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Laura E Wright Heflin
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EFFECT OF DIETARY PROTEIN AND CARBOHYDRATE LEVELS ON
WEIGHT GAIN AND ORGAN PRODUCTION IN THE SEA URCHIN
LYTECHINUS VARIEGATUS

by

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A THESIS

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2010

EFFECT OF DIETARY PROTEIN AND CARBOHYDRATE LEVELS ON WEIGHT
GAIN AND ORGAN PRODUCTION IN THE SEA URCHIN *LYTECHINUS*
VARIEGATUS

LAURA E. WRIGHT

BIOLOGY

ABSTRACT

The development of sustainable sea urchin aquaculture techniques will be important for the sea urchin industry. To successfully culture urchins in captivity, an appropriate commercial diet must be developed. *Lytechinus variegatus* (20 g wet weight, 40 mm diameter) were fed formulated feeds with eight different protein (ranging from 12 to 36%) and carbohydrate (ranging from 21 to 39 %) levels, with protein:energy ratios ranging from 39 to 96 mg protein kcal⁻¹ and energy levels ranging from 2380 to 3749 calories gram⁻¹. For each sea urchin (n = 8 per treatment), a sub-satiation ration of 1.5% of the average body weight was fed daily for 9 weeks.

Total weight gain and dry matter production were directly related to dietary protein level and protein:energy ratio, but were not affected by carbohydrate at the levels provided. Maximal wet weight gain and dry matter production were observed at dietary protein levels above 12% and protein:energy ratios above 60-68 mg protein kcal⁻¹. Gonad wet weight gain and gonad dry matter production were closely correlated with dietary protein level and, to a lesser extent, protein:energy ratio, but not carbohydrate levels. Gonad wet weight gain and dry matter production were lowest in individuals fed diets with 12% protein, regardless of energy or carbohydrate level.

Relative growth of calcareous tissues (test and Aristotle's lantern) indicated test wet weight gain and test dry matter production varied directly with dietary protein level,

total dietary energy, and protein:energy ratio. However, wet weight gain and dry matter production of the Aristotle's lantern did not vary with nutritional composition of the diets. Trend analysis indicated that Aristotle's lantern index was inversely correlated with dietary protein level, protein:energy ratio, and dietary energy level, suggesting protein and/or energy are primary determinants of the lantern index under the conditions of this study. Differences in lantern index are attributed to variations in test weight to dietary nutrient levels. These data suggest that these organs respond differentially to changes in protein, carbohydrate, and energy at this life stage.

Keywords: protein, carbohydrate, nutrition, plasticity, *Lytechinus variegatus*, sea urchin

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CHAPTER 1

EFFECT OF DIETARY PROTEIN AND CARBOHYDRATE LEVELS ON WEIGHT GAIN AND ORGAN PRODUCTION IN THE SEA URCHIN *LYTECHINUS VARIEGATUS*

BACKGROUND

Demand for sea urchin roe is increasing worldwide, leading to overfishing of wild sea urchin populations. To avoid continued depletion of these populations, it is desirable to develop sustainable aquaculture of sea urchins. One important aspect of successful aquaculture is the development of an appropriate feed. A nutritionally-complete and cost effective feed will maximize production and efficiency while minimizing production cost and environmental impact. To develop a nutritionally-complete feed, daily dietary requirements for both macronutrients and micronutrients must be determined. Animals require macronutrients in the form of proteins, lipids, and carbohydrates. Proteins provide amino acids that are essential for growth, maintenance, and repair of tissues and proteins can also be used for energy. Dietary carbohydrates are a primary source of energy for sea urchins (Marsh and Watts, 2007).

In the wild sea urchins feed on a wide variety of plant and animal material, including, but not limited to, algae, sea grass, coral, and bivalves (De Ridder and Lawrence, 1982). They are opportunistic omnivores, and their consumption habits vary according to season, habitat, and availability (De Ridder and Lawrence, 1982; Lawrence

et al., 2007). Harvesting natural forages for use in sea urchin aquaculture is impractical because of seasonal variations in quality and availability, excessive costs of harvest, short shelf life, and adverse effects on the environment, (Lawrence et al., 2001; Lawrence and Lawrence, 2003). However, studying the nutritional content of plants and animals consumed by sea urchins in the wild can be helpful in the preparation of a formulated diet. Several studies have shown that individuals fed a formulated diet often grow larger (Cook et al., 1998; Fernandez and Bourdoursque, 1998; Robinson et al., 2002; Lawrence and Lawrence, 2003; Otero-Villanueva et al., 2004; Chang et al., 2005; Daggett et al., 2005; Taylor, 2006; Cook et al., 2007) and produce larger gonads than individuals fed a natural forage (Fernandez and Caltagirone, 1994; de Jong-Westman et al., 1995; Grosjean et al., 1996; Lawrence et al., 1997; Barker et al., 1998; Cook et al., 1998; McBride et al., 1999; Lawrence, 2001; Olave et al., 2001; Spirlet et al. 2001; Pearce et al., 2002a, 2002b; Robinson et al., 2002; Lawrence and Lawrence, 2003; Otero-Villanueva et al., 2004; Chang et al., 2005; Kennedy et al., 2005; Schlosser et al., 2005; Taylor, 2006; Woods et al., 2008). Senatra et al. (2005) reported contradictory results in the gonadal growth of *Heliocidaris erythrogramma*, but this may have been due to poor digestibility of nutrients or nutritional deficiencies in the formulated diets used (Lawrence et al., 2007).

Despite their potential to produce marketable gonads in a short time period, formulated feeds that are currently available have limited application for use in aquaculture facilities because roe harvested from individuals fed prepared feeds is often of lesser quality (taste, color, firmness, and texture) than that of individuals fed a natural diet (Barker et al., 1998; Grosjean et al., 1998; Watts et al., 1998; Lawrence et al., 2001; Pearce et al., 2002a, 2002b; Robinson et al., 2002; Lawrence and Lawrence, 2003;

McBride et al., 2004; Kennedy et al., 2005; Woods et al., 2008). These studies suggest that further research is needed before formulated sea urchin feeds can be used successfully for commercial roe production.

One of the most necessary and expensive components of a formulated feed is protein. Protein is a vital macronutrient and is required by all eukaryotic organisms, including sea urchins, to maintain proper physiological functions (Marsh and Watts, 2007). In sea urchins, protein most likely has a crucial role in many biological processes, including reproduction, early development, growth, and repair and maintenance of body tissues. Elevated dietary protein levels have been associated with lower feed intake (Frantzis and Gremare, 1992; Fernandez and Bourdouresque, 1998; McBride et al., 1998; Meidel and Scheibling, 1999; Agatsuma, 2000; Fernandez and Bourdouresque, 2000; Hammer et al., 2004; Daggett et al., 2005; Hammer et al., 2006), increased somatic growth (Fernandez, 1997; Cook et al. 1998; Fernandez and Bourdouresque, 1998; Fernandez and Pergent, 1998; Meidel and Scheibling, 1999; Agatsuma 2000; Akiyama, 2001; Hammer et al., 2004; Hammer et al., 2006; Taylor, 2006), and increased gonad growth (de Jong-Westman et al., 1995; Fernandez, 1997; Barker et al., 1998; Cook et al., 1998; Meidel and Scheibling, 1999; Schlosser et al., 2005; Pearce et al., 2002b; Hammer et al., 2004; Chang et al., 2005; Hammer et al., 2006; Marsh and Watts, 2007; Woods et al., 2008), but findings from several studies have also suggested that there is a maximal protein level, above which there is no further increase in the rate of somatic or gonadal growth (McBride et al., 1998; Kennedy et al., 2005; Senaratna et al., 2005; Hammer et al., 2006; Marsh and Watts, 2007).

Intake of excess dietary protein can be problematic. Metabolism of proteins is energetically costly, and protein yields less net energy in comparison to carbohydrates (Marsh and Watts, 2007). Previous studies have also shown that high protein levels in manufactured feeds impart a bitter flavor to roe (Pearce et al., 2002b; Woods et al., 2008), and it was suggested that different amino acid contents of protein sources affect flavor of sea urchin roe differently (Hoshikawa, 1998; Lawrence et al., 2001; Murata et al., 2001, 2002; Pearce et al. 2002a, 2002b; Robinson et al., 2002; Senaratna et al., 2005; Woods et al. 2008). Additionally, nitrogenous waste from protein is a pollutant in water systems (Basuyaux and Mathiau, 1998). Therefore, it is important that dietary protein requirements are satisfied but not exceeded when preparing a commercial sea urchin diet.

Exact dietary protein requirements for sea urchins have not been established, but previous studies have shown variations in dietary protein requirements among species and age classes. McBride et al. (1998) reported highest production in *Strongylocentrotus franciscanus* at protein levels of 40%. *Pseudocentrotus depressus* fed a purified feed had the most test growth at protein levels between 20-50% and the highest feed efficiency at protein levels between 20-40% (Akiyama et al., 2001). Hammer et al. (2006) reported optimal feed efficiency for adult *Lytechinus variegatus* at a protein level of 20% and recorded increased mortality with a 9% protein level. Likewise, juvenile *L. variegatus* have been shown to require a minimum of 21% protein for optimal growth and survivorship (Hammer et al., 2004). A study by Pearce et al. (2002b) found that both adult and juvenile *Strongylocentrotus droebachiensis* require around 20% dietary protein. Hammer et al. (2004) also showed that increased intake of a lower protein diet would

increase gonad growth in *L. variegatus*. Both the quantity and quality of proteins must still be evaluated in many sea urchin species.

In addition to a source of amino nitrogen, animals require energy to maintain the processes necessary for survival. In eukaryotes, the majority of energy is used for protein metabolism (Marsh and Watts, 2007), so it is reasonable to conclude that individuals must not only have adequate dietary protein but must also have the energy required for protein anabolism. While amino acids derived from dietary proteins can be catabolized for cellular energy, the process is inefficient (Marsh and Watts, 2007) and energetically costly. It has been suggested that sea urchins may not be able to obtain dietary energy from lipids due to the low oxygen content in their tissues. However, carbohydrates are easily processed by sea urchins and are a much more efficient energy source than protein in these species (Marsh and Watts, 2007). An important function of carbohydrates is to provide chemical energy for an organism (Wilson, 1979), and many animals, including sea urchins, apparently use carbohydrates as their primary energy source (Marsh and Watts, 2007).

Recent studies by Taylor (2006) and Hammer (2006) suggest that sea urchins may adjust feed intake to satisfy an energy requirement. Juvenile *L. variegatus* fed a lower energy feed consumed significantly more feed and, as a consequence, consumed significantly more protein than individuals receiving feed with an equivalent protein level but a higher energy level (Taylor, 2006). A similar trend was seen in adult *L. variegatus*. Hammer (2006) observed an inverse relationship between intake and energy level of feed regardless of decreases in protein intake. Otero-Villanueva et al. (2004) found that *Psammechinus miliaris* consumed significantly less of a high energy (18 kJ g^{-1}) salmon

feed than of a low energy algal diet (9 kJ g^{-1}). Lawrence et al. (in press) saw similar feed intake trends in *Strongylocentrotus intermedius* fed two isocaloric feeds with different levels of protein and carbohydrates. However, variations in protein intake were not observed to have a significant effect on growth or production (Lawrence et al., in press), suggesting that protein was in adequate supply in both diets. Decreased protein intake resulting from energy satiation led to decreased somatic growth in both adult (Hammer 2006) and juvenile (Taylor, 2006) *L. variegatus*; in adult sea urchins, decreased organ production was also observed (Hammer, 2006). Fernandez and Pergent (1998) reported similar results for somatic growth and gonad index in 20-25mm *Paracentrotus lividus* fed a low energy vegetable-based diet as compared to *P. lividus* fed animal-based and mixed diets with higher levels of dietary energy. These data suggest that individuals receiving a high energy feed may become prematurely satiated and may not consume adequate protein and/or other nutrients required for growth and maintenance. Field populations of *Tripneustes ventricosus* have been reported to compensate for low dietary protein levels by adjusting calorie:protein ratio through selective nutrient absorption (reviewed in Lawrence and Lane, 1982).

Conversely, individuals receiving adequate protein but low levels of dietary carbohydrates also exhibit decreased growth and production, suggesting that dietary protein will be used as an additional energy source when carbohydrate levels are limiting (Schlosser et al., 2005; Hammer et al., 2006). Decreased survival has also been observed in *S. droebachiensis* when dietary protein levels are inadequate to compensate for low carbohydrate levels (de Jong-Westman et al., 1995).

Sea urchins have a low respiration rate (Lawrence and Lane, 1982). They do not thermoregulate and are relatively sedentary, suggesting that their energy requirement should be low in comparison to other animals. Therefore, it would be reasonable to expect that a nutritionally-complete sea urchin feed would have a higher protein: energy ratio as compared to many other animals. Data collected by Taylor (2006) support this assumption; juvenile *L. variegatus* fed formulated diets containing 104-112 mg protein kcal⁻¹ experienced higher somatic growth than individuals receiving feed containing 82 mg protein kcal⁻¹. Hammer (2006) saw similar results in adult *L. variegatus* fed isocaloric feeds with varying protein: energy ratios. *Strongylocentrotus intermedius* fed isocaloric diets with varying protein: carbohydrate ratios had higher gonad organic matter production with a higher protein:energy ratio, but showed no difference in wet weight or in wet test, lantern gut, or gonad index (Lawrence et al., in press), suggesting that the lower protein:energy ratio diets were adequate for growth and production in this species.

To date, the previous studies examining the relationship between dietary protein and dietary energy requirements in sea urchins have not provided a conclusive understanding of this interaction. This information will be important in the formulation of a diet that will provide adequate protein for optimal growth and production, yet spare protein as an energy source. This study examines the effect of eight formulated diets with different dietary protein and carbohydrate levels fed to the omnivorous sea urchin *L. variegatus* for nine weeks. Weight gain and dry matter production were evaluated among dietary treatments. Trend analyses were examined in terms of percent protein, percent carbohydrate, total energy, protein:carbohydrate ratio, and protein:energy ratio to determine which of these factors is correlated with weight gain and production.

Hypothesis

We hypothesize that total weight and the weights of component organs will increase with increasing levels of dietary protein until a protein level maximizing weight gain was reached. We further hypothesize that total weight and the weights of component organs will increase with increasing dietary carbohydrate levels until a carbohydrate level maximizing weight gain is reached.

Summary and Justification

The development of sea urchin aquaculture facilities will help satisfy the increasing commercial demand for sea urchin roe while conserving natural populations. Large-scale commercial rearing of sea urchins in a controlled environment with a standardized diet will yield roe that is easily harvested and is consistent in quality, cost and availability. Additionally, the development of sea urchin aquaculture techniques will prevent overfishing, allowing natural populations to potentially recover. From an economic standpoint, a feed suitable for commercial use should produce optimal growth in the shortest time possible with least cost.

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CHAPTER 2

EFFECT OF DIETARY PROTEIN AND CARBOHYDRATE LEVELS ON WEIGHT GAIN AND GONAD PRODUCTION IN THE SEA URCHIN *LYTECHINUS VARIEGATUS*

INTRODUCTION

The development of sustainable sea urchin aquaculture techniques will be important for the sea urchin industry. To successfully culture urchins in captivity, an appropriate commercial diet must be developed. A commercial diet must be cost-effective and must satisfy the nutritional requirements of individuals. Formulated diets that have been tested for sea urchins have had limited application for use in sea urchin culture. They are often not cost effective, and roe harvested from individuals fed these diets is often of lesser quality (taste, color, firmness, and texture) than that of individuals fed a natural diet (Barker et al., 1998; Grosjean et al., 1998; Watts et al., 1998; Lawrence et al., 2001; Pearce et al., 2002a, 2002b; Robinson et al., 2002; Lawrence and Lawrence, 2003; McBride et al., 2004; Kennedy et al., 2005; Woods et al., 2008; Watts et al., 2010). These studies suggest that further research is needed before formulated sea urchin diets can be used successfully for commercial roe production.

Protein is one of the most necessary and costly nutrients of most aquatic animal diets. Adequate provision of dietary protein has been shown to lower feed intake (Frantzis and Gremare, 1992; Fernandez and Bourdoursque, 1998; McBride et al., 1998; Meidel and Scheibling, 1999; Agatsuma, 2000; Fernandez and Bourdoursque, 2000;

Hammer et al., 2004; Daggett et al., 2005; Hammer et al., 2006), increase growth (Fernandez, 1997; Cook et al. 1998; Fernandez and Bourdoursque, 1998; Fernandez and Pergent, 1998; Meidel and Scheibling, 1999; Agatsuma 2000; Akiyama, 2001; Hammer et al., 2004; Hammer et al., 2006a; Taylor, 2006), and increase roe production (de Jong-Westman et al., 1995a; Fernandez, 1997; Barker et al., 1998; Cook et al., 1998; Meidel and Scheibling, 1999; Schlosser et al., 2005; Pearce et al., 2002b; Hammer et al., 2004; Chang et al., 2005; Hammer et al., 2006a; Marsh and Watts, 2007; Woods et al., 2008). However, we hypothesize there is a maximal protein level above which there is no further increase in the rate of somatic or gonadal growth (McBride et al., 1998; Kennedy et al., 2005; Senaratna et al., 2005; Hammer et al., 2006a; Marsh and Watts, 2007).

Metabolism of proteins as an energy source is energetically inefficient (Marsh and Watts, 2007) and nitrogenous waste is a pollutant in water systems (Basuyaux and Mathieu, 1999). Furthermore, high protein levels (Pearce et al., 2002b; Woods et al., 2008) and possibly the presence of specific amino acids (Hoshikawa, 1998; Lawrence et al., 2001; Murata et al., 2001, 2002; Pearce et al. 2002a, 2002b; Robinson et al., 2002; Senaratna et al., 2005; Woods et al. 2008) have an adverse effect on the quality of sea urchin roe. Therefore, a formulated diet should provide individuals with adequate protein for maximal growth and production, but excess protein intake should be avoided. Exact dietary protein requirements for sea urchins have not yet established but, as with other animals, requirements have been shown to vary among species and age classes. Studies indicate that dietary protein requirements are probably between 20-40% for most species (McBride et al., 1998; Akiyama et al., 2001; Pearce et al., 2002b; Hammer et al., 2004; Hammer et al., 2006a).

In addition to a source of amino nitrogen, urchins require energy to maintain the processes necessary for survival. Carbohydrates are easily processed by sea urchins and are a much more efficient energy source than protein (Marsh and Watts, 2007). Numerous carbohydrases have been identified in the sea urchin gut (Lawrence et al., 2007). Sea urchins have varying levels of enzymes for hydrolyzing starch, glycogen, and algal polysaccharides (reviewed by Lawrence et al., 2007). Urchins are apparently unable to directly process cellulose; however, they have enzymes for the digestion of cellobiose (Lawrence et al., 2007). The variety of carbohydrases in the digestive tract suggests that sea urchins can utilize carbohydrates from a wide array of sources.

Although carbohydrates appear to be the primary energy source of sea urchins, they also have the ability to process and store phospholipids (Gibbs et al., 2009). Phospholipase has been reported in the sea urchin gut (Lawrence et al., 2007). A requirement for dietary phospholipids in sea urchins has not been determined. Data indicate that sea urchin growth is enhanced by low levels of dietary phospholipids (Gibbs et al., 2009); however, excess dietary phospholipids inhibit growth in juvenile *L. variegatus* (Gibbs et al., 2009).

Recent studies indicate that sea urchins may adjust feed intake to satisfy an energy requirement regardless of other nutrient levels (Otero-Villanueva et al., 2004; Hammer, 2006; Taylor, 2006; Lawrence et al., in press). In some cases, decreased protein intake resulting from energy satiation led to decreased somatic growth and organ production in adult sea urchins (Fernandez and Pergent, 1998; Hammer 2006) and decreased growth in juvenile sea urchins (Taylor, 2006). Consequently, feed intake must be measured to accurately determine dietary requirements for these nutrients. Others were

reported to compensate for an imbalance in calorie: protein ratio by selective nutrient absorption (reviewed in Lawrence and Lane, 1982). In cases where dietary carbohydrate levels are limiting, sea urchins may use dietary protein as an additional energy source, thus, decreasing growth and production (Schlosser et al., 2005; Hammer et al., 2006).

Sea urchins have a low respiration rate (Lawrence and Lane, 1982); they do not thermoregulate and are relatively sedentary, suggesting that their energy requirements are low in comparison to other animals. Therefore, a suitable sea urchin diet would most likely have a high protein: energy ratio. Taylor (2006) found that juvenile *L. variegatus* fed formulated diets containing 104-112 mg protein kcal⁻¹ experienced higher overall growth than individuals receiving feed containing 82 mg protein kcal⁻¹. Hammer (2006) saw similar results in adult *L. variegatus*; sea urchins fed diets with high protein: energy ratios had significantly higher weight gain than individuals fed diets with lower protein: energy ratios. Gonad production in these same individuals correlated directly with protein:energy ratios (Hammer, 2006). *Strongylocentrotus intermedius* fed isocaloric diets with varying protein:carbohydrate ratios had higher gonad organic matter production with a higher protein:energy ratio, but showed no difference in wet weight or in wet test, lantern gut, or gonad index (Lawrence et al., in press), suggesting that the lower protein:energy ratio diets were adequate for growth and production in this species.

To date, few studies have examined the relationship between dietary protein and dietary energy requirements in sea urchins, but it appears that understanding this relationship may be an important step in the formulation of a feed suitable for sustainable sea urchin aquaculture. The purpose of this study is to examine the effect of variations in dietary protein and carbohydrate level, protein:energy ratio, protein:carbohydrate ratio,

and total dietary energy level on organismal growth and roe production in the sea urchin *Lytechinus variegatus*.

MATERIALS AND METHODS

Collection and Initial Measurements

Adult *Lytechinus variegatus* (ca. 19.5 ± 2.01 g initial wet weight) were collected from St. Joseph's Bay (30°N, 85.5°W), FL and transported to Texas AgriLIFE Mariculture Research Laboratory in Port Aransas, Texas. Nineteen individuals were randomly selected for initial evaluation. Individuals were weighed (to the nearest mg) and dissected with a circular incision around the peristomial membrane on the oral surface. The gut (esophagus, stomach, and intestine combined), gonads, and Aristotle's lantern were removed. The gut was cleaned in seawater to remove remaining food pellets, and organs were blotted on a clean paper towel to remove excess water. Wet weights were recorded for gut, gonad, test, and Aristotle's lantern. Organs (test, Aristotle's lantern, gut, and gonad) were dried in a 60°C oven for 48 hours to constant weight, and dry weights were recorded. Mean dry organ and total dry weights (the sum of the organ dry weights) were calculated for the initial sub-sample and used as estimated initial dry organ and total dry weights for the remaining 64 urchins. Initial wet weights were recorded for remaining urchins, and these individuals were assigned at random to one of eight dietary treatments (n=8 per diet).

Culture Conditions

Experimental urchins were held in a semi-recirculating system with both mechanical and biological filtration and UV sterilization. The culture system (2400 L) was comprised of 72 interconnected 20 L fiberglass tanks containing water distributed from a central sump, 16 of which were used for this feed trial. Each tank held four cylindrical plastic mesh cages (12 cm dia., 30 cm height, and a 4 mm open mesh). Each plastic cage was inserted into a PVC coupling (11.5 cm I.D.) and elevated with PVC spacers to allow unimpeded water circulation throughout the cage. Each cage housed one individual.

Water volume in each tank was maintained by a central standpipe, and natural seawater was supplied to each mesh enclosure at a ca. rate of 25 L hr⁻¹ (water exchange rate of 3000% per day). Fresh seawater was passed through a stratified sand filter and a Diamond water filter (Diamond Water Conditioning, Horton, WI), and water in the entire culture system was exchanged in the system at a rate of 10% per day. Water quality parameters were determined by color metric analysis.

