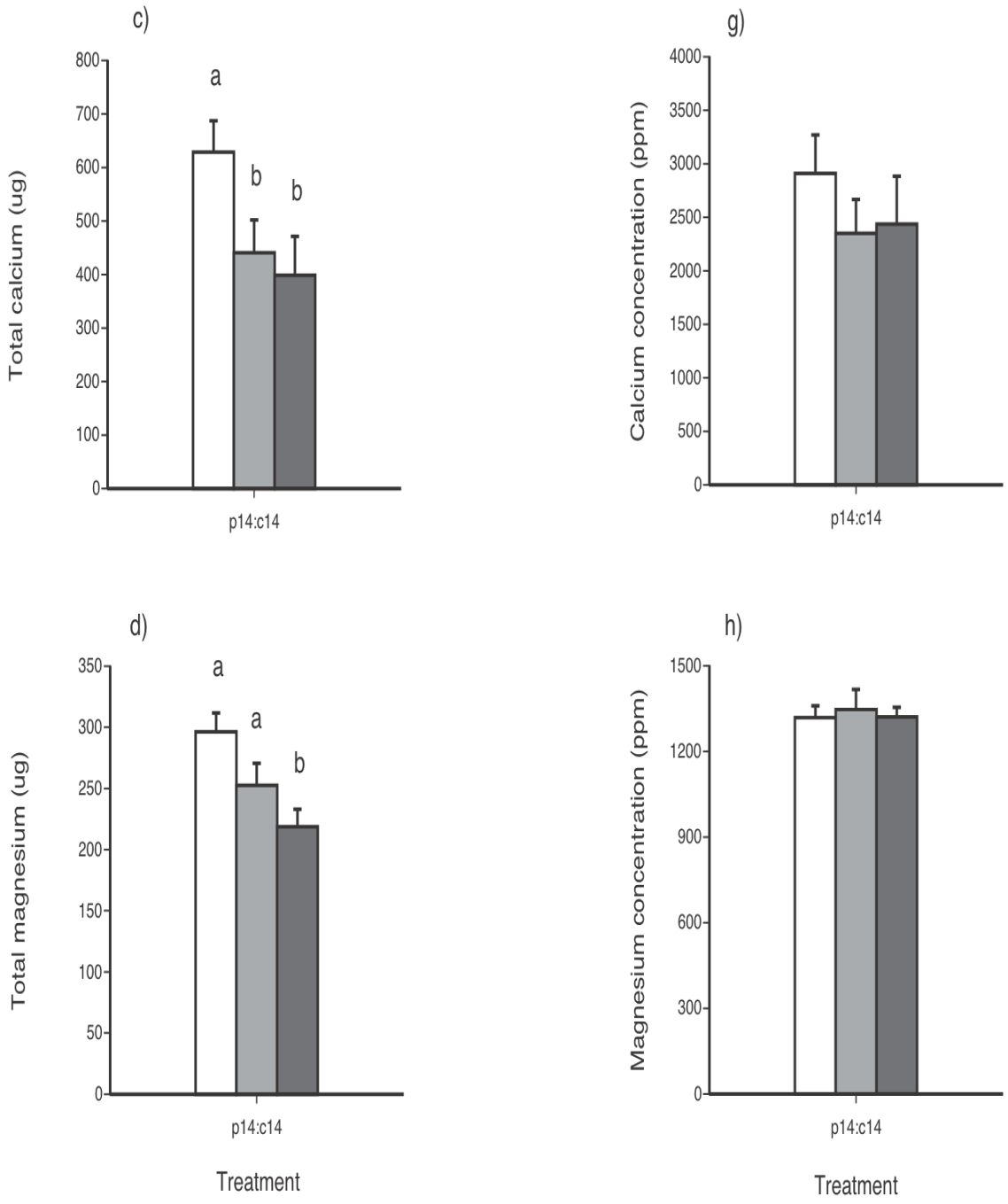


Figure 2.10 continued.

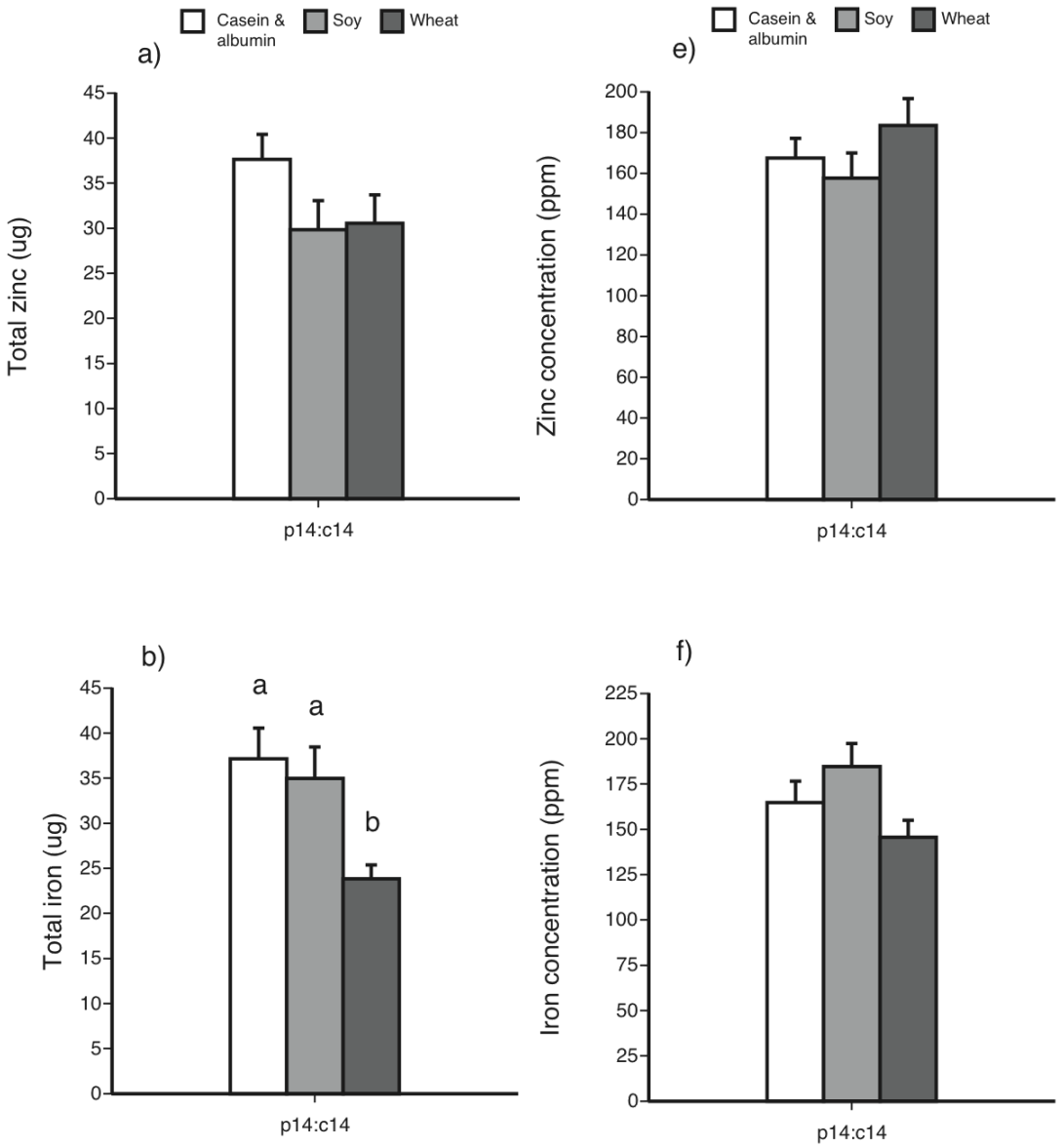


Figure 2.11 The left column contains total amounts (μg) of each catalytic element that was present at end of experiment 2 (LS means ±SE). The right column shows the concentration of the catalytic elements (ppm) (LS means ± SE).