Feed and Feed Preparation

Eight semi-purified diets were formulated and produced using both purified and practical ingredients. Levels of dietary protein and carbohydrate (Table 1, Table 2) ranged from 12 to 36 % protein (using a purified protein source) and 21 to 39% carbohydrate (using a purified starch source). Total levels of protein and carbohydrate were adjusted with acid washed diatomaceous earth which is known to have no effect on sea urchins at these levels (unpublished data). All other nutrients remained constant

among treatments. The proximate components are shown in Table 2. Dry ingredients were mixed with a PK twin shell® blender (Patterson-Kelley Co., East Stroudsburg, PA) for 10 minutes. Dry ingredients were then transferred to a Hobart stand mixer (Model A-200, Hobart Corporation, Troy, OH) and blended for 40 minutes. Liquid ingredients were added, and the mixture was blended for an additional 10 minutes to a mash-like consistency. The diets were extruded using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Feed strands were separated and dried on wire trays in a forced air oven (35°C) for 48 hours. Final moisture content of all feed treatments was 8–10%. Feed was stored in air-tight storage bags at 4°C until used.

Table 1. Calculated protein and carbohydrate levels (as fed), total energy, protein: energy, and protein: carbohydrate ratios in each of the eight diets tested.

Protein (%)	Carbohydrate (%)	Total Energy (cal/g)	Protein:Energy (mg P/kcal)	Protein:Carbohydrate Caloric Ratio
36	21	3749	95	1.7
28	30	3299	76	0.93
19	21	2783	68	0.90
19	30	3130	60	0.63
19	39	3478	54	0.49
12	21	2380	50	0.57
12	30	2727	44	0.40
12	39	3075	39	0.31

Table 2. Proximate composition of the formulation^a used to produce diets varying in protein and carbohydrate levels.

	12% P: 21% C	12% P: 30% C	12% P: 39% C	19% P: 21% C	19% P: 30% C	19%P: 39% C	28% P: 30% C	36% P: 21% C
Crude protein (%)	12	12	12	19	19	19	28	36
Carbohydrate (%)	21	30	39	21	30	39	30	21
Fiber (%)	4.5	4.5	4.5	4.5	4.5	4.5	4.6	4.6
Diatomaceous Earth (DE, %)	27	18	9	19	10	1	0	0
Non-DE Ash (%)	24	24	24	24	24	24	24	25
Crude fat (%)	7	7	7	7	7	7	7	7

All values are approximate, calculated, and on an “as fed” basis unless otherwise indicated.

a All diets contain up to 28% marine ingredients, 28.7% plant ingredients, 1.1% carotenoids, 0.7% vitamin premix, 24 % mineral mix, 7.2% binder and antifungal-antioxidant.

Feeding Rate

Each sea urchin was proffered a limiting daily ration equal to 1.5% (sub-satiation) of the initial average wet body weight. Approximately 1.5% of wet body weight per day is a sub-satiation ration for adult *Lytechinus variegatus* of this size class, whereas a ration equivalent to or above 3% of body weight is *ad libitum* (unpublished data). Feeding at sub-satiation ensured that urchins consumed all of their food in a 24 hour period and allowed for direct measure of feed intake. A sub-satiation feeding regime also prevented individuals from compensating for a dietary deficiency by increasing consumption. Individuals were weighed every three weeks and feed rations were adjusted to be equivalent to 1.5% of the average body weight (Table 3). Feed intake of the presented diet was confirmed by direct observation. Feces were siphoned out just prior to feeding each day.

Daily feeding rate was calculated as:

$$(1) \text{Average wet weight of individuals (g)} \times 0.015$$

Protein:energy ratio of each feed was calculated as:

$$(2) \text{ Protein (mg) / energy content (kcal)}$$

Total energy content of each feed (per g) was calculated based on the methods of Phillips (1972):

$$(3) \% \text{ protein} / 100 \times 5650 (\text{cal g}^{-1}) + \% \text{ carbohydrate} / 100 \times 4000 (\text{cal g}^{-1}) + \% \text{ lipid} / 100 \times 9450 (\text{cal g}^{-1})$$

Final Dissection

After nine weeks, urchins were photographed and dissected by making a circular incision around the peristomial membrane. The gut (esophagus, stomach and intestine) and gonad were separated, cleaned, and blotted on paper towels. Each organ was placed in a separate pre-weighed aluminum weigh pan, and wet weight of each organ was recorded. Tissues were dried in a 60°C oven for 48 hours to constant weight, and dry weights were recorded.

Weight Gain and Production

Individuals were weighed every three weeks. Wet weight gain over the 9-week period was calculated as:

$$(4) \text{ Final wet weight (g) - initial wet weight (g)}$$

Estimated total dry matter production was calculated as:

$$(5) \text{ Final dry weight (g) - average initial dry weight (g)}$$

Estimated protein efficiency ratio (PER) for each individual was calculated as:

$$(6) \text{ Dry matter produced (g) / dry weight protein consumed (g)}$$

Production efficiency for each individual was calculated as:

$$(7) [Final \text{ dry weight (g)} - initial \text{ dry weight (g)} / dry \text{ feed intake (g)}] \times 100$$

Estimated organ (gut and gonad) dry matter production for each individual was calculated as:

$$(9) Final \text{ dry weight of organ (g)} - initial \text{ average dry weight of organ (g)}$$

Final dry organ gut and gonad index was calculated for each individual as:

$$(10) Final \text{ dry weight of organ (g)} / final \text{ dry weight of individual (g)} \times 100$$

Feed Conversion Ratio (FCR) for each individual was calculated as:

$$(11) Total \text{ feed consumed (g, as fed)} / wet \text{ weight gain (g)}$$

Statistics

Statistical analyses were performed to compare total wet weight gain, dry matter production, organ wet weight, and organ production among diets. All statistical comparisons were performed using SAS System 9.2. A *p* value of <0.05 was considered statistically significant. Data were tested for normality with a Kolmogorov-Smirnov test and for equality of variance using a Levene's test. Data were found to be normal, so data were analyzed with ANOVA using the GLM procedure. If a significant difference (*p*<0.05) was detected, a Duncan's Multiple Range Test was used to compare means.

Data were evaluated by trend analysis in terms of five factors: Total energy content of treatment (cal g^{-1}), protein:energy ratio (mg P kcal^{-1}), protein:carbohydrate ratio (mg P mg C^{-1}), % protein, and % carbohydrate. Associations were modeled with 2nd degree polynomial functions. R^2 values (from 2nd degree polynomials) were determined to show the correlation between each factor and the parameter being tested.

RESULTS

Water Quality

Water conditions were maintained as follows: 32 ± 0.5 ppt salinity, $22 \pm 2^\circ\text{C}$, D.O. 7 ± 2 ppm., ammonia 0 ppm, nitrite 0 ppm, nitrate 0 ppm, and pH 8.2. A 12:12 light:dark photoperiod was maintained.

Feed Intake

All individuals were fed a limiting ration (1.5% of body weight). As a result, feed intake did not vary among treatments.

Weight Gain and Production

Wet weights did not differ significantly among treatments at time 0. Urchins in all dietary treatments grew over the course of the 9-week study. Total wet weight gain was most strongly correlated to protein:energy ratio ($R^2=0.8372$, Fig. 1a). Diets with the highest ($\geq 68.6 \text{ mg P kcal}^{-1}$) protein:energy ratios supported the most wet weight gain at the end of 9 weeks (Table 3). The lowest wet weight gain was seen in individuals fed

diets with low (≤ 54.9 mg p kcal⁻¹) protein:energy ratios (Table 3). Dietary protein level correlated positively with wet weight gain ($R^2=0.8030$, Fig. 1b) over the range used in this study, but protein:energy ratio showed the highest rate of correlation ($R^2=0.8372$, Fig. 1a). There was minimal correlation between dietary carbohydrate level and wet weight gain ($R^2=0.2121$, Fig. 1c) or total energy and wet weight gain ($R^2=0.4137$, Fig. 1e). Protein:carbohydrate ratio was a better predictor of total wet weight gain than either dietary carbohydrate level or total energy ($R^2=0.8210$, Fig. 1d). However, the association between wet weight gain and protein:carbohydrate ratio was less than that of protein:energy ratio.

Diets with protein:energy ratios between 40-55 mg P kcal⁻¹ showed uniformly low production (Fig. 2a). Production was somewhat higher among diets with protein:energy ratios between 60-70 mg P kcal⁻¹, but the highest protein:energy ratios (≥ 68.56 mg P kcal⁻¹) supported the most dry matter production at the end of 9 weeks (Fig. 2a). Dietary protein level was a good predictor of dry matter production ($R^2=0.8746$, Fig. 2b). Total dry matter production did not differ significantly among urchins receiving the 12% protein diets and the 19:39% protein:carbohydrate diet after 9 weeks (Table 3), but urchins receiving the 12:21% protein:carbohydrate diet had significantly lower dry matter production than urchins receiving the 19:21, 28:30, and 36:21% protein:carbohydrate diets ($p<0.001$, Table 3). After 9 weeks, dry matter production of urchins receiving the 19:21% protein:carbohydrate diet was not significantly different than urchins receiving the 12:39, 19:39, 28:30 or the 36:21% protein:carbohydrate diets, but was significantly higher than that of urchins receiving both the 12:21% protein:carbohydrate and the 12:30% protein:carbohydrate diets ($p<0.001$, Table 3). Dietary carbohydrate level by

itself was not a good predictor of dry matter production ($R^2=0.1194$, Fig. 2c). Both protein:carbohydrate ratio ($R^2=0.6979$) and total energy ($R^2=0.6182$) were weakly correlated with dry matter production (Fig. 2d, 2e).

Gut Analysis

There were significant differences in wet gut weight gain among dietary treatments ($p=0.019$, Table 4). However, none of the dietary factors that were examined (protein level, carbohydrate level, protein:energy ratio, protein:carbohydrate ratio, or total energy) were an accurate predictor of wet gut weight gain (Fig. 3) or gut dry matter production (Fig. 4). Gut dry matter production was significantly higher in urchins receiving the 36:21% protein:carbohydrate diet than in all other diets except the 19:21% protein:carbohydrate diets ($p=0.001$, Table 4). There was no difference in gut dry matter production among the 12% protein diets, the 19:30% protein:carbohydrate diet or the 28:30% protein:carbohydrate diet (Table 4).

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Gonad Analysis

Gonad wet weight gain was directly related to dietary protein levels ($R^2 = 0.9760$, Fig. 5b) and, to a lesser extent, protein:energy ratio ($R^2 = 0.8540$, Fig. 5a). Gonad wet weight gain did not vary among urchins fed the 36:21% protein:carbohydrate, the 28:30% protein:carbohydrate, and the 19% protein diets (Table 5); however, gonad wet weight gain was significantly lower in urchins fed the 12% protein diets ($p<0.001$, Fig. 5b). Gonad wet weight gain was not correlated to dietary carbohydrate level ($R^2 = 0.0653$, Fig. 5c). Protein:carbohydrate ratio ($R^2=0.6829$, Fig. 5d) and total energy ($R^2=0.6617$, Figure 5e) were moderate predictors of gonad wet weight gain.

Gonad dry matter production was significantly higher in urchins receiving the 36:21% protein:carbohydrate and the 28:30% protein:carbohydrate diets than in the 12:21 or 12:30% protein diets after 9 weeks ($p=0.0002$, Table 5). Gonad dry matter production varied directly with dietary protein level ($R^2=0.8354$, Fig. 6b) and was moderately correlated with protein:energy ratio ($R^2 = 0.5797$, Fig 6a), protein:carbohydrate ratio ($R^2 = 0.4526$, Fig. 6d), and total energy ($R^2 = 0.6958$, Fig. 6e). Gonad dry matter production did not vary with carbohydrate level ($R^2 = 0.0831$).

Production Efficiency

Production Efficiency (PE) did not vary among urchins fed the 12% diets (Table 6); however, PE of individuals fed the 12:21% and 12:30% protein:carbohydrate diets was significantly lower than individuals fed the 19:21%, 28%, and 36% protein diets ($p=0.0237$, Table 6). At the levels used in this study, PE was correlated with both dietary protein levels ($R^2=0.8314$, Fig.8b) and protein:energy ratio ($R^2=0.6468$, Fig. 8a) but not by dietary carbohydrate level ($R^2=0.0888$, Fig 8c).

Protein Efficiency Ratio

Protein efficiency ratio (PER) did not vary significantly among urchins fed the 12% protein diets (Table 6). PER among urchins fed the 19% protein diets did not vary from each other but were significantly lower than that of individuals fed the 12% protein diets ($p<0.0001$, Table 6). PER was significantly lower in urchins fed the 36:21% protein:carbohydrate diet than urchins in all other dietary treatments except the 28:30% protein:carbohydrate diet ($p<0.0001$, Table 6).

Feed Conversion Ratio

Feed conversion ratio (FCR) did not vary significantly among the 12% protein diets and the 19:30 and 19:39% protein:carbohydrate diets (Table 6). FCR was significantly lower in urchins fed the 19:21, 28:30, and 36:21% protein:carbohydrate diets than in the 12:21% dietary treatment ($p=0.0169$, Table 6). Trend analysis showed a correlation between feed conversion ratio and dietary protein:energy ratio ($R^2 = 0.6453$, Fig. 7a). At the levels used in this study, dietary protein correlated directly with feed conversion ratio ($R^2 = 0.7229$, Fig. 7b). Dietary carbohydrate (at the range used in this study) was not correlated to dietary feed conversion ratio ($R^2 = 0.1058$, Fig. 7b).

Table 3: Mean total wet weight gain and dry matter production of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein:energy ratios (P:E), total energy (TE), and protein:carbohydrate ratios (P:C). P:E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P:C represents protein:carbohydrate ratio (mg/mg). Letters indicate statistical differences among diets ($P < 0.05$).

% Protein	% Carbohydrate	P:E (mg P/kcal)	TE (cal/g)	P:C	Final Wet Weight (g)	Wet Weight Gain (g)		Final Dry Weight (g)	Dry Matter Production (g)	
12	21	50.79	2380	0.57	42.02 +/- 2.37	22.74 +/- 2.30	D	9.18 +/- 0.35	4.98 +/- 0.35	D
12	30	44.32	2728	0.40	42.69 +/- 1.54	23.59 +/- 1.09	CD	9.79 +/- 0.37	5.59 +/- 0.37	CD
12	39	39.31	3075	0.31	44.51 +/- 2.02	24.68 +/- 1.75	CD	10.04 +/- 0.39	5.84 +/- 0.39	BCD
19	21	68.56	2783	0.90	51.53 +/- 1.53	32.43 +/- 1.31	AB	11.04 +/- 0.36	6.84 +/- 0.36	AB
19	30	60.95	3131	0.63	47.74 +/- 1.77	28.30 +/- 1.44	BC	10.92 +/- 0.49	6.72 +/- 0.49	CD
19	39	54.85	3478	0.49	43.86 +/- 2.47	24.53 +/- 2.06	CD	10.10 +/- 0.44	5.89 +/- 0.44	BCD
28	30	76.88	3647	0.93	53.11 +/- 1.65	33.68 +/- 1.13	A	12.09 +/- 0.29	7.89 +/- 0.29	A
36	21	95.57	3749	1.71	55.04 +/- 1.39	34.79 +/- 1.39	A	12.20 +/- 0.30	8.00 +/- 0.30	A

Initial average wet weight was 19.28+/-2.37 g.

Initial average dry weight was 4.20+/-0.08 g.

Table 4: Mean final gut wet weight gain, dry gut index, and gut dry matter production of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein:energy ratios (P:E), total energy (TE), and protein:carbohydrate ratios (P:C).

P:E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P:C represents protein:carbohydrate ratio (mg/mg). Letters indicate statistical differences among diets ($P < 0.05$).

% Protein	% Carbohydrate	P:E (mg P /kcal)	TE (cal/g)	P:C	Final Wet Gut Weight (g)	Wet Gut Weight Gain (g)		Dry Gut Index (%)	Final Dry Gut Weight (g)	Dry Gut Production (g)	
12	21	50.8	2380	0.57	1.08 +/- 0.05	0.78 +/- 0.05	AB	2.99 +/- 0.00	0.27 +/- 0.01	0.23 +/- 0.01	BC
12	30	44.3	2728	0.40	1.08 +/- 0.06	0.78 +/- 0.06	AB	2.74 +/- 0.00	0.27 +/- 0.01	0.23 +/- 0.01	CD
12	39	39.3	3075	0.31	1.09 +/- 0.03	0.79 +/- 0.03	AB	2.73 +/- 0.00	0.27 +/- 0.01	0.23 +/- 0.01	CD
19	21	68.6	2783	0.90	1.26 +/- 0.09	0.96 +/- 0.09	A	2.90 +/- 0.00	0.32 +/- 0.02	0.28 +/- 0.02	AB
19	30	60.9	3131	0.63	1.16 +/- 0.05	0.86 +/- 0.05	A	2.61 +/- 0.00	0.28 +/- 0.01	0.24 +/- 0.01	BC
19	39	54.9	3478	0.49	0.96 +/- 0.05	0.66 +/- 0.05	B	2.33 +/- 0.00	0.23 +/- 0.01	0.19 +/- 0.01	D
28	30	76.9	3647	0.93	1.17 +/- 0.07	0.87 +/- 0.07	A	2.35 +/- 0.00	0.28 +/- 0.02	0.24 +/- 0.02	BC
36	21	95.6	3749	1.71	1.27 +/- 0.06	0.97 +/- 0.06	A	2.70 +/- 0.00	0.33 +/- 0.01	0.29 +/- 0.01	A

Initial average wet gut weight was 0.30+/-0.01

Initial average dry gut weight was 0.04+/-0.01

Table 5: Mean final gonad wet weight gain, dry gonad index, and gonad dry matter production of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein:energy ratios (P:E), total energy (TE), and protein:carbohydrate ratios (P:C). P:E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P:C represents protein:carbohydrate ratio (mg/mg). Letters indicate statistical differences among diets ($P < 0.05$).

% Protein	% Carbohydrate	P:E (mg P/kcal)	TE (cal/g)	P:C	Final Wet Gonad Weight (g)	Wet Gonad Weight Gain (g)		Dry Gonad Index (%)	Final Dry Gonad Weight (g)	Dry Gonad Production (g)	
12	21	50.8	2380	0.57	4.07 +/- 0.15	3.65 +/- 0.15	B	11.88 +/- 0.01	1.08 +/- 0.04	0.99 +/- 0.04	D
12	30	44.3	2728	0.40	4.73 +/- 0.45	4.30 +/- 0.45	B	13.95 +/- 0.01	1.39 +/- 0.14	1.30 +/- 0.14	CD
12	39	39.3	3075	0.31	4.62 +/- 0.20	4.19 +/- 0.20	B	14.88 +/- 0.01	1.49 +/- 0.08	1.40 +/- 0.08	BCD
19	21	68.6	2783	0.90	6.63 +/- 0.69	6.20 +/- 0.69	A	15.99 +/- 0.01	1.77 +/- 0.17	1.69 +/- 0.17	ABC
19	30	60.9	3131	0.63	6.47 +/- 0.61	6.05 +/- 0.61	A	16.29 +/- 0.01	1.79 +/- 0.16	1.70 +/- 0.16	ABC
19	39	54.9	3478	0.49	6.25 +/- 0.75	5.83 +/- 0.75	A	15.93 +/- 0.01	1.63 +/- 0.22	1.54 +/- 0.22	BC
28	30	76.9	3647	0.93	7.47 +/- 0.65	7.05 +/- 0.65	A	17.41 +/- 0.02	2.08 +/- 0.20	1.99 +/- 0.20	A
36	21	95.6	3749	1.71	7.32 +/- 0.44	6.89 +/- 0.44	A	15.61 +/- 0.01	1.91 +/- 0.08	1.82 +/- 0.08	AB

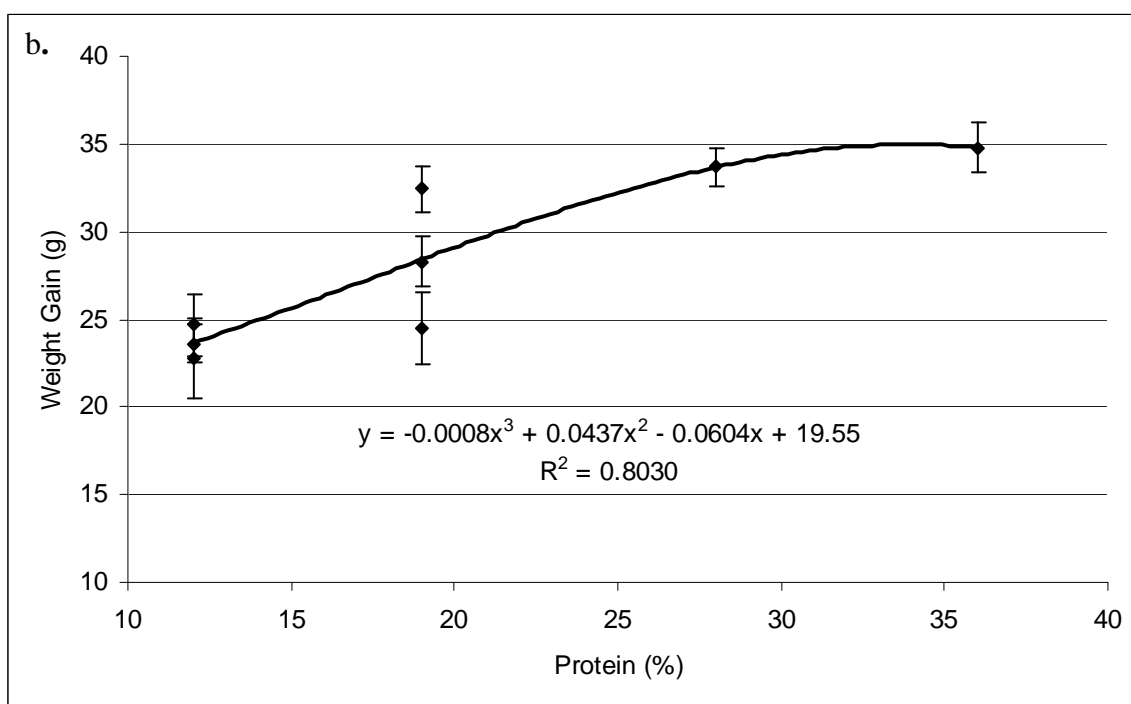
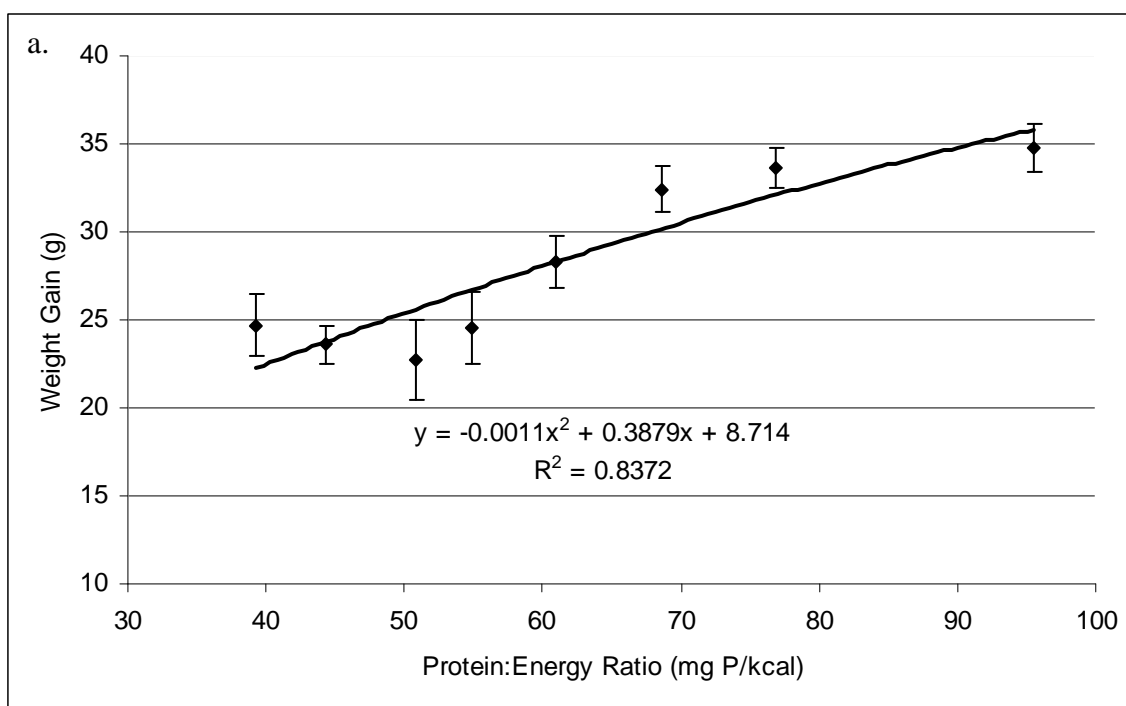
Initial average wet gonad weight was 0.42+/-0.08