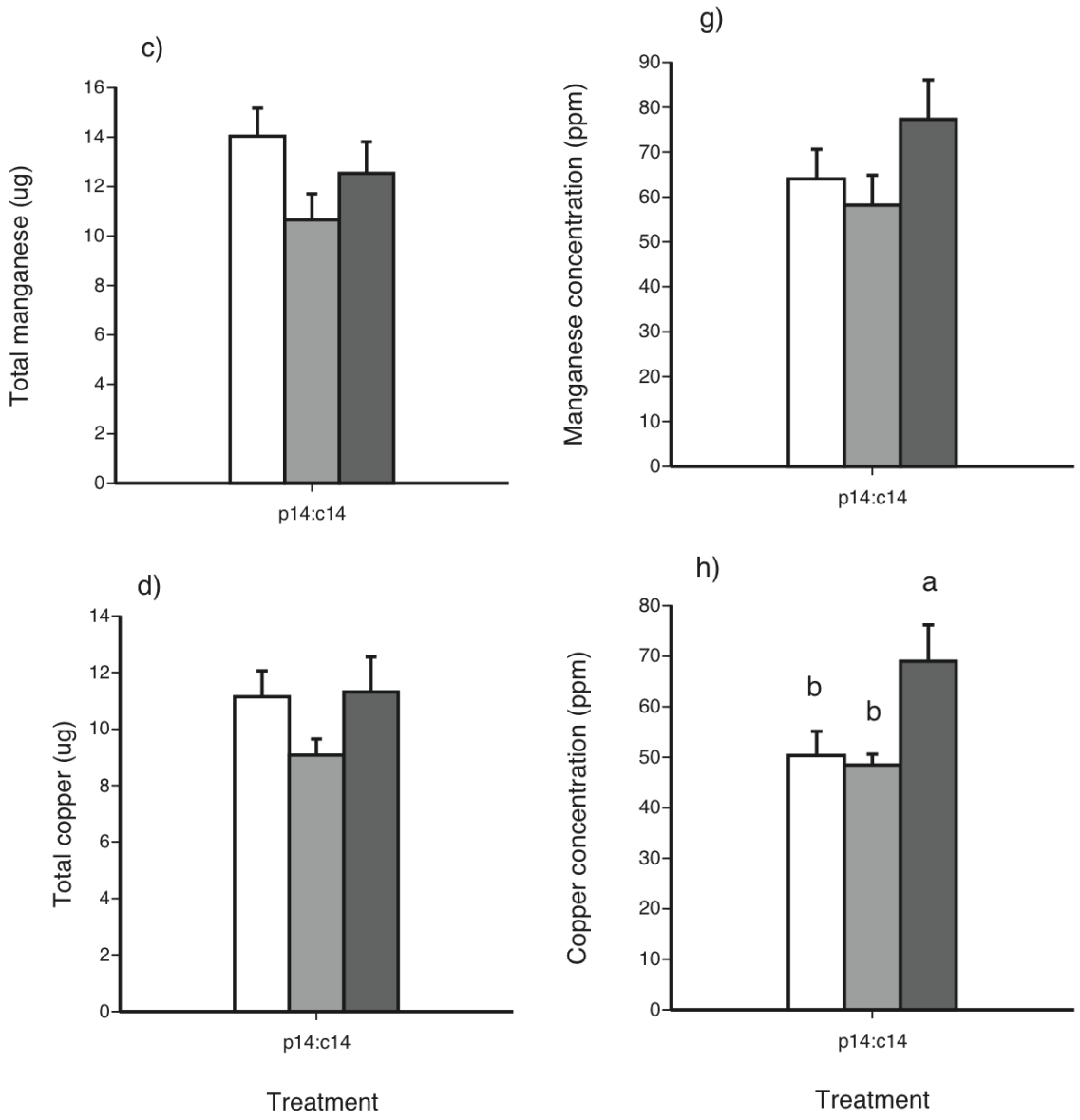


Figure 2.11 continued.

Figure 2.11 shows the catalytic elements, only the total amount of iron and the concentration of copper were the only catalytic elements to be significantly affected by the protein source. The total amounts of iron were equally high for grasshoppers fed the casein plus albumin and soy proteins, with significantly lower levels being observed for the individuals fed wheat protein. The highest concentration of copper was seen in grasshoppers fed wheat protein, and equally on both the casein plus albumin and the soy proteins.

DISCUSSION

Insect herbivores that feed on a variety of different plants encounter different types of protein, and this paper shows for the first time in grasshoppers that different amino acid profiles influence grasshopper performance and elemental profile. In our first experiment we explored the effect of protein type across three different protein-carbohydrate ratios: p7:c35, p21:c21, and p35:c7. The p21:c21 diet had the optimal protein-carbohydrate (p:c) ratio for *S. americana* nymphs (Chapter II), although the soy protein, compared to the control diet (casein and albumin) and the wheat protein diet, contained a significantly lower amount of total nitrogen (8% in the soy protein versus 14% in both the casein plus albumin protein and the wheat protein). This difference is important because nitrogen is a known growth-limiting nutrient for insects (Chapman 1998, Sterner & Elser 2002, and Fagan 2002). Interestingly the nitrogen content of the diet did not significantly affect total consumption, nor the protein-carbohydrate intake, but grasshoppers on the p21:c21 soy protein diets did ingest significantly less total nitrogen than did grasshoppers on the control and wheat protein equivalent diets. On the three p21:c21 diets it was clear that

protein quality, measured as amino acid profile, and not nitrogen quantity, had a major influence on performance. Grasshoppers on the control and soy protein p21:c21 diets had similar developmental rates and mass gain, and both of these measures of performance were superior compared to those on the p21:c21 wheat protein diet.

The key difference in amino acid profile, at least in terms of essential amino acids, between these three protein types appears to be the level tryptophan, which occurs at very low levels in soy protein, and is virtually non-existent in wheat protein (Figure 2.1). The low levels in soy protein do not appear to have a negative effect on grasshopper development on the p21:c21 diets. However, low levels of tryptophan in soy protein may have a greater effect when total protein content is low. Tryptophan is an essential amino acid that is needed for molting (Chapman 1998), and survival on the soy p7:c35 diet was very low, while development time and mass gain were greatly extended, and lower, respectively, compared to the control p7:c35 diet. The low levels of tryptophan in the wheat protein, plus low levels of other important essential amino acids (e.g. lysine), likely was the cause of complete mortality for grasshoppers reared on the wheat p7:c35 diet. Amino acid profiles have been shown to influence development in past insect studies as well (Broadway & Duffey 1988).