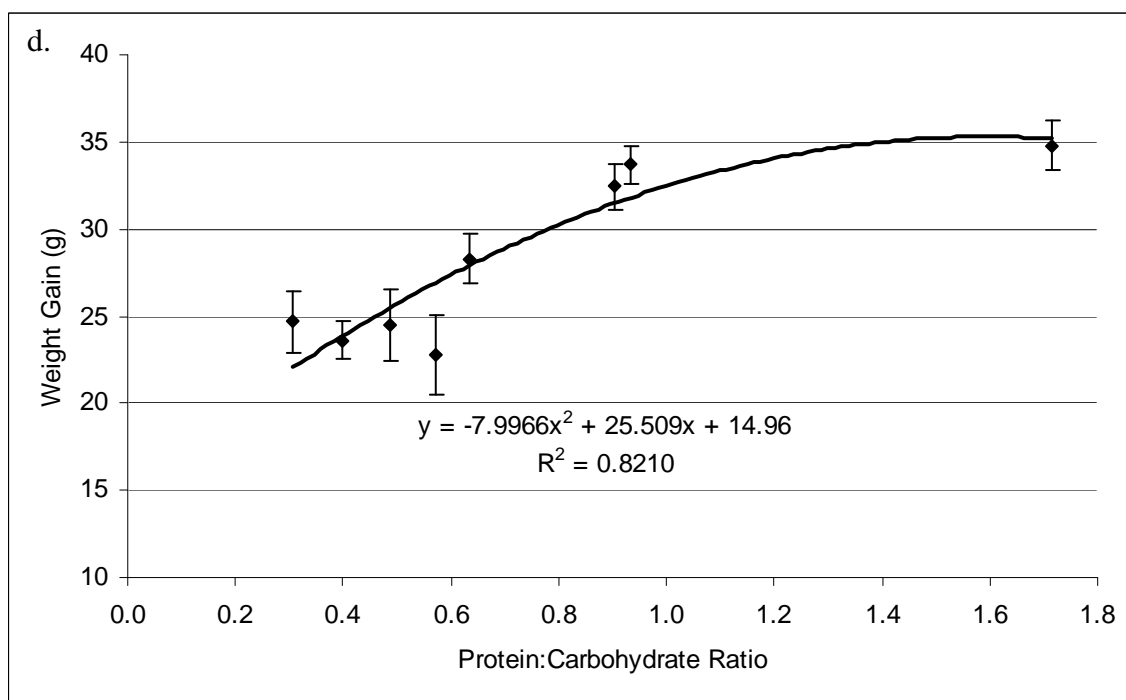
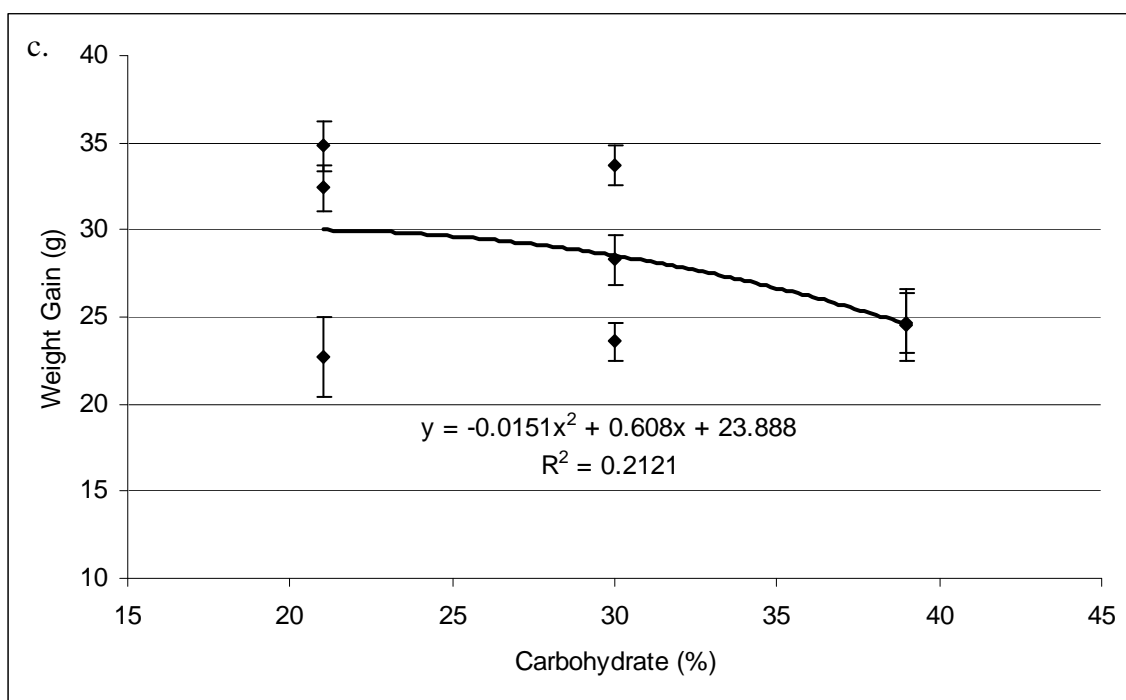
Initial average dry gonad weight was 0.09+/-0.02

Table 6: Mean feed conversion ratio, production efficiency, and protein efficiency ratio of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein:energy ratios (P:E), total energy (TE), and protein:carbohydrate ratios (P:C).

P:E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P:C represents protein:carbohydrate ratio (mg/mg). Letters indicate statistical differences among diets ($P < 0.05$).

% Protein	% Carbohydrate	P:E (mg P/kcal)	TE (cal/g)	P:C	Feed Conversion Ratio		Production Efficiency (%)		Protein Efficiency Ratio	
12	21	50.79	2380	0.57	1.27 +/- 0.16	A	20.67 +/- 1.65	C	1.58 +/- 0.13	A
12	30	44.32	2728	0.40	1.12 +/- 0.05	AB	23.55 +/- 1.55	C	1.80 +/- 0.12	A
12	39	39.31	3075	0.31	1.12 +/- 0.08	AB	23.86 +/- 1.60	ABC	1.82 +/- 0.12	A
19	21	68.56	2783	0.90	0.89 +/- 0.04	B	26.30 +/- 1.40	AB	1.27 +/- 0.07	B
19	30	60.95	3131	0.63	1.01 +/- 0.06	AB	26.04 +/- 1.68	ABC	1.25 +/- 0.08	B
19	39	54.85	3478	0.49	1.09 +/- 0.09	AB	24.69 +/- 1.83	ABC	1.19 +/- 0.09	BC
28	30	76.88	3647	0.93	0.89 +/- 0.03	B	28.79 +/- 1.12	A	0.94 +/- 0.04	CD
36	21	95.57	3749	1.71	0.90 +/- 0.03	B	28.17 +/- 1.00	AB	0.72 +/- 0.03	D





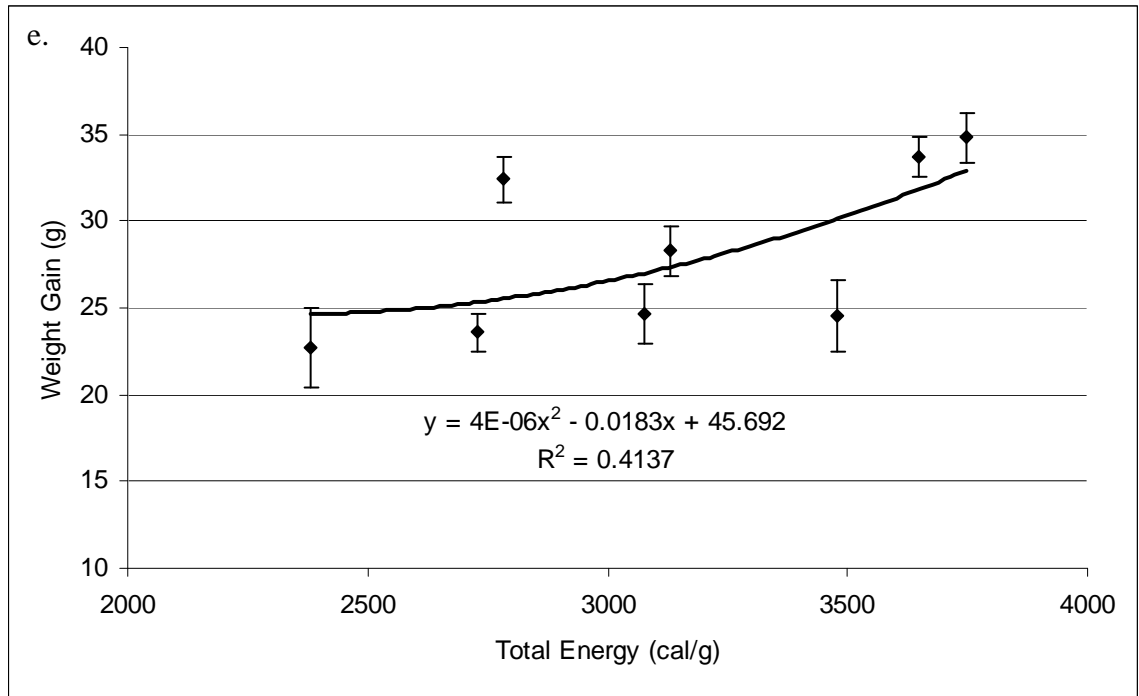
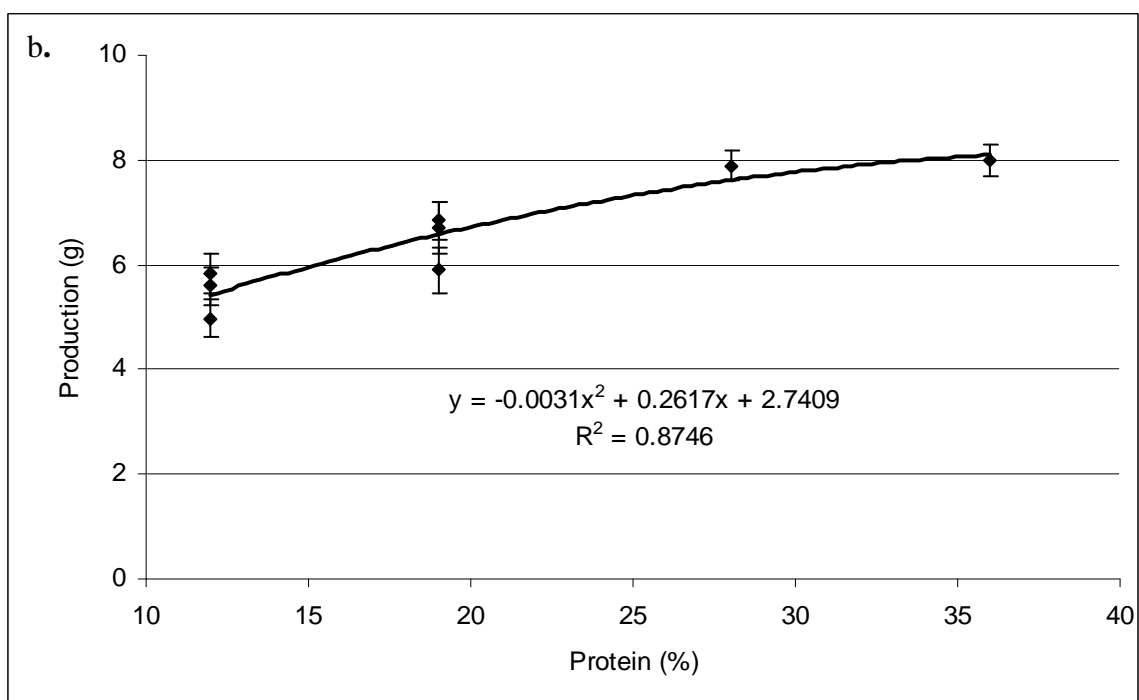
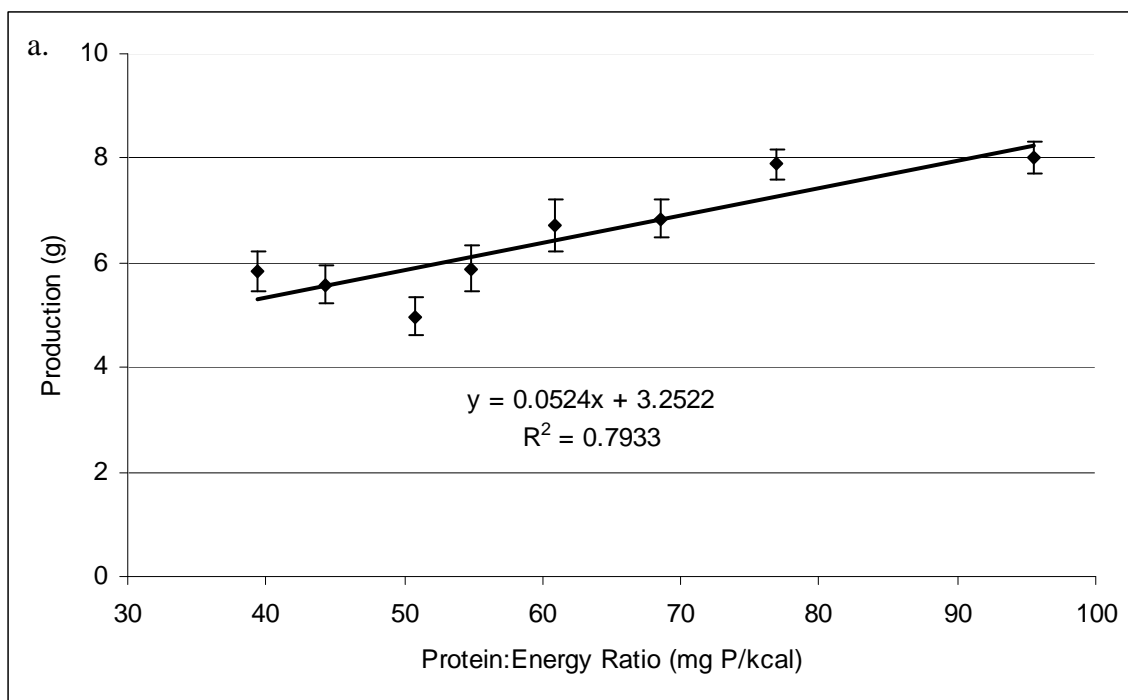
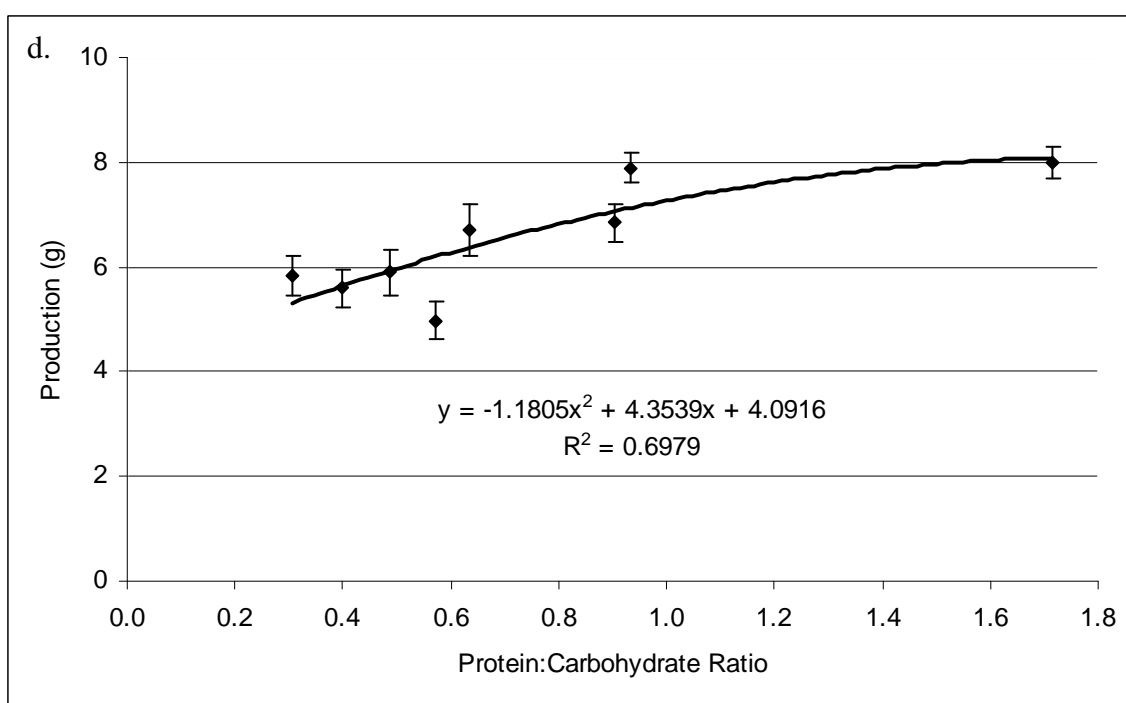
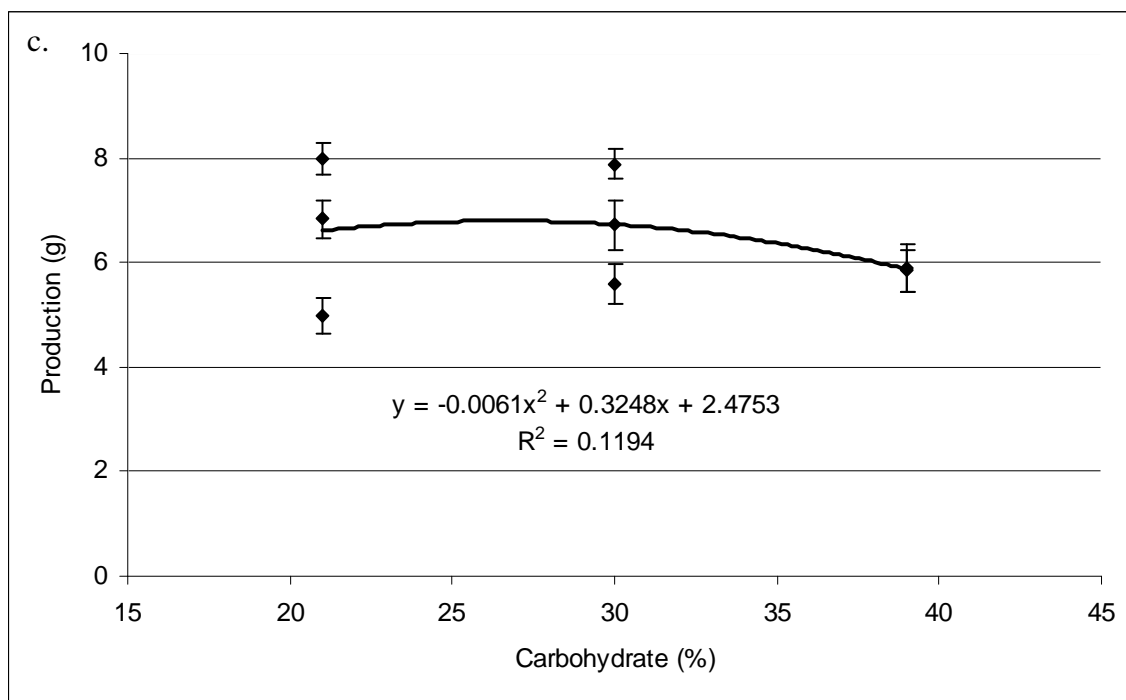


Figure 1. Trend analysis of total wet weight gain (g) vs. dietary (a) protein:energy ratio, (b) protein level, (c) carbohydrate level, (d) protein:carbohydrate ratio, and (e) energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).





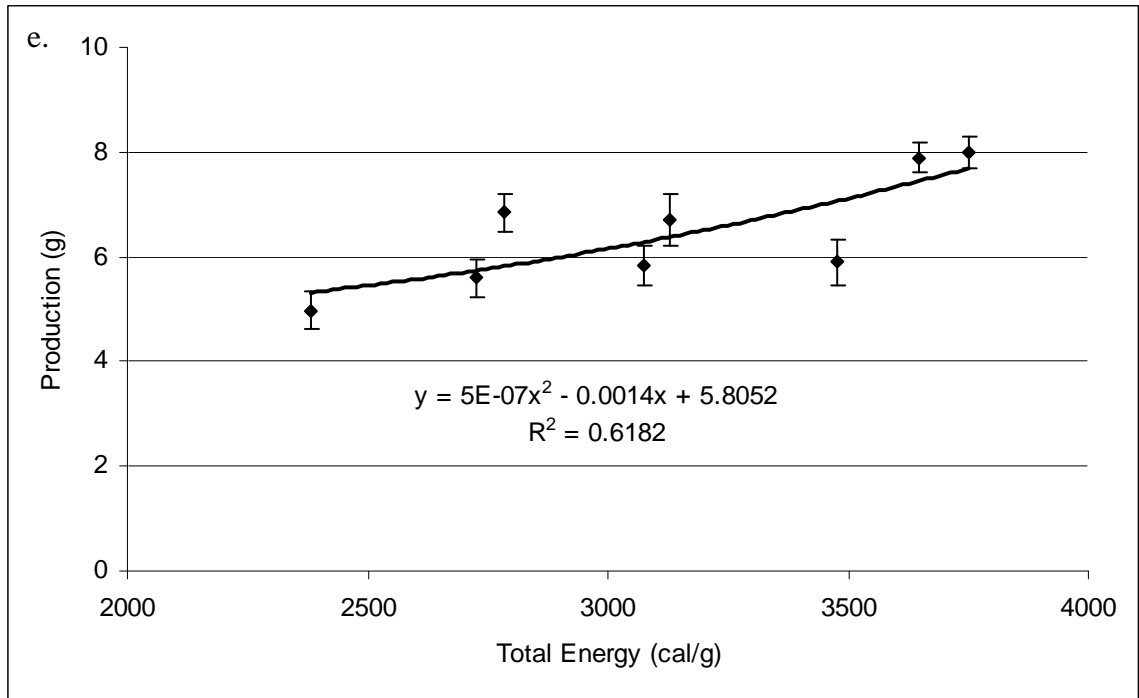
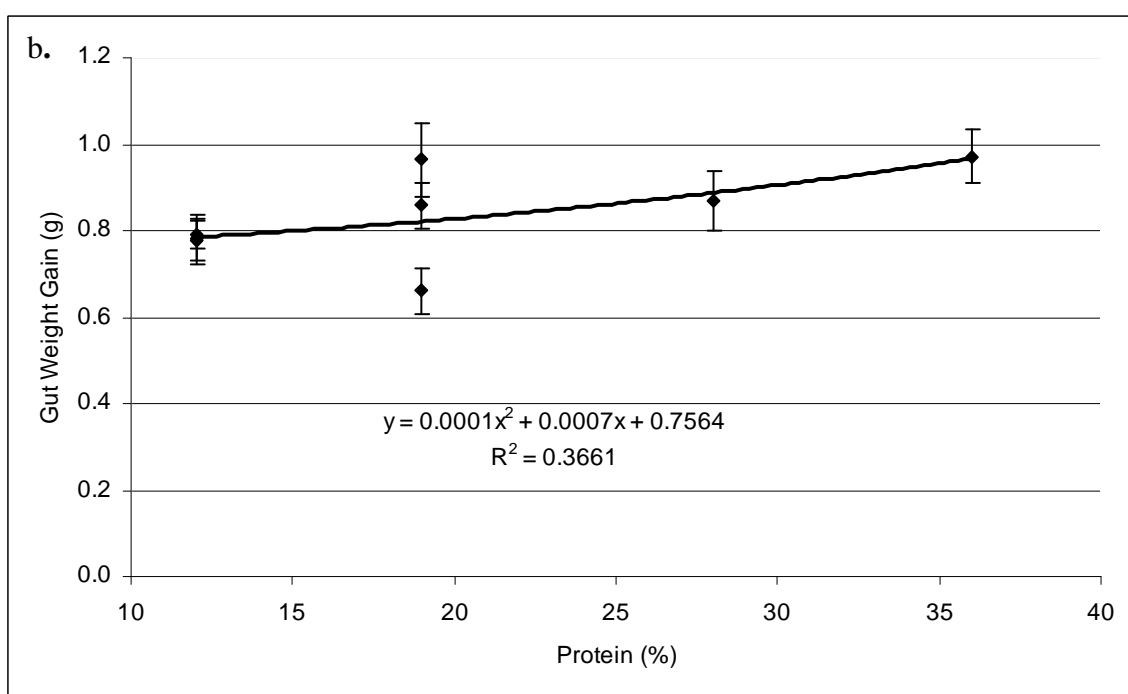
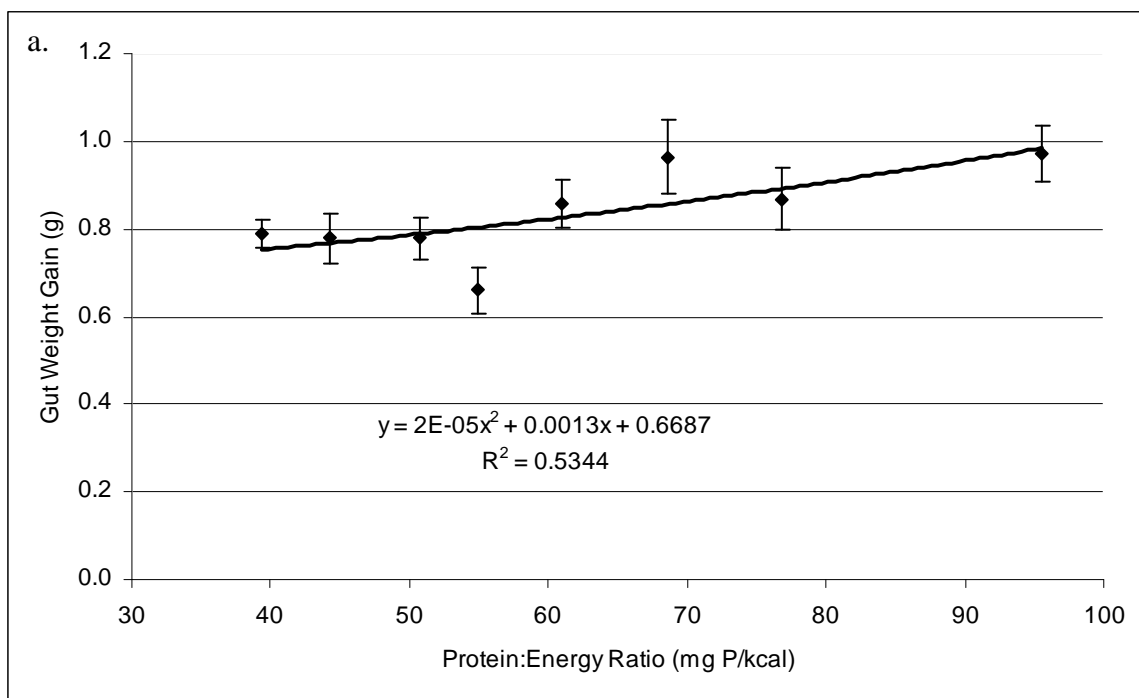
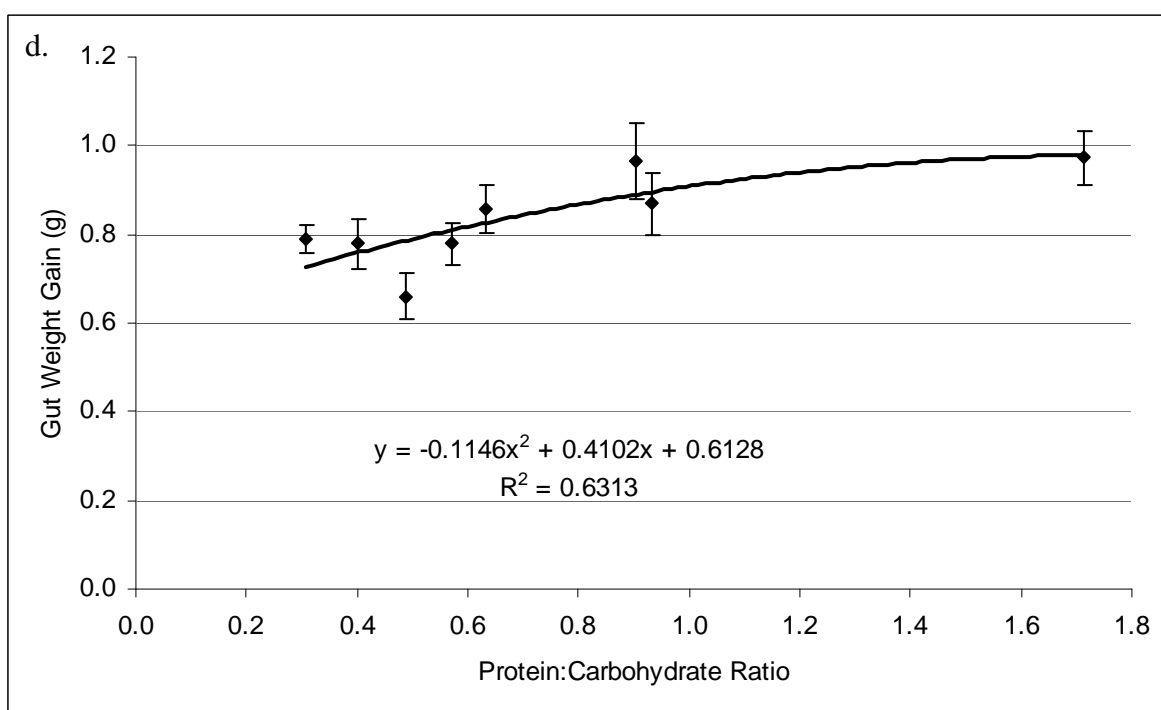
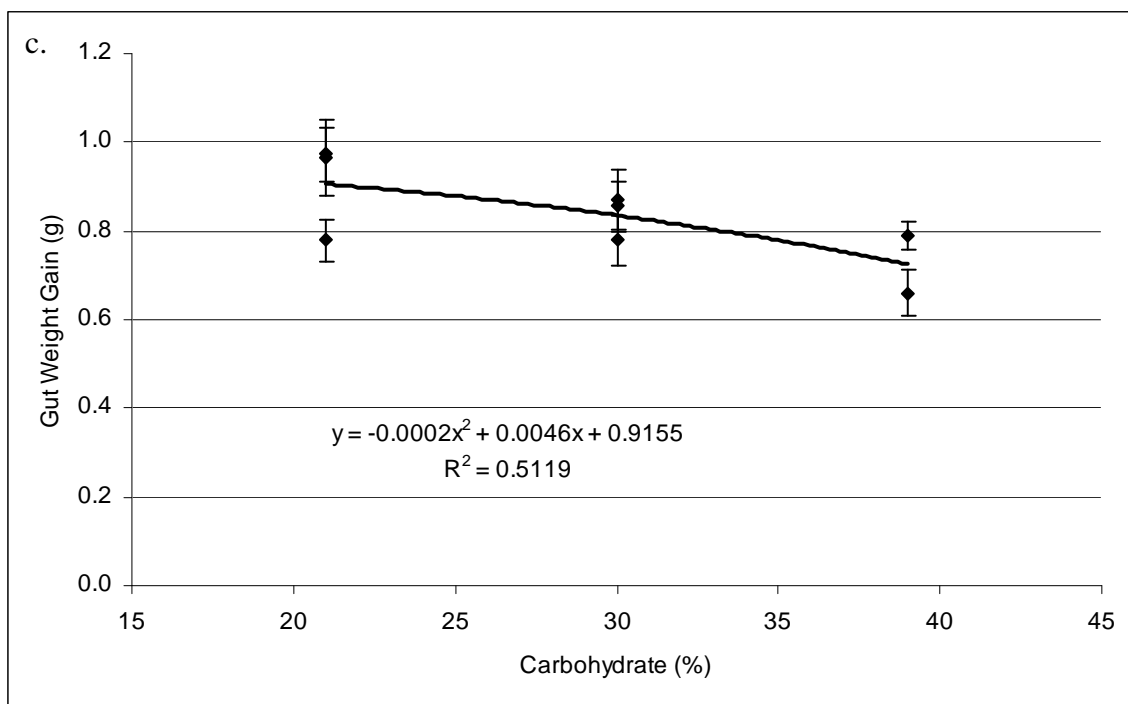


Figure 2. Trend analysis of dry matter production (g) vs. dietary (a) protein:energy ratio, (b) protein level, (c) carbohydrate level, (d) protein:carbohydrate ratio, and (e) energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).





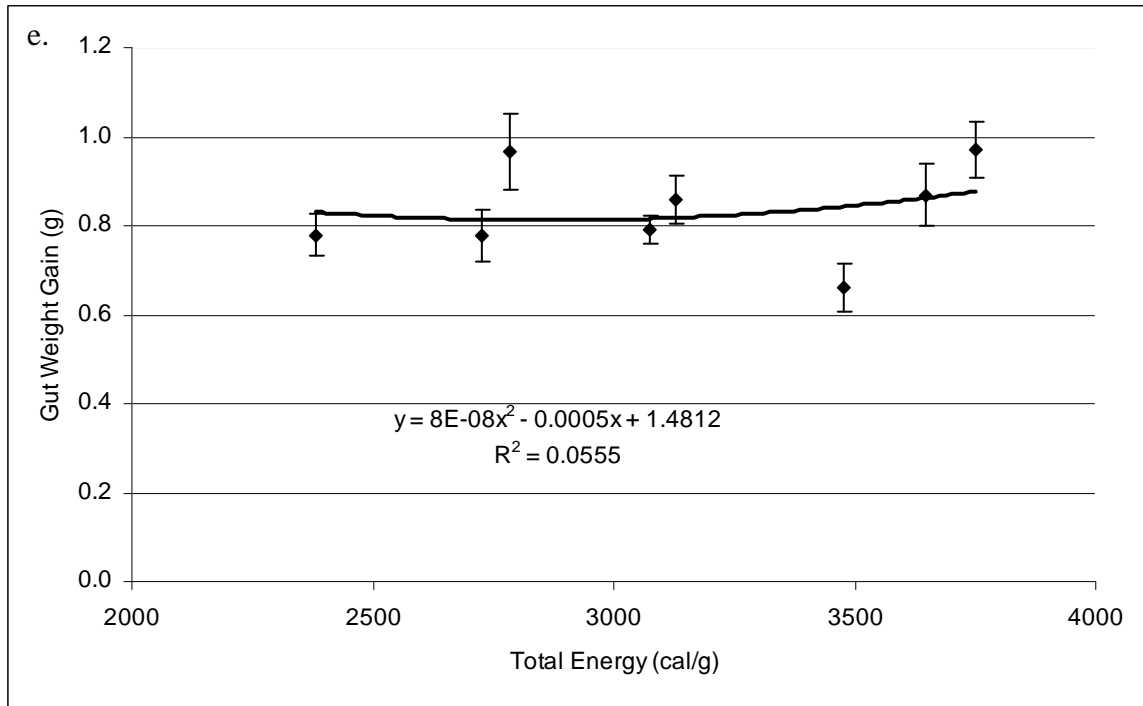
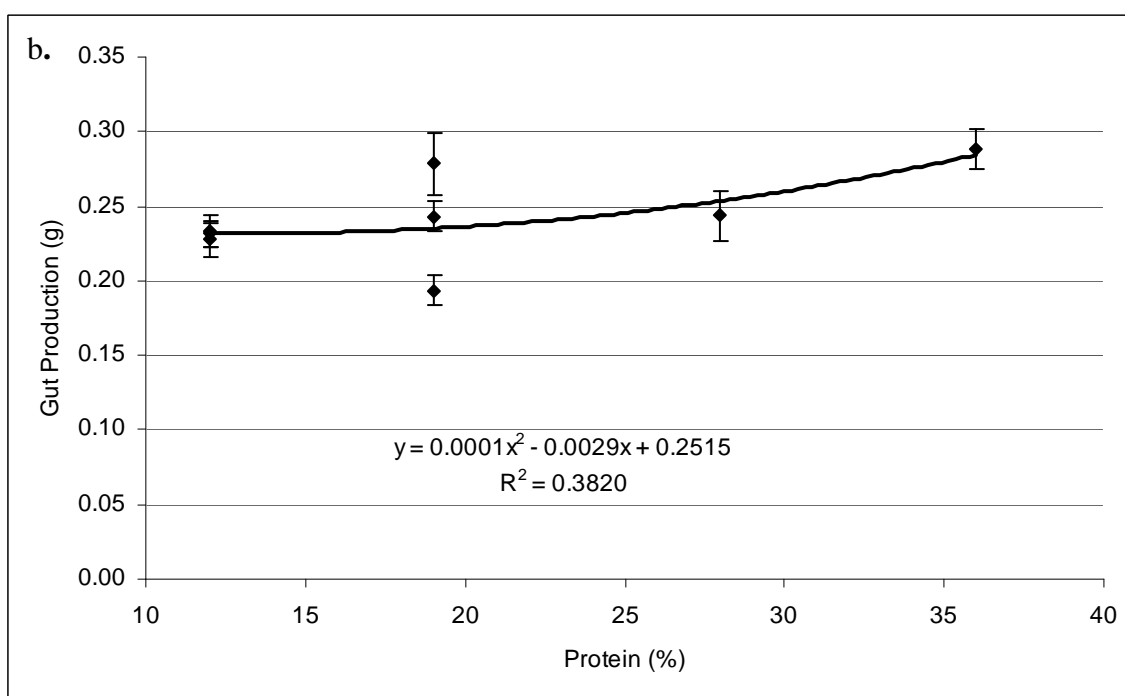
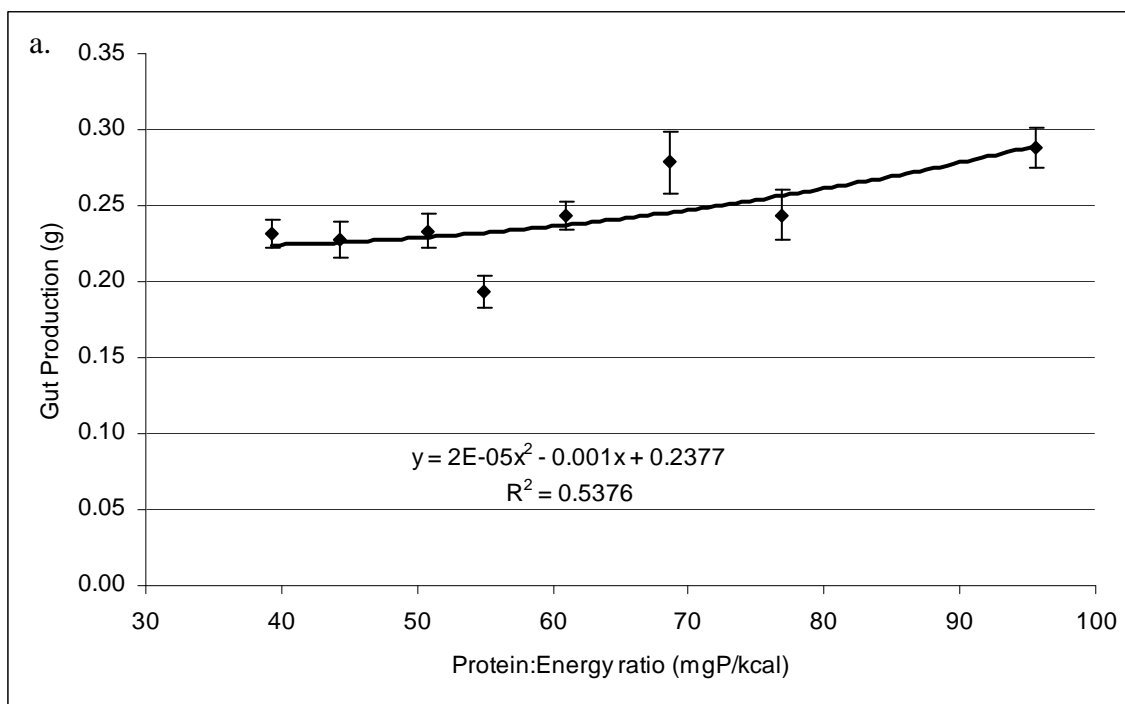
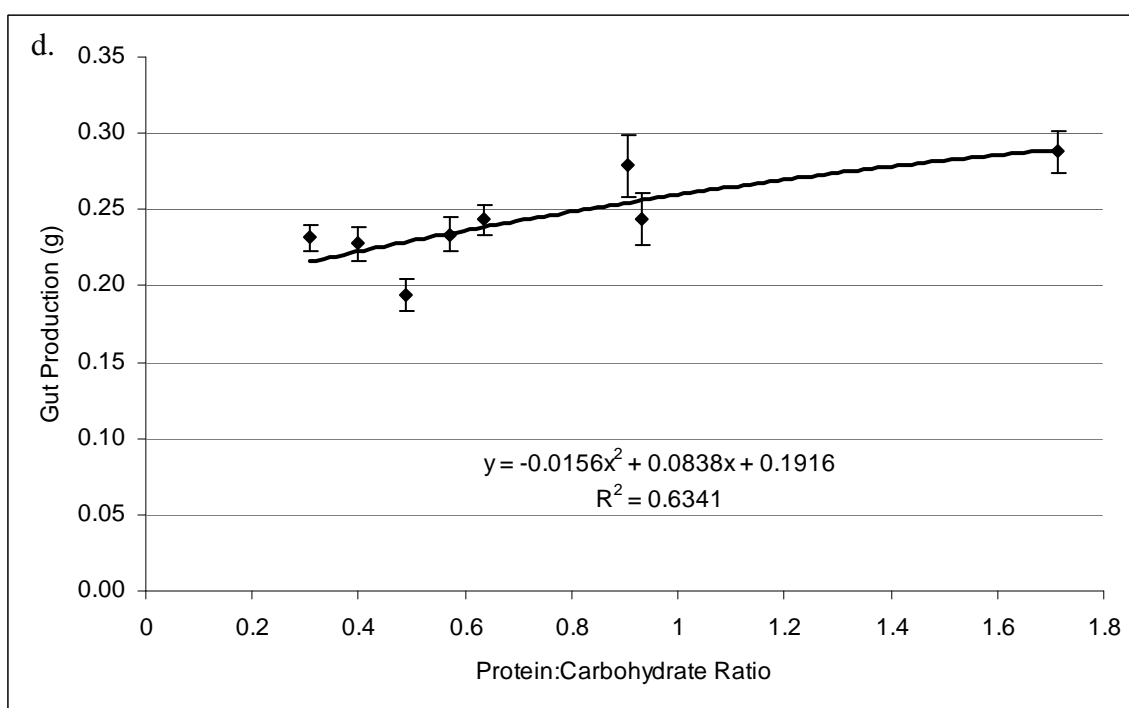
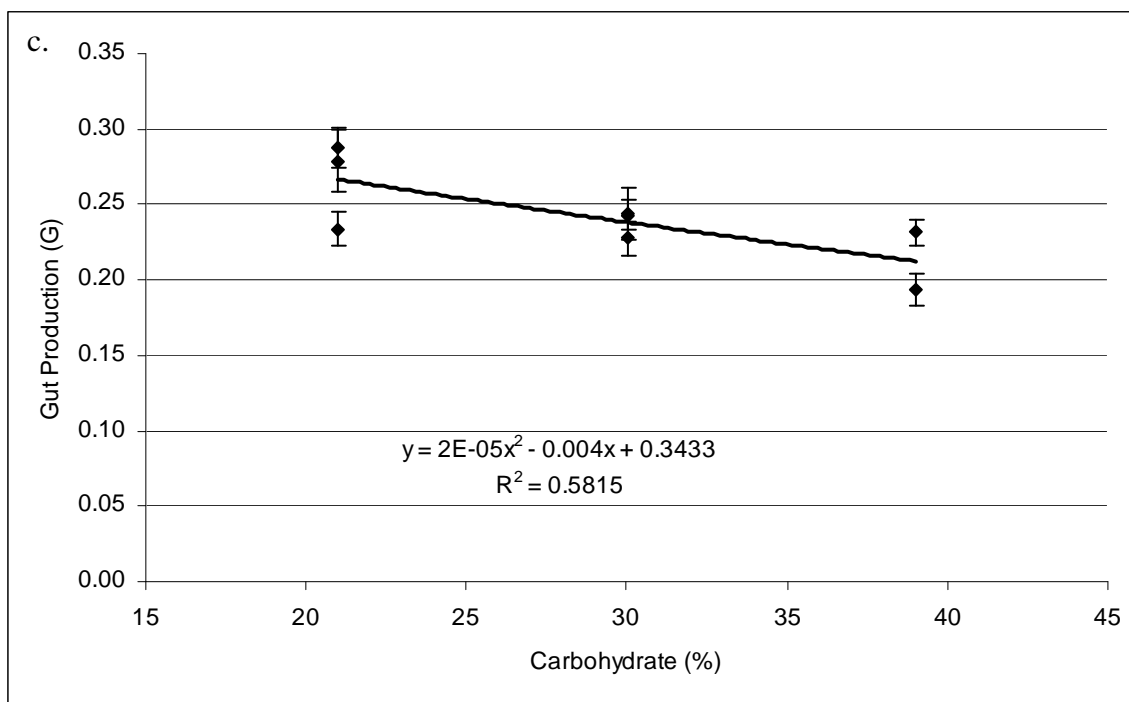


Figure 3. Trend analysis of gut wet weight gain (g) vs. dietary (a) protein:energy ratio, (b) protein level, (c) carbohydrate level, (d) protein:carbohydrate ratio, and (e) energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).