When grasshoppers were presented with the three high protein diets (p35:c7), consumption was equal on the control and wheat protein treatments, and significantly elevated on the soy protein treatment. Here, however, development was similar across the three diets, although mass gain was much higher on the soy diets compared to the control and wheat protein diets. In previous studies, using casein as the protein source in grasshopper diets, mass gain was optimal on diets with balanced p:c ratios, and dropped

off symmetrically on both sides of the optimal p:c ratio as the diets became more imbalanced (e.g. Chapter II). This pattern was not, however, observed on the diets with soy protein – instead mass gain tended to increase on the soy protein treatments as protein content (and nitrogen content) increased. Because grasshoppers on the p35:c7 diets ate similar total amounts of nitrogen (Figure 2.2b), this result suggests that increased intake of some individual amino acids may enhance growth.

In terms of body elemental levels and concentrations, two key patterns were observed. First, the two most easily influenced elements, carbon and nitrogen, showed tight regulation across all treatments and protein types even with the differences in the nitrogen concentration in the protein sources and the low amounts of some amino acids. The similar nitrogen content in the body of grasshoppers from the two imbalanced diets is not surprising because grasshoppers on these treatments ingested similar total amounts of nitrogen. However, similar nitrogen intake was not the case on the p21:c21 diets. This suggests that grasshoppers on the soy p21:c21 diets were highly efficient as using ingested nitrogen (Zanatto et al. 1993).

The second interesting trend was that only on the balanced diets, where compensatory responses with respect to nitrogen intake were not observed, were differences in absolute amounts of elements (P, K, Na, Mg, Zn, and Fe) and concentrations of elements (Na, Zn, Fe, and Cu) observed. Differences in absolute amounts may simply be a size effect (generally those grasshoppers on the casein plus albumin diet), but ultimately grasshoppers, regardless of diet protein type, showed strict homeostasis with respect to P, K, and Mg (Figures 2.4 & 2.5). With respect to K body concentrations, they may be higher on the casein plus albumin and soy protein diets than

on the wheat due to unique interactions with specific amino acids (Standifer 1967). There was, however, no clear pattern between protein type and elemental body concentrations of the metals Zn, Fe, and Cu, which suggests that food amino acid profile may influence metal absorption, incorporation and regulation in a complex manner.

Having explored the effects of different proteins and different p:c ratios, keeping total macronutrient content constant (at 42%), the second experiment was designed to examine how nutrient dilution of the different proteins influenced performance and organismal stoichiometry. In contrast to the p21:c21 diets, grasshoppers on the p14:c14 diets did not eat similar total amounts of food – grasshoppers on the soy protein ate more food compared control and wheat protein fed grasshoppers, a result that could be explained by the available nitrogen differences when soy is compared to casein plus albumin and wheat protein. Despite generally similar total amounts of nitrogen being ingested on the three p14:c14 treatments, development was significantly longest on the wheat protein, likely due to the lack of tryptophan. Decreased intake of tryptophan on the wheat treatment also likely explains why dry mass gain for grasshoppers fed the wheat protein shows was low compared to the other two p14:c14 treatments. In general, the effect of protein quality on grasshopper performance was consistent between the two balanced ratio treatments (p21:c21 and p14:c14), which suggests that for grasshopper in the field, not only is protein quantity important in terms of influencing fitness, but so too is the type of protein ingested.

In terms of stoichiometry, patterns with respect to both absolute body amounts and body concentrations of elements differed across the p14:c14 treatments. Most notably, and in contrast to the p21:c21 treatments, the two most easily influenced

elements carbon and nitrogen, did not show tight regulation even though the ratio or protein to carbohydrate stayed the same. The key response here was that nitrogen concentration decreased, and carbon concentration increased on the soy and wheat diets compared to the control diet. These results suggest that when key amino acids (e.g. tryptophan) become limiting, homeostatic processes with respect to key limiting nutrient elements can begin to breakdown. In terms of other elements not correlated with protein or digestible carbohydrate, total absolute amounts and concentrations of elements followed patterns similar to those observed for the p21:c21 diets.

Overall, we see that the protein quality has a significant effect on grasshopper growth and elemental composition. Further experiments should be conducted to determine at what levels amino acids need to be present to alter elemental content of organism. Studies should also investigate whether or not a natural food can be altered while growing and what effects would be seen in individuals that feed on these food sources.

CHAPTER IV

CONCLUSION

These experiments have provided insight into specific nutrient needs of *Schistocerca ameircana* and the effects nutritionally different foods have on the grasshopper's elemental composition. I utilized the "Geometric Framework" to explore how protein-carbohydrate ratio and total macronutrient content affect the ecological stoichiometry of an organism, and hypothesized that by creating a situation in the lab mimicking a natural environment, where grasshoppers have the ability to choose their food, I would see similar levels of growth, food consumption, and elemental levels. Secondly, I hypothesized that when a grasshopper is forced to ingest only one food, the total macronutrient content, ratio, and also the protein quality of that food would affect the growth, total food consumption, and the elemental composition of the insects. Analyses show that when insects are given a choice of foods, they do show statistically similar levels in all measured tests. While insects raised on diets whose macronutrient content and ratio was changed, but the protein quality was the same across all treatments, showed surprising strict homeostatic control over the majority of the elements tested. This creates interesting questions regarding the regulatory and post-ingestive physiological interactions that occur to a food after it is ingested by an insect. Insects that were fed diets varying in macronutrient ratio and content and which had the protein quality changed throughout the study showed that the protein quality is a significant factor when the elemental composition and the growth and consumption of individuals is investigated.