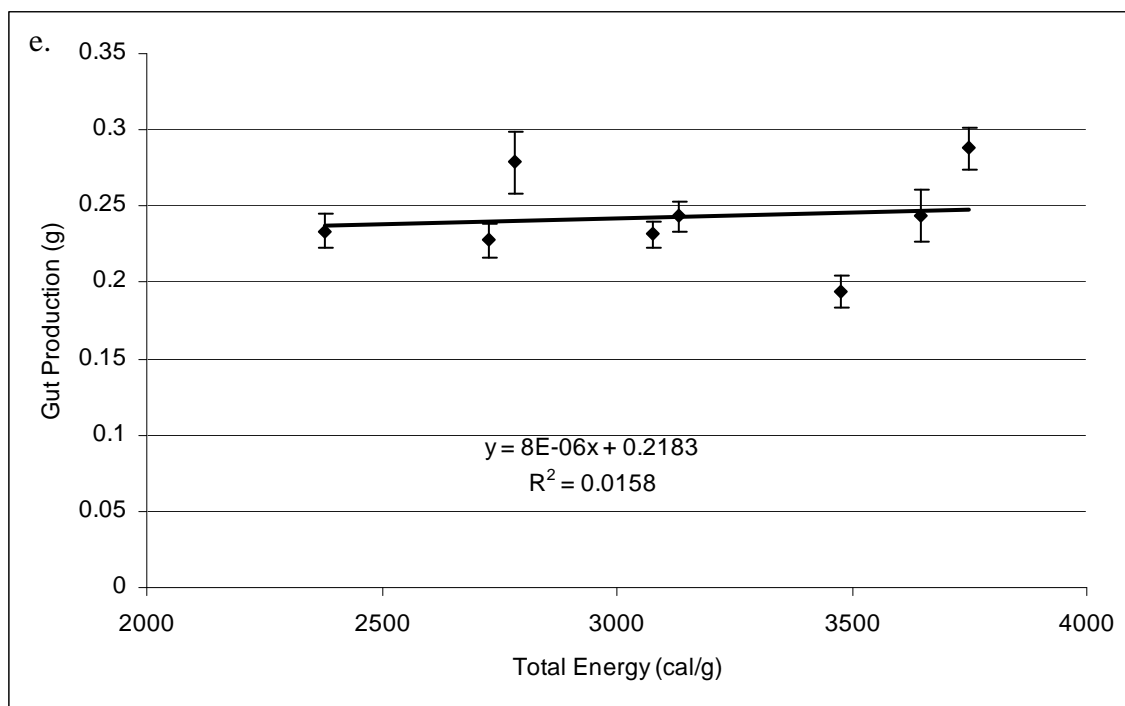
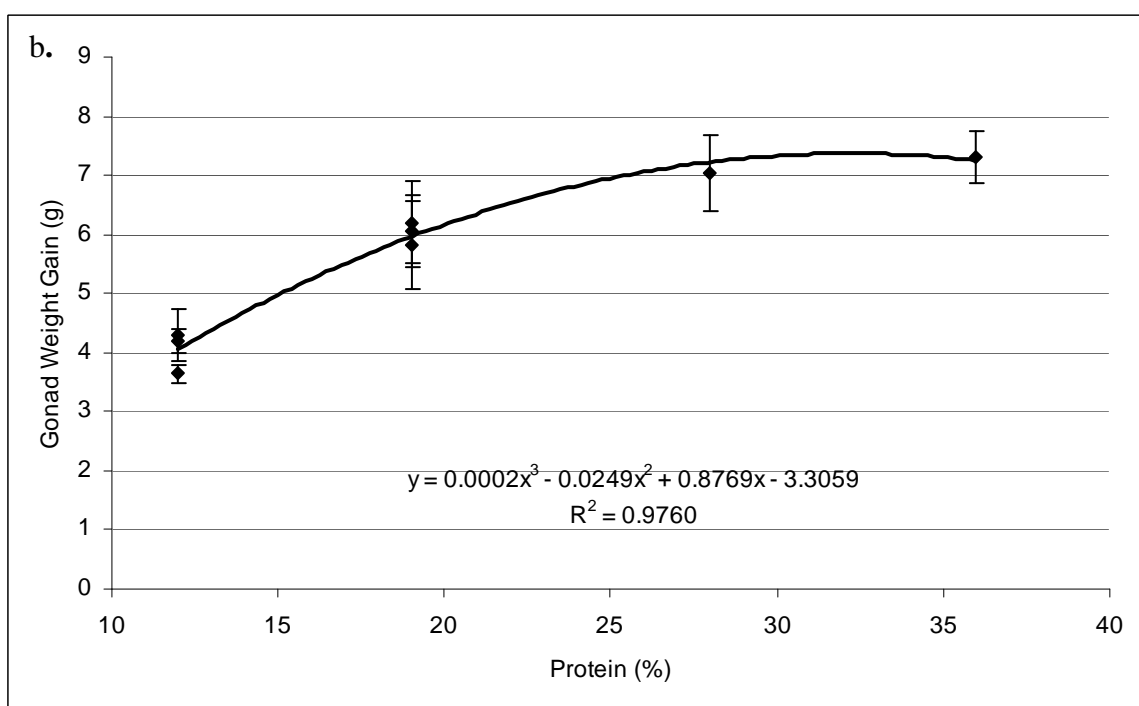
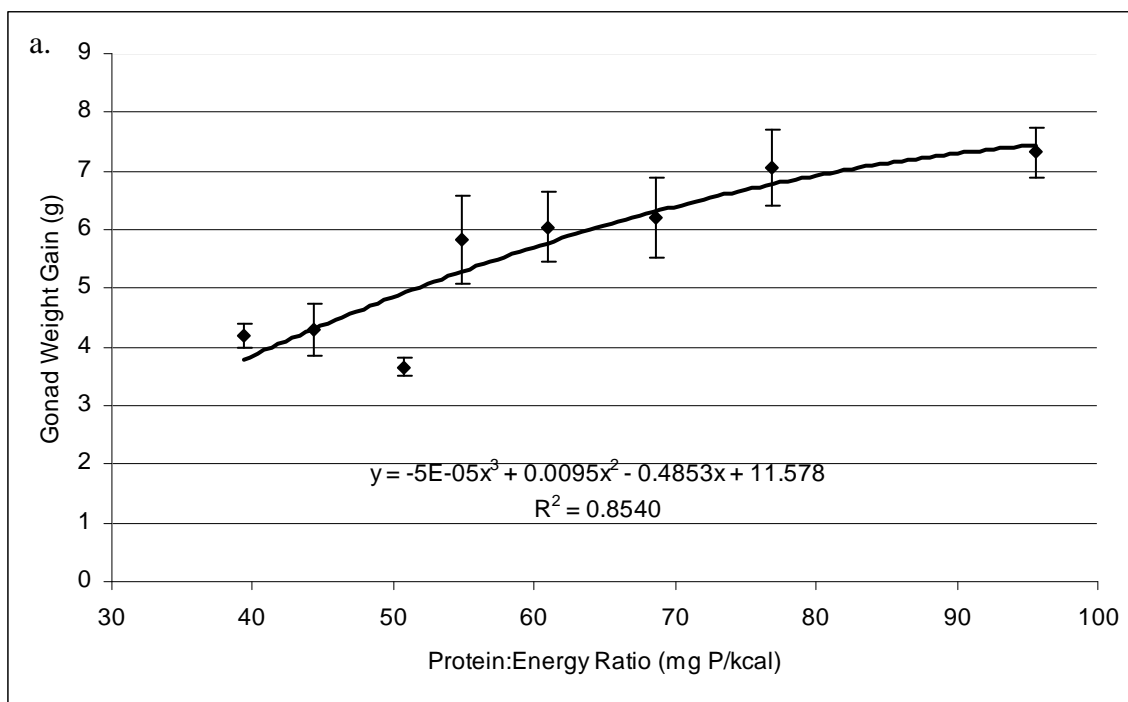
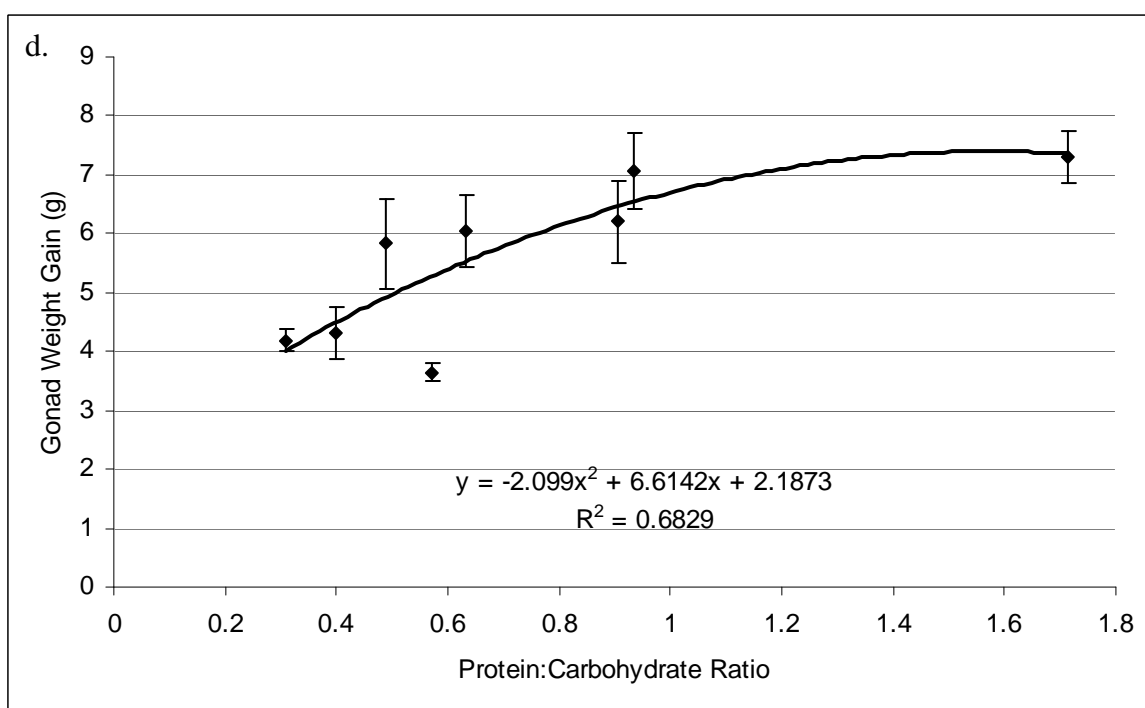
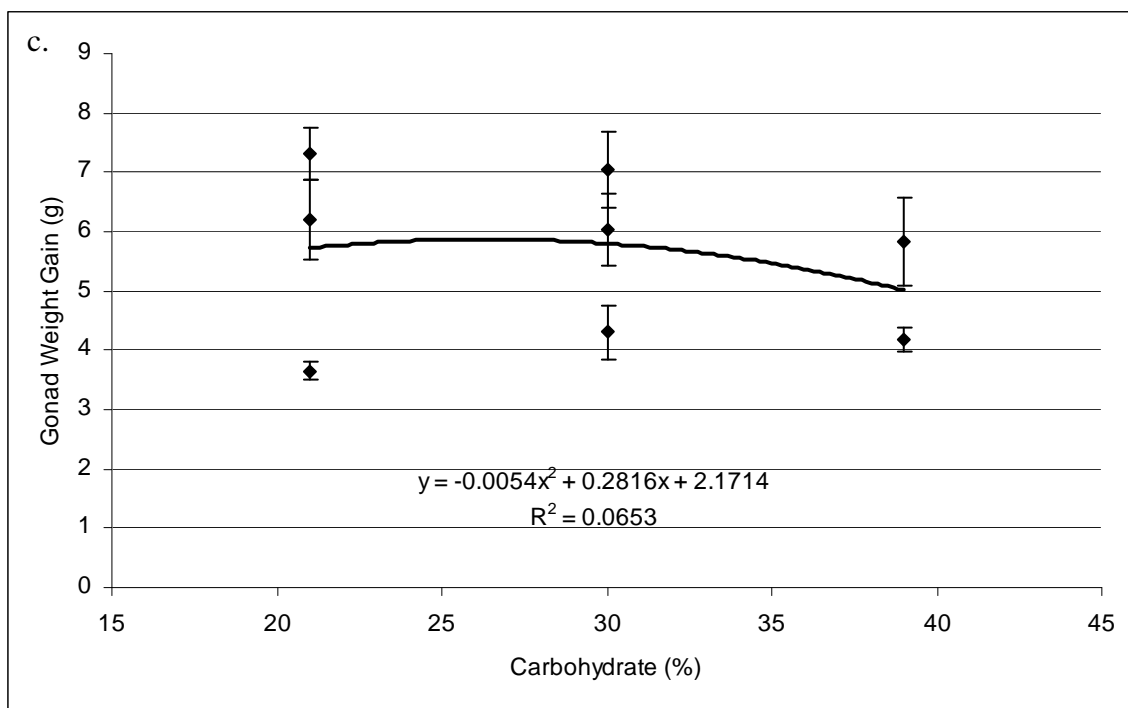


Figure 4. Trend analysis of gut dry matter production (g) vs. dietary (a) protein:energy ratio, (b) protein level, (c) carbohydrate level, (d) protein:carbohydrate ratio, and (e) energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).





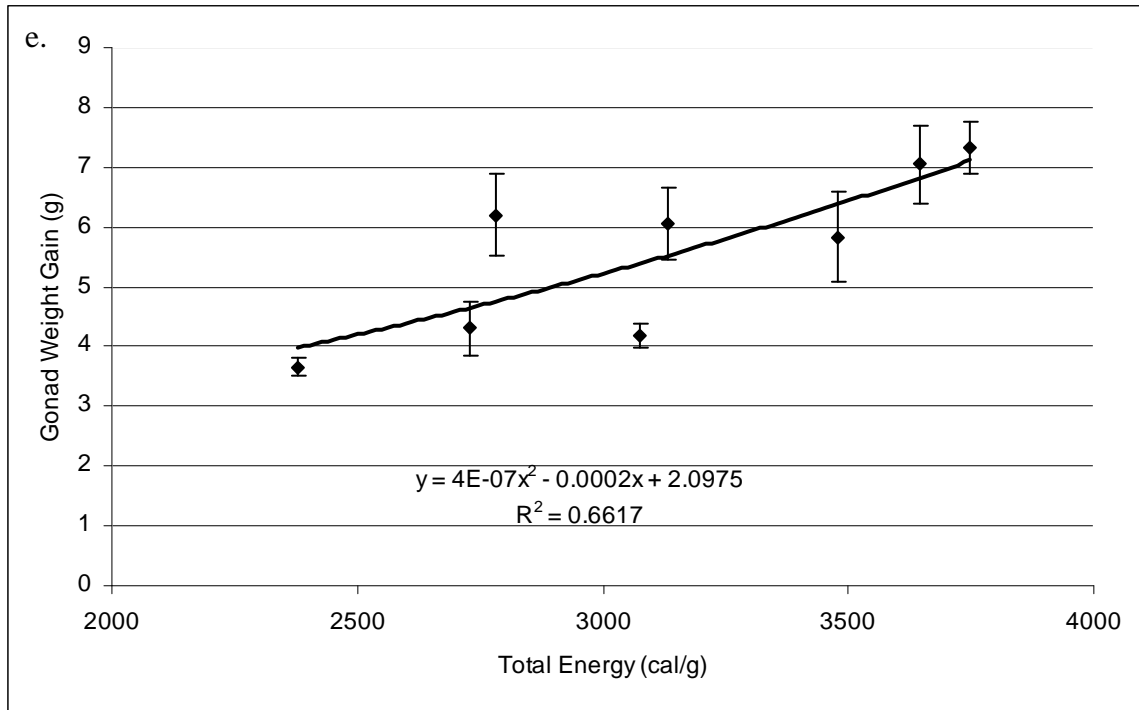
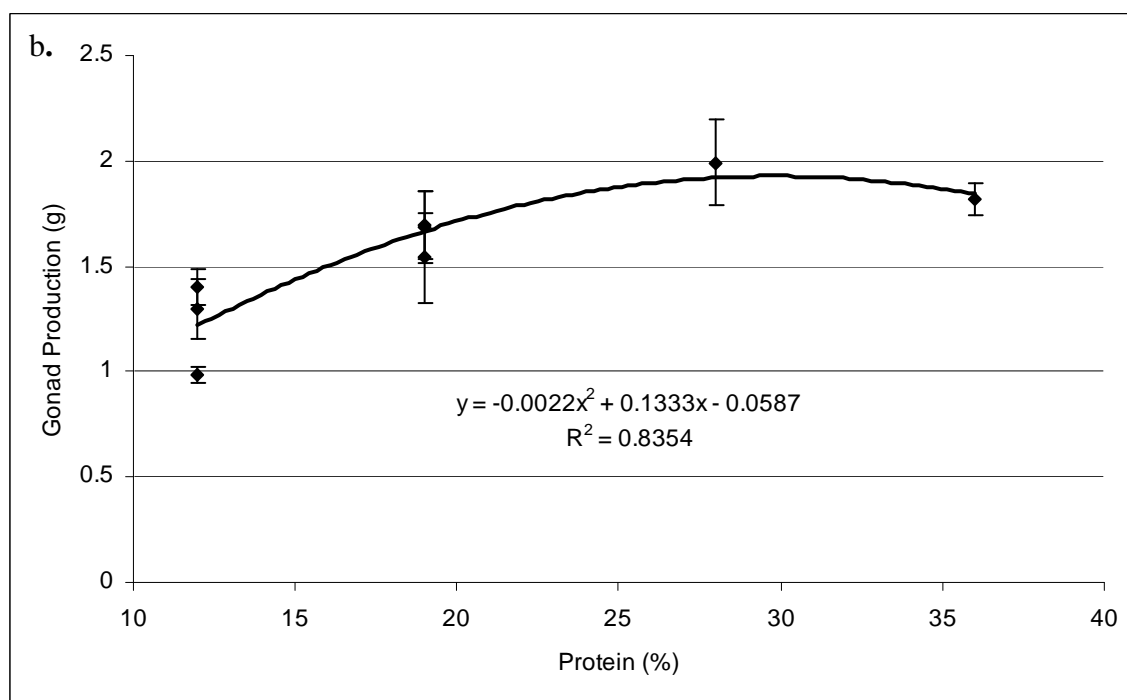
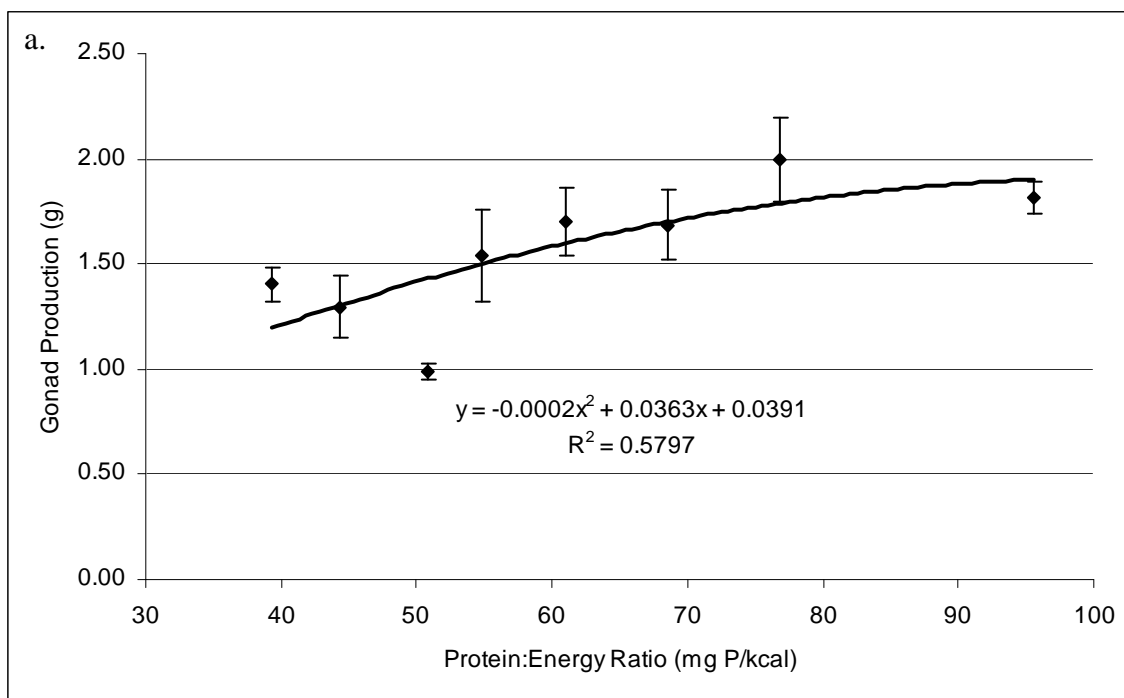
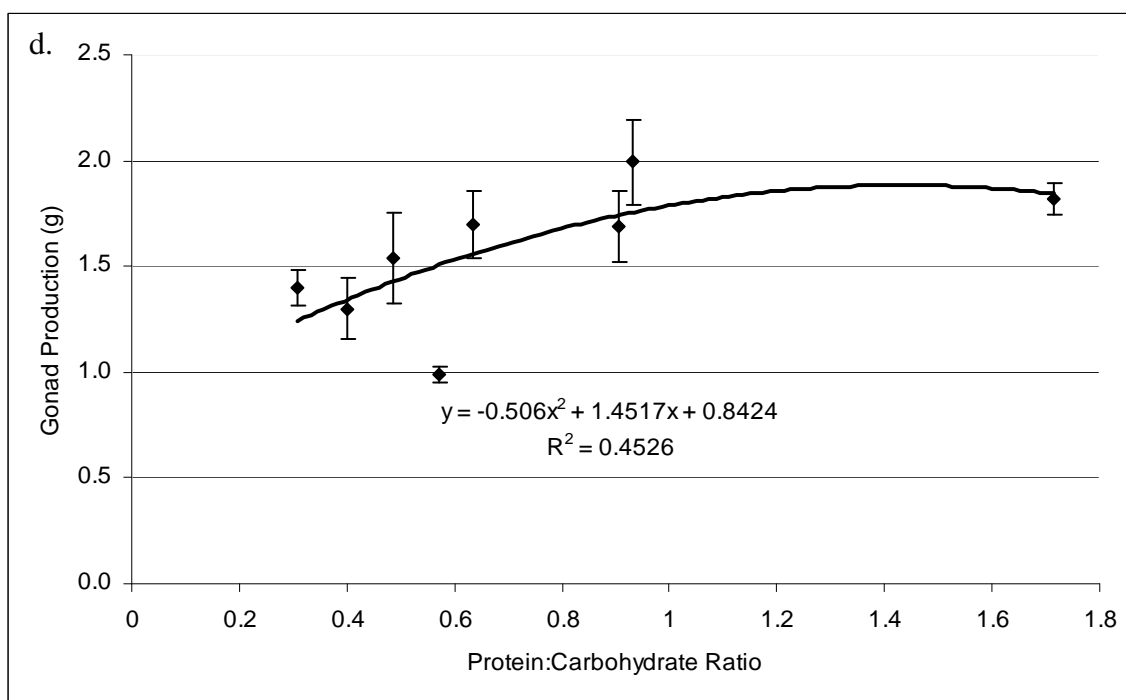
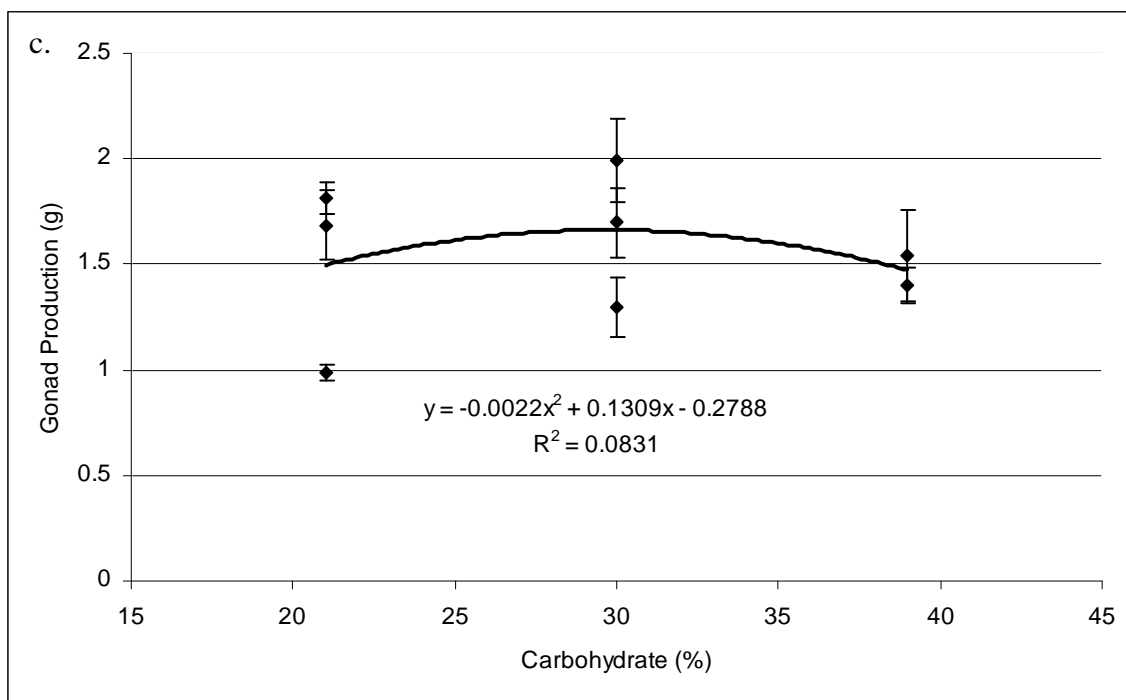


Figure 5. Trend analysis of gonad wet weight gain (g) vs. dietary (a) protein:energy ratio, (b) protein level, (c) carbohydrate level, (d) protein:carbohydrate ratio, and (e) energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).





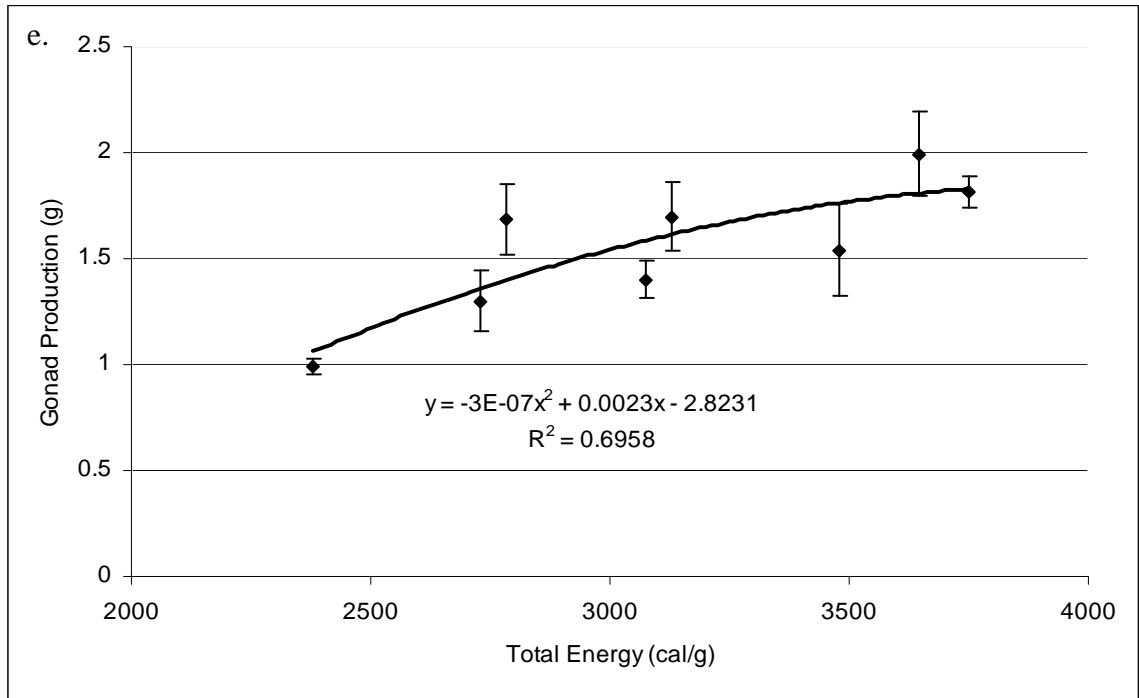
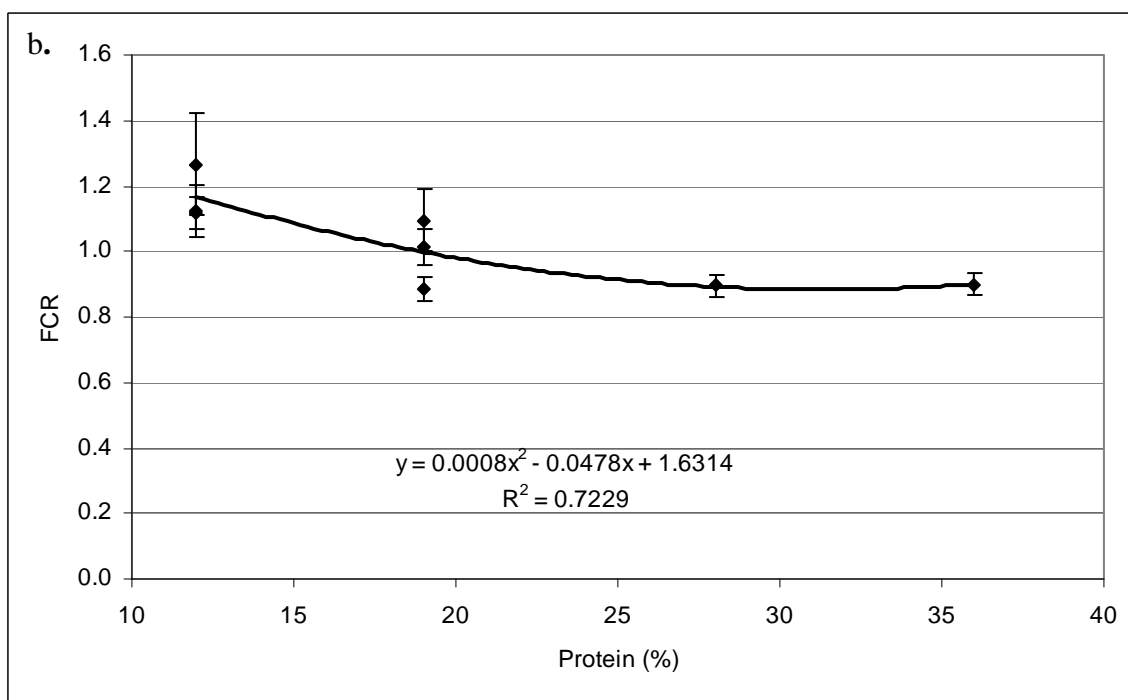
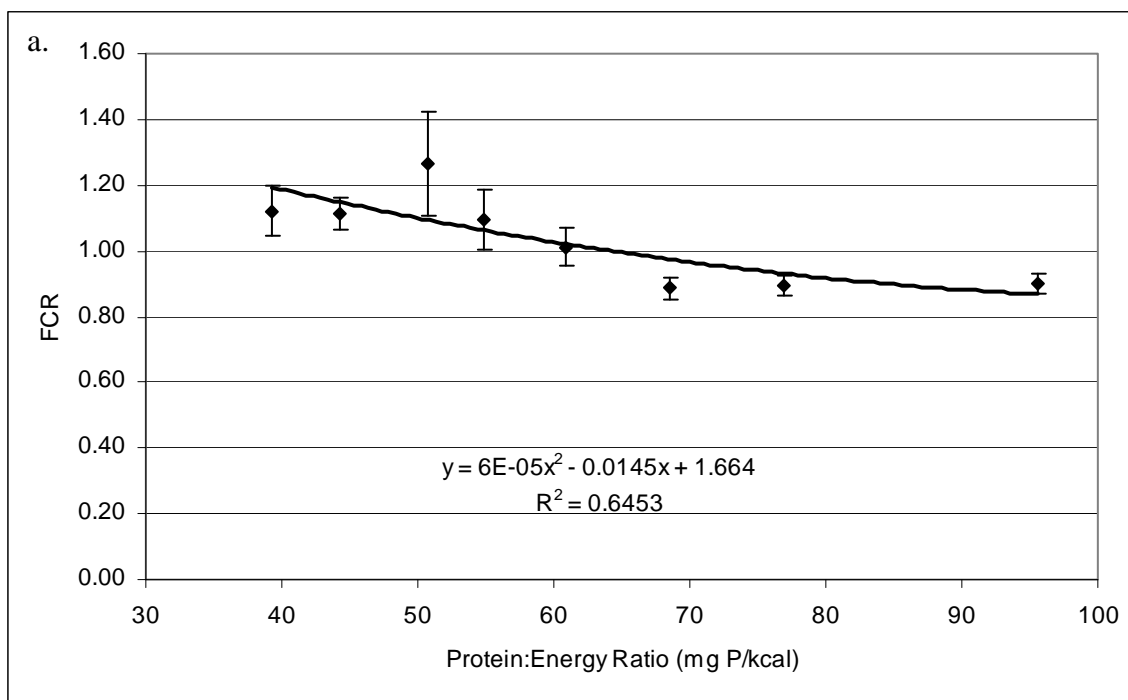


Figure 6. Trend analysis of gonad dry matter production (g) vs. dietary (a) protein:energy ratio, (b) protein level, (c) carbohydrate level, (d) protein:carbohydrate ratio, and (e) energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).



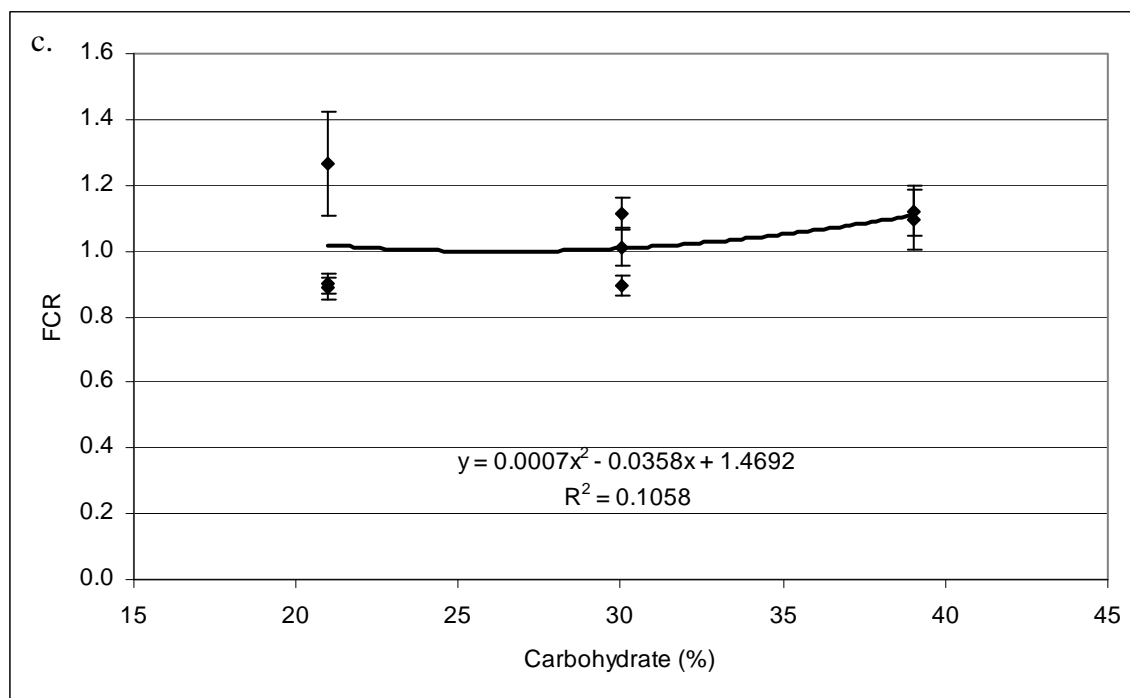
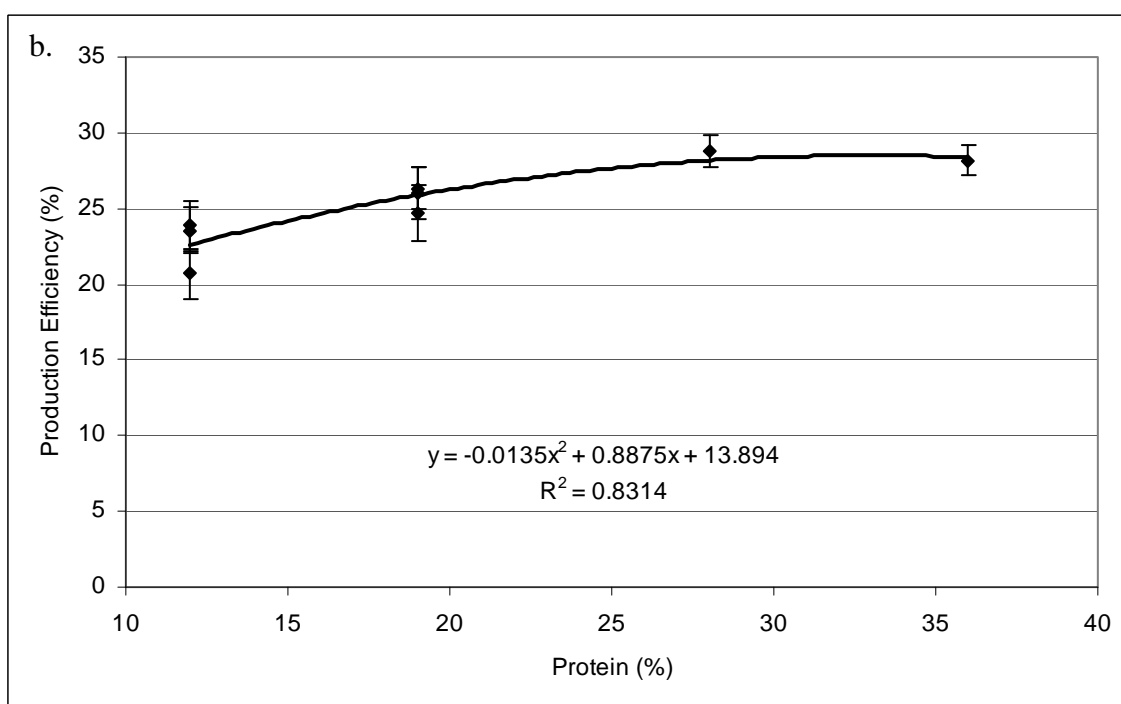
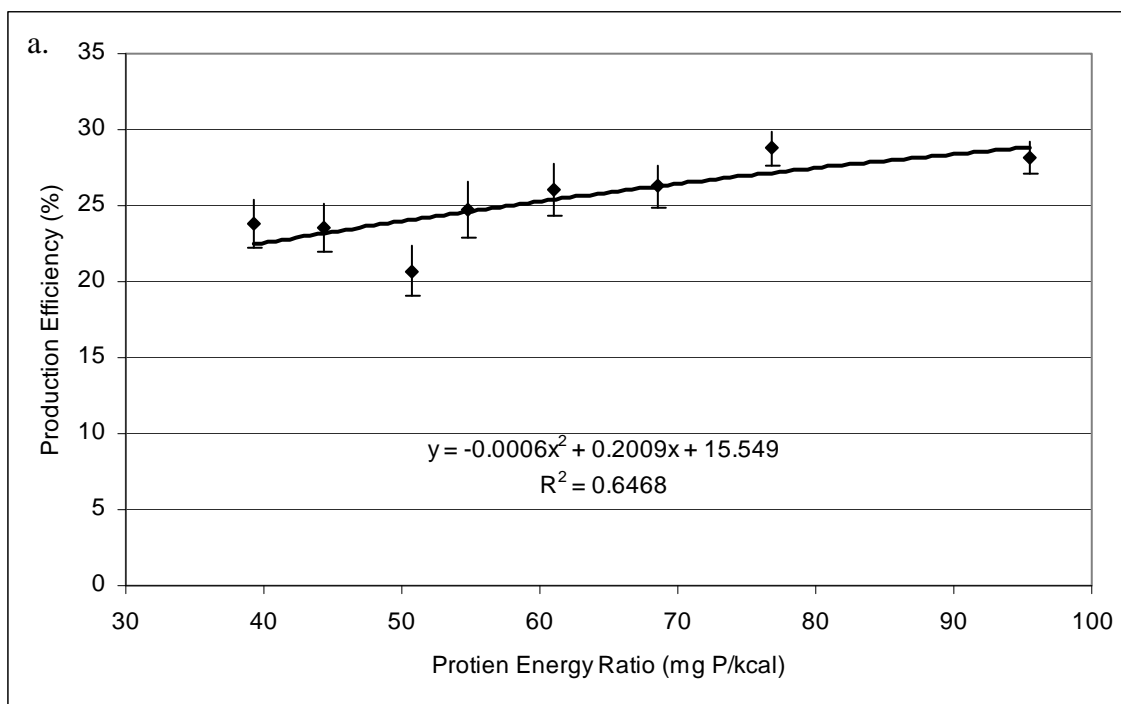


Figure 7. Trend analysis of feed conversion ratio (FCR) vs. dietary (a) protein:energy ratio, (b) protein level, (c) carbohydrate level of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).



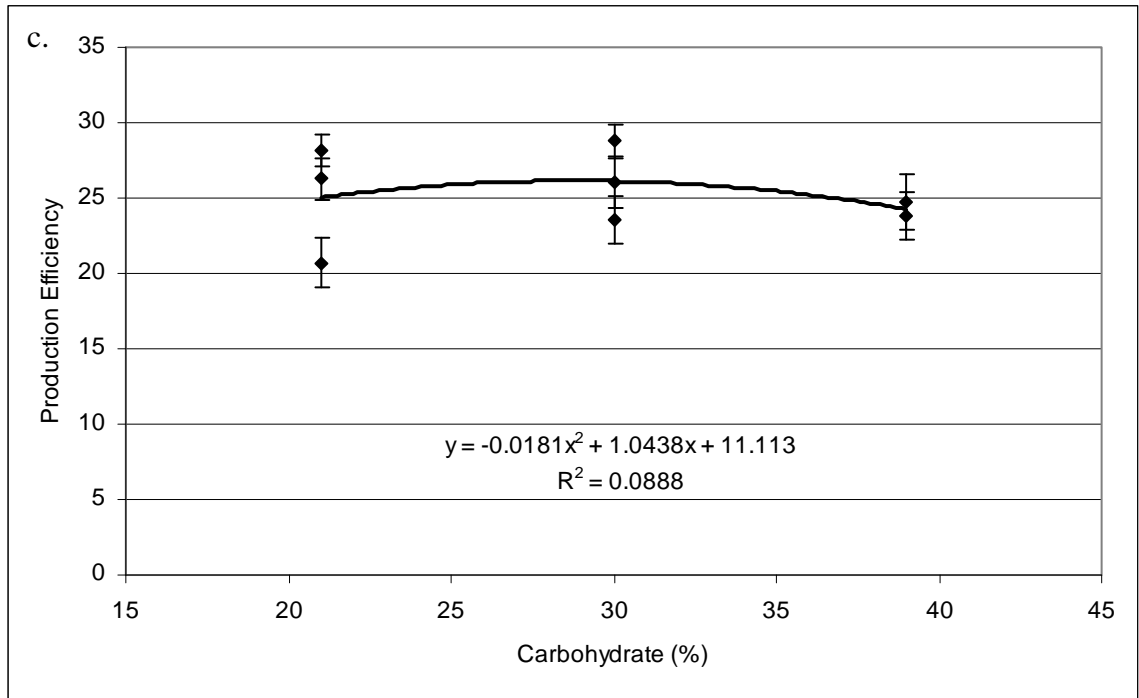


Figure 8. Trend analysis of production efficiency (PE) vs. dietary (a) protein:energy ratio, (b) protein level, and (c) carbohydrate level of *L. variegatus* fed one of eight semi-purified diets for 9 weeks. Values represent means \pm SE. (n = 5-8 individuals per treatment).

Discussion

Water quality parameters maintained in this study were within the ranges suitable for sea urchins (Basuyaux and Mathieu, 1998).

Direct observation indicated that feed rations were below satiation level for *L. variegatus* in this study. Previous studies have shown that sea urchins will adjust feed

intake to satisfy nutritional requirements (McBride et al., 1998; Fernandez and Boudouresque, 2000; Wallace, 2001; Taylor, 2006). Consequently, feeding at sub-satiation ensured that all individuals consumed equal amounts of feed and that urchins were not able to compensate for nutritional deficiencies in the diets by increasing consumption.

Determination of dietary protein and energy requirements for optimal growth and production in sea urchins is a complex challenge. In this study, observed limitations in weight gain and dry matter production based on suggested limitations in protein content may in fact be attributed to limitations in essential amino acids, as indispensable amino acids have not been identified in sea urchins.

In the current study, total dietary protein and carbohydrate levels were adjusted by varying levels of diatomaceous earth (DE), which has been shown to have no effect on sea urchins in the range of DE used in this study (unpublished data). Thus, any differences in the measured parameters seen among dietary treatments at the end of 9 weeks can be directly attributed to variations in the amount of protein or carbohydrate consumed. Carbohydrates are the preferential energy source for many animals and sea urchins are most likely no exception (Marsh and Watts, 2007). As such, formulated diets should supply enough energy from dietary carbohydrates to fulfill the energetic requirements of sea urchins so that more expensive nutrients like protein will be spared. In this study, carbohydrate levels varied from 21-39% among diets. Carbohydrate consumption was greatest in urchins fed the 12:39 and 19:39% protein:carbohydrate diets, but no differences in total growth in terms of wet weight gain or dry matter production were observed when carbohydrate levels were lowered to 21 or 30%,

regardless of protein content. This suggests that dietary protein was spared as an energy source. We can conclude that carbohydrate energy was not limiting in any of the diets, indicating that *L. variegatus* at this life stage and under these conditions are unlikely to require dietary carbohydrate levels in excess of 21%.

Previous studies with *L. variegatus* (Hammer, 2006; Taylor, 2006; Gibbs et al., 2009), *Psammechinus miliaris* (Otero-Villanueva et al., 2004) and *Paracentrotus lividus* (Fernandez and Pergent, 1998) have observed that sea urchins fed a high energy diet may become satiated and may not consume adequate quantities of other nutrients that might be necessary for optimal growth and development. Other marine and fresh water organisms have also been observed to adjust feed intake levels according to the level of dietary carbohydrates. Feed intake by channel catfish (reviewed by Gatlin, 1986) was limited by increased dietary energy, as was that of rainbow trout (Boujard and Medale, 1994), tilapia (Bowen et al., 1995), and shrimp (Siccardi, 2006; Davis and Arnold, 1995). Urchins in this study could not adjust feed intake to increase, consequently, carbohydrate or energy intake could not be adjusted for the dietary level proffered

Previous have shown that dietary protein levels affect somatic growth of sea urchins (Fernandez, 1997; Cook et al. 1998; Fernandez and Bourdouresque, 1998; Fernandez and Pergent, 1998; Meidel and Scheibling, 1999; Agatsuma 2000; Akiyama, 2001; Hammer et al., 2004; Hammer et al., 2006a; Taylor, 2006). Trend analysis indicated that dietary protein levels in this study had a proportional effect on growth and production in sea urchins; however, above 28% protein the relationship was asymptotic, indicating that dietary protein was in excess at the highest levels provided. *Lytechinus variegatus* fed the 36:21% protein:carbohydrate diet consumed the most protein;

however, wet weight gain and dry matter production in urchins fed the 19:21% protein:carbohydrate diet was equivalent to that of urchins fed the or 28:30 or 36:21% protein:carbohydrate diets, further suggesting that a dietary protein levels of 28% or higher may exceed the protein requirements of *L. variegatus* at this life stage. Unlike individuals fed the 19:21% protein:carbohydrate diet, urchins fed the 19:30 or 19:39% protein:carbohydrate diets had lower wet weight gain and dry matter production than those fed the diets with protein levels of 28 or 36%. Since consumption rates did not vary among diets, we suggest that the increased carbohydrate levels in the 19:30 and 19:39% protein:carbohydrate diets (a reduced protein:energy ratio) may have adversely affected digestibility of the feed and/or in some way inhibited the absorption or utilization of other dietary nutrients via an undetermined mechanism. *Tripneustes ventricosus* have been observed to selectively absorb nutrients (reviewed in Lawrence and Lane, 1982).

These data suggest that dietary protein levels of 19% are adequate for organismal growth of *L. variegatus* at this life stage, as long as carbohydrate levels are not excessive. Previous studies using *L. variegatus* and other species corroborate these findings. Hammer et al. (2006a) reported that growth of adult *L. variegatus* fed a 20:23% protein:carbohydrate diet was comparable to that of urchins fed a 31:12% diet. Somatic growth of *S. droebachiensis* was maximized at dietary protein levels of 19-20% (Pearce et al., 2002b; Kennedy and Robinson, 2005). Akiyama (2001) concluded that 20% protein was optimal in a purified diet for *P. depressus*. However, Hammer (2004) reported reduced growth and survivorship in small *L. variegatus* fed a formulated diet with 19% protein (as compared to 27%). Sea urchin growth can be described relatively well by the Tanaka growth model (Ebert, 1997; McShane and Anderson, 1997), which

consists of slow initial growth followed by a period of exponential growth and then a period of slow but constant growth. It is reasonable to assume that sea urchins may require different levels of dietary protein and/or carbohydrate at different life stages. Juvenile sea urchins such as those used by Wallace (2001) should be in the exponential growth phase and, thus, may have a higher requirement for dietary protein.

The ratio of dietary protein to dietary carbohydrate did correlate to both wet weight gain and dry matter production. However, the correlation appeared to be largely an effect of dietary protein level, consequently, protein:carbohydrate ratios may have limited value in explaining changes in these growth parameters.

Under the conditions of this study, total dietary energy (energy from protein, carbohydrates, and lipids) was not a good predictor of urchin growth in terms of wet weight gain and dry matter production. Lipid levels did not vary among diets, thus, energy from lipids was the same among diets. Although urchin growth was correlated highly with dietary protein levels, total energy content of diets did not vary directly with dietary protein levels, and thus total energy limited in its ability to predict growth. Comparison of dietary protein level to total dietary energy (protein:energy ratio) yielded a stronger relationship with growth and dry matter production. To date, few studies have examined the effect of protein:energy ratio on sea urchin growth, but it is known that protein:energy ratio affects both shrimp and fish. Bautista (1986) studied protein:energy ratios in penaeid shrimp and found that the most weight gain and lowest mortality rate occurred at protein:energy ratios between 120-174 mg p kcal⁻¹. When dietary protein levels were raised from 40 to 50%, individuals did not grow as well unless energy levels were also raised (Bautista, 1986), indicating that not just level of dietary protein, but the

ratio of dietary protein to dietary energy, is an important consideration when evaluating the nutritional requirements of penaeid shrimp. Recent studies suggest that dietary protein:energy ratio may influence growth and production in sea urchins in a similar manner (Hammer, 2006; Taylor, 2006). Due to their sedentary lifestyle and low respiration rate, energy requirements of sea urchins are low (Lawrence and Lane, 1982; Marsh and Watts, 2007). As such, diets with high protein:energy ratios would be expected to provide the greatest growth and production. In this study, urchins fed the diets with the highest protein:energy ratios had the highest total wet weight gain and total dry matter production. However, since diets with protein:energy ratios of higher than $68.56 \text{ mg p kcal}^{-1}$ did not further increase growth and production, it can be assumed that there may be an optimal protein:energy ratio (around $69 \text{ mg p kcal}^{-1}$) for *L. variegatus* at this life stage. Dietary protein:energy levels above optimal, while not detrimental from a nutritional standpoint, may not further enhance growth and production and may, in fact, be disadvantageous in terms of cost and pollution.