These experiments allowed me to use the GF and ES to complete studies which have no been done anywhere else until now. Working with grasshoppers allowed me to use insects that grow quickly, eat easily measurable amounts of food, and can be reared and used in the laboratory under a suite of conditions. This work focused on exploring the twelve elements of interest (C, N, P, S, K, Na, Ca, Mg, Zn, Fe, Mn, and Cu) and how macronutrient content and protein quality can affect the movement of these elements from a food source and their ultimate fate once ingested. Future research should focus on single or combinations of amino acid, differences in carbohydrate importance, and differences in carbon available to an insect (C in cellulose, lignin, carbohydrates, etc.). Individual element/minerals should also be manipulated in diets to study possible changes in physiological function in insects.

Future research should also use the entire life span of organisms and determine what possible elemental affects can occur over a life time. It would be interesting to see if adults are more or less fecund if feed certain proteins or combinations of nutrients. This would be important agriculturally if a plant could be genetically altered to deter insect feeding, while not harming its physiological integrity or food value. A number of other outstanding questions also remain. Results from my experiments demonstrated the ability to study nutrient regulation and elemental composition changes in a generalist insect herbivore when fed a variety of foods with different total macronutrient content levels, different protein-carbohydrate ratios, and when these foods were changed further by having different qualities of protein. This study provides a starting point for future research investigating the importance of elemental composition to other organisms, and regarding other food sources.

REFERENCES

Abbot, P., Grinath, J., Brown, J., Peeden, E., Erickson, D. & Billick, I. (2008) Insect herbivore stoichiometry: the relative importance of host plants and ant mutualists. *Ecological Entomology* **33**, 497-502.

Anderson, T.R., Boersma, M. & Raubenheimer, D. (2004) Stoichiometry: linking elements to biochemicals. *Ecology* **85**, 1193-1202.

Anderson, T.R., Hessen, D.O., Elser, J.J. & Urabe, J. (2005) Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. *American Naturalist* **165**, 1-15.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* **47**, 817-844.

Barker, D., Fitzpatrick, M.P. & Dierenfeld, E.S. (1998) Nutrient composition of selected whole invertebrates. *Zoo Biology* **17**, 123-134.

Behmer, S.T. (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology* **54**, 165-187.

Behmer, S.T. & Joern, A. (2008) Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 1977-1982.

Behmer, S.T., Cox, E., Raubenheimer, D. & Simpson, S.J. (2003) Food distance and its effect on nutrient balancing in a mobile insect herbivore. *Animal Behaviour* **66**, 665-675.

Behmer, S.T., Lloyd, C.M., Raubenheimer, D., Stewart-Clark, J., Knight, J., Leighton, R.S., Harper, F.A. & Smith, J.A.C. (2005) Metal hyperaccumulation in plants: mechanisms of defence against insect herbivores. *Functional Ecology* **19**, 55-66.

Behmer, S.T., Raubenheimer, D. & Simpson, S.J. (2001) Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Animal Behaviour* **61**, 995-1005.

Behmer, S.T., Simpson, S.J. & Raubenheimer, D. (2002) Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. *Ecology* **83**, 2489-2501.

Benrey, B. & Denno, R. F. (1997) The slow-growth–high-mortality hypotheses: a test using the cabbage butterfly. *Ecology* **78,** 987–999 (1997).

Bernays, E.A. (1998) The value of being a resource specialist: behavioral support for a neural hypothesis. *American Naturalist* **151**, 451-464.

Bernays, E.A. & Bright, K.L. (2001) Food choice causes interrupted feeding in the generalist grasshopper *Schistocerca americana*: further evidence for inefficient decision-making. *Journal of Insect Physiology* **47**, 63-71.

Bernays, E. A., Bright, K. L., Gonzalez, N., and Angel, J. E. (1994). Dietary mixing in grasshoppers: tests of two hypotheses. *Ecology* **75:** 1997–2006.

Bertram, S.M., Bowen, M., Kyle, M. & Schade, J.D. (2008) Extensive natural intraspecific variation in stoichiometric (C : N : P) composition in two terrestrial insect species. *Journal of Insect Science* **8**, 26.

Bertram, S.M., Schade, J.D. & Elser, J.J. (2006) Signalling and phosphorus: correlations between mate signalling effort and body elemental composition in crickets. *Animal Behaviour* **72**, 899-907.

Block, R. J., and H. H. Mitchell (1946) The correlation of the amino acid composition of proteins with their nutritive value. *Nutrition Abstr. Rev.*, **16**: 249.