Production efficiency does not include weight associated with coelomic fluid. Consequently, production efficiency may be a more accurate measure of sea urchin dry matter production than FCR. Production efficiency among sea urchins fed the 12% protein diets was generally lower than that of individuals fed diets with 19%, 28%, or 36% protein. This further supports our hypothesis that under these study conditions, 12% protein, while adequate for maintenance and survival, was insufficient for maximal weight gain. These data suggest that carbohydrate (over the range tested) did not affect the use of protein in the diet as an energy source. These data further suggest that FCR and/or protein efficiency calculations may potentially be used by investigators and

aquaculturists to calculate dietary rations that are suitable for either maintenance or growth, depending on which is desired.

Protein efficiency ratio (PER) in sea urchins has been reported to vary with both season and with dietary energy levels (Schlosser et al. 2005). Hammer (2006) observed no difference in PER of adult *L. variegatus* among diets with high carbohydrate levels. However, when dietary energy from carbohydrates was limiting, PER decreased with increasing dietary protein levels, suggesting that protein was metabolized as an energy source (Hammer et al., 2006b). Under the conditions of this study, PER varied inversely with dietary protein level, but was not affected by dietary carbohydrate level over the range tested. This further suggests that carbohydrate levels among these diets were sufficient for a protein-sparing effect. However, these data indicate that when protein was a limiting factor for growth and production, it was used more efficiently for tissue production. This decrease in efficiency at high dietary protein levels may be attributed to the energetic cost of processing protein.

Food conversion ratio (FCR) is typically low in sea urchins (Hammer et al. 2004, Hammer, 2006). This is partially attributed to the fact that FCR calculations include the weight associated with the large volume of coelomic fluid which fills the body cavity of sea urchins (Hammer et al., 2004). Regardless, *L. variegatus* fed diets with high protein levels typically have a comparatively low FCR. Hammer (2006) reported FCRs as low as 0.56 in adult *L. variegatus* fed a high protein:high carbohydrate diet. FCRs calculated in the current study were generally higher than those reported by Hammer (2006), and most likely represent an increase in maintenance cost relative to energy available for growth when diets are sub-satiating. Individuals fed the 28% and 36% protein diets were more

efficient at converting food to biomass (although differences were not always significant) than those fed the 12% and 19% protein diets (Table 3). Sea urchins fed at 12% protein had comparatively high FCRs, indicating that a dietary protein level of 12%, while adequate for maintenance, contained inadequate dietary protein for efficient biomass increase.

The general shape of the sea urchin gut can be described as that of a tubular duct which begins in the Aristotle's lantern with the pharynx and loops in a counterclockwise pattern throughout the coelomic cavity and exits the body at the anus on the aboral surface (De Ridder and Jangoux, 1982). The digestive tract of the sea urchin is lined with an inner endothelial layer composed of simple columnar cells called enterocytes (Jangoux, 1982). Enterocytes serve, not only for nutrient absorption, but also for short term nutrient storage (Jangoux, 1982; Bishop and Watts, 1992; Hammer et al., 2006b; Gibbs et al., 2009). Typically, variations in gut size in sea urchins are in response to food availability instead of food quality (Hammer et al., 2006b; Bishop and Watts, 1992). Over the ranges tested in the current study, dietary carbohydrate and protein did not result in any changes in gut weight or gut production of individuals under the conditions of this study. Consequently, no other factor examined in this study (protein:carbohydrate ratio, total energy, or protein:energy ratio) affected gut production or growth. This is consistent with findings of other studies involving adult *S. franciscanus* (McBride et al., 1998) and adult *L. variegatus*, in which variations in dietary nutrients affected the biochemical composition of the gut but not gut mass or gut dry matter production (Hammer et al., 2006). Biochemical analysis was not performed on individuals in the current study, so it

is unknown whether or not variations in nutrients affected the composition of the gut tissue.

Sea urchin gonads are unique from those of other animals in that they are not only used for reproductive purposes but also for long term nutrient storage (Walker, 1982; Fernandez, 1997) As such, urchin gonads are made up of both gametic cells and storage cells called nutritive phagocytes. Although dietary carbohydrates appear to be stored primarily in the gonads (Marsh and Watts, 2007), under the conditions of this study, variations in dietary carbohydrate levels did not appear to affect the wet weight gain or dry matter production of the gonads. Schlosser et al. (2005) found decreased gonad production in *P. lividus* fed low (presumably inadequate) carbohydrate algal diets as compared to urchins fed a prepared diet with adequate carbohydrate energy, further suggesting that the range of dietary carbohydrate levels tested in the current study were adequate in all diets.

Dietary protein levels are often directly correlated with gonad production (de Jong-Westman et al., 1995; Fernandez, 1997; Barker et al., 1998; Cook et al., 1998; Meidel and Scheibling, 1999; Schlosser et al., 2005; Pearce et al., 2002b; Hammer et al., 2004; Chang et al., 2005; Hammer et al., 2006a; Marsh and Watts, 2007; Woods et al., 2008), and can possibly influence fecundity of individuals (Hammer et al., 2006b). Under the conditions of this study, wet gonad weight and, to a lesser extent, dry matter production were affected by dietary protein levels. Protein levels above 19% did not appear to further increase gonad weight gain or production, again suggesting that 19% may be close to an optimum level of dietary protein for *L. variegatus* at this life stage. Olave et al. (2001) found that gonad production in *L. albus* was higher at dietary protein

levels of 20% than at levels of 11 and 17%, but no diets with protein levels higher than 20% were examined for comparison. Gonadal growth of *S. droebachiensis* was maximized at 19-20% protein (Pearce et al., 2002b, McBride et al., 1998; de Jong-Westman, 1995). Adult *P. lividus* fed a 29% protein diet had significantly higher gonad index than those fed a 13% protein diet but the gonad index of individuals fed the 29% protein diet was not different than that of individuals fed a 47% protein diet (Fernandez et al., 1997), suggesting that dietary protein levels of 47% are excessive for *P. lividus*. Akiyama et al. (2001) found no statistical difference in gonad index among *P. depressus* fed diets with protein levels of 10, 20, 30 and 40%, but comparison of the somatic growth data shows that individuals fed the 10% diet were significantly smaller than individuals fed the higher protein diets, suggesting that gonad production was less in individuals fed the 10% protein feed. *L. variegatus* fed diets with 20% or 31% protein had significantly larger gonads at 32 days than urchins fed a diet with a 9% protein level, although at 65 days, there were no differences in gonad size between the 9, 20 and 31% protein diets (Hammer et al., 2006a).

Both high protein levels and protein source can adversely affect roe quality (Pearce et al., 2002a 2002b; Woods et al., 2008; Hoshikawa, 1998; Lawrence et al., 2001; Murata et al., 2001, 2002; Robinson et al., 2002; Senaratna et al., 2005; Woods et al. 2008). Additionally, protein is one of the most costly feed ingredients and excess protein contributes greatly to water fouling. As such, culturists must find the balance between optimal roe yield and cost savings. Results from this and other studies suggest that dietary protein levels around 19-20% may be optimal for use in aquaculture.

Nutritional studies have greatly increased knowledge of sea urchin nutritional requirements and sea urchin responses to variations in nutrients and nutrient ratios, but there is still much to learn (Watts et al., 2010). In addition to levels of dietary protein and carbohydrate, sea urchin growth and gonad production can vary in response to changing season and temperature (Hill and Lawrence, 2006; Gibbs et al., 2007; Lawrence et al., 2009), water quality (Basuyaux and Mathieu, 1999), life stage (Pearce et al., 2004; Watts et al., 2010), and other essential nutrients (Jones, 2007; Gibbs et al., 2009; Trawick, 2009; Watts et al., 2010). The development of large-scale sea urchin aquaculture techniques will depend upon our ability to answer questions surrounding these and many other nutritional issues.

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Chapter 3

FOOD QUALITY AFFECTS NUTRIENT ALLOCATION TO THE TEST AND ARISTOTLE'S LANTERN IN THE SEA URCHIN *LYTECHINUS VARIEGATUS*

INTRODUCTION

Natural environments are highly variable. To maximize fitness, organisms need the ability to adjust not only from generation to generation, but also at the phenotypic level with changes (plasticity) occurring within the lifetime of one individual (Newman, 1992; Scheiner, 1993; DeWitt, 1998). The energetic cost associated with maintaining plasticity of traits is higher than that of maintaining a fixed trait (Newman, 1992; Scheiner, 1993, DeWitt, 1998; Scheiner and Berrigan, 1998; Lau et al., 2009). However, the extra energetic investment may be worthwhile if survival of the individual is increased. Phenotypic plasticity is important in plants (Schlichting, 1986) and is probably also important in animals that are either sessile or unable to travel quickly to a habitat with more desirable conditions (Ebert, 1996; Russell, 1998). Given the longevity of phylum echinodermata and the sedentary nature of the individuals within it, a high degree of plasticity would be predicted.

Phenotypic plasticity is an adaptive response to an environmental stress or change (Ebert, 1980; Newman, 1992; Russell, 1997; Miner, 2005). Low food quality and low food availability are both stressors that lead to differential phenotypic expression in both

adult (Fernandez and Boudouresque, 1997; Ebert, 1996; Guillou, 2000) and larval echinoderms (Hart and Strathmann, 1994; Sewell et al., 2004; Miner, 2005; Podolsky and McAlister, 2005; Reitzel and Heyland, 2007; Morgan 2008). In echinoderm larvae, plasticity is often observed in structures used to procure, ingest, or digest food (Hart and Strathmann, 1994). The larval forms of many echinoderms have arms with ciliated bands that are used to collect food from the water column (Hart and Strathmann, 1994). Elongation of the arms and, consequently, the ciliated band has been observed under low food conditions in larvae of sea cucumbers (Morgan, 2008), sand dollars (Hart and Strathmann, 1994; Miner, 2007; Reitzel and Hayland, 2007), brittle stars (Podolsky and McAlister, 2005), and sea urchins (Strathmann et al., 1992; McEdward and Herrera, 1999; Sewell et al., 2004; Miner, 2005, 2007). Sea star larvae do not have arms until late larval stages. Nevertheless, larvae of the sea star *Pisaster ochraceus* adapt to low food conditions by developing a comparatively large mouth and stomach and a wider and longer body than well-fed larvae (Hart and Strathmann, 1994; George, 1999). In sea urchin larvae, an inverse relationship between stomach size and arm length has been observed for *Strongylocentrotus franciscanus*, *Strongylocentrotus purpuratus*, and *Paracentrotus lividus* (Strathmann et al, 1992; Miner, 2005). The larger gut of well-fed larvae was originally attributed to distention by food (Miner, 2005). However, data indicate that structural changes occur before the larvae are mature enough to feed, suggesting that environmental cues may direct individuals to preferentially divert resources towards increasing the ability to procure nutrients when food is limited (Miner, 2005). To offset the energetic cost of increased arm length, gut tissue, which is costly for the individual to build and maintain, is sacrificed (Miner, 2005).

The plastic response of adult echinoderms under varying food conditions is similar to that of larvae. However, adults must also invest energy in reproduction. Under conditions of low food or low food quality many adult echinoderms will alter the allocation of resources used for gonadal or gametic growth and development (Lawrence and Lane, 1982; reviewed by Chia and Walker, 1991; Hendler, 1991; Lawrence et al. 1997; Minor and Scheibling, 1997; George, 1999; Guillou, 2000; Meidel and Scheibling, 1999; Olave et al., 2001; Spirlet et al., 2001; Pearce et al., 2002a, 2002b; Otero-Villanueva et al., 2003; Senaratna et al., 2005; Pita, 2008; Woods et al., 2008). Reproductive adaptations vary among echinoderms and may affect the age at which individuals reach sexual maturity (McShane and Anderson, 1997), the quantity and size of eggs produced (Lasker and Giese, 1954; George, 1999), the quality of gametes (Lasker and Giese, 1954; George, 1999), seasonal spawning cycles (Hendler, 1991) and gonad production (Lasker and Giese, 1954; Ebert, 1968; Dix, 1972; Fernandez and Boudouresque, 1997; Beddingfield and McClintock, 1998; Guillou, 1999; Martínez-Pita, 2008; Woods et al., 2008; Lau et al., 2009).

Plasticity of the feeding apparatus has been observed in several echinoderm species (reviewed by Levitan, 1991). Under food limiting conditions, the lantern has been reported to be comparatively larger than when food is plentiful (Ebert, 1968, 1980; Black et al., 1984; Levitan, 1991; Fernandez and Boudouresque, 1997; McShane and Anderson, 1997; Hagen, 2007; Lau et al., 2009). Some studies suggest that the difference in lantern index is, in part, due to remodeling of the test and lantern, resulting in decreased test size and a comparatively large lantern (Ebert, 1968, 1980; Levitan, 1991; Guillou, 2000). Decrease in test size is often attributed to re-absorption of organic material stored in the

body wall (Constable, 1993; Guillou, 2000) Ebert (1996) also suggested that a decrease in test diameter may contribute to an increase in fitness of an individual because a smaller body wall requires fewer resources for basic maintenance and upkeep. Ebert (1968) reported low availability of food for *Strongylocentrotus purpuratus* resulted in a decrease in test diameter and an increase in lantern index. However, these results conflicted with those of Fansler (reviewed by Ebert, 1996, 2004), who reported variations in lantern index among *S. purpuratus* held under varying food conditions, but observed no decrease in test diameter. Ebert (2004) later re-examined data from the 1968 study and concluded that for that particular study, the differences in test diameter were probably a result of human error rather than a decrease in test diameter. Regardless, a decrease in test diameter has been observed in several other species (reviewed by Ebert, 2004).

Diadema setosum starved for one year were reported to have remodeled the calcareous tissue of both the test and Aristotle's lantern (as evidenced by the absence of a tetracycline marker), creating a lantern that was larger relative to test diameter than that of fed individuals (Ebert, 1980). A similar study with *Diadema antillarum* found that when food was slightly limiting the test shrank while the demipyramids of the Aristotle's lantern continued to grow at a decreased rate (Levitan, 1991). However, when *D. antillarum* were held under severely limiting conditions, the test was still remodeled, but the lantern either stopped growing altogether or shrank slightly, resulting in a lantern that was relatively large in comparison to the test (Levitan, 1991). Test indices of *Paracentrotus lividus* held in a seagrass bed with abundant high-quality food differed significantly from those of individuals held in a pebble field where food was scarce and of poor nutrient value (Fernandez and Boudouresque, 1997). A decreased lantern index

was also observed when quality food was readily available (Fernandez and Boudouresque, 1997). McShane and Anderson (1997) used tag-recapture methods to determine that *Evechinus chloroticus* from a food-rich habitat grow larger and have comparatively smaller lanterns than those from a low-food habitat. A study with *Strongylocentrotus droebachiensis* showed no significant differences in relative lantern size or test diameter among urchins fed various algae for six weeks (Russell, 1997). However, Minor and Scheibling (1997) observed plasticity in the lantern of *S. droebachiensis* during a 12 week study. Russell (1998) reports that others have also observed plasticity in the lantern of *S. droebachiensis* and conceded that 6 weeks may have been an insufficient time for change to occur at a noticeable level. Likewise, *Lytechinus variegatus* starved for 2.5 months re-absorbed organic matter from the body wall but did not shrink in terms of test diameter, possibly because of the short experiment time (Lares, 1998). Lantern index was not reported for these individuals.

The functional significance of a comparatively large jaw apparatus is not entirely understood. It has been suggested that the increased size may, in part, be an effect of increased size of the muscles associated with the jaws due to increased scraping effort (Ebert, 1980). Another explanation is that a large lantern may help an individual procure more food because it allows the urchin to graze upon a larger surface area (Ebert, 1980, 1996; Black, 1984; Minor and Scheibling 1997; Lau et al., 2009). Among *Echinometra mathaei* held under identical conditions, those with a relatively large lantern were found to be more efficient food gatherers than those with a relatively small lantern (Black et al., 1984). It was also observed that *E. mathaei* with a large lantern index were able to eat higher quantities of coralline algae, considered a difficult foodstuff to eat (Black et al.,

1984). A comparison of *Strongylocentrotus pallidus*, a seldom-studied species with a large lantern index, and *S. drobachiensis*, a destructive grazer with a low lantern index, showed similar results in that individuals with a relatively large lantern were able to eat harder, more unwieldy prey than individuals with a relatively small lantern (Hagen, 2007). However, Hagen (2007) reports that individuals with a larger lantern did not consume more total food than those with a small lantern.

While differential resource allocation must certainly increase the fitness of sea urchins, plasticity of Aristotle's lantern is not fully understood. Additional studies are needed before we understand which environmental cues induce individuals to direct resources toward manufacture of a large jaw apparatus and exactly how these changes occur. The advantage to the individual must be great enough to outweigh the cost of both maintaining plasticity of the trait and undergoing the morphological changes. However, it remains unclear as to the exact nature of the benefit and whether it bestows a single advantage upon an individual or is multifaceted.

Although other studies have evaluated gross food quantity in affecting lantern/test size, the effect of specific nutrients has only been investigated in a single report. Jones et al. (2010) suggested that dietary manipulation of the mineral selenium affected the ratio of lantern weight to test weight, suggesting specific nutrients may affect phenotypic variation in lantern and/or test size. Consequently, those nutrients which contribute to energy production or utilization may ultimately affect body organ allometry. In sea urchins, protein and carbohydrates are the two primary energy sources used for maintenance and growth (Marsh and Watts, 2007). Protein, carbohydrate, and their inherent value as energy sources affected total body weight gain in *L. variegatus* (Chapter

2), and dietary changes in their profiles could potentially affect organ growth in the test and lantern. The purpose of this study is to examine the effect of variations in dietary protein and carbohydrate level, protein:energy ratio, protein:carbohydrate ratio, and total dietary energy level on growth and relative size of the test and Aristotle's lantern in the sea urchin *Lytechinus variegatus*.

MATERIALS AND METHODS

Collection and Initial Measurements

Adult *Lytechinus variegatus* (ca. 19.5 ± 2.01 g initial wet weight) were collected from St. Joseph's Bay (30°N, 85.5°W), FL and transported to Texas AgriLIFE Mariculture Research Laboratory in Port Aransas, TX. Nineteen individuals were randomly selected for initial evaluation. Individuals were weighed (to the nearest mg) and photographed for diameter measurements (to the nearest mm) using ImageJ® software. Urchins were dissected with a circular incision around the peristomial membrane on the oral surface. The gut, gonads and Aristotle's lantern (with muscles and pharynx) were removed. The interior surfaces of the test were scraped with a spatula to remove any remaining soft tissue, and both test and lantern were blotted to remove excess moisture. Wet weights were recorded for test and Aristotle's lantern. Organs were dried in a 60°C oven for 48 hours, and dry weights were recorded. Mean dry organ and total dry weights were calculated for the initial sub-sample population and used as an estimate for initial dry organ and total dry weights for the remaining 64 urchins used in the study. Initial wet weights and diameters were recorded for the remaining urchins. These individuals were assigned at random to one of eight dietary treatments (n=8 per diet).

Culture Conditions

Experimental urchins were held in a semi-recirculating system with both mechanical and biological filtration and UV sterilization. The culture system (2400 L) was comprised of 72 interconnected 20 L fiberglass tanks containing water distributed from a central sump, 16 of which were used for this feed trial. Each tank held four cylindrical plastic mesh cages (12 cm dia., 30 cm height, and a 4 mm open mesh). Each plastic cage was inserted into a PVC coupling (11.5 cm I.D.) and elevated with PVC spacers to allow unimpeded water circulation throughout the cage. Each cage housed one individual.

Water volume in each tank was maintained by a central standpipe, and natural seawater was supplied to each mesh enclosure at a ca. rate of 25 L hr⁻¹ (water exchange rate of 3000% per day). Fresh seawater was passed through a stratified sand filter and a Diamond water filter (Diamond Water Conditioning, Horton, WI). Water in the entire culture system was exchanged at a rate of 10% per day. Water quality parameters were determined by color metric analysis.

Feed and Feed Preparation

Eight semi-purified diets were formulated and produced using both purified and practical ingredients. Levels of dietary protein and carbohydrate (Table 1, Table 2) ranged from 12 to 36 % protein and 21 to 39% carbohydrate. Levels were adjusted with acid washed diatomaceous earth, which does not have an affect on sea urchins at the levels used in these diets (unpublished data). All other nutrients were constant among treatments. Dry ingredients were mixed with a PK twin shell® blender (Patterson-Kelley

Co., East Stroudsburg, PA) for 10 minutes. Dry ingredients were then transferred to a Hobart stand mixer (Model A-200, Hobart Corporation, Troy, OH) and blended for 40 minutes. Liquid ingredients were added, and the mixture was blended for an additional 10 minutes to a mash-like consistency. The diets were extruded using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Feed strands were separated and dried on wire trays in a forced air oven (35°C) for 48 hours. Final moisture content of all feed treatments was 8–10%. Feed was stored in air-tight storage bags at 4°C until used.

Table 1. Calculated protein and carbohydrate levels (as fed), total energy, protein: energy, and protein: carbohydrate ratios in each of the eight diets tested.

Protein (%)	Carbohydrate (%)	Total Energy (cal/g)	Protein:Energy (mg P/kcal)	Protein:Carbohydrate Caloric Ratio
36	21	3749	95	1.7
28	30	3299	76	0.93
19	21	2783	68	0.90
19	30	3130	60	0.63
19	39	3478	54	0.49
12	21	2380	50	0.57
12	30	2727	44	0.40
12	39	3075	39	0.31

Table 2. Proximate composition of the formulation^a used to produce diets varying in protein and carbohydrate levels.

	12% P: 21% C	12% P: 30% C	12% P: 39% C	19% P: 21% C	19% P: 30% C	19%P: 39% C	28% P: 30% C	36% P: 21% C
Crude protein (%)	12	12	12	19	19	19	28	36
Carbohydrate (%)	21	30	39	21	30	39	30	21
Fiber (%)	4.5	4.5	4.5	4.5	4.5	4.5	4.6	4.6
Diatomaceous Earth (DE, %)	27	18	9	19	10	1	0	0
Non-DE Ash (%)	24	24	24	24	24	24	24	25
Crude fat (%)	7	7	7	7	7	7	7	7

All values are approximate, calculated, and on an “as fed” basis unless otherwise indicated.

a All diets contain up to 28% marine ingredients, 28.7% plant ingredients, 1.1% carotenoids, 0.7% vitamin premix, 24 % mineral mix, 7.2% binder and antifungal-antioxidant.

Feeding Rate

Each sea urchin was proffered a limiting daily ration equal to 1.5% (sub-satiation) of the initial average wet body weight. Approximately 1.5% of wet body weight per day is a sub-satiation ration for adult *Lytechinus variegatus* of this size class, whereas a ration equivalent to or above 3% of body weight is *ad libitum* (unpublished data). Feeding at sub-satiation ensured that urchins consumed all of their food in a 24 hour period and allowed for direct measure of feed intake. A sub-satiation feeding regime also prevented individuals from compensating for a dietary deficiency by increasing consumption. Individuals were weighed every three weeks and feed rations were adjusted to be equivalent to 1.5% of the average body weight (Table 3). Feces were siphoned out just prior to feeding each day.

Daily feeding rate was calculated as:

$$(1) \text{ Wet weight of individual (g) } \times 0.015$$

Protein:energy ratio of each feed was calculated as:

$$(2) \text{ Protein (mg) / energy content (Kcal)}$$

Total energy content of each feed was calculated based on the methods of Phillips (1972):

$$(3) \% \text{ protein} / 100 \times 5650 (\text{cal g}^{-1}) + \% \text{ carbohydrate} / 100 \times 4000 (\text{cal g}^{-1}) + \% \text{ lipid} / 100 \times 9450 (\text{cal g}^{-1})$$

After nine weeks, urchins were dissected as previously described.