Boersma, M. & Elser, J.J. (2006) Too much of a good thing: On stoichiometrically balanced diets and maximal growth. *Ecology* **87**, 1325-1330.

Boswell, A.W., Provin, T., & Behmer, S.T. (2008) The relationship between body mass and elemental composition in nymphs of the grasshopper *Schistocerca americana*. *Journal of Orthoptera Research* **17(2)**, 207-313.

Broadway, R.M. and S.S. Duffey (1988) Duffey, Plant proteinase inhibitors: the effect of plant protein quality on insect physiology and the toxicity of plant proteinase inhibitors, *Journal of Insect Physiology* **34,** 1111–1117.

Chapman, R. F. (1998). *The Insects Structure and Function*. English Universities Press: London.

Cohen, A.C. (2004) *Insect Diets: Science and Technology*. CRC Press, Boca Raton, FL.

Cross, W.F., Wallace, J.B. & Rosemond, A.D. (2007) Nutrient enrichment reduces constraints on material flows in a detritus-based food web. *Ecology* **88**, 2563-2575.

Dadd, R.H. (1961) The nutritional requirements of locusts: requirements for vitamins of the B-complex. *Journal of Insect Physiology* **6**, 1-12.

Damodaran, S., (1996) Amino acids, peptides, and proteins, *Food Chemistry*, 3rd ed., (ed.Fennema, O. R.) Marcel Dekker Inc., New York., pp. 322-425.

Danner, B., and A. Joern. 2003. Resource-mediated impact of spider predation risk on performance in the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae).*Oecologia* **137**, 352–359.

Elser, J. (2006) Biological stoichiometry: a chemical bridge between ecosystem ecology and evolutionary biology. *American Naturalist* **168**, S25-S35.

Elser, J.J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W., Schade, J., Hood, J. & Sterner, R.W. (2003) Growth rate-stoichiometry couplings in diverse biota. *Ecology Letters* **6**, 936-943.

Elser, J.J., Hayakawa, K. & Urabe, J. (2001) Nutrient limitation reduces food quality for zooplankton: daphnia response to seston phosphorus enrichment. *Ecology* **82**, 898-903.

Elser, J.J., Sterner, R.W., Galford, A.E., Chrzanowski, T.H., Findlay, D.L., Mills, K.H., Paterson, M.J., Stainton, M.P. & Schindler, D.W. (2000) Pelagic C : N : P stoichiometry in a eutrophied lake: responses to a whole-lake food-web manipulation. *Ecosystems* **3**, 293-307.

Erasmus, L.J., Botha, P.M., Cruywagen, C.W. & Meissner, H.H. (1994) Amino-acid profile and intestinal digestibility in dairy-cows of rumen-undegradable protein from various feedstuffs. *Journal of Dairy Science* **77**, 541-551.

Evans-White, M.A., Stelzer, R.S. & Lamberti, G.A. (2005) Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. *Freshwater Biology* **50**, 1786-1799.

Fagan, W.F. & Denno, R.F. (2004) Stoichiometry of actual vs. potential predator-prey interactions: insights into nitrogen limitation for arthropod predators. *Ecology Letters* **7**, 876-883.

Fagan, W.F., Siemann, E., Mitter, C., Denno, R.F., Huberty, A.F., Woods, H.A. & Elser, J.J. (2002) Nitrogen in insects: implications for trophic complexity and species diversification. *American Naturalist* **160**, 784-802.

Frausta da Silva, J. J. R., and R. J. P. Williams. 1991. The biological chemistry of the elements: the inorganic chemistry of life. Clarendon Press, Oxford, England.8:369–384.

Frost, P.C., Cherrier, C.T., Larson, J.H., Bridgham, S. & Lamberti, G.A. (2007) Effects of dissolved organic matter and ultraviolet radiation on the accrual, stoichiometry and algal taxonomy of stream periphyton. *Freshwater Biology* **52**, 319-330.

Frost, P.C., Evans-White, M.A., Finkel, Z.V., Jensen, T.C. & Matzek, V. (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos* **109**, 18-28.

Frost, P.C., Xenopoulos, M.A. & Larson, J.H. (2004) The stoichiometry of dissolved organic carbon, nitrogen, and phosphorus release by a planktonic grazer, Daphnia. *Limnology and Oceanography* **49**, 1802-1808.

Gillooly, J.F., Allen, A.P., Brown, J.H., Elser, J.J., Del Rio, C.M., Savage, V.M., West, G.B., Woodruff, W.H. & Woods, H.A. (2005) The metabolic basis of whole-organism RNA and phosphorus content. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 11923-11927.

Grover, C.D., Kay, A.D., Monson, J.A., Marsh, T.C. & Holway, D.A. (2007) Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. *Proceedings of the Royal Society B-Biological Sciences* **274**, 2951-2957.

Hagele, B.F. & Rowell-Rahier, M. (1999) Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution? *Oecologia* **119**, 521-533.

Haggstrom, H. & Larsson, S. (1995) Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. *Oecologia* **104**, 308-315.

Hahn, D.A. (2005) Larval nutrition affects lipid storage and growth, but not protein or carbohydrate storage in newly eclosed adults of the grasshopper Schistocerca americana. *Journal of Insect Physiology* **51**, 1210-1219.

Harvey, A. W. (1981) A reclassification of the *Schistocerca americana* complex (Orthoptera: Acrididae). Acrida **10**, 61-67.

Havlin, J.L. & Soltanpour, P.N. (1980) A nitric-acid plant-tissue digest method for use with inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* **11**, 969-980.

Hillebrand, H., Frost, P. & Liess, A. (2008) Ecological stoichiometry of indirect grazer effects on periphyton nutrient content. *Oecologia* **155**, 619-630.

Huberty, A.F. & Denno, R.F. (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* **85**, 1383-1398.

Huberty, A.F. & Denno, R.F. (2006) Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. *Oecologia* **149**, 444-455.

Jaenike, J. & Markow, T.A. (2003) Comparative elemental stoichiometry of ecologically diverse drosophila. *Functional Ecology* **17**, 115-120.

Joern, A. and Behmer, S. T. (1997) Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia **112**, 201-208.

Joern, A. & Behmer, S.T. (1998) Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. *Ecological Entomology* **23**, 174-184.

Karimi, R. & Folt, C.L. (2006) Beyond macronutrients: element variability and multielement stoichiometry in freshwater invertebrates. *Ecology Letters* **9**, 1273-1283.

Kay, A.D., Rostampour, S. & Sterner, R.W. (2006) Ant stoichiometry: elemental homeostasis in stage-structured colonies. *Functional Ecology* **20**, 1037-1044.

Kilby, B.A. & Neville, E. (1957) Amino-acid metabolism in locust tissues. *Journal of Experimental Biology* **34**, 276-289.

Knorr, I.B., Naumova, E.N., Trounova, V.A. & Zolotarev, K.V. (1998) Migration of chemical elements in food chains and ontogeny phases of meadow moth in the remote migration of butterflies. *Nuclear Instruments & Methods in Physics Research Section a-Accelerators Spectrometers Detectors and Associated Equipment* **405**, 550-552.

Kuitert, L. C. & Conkin, R. V., (1952) Biology of the American grasshopper in the southeastern United States. *Florida Entomology* **35**, 22-33.

Lasztity, B. (1984) Development and nutrient turnover during the vegetation eriod as affected by fertilization in sunflower .3. Nutrient ratios. *Novenytermeles* **33**, 565-571.

Lee, K.P. (2007) The interactive effects of protein quality and macronutrient imbalance on nutrient balancing in an insect herbivore. *Journal of Experimental Biology* **219**, 3236-3244.

Leland, H.V., Fend, S.V., Dudley, T.L. & Carter, J.L. (1989) Effects of copper on species composition of benthic Insects in a Sierra-Nevada, California, Stream. *Freshwater Biology* **21**, 163-179.

Levy, R. & Cromroy, H.L. (1973) Concentration of some major and trace-elements in 41 species of adult and immature insects determined by atomic-absorption spectroscopy. *Annals of the Entomological Society of America* **66**, 523-526.

Loveridge, J.P. (1973) Age and the changes in water and fat content of adult laboratory-reared *Locusta migratoria migratorioides* R. and F. *Rhod. J. Agric. Res.* **11**. 131–143.

Matsumura, M., Trafelet-Smith, G.M., Gratton, C., Finke, D.L., Fagan, W.F. & Denno, R.F. (2004) Does intraguild predation enhance predator performance? A stoichiometric perspective. *Ecology* **85**, 2601-2615.

Mcgeehan, S.L. & Naylor, D.V. (1988) Automated instrumental analysis of carbon and nitrogen in plant and soil samples. *Communications in Soil Science and Plant Analysis* **19**, 493-505.

Mulder, K. & Bowden, W.B. (2007) Organismal stoichiometry and the adaptive advantage of variable nutrient use and production efficiency in daphnia. *Ecological Modelling* **202**, 427-440.

Perkins, M.C., Woods, H.A., Harrison, J.F. & Elser, J.J. (2004) Dietary phosphorus affects the growth of larval *Manduca sexta*. *Archives of Insect Biochemistry and Physiology* **55**, 153-168.

Raubenheimer, D. & Simpson, S.J. (1990) The effects of simultaneous variation in protein, digestible carbohydrate and tannic-acid on the feeding-behavior of larval *Locusta migratoria* (L) and *Schistocerca gregaria* (Forskal) .1. Short-Term Studies. *Physiological Entomology* **15**, 219-233.

Raubenheimer, D. & Simpson, S.J. (1994) The analysis of nutrient budgets. *Functional Ecology* **8**, 783-791.

Raubenheimer, D. & Simpson, S.J. (1995) Constructing nutrient budgets. *Entomologia Experimentalis Et Applicata* **77**, 99-104.

Raubenheimer, D. & Simpson, S.J. (1996) Meeting nutrient requirements: The roles of power and efficiency. *Entomologia Experimentalis Et Applicata* **80**, 65-68.

Raubenheimer, D. & Simpson, S.J. (1997) Integrative models of nutrient balancing: application to insects and vertebrates. *Nutrition Research Reviews* **10**, 151-179.

Raubenheimer, D. & Simpson, S.J. (2003) Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology* **206**, 1669-1681.

Raubenheimer, D. & Simpson, S.J. (2004) Organismal stoichiometry: quantifying non-independence among food components. *Ecology* **85**, 1203-1216.

Rothlisberger, J.D., Baker, M.A. & Frost, P.C. (2008) Effects of periphyton stoichiometry on mayfly excretion rates and nutrient ratios. *Journal of the North American Benthological Society* **27**, 497-508.

Schade, J.D., Kyle, M., Hobbie, S.E., Fagan, W.F. & Elser, J.J. (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. *Ecology Letters* **6**, 96-10.

Schmitz, O.J. (2006) Scaling from plot experiments to landscapes: studying grasshoppers to inform forest ecosystem management. *Oecologia*, **145**, 225–234.

Schmitz, O.J. (2007) Predator diversity and trophic interactions. *Ecology*, **88**, 2415–2426.

Schmitz, O.J. (2008) Effects of predator hunting mode on grassland ecosystem function. *Science*, **319**, 952–954.

Schmitz, O.J., Kriva, V. & Ovadia, O. (2004) Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, **7**, 153–163.

Schoonhoven, L.M. (2005) Insect-plant relationships: the whole is more than the sum of its parts. *Entomologia Experimentalis Et Applicata* **115**, 5-6.

Scriber, J.M. & Slansky, F. (1981) The nutritional ecology of immature insects. *Annual Review of Entomology* **26**, 183-211.

Scriber, J.M. & Slansky, F. (1993) Nutritional Ecology and Evolutionary Entomology - a Citation-Classic Commentary on the Nutritional Ecology of Immature Insects by Scriber,J.M., and Slansky,F. *Current Contents/Agriculture Biology & Environmental Sciences* **8**, 8.

Simpson, S.J. & Raubenheimer, D. (1993) A multilevel analysis of feeding-behavior - the geometry of nutritional decisions. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **342**, 381-402.

Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T. & Raubenheimer, D. (2004) Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour* **68**, 1299-1311.

Standifer, L. N. (1967). A comparison of the protein quality of pollens for growth-stimulation of the hypopharyngeal glands and longevity of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Insectes Sociaux **14**:415–426.

Sterner, R.W. & Elser, J.J. (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere.

Studier, E.H. & Sevick, S.H. (1992) Live mass, water-content, nitrogen and mineral levels in some insects from South-central Lower Michigan. *Comparative Biochemistry and Physiology a-Physiology* **103**, 579-595.

Studier, E.H., Keeler, J.O. & Sevick, S.H. (1991) Nutrient composition of caterpillars, pupae, cocoons and adults of the eastern tent moth, *Malacosoma americanum* (Lepidoptera, Lasiocampidae). *Comparative Biochemistry and Physiology a-Physiology* **100**, 1041-1043.

Swaisgood, H.E., 1993. Review and update of casein chemistry. *J. Dairy Sci.* **76**, pp. 3054–3061

Thorne, A.D., Pexton, J.J., Dytham, C. & Mayhew, P.J. (2006) Small body size in an insect shifts development, prior to adult eclosion, towards early reproduction. *Proceedings of the Royal Society B-Biological Sciences* **273**, 1099-1103.

Vanderza.Es (1974) Development, significance, and application of artificial diets for insects. *Annual Review of Entomology* **19**, 139-160.

Van Loon, J.J.A., Casas, J. & Pincebourde, S. (2005) Nutritional ecology of insect-plant interactions: persistent handicaps and the need for innovative approaches. *Oikos* **108**, 194-201.

Waldbauer, G.P. & Friedman, S. (1991) Self-Selection of Optimal Diets by Insects. *Annual Review of Entomology* **36**, 43-63.

Watts, T., Woods, H.A., Hargand, S., Elser, J.J. & Markow, T.A. (2006) Biological stoichiometry of growth in *Drosophila melanogaster*. *Journal of Insect Physiology* **52**, 187-193.

Woods, H. A., Makino, W., Cotner, J. B., Hobbie, S. E., Harrison, J. F., Acharya, K. & Elser, J. J. 2003. Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology*, **17,** 237-245.

Zanotto, F. P., S. J. Simpson & D. Raubenheimer, 1993. The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary protein and carbohydrate. Physiological Entomology **18**: 425–434.

Zera, A.J., Mole, S. & Rokke, K. (1994) Lipid, carbohydrate and nitrogen-content of long-winged and short-winged *Gryllus firmus* - implications for the physiological cost of flight capability. *Journal of Insect Physiology* **40**, 1037-1044.

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