Growth

To measure test diameters, urchins were photographed and diameters were measured (mm) using ImageJ® software. Test diameters did not differ among treatments at time 0. Test diameter was measured every three weeks. Diameter increase was calculated as:

$$(4) \text{ Final diameter (mm)} - \text{initial diameter (mm)}$$

Estimated Aristotle's lantern and test dry matter production were calculated for each individual as:

$$(5) \text{ Final dry weight of organ (g)} - \text{initial average dry weight of organ (g)}$$

Final dry Aristotle's lantern and test indices to final dry weight of the individual were calculated as:

$$(6) \text{ Dry weight of organ (g) / dry weight of individual (g)} \times 100$$

Final dry Aristotle's lantern to final dry test index was calculated as:

$$(7) \text{ Dry weight of Aristotle's lantern (g) / dry weight of test (g)} \times 100$$

Percent Organic Matter of Test and Spines

For each individual, a ¼ section of the dry test was analyzed, with oral and aboral plates and spines removed from a lateral quadrant (not including the peristomial membrane). Test plates and spines were placed separately in pre-weighed crucibles. Initial weight of the combined crucible and tissue was recorded, and tissues were placed in a muffle furnace at 500°C for 4 hours. After cooling, the combined weight of the crucible and tissue was recorded, and the percent organic matter was calculated.

Statistics

Statistical analyses were performed to compare mean test and Aristotle's lantern wet weight gain and dry matter production, and percent organic matter of the test and spines. All statistical comparisons were performed using SAS System 9.2. A *p* value of <0.05 was considered statistically significant. Data were tested for normality with a Kolmogorov-Smirnov test and for equality of variance using a Levene's test. Data were found to be normal and were analyzed by ANOVA using the GLM procedure to determine whether there were any dietary effects. If a significant difference ($p < 0.05$) was detected, a Duncan's Multiple Range Test was used to compare means. Aristotle's lantern and test indices were evaluated by trend analysis in terms of five factors: Total energy content of treatment (cal g^{-1}), protein:energy ratio (mg P kcal^{-1}), protein:carbohydrate ratio (mg P mg C^{-1}), % protein, and % carbohydrate. Associations were modeled with 2nd degree polynomial functions. R^2 values (from 2nd degree polynomials) were determined to show the correlation between each factor and the parameter being tested.

RESULTS

Water Quality

Water conditions were maintained as follows: 32 ± 0.5 ppt salinity, $22 \pm 2^\circ\text{C}$, D.O. 7 ± 2 ppm., ammonia 0 ppm, nitrite 0 ppm, nitrate 0 ppm, and pH 8.2. A 12:12 light:dark photoperiod was maintained.

Consumption

All individuals were fed a sub-satiation ration (1.5% of average body weight). This was verified by visual observation. There was no feed remaining when sea urchins were fed the following day.

Test Analysis

Increase in diameter of urchins receiving the 12:21% protein:carbohydrate diet were significantly less at 9 weeks than that of urchins receiving the 19:21% protein:carbohydrate, the 28:30% protein:carbohydrate and the 36:21% protein:carbohydrate diets ($p < 0.003$, Table 3). After 9 weeks, the increase in diameter among urchins receiving the 12% protein diets did not vary significantly from each other ($p < 0.003$, Table 3). Increase in diameter among the 19% protein diets did not vary from each other after 9 weeks ($p < 0.003$, Table 3). Increase in diameter of individuals fed the 36:21% protein:carbohydrate diet was significantly greater than that of urchins fed the 19:39% protein carbohydrate diet after 9 weeks ($p = 0.003$, Table 3), but not those fed the 28% protein.

After 9 weeks, test wet weight gain was significantly lower in urchins receiving the 12% protein diets than in the 36 and 28% protein diets ($p < 0.0001$, Table 3). Test wet weight gain did not differ among individuals fed the 12:30% protein:carbohydrate; the 12:39% protein:carbohydrate and the 19% protein diets after 9 weeks (Table 3). Test wet weight gain was closely correlated to dietary protein level ($R^2 = 0.9310$, Fig. 1b) and, to a lesser extent, protein:energy ratio ($R^2 = 0.8690$, Fig. 1a). Protein:carbohydrate ratio was a moderately good predictor of test wet weight ($R^2 = 0.7718$, Fig. 2d), as was total energy ($R^2 = 0.6415$, Fig. 1e) but dietary carbohydrate levels did not correlate with test wet weight gain ($R^2 = 0.1396$, Fig. 1c).

After 9 weeks, test dry matter production of individuals receiving 36:21% protein:carbohydrate and the 28:30% protein:carbohydrate diets was significantly higher than that of individuals receiving the 12 % protein and the 19:39% protein:carbohydrate diets ($p < 0.0001$, Table 3). Test dry matter production did not vary significantly among individuals fed the 12% protein, the 19:30% protein:carbohydrate, and the 19:39% protein:carbohydrate diets (Table 3). However, test dry matter production of individuals fed the 12:21% protein:carbohydrate diet was significantly less than test dry matter production of individuals fed the 19:21% protein:carbohydrate diet ($p < 0.0001$, Table 3). After 9 weeks, individuals fed the 36% protein diets had significantly higher test dry matter production than individuals in all other treatments except the 28% protein diet ($p < 0.0001$, Table 3). Test dry matter production was closely correlated with dietary protein level ($R^2 = 0.9569$, Fig. 2b) but was not affected by dietary carbohydrate level ($R^2 = 0.0102$, Fig. 2c). Test dry matter production varied proportionally with protein:energy ratio ($R^2 = 0.7681$, Fig. 2a), protein:carbohydrate ratio ($R^2 = 0.6240$, Fig. 2d), and total

energy ($R^2=0.8442$, Fig. 2e), but none of these factors were as closely correlated as dietary protein level.

Percent organic matter of the test varied among dietary treatments (Table 4). Tests of individuals receiving the 19% protein diets had significantly higher percent organic matter than tests of individuals receiving the 12% diets ($p<0.001$, Table 4). Percent organic matter of tests was significantly higher among individuals receiving the 19:30% and 19:39% protein:carbohydrate diets than among individuals receiving the 28% protein diet ($p<0.0001$, Table 4). Dietary protein level was correlated with test percent organic matter ($R^2=0.6525$, Figure 3b). Protein:energy ratio was a moderate predictor of test percent organic matter ($R^2=0.4516$, Fig. 3a). Test percent organic matter was not affected by dietary carbohydrate level ($R^2=0.024$, Fig. 3c).

Spine Analysis

Percent organic matter of the spines varied among dietary treatments (Table 4). Spines of individuals fed the 19:21% protein:carbohydrate and 36:21% protein:carbohydrate diets had a significantly higher percent organic matter than spines of individuals fed the 12:30% protein:carbohydrate, 12:39% protein:carbohydrate, and the 28:30% protein:carbohydrate diets (Table 4). Percent organic matter of the spines did not seem to correlate to total dietary energy ($R^2=0.0969$, Fig. 4e) and was not closely correlated with dietary protein ($R^2=0.2286$). All other factors examined (protein:energy ratio, dietary carbohydrate level, and protein:carbohydrate level) were positively correlated with percent organic matter of the spines (Fig. 4a-d).

Lantern Analysis

After 9 weeks, wet weight gain of the lantern did not vary among treatments (Table 5) and, consequently, was not affected by any of the dietary factors examined (protein:energy ratio, dietary protein level, dietary carbohydrate level, protein:carbohydrate level, and total energy, Fig 5a-e). Dry matter production of the lantern did not vary among treatments (Table 5) or among dietary factors (protein:energy ratio, dietary protein level, dietary carbohydrate level, and protein:carbohydrate level, total energy, Fig. 6a-e).

Dry lantern indices varied among dietary treatments whether the index was relative to dry test weight (L/T) or to total sea urchin dry weight (L/DWt) (Table 5). Lantern weight to test weight index did not vary between the 12% and 19% protein diets (Table 5). However, L/T index was significantly greater in the 28% protein and 36% protein diets than in all other dietary treatments except the 19:39% protein:carbohydrate diet ($p=0.007$, Table 5). L/T index was highly correlated with dietary protein level ($R^2=0.9118$, Fig.6b), and to a lesser extent with dietary protein:energy ratio ($R^2=0.8319$, Fig. 7a) and total dietary energy ($R^2=0.8363$, Fig. 7e).

The lantern index calculated as L/DWt, lantern index did not vary among individuals in the 12% protein and 19% protein diets after 9 weeks (Table 5). No significant differences in L/DWt index were detected between 28% protein diet and the 36% protein diet (Table 5). The L/DWt index of individuals fed the 28% protein:carbohydrate and the 36% protein:carbohydrate diets did not vary significantly from individuals fed the 19:21% protein:carbohydrate or the 19:39% protein:carbohydrate diets, yet L/DWt index was significantly less in individuals fed the

28% protein and the 36% protein:carbohydrate diets than in individuals fed the 19:30% protein:carbohydrate diets ($p < 0.0007$, Table 5). As with L/T, L/DWt index was highly correlated with dietary protein levels ($R^2 = 0.9920$, Fig. 8b) and, to a lesser extent, protein:energy ratio ($R^2 = 0.8706$, Fig. 8a) and total dietary energy ($R^2 = 0.8428$, Fig. 8e).

Table 3: Mean final test wet weight, test wet weight gain, final dry test index, final test production and test diameter increase of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein:energy ratios (P:E), total energy (TE), and protein:carbohydrate ratios (P:C). P:E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P:C represents protein:carbohydrate ratio (mg/mg). Letters indicate statistical differences among diets (P<0.05).

% Protein	% Carbohydrate	P:E (mg P/kcal)	TE (cal/g)	P:C	Final Wet Test Weight (g)	Wet Test Weight Gain (g)	Final Dry Test Weight (g)	Dry Test Production (g)	Initial Diameter (mm)	Final Diameter (mm)	Diameter Increase (mm)
12	21	50.8	2380	0.5	13.72	6.87 +/-	7.19 +/-	3.50 +/-	38.75 +/-	49.94 +/-	11.19 +/-
				7	+/- 0.69	0.69	D	0.33	0.33	D	0.91
12	30	44.3	2728	0.4	14.65	7.80 +/-	7.46 +/-	3.77 +/-	38.22 +/-	49.96 +/-	11.74 +/-
				0	+/- 0.57	0.57	CD	0.24	0.24	CD	0.82
12	39	39.3	3075	0.3	14.57	7.72 +/-	7.58 +/-	3.89 +/-	38.15 +/-	50.38 +/-	12.23 +/-
				1	+/- 0.65	0.65	CD	0.33	0.33	CD	0.69
19	21	68.6	2783	0.9	16.60	9.75 +/-	8.26 +/-	4.56 +/-	38.07 +/-	52.47 +/-	14.39 +/-
				0	+/- 0.68	0.68	BC	0.29	0.29	BC	0.76
19	30	60.9	3131	0.6	16.39	9.54 +/-	8.14 +/-	4.44 +/-	38.09 +/-	50.89 +/-	12.80 +/-
				3	+/- 0.90	0.90	BC	0.36	0.36	D	0.88
19	39	54.9	3478	0.4	15.31	8.46 +/-	7.60 +/-	3.91 +/-	38.68 +/-	51.02 +/-	12.63 +/-
				9	+/- 0.41	0.41	CD	0.25	0.25	CD	1.43
28	30	76.9	3647	0.9	18.22	11.37	9.08 +/-	5.39 +/-	38.00 +/-	53.47 +/-	15.47 +/-
				3	+/- 0.97	+/- 0.97	AB	0.42	0.42	AB	1.17
36	21	95.6	3749	1.7	18.84	11.99	9.31 +/-	5.61 +/-	39.07 +/-	55.37 +/-	16.30 +/-
				1	+/- 0.57	+/- 0.57	A	0.25	0.25	A	1.22

Initial average wet test weight was 6.85 +/- 0.17 g

Initial average dry test weight was 3.65 +/- 0.33 g

Table 4: Mean test and spine organic matter (%) of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein:energy ratios (P:E), total energy (TE), and protein:carbohydrate ratios (P:C). P:E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P:C represents protein:carbohydrate ratio (mg/mg). Letters indicate statistical differences among diets ($P < 0.05$).

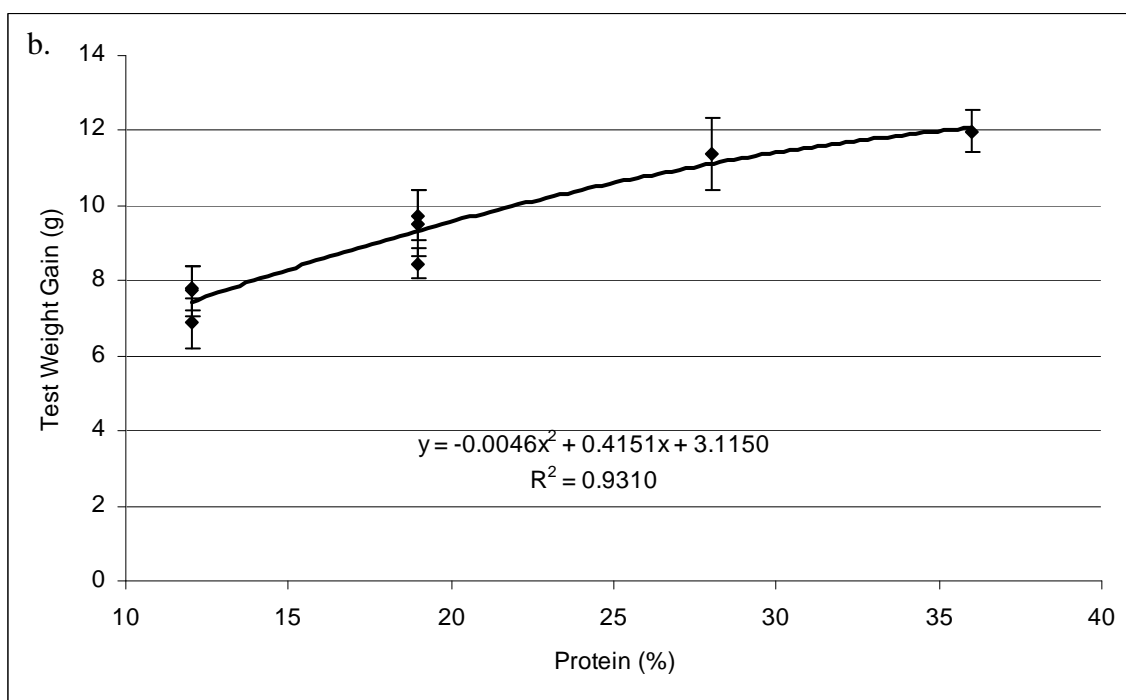
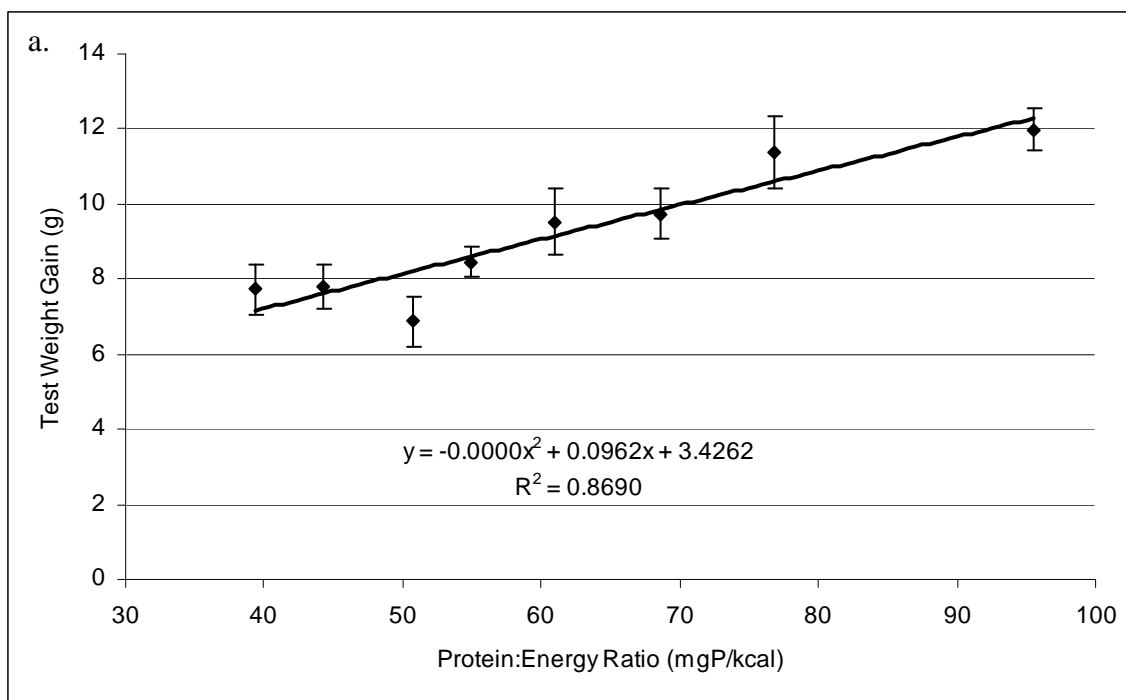
% Protein	% Carbohydrate	P:E (mg P/kcal)	TE (cal/g)	P:C	Test Organic Matter (%)		Spine Organic Matter (%)	
12	39	39.3	3075	0.31	12.36 +/- 0.34	EF	10.04 +/- 0.29	B
12	30	44.3	2728	0.40	12.78 +/- 0.19	DEF	10.02 +/- 0.22	B
12	21	50.8	2380	0.57	12.16 +/- 0.28	F	10.92 +/- 0.32	AB
19	39	54.9	3478	0.49	14.26 +/- 2.03	AB	10.65 +/- 0.42	AB
19	30	60.9	3131	0.63	14.54 +/- 0.42	A	10.97 +/- 0.25	AB
19	21	68.6	2783	0.90	14.01 +/- 0.22	ABC	11.43 +/- 0.34	A
28	30	76.9	3647	0.93	13.23 +/- 0.24	CDE	10.28 +/- 0.42	B
36	21	95.6	3749	1.71	13.51 +/- 0.16	BCD	11.40 +/- 0.38	A

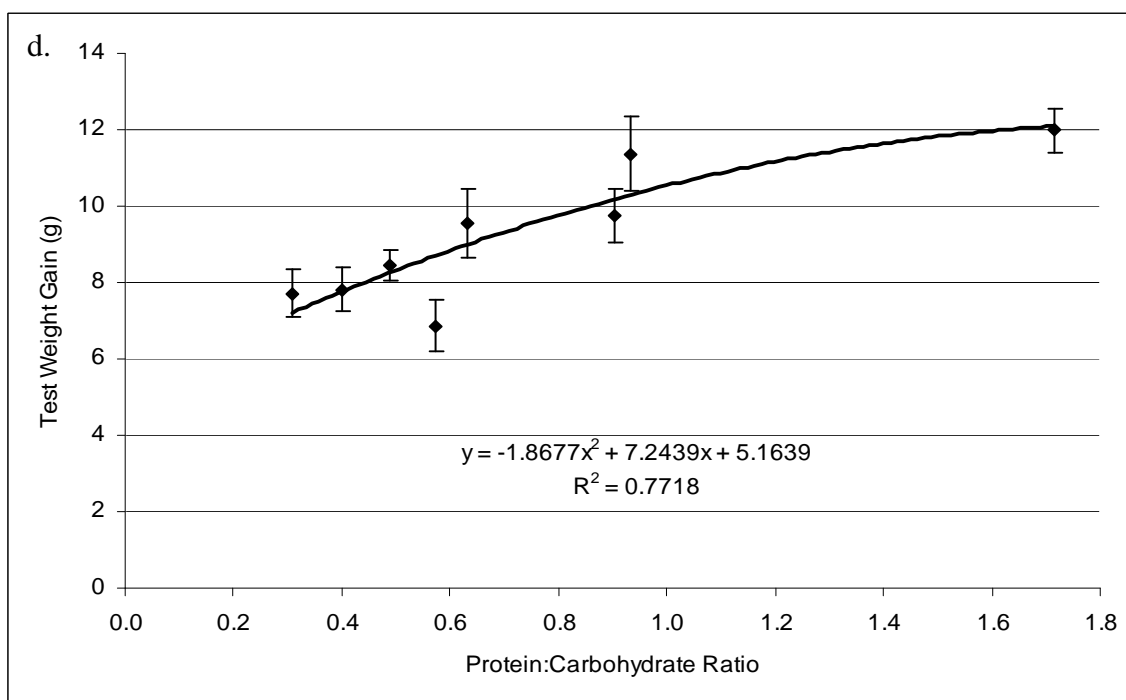
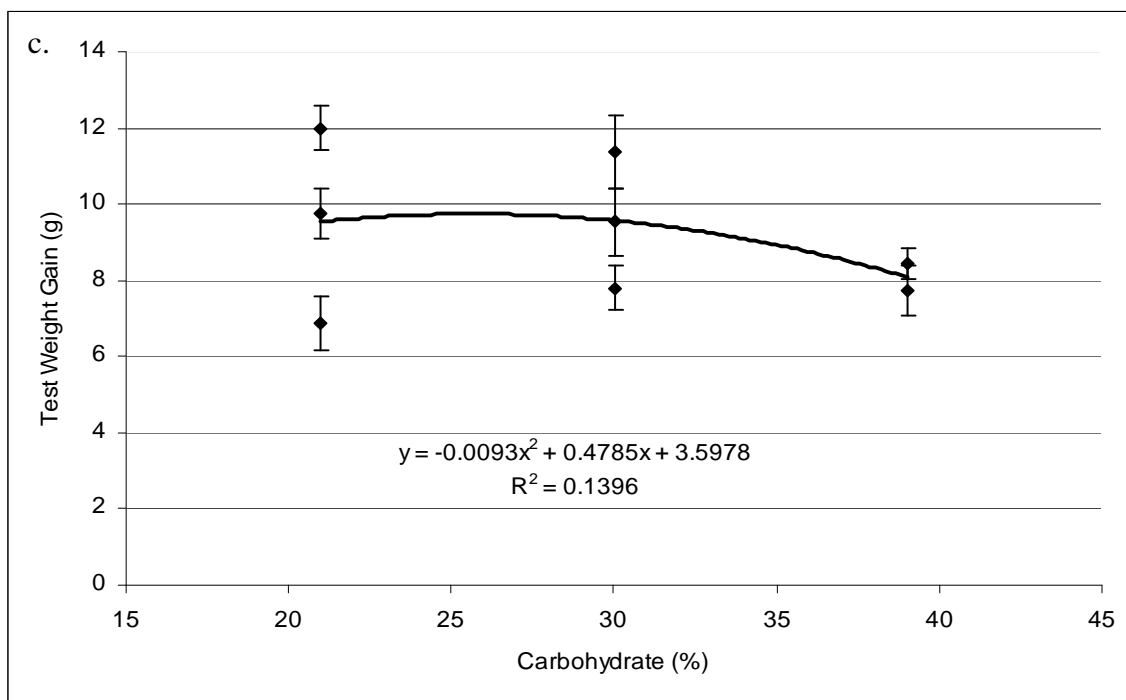
Table 5: Mean final lantern wet weight, lantern wet weight gain, lantern production, dry lantern/dry total weight (L/DWt) index, dry lantern/dry test index (L/T, \pm SE) of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein:energy ratios (P:E), total energy (TE), and protein:carbohydrate ratios (P:C). P:E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P:C represents protein:carbohydrate ratio (mg/mg). Letters indicate statistical differences among diets ($P < 0.05$).

% Protein	% Carbohydrate	P:E (mg P/kcal)	TE (cal/g)	P:C	Final Wet Lantern Weight (g)	Wet Lantern Weight Gain (g)	Final Dry Lantern Weight (g)	Dry Lantern Production (g)	Lantern Index (L/DWt, %)	Lantern Index (L/T, %)
12	21	50.8	2380	0.57	1.07 +/- 0.09	0.34 +/- 0.09	0.64 +/- 0.05	0.22 +/- 0.05	6.96 +/- 0.00	8.93 +/- 0/60
12	30	44.3	2728	0.40	1.19 +/- 0.05	0.45 +/- 0.05	0.67 +/- 0.01	0.25 +/- 0.01	6.91 +/- 0.00	9.04 +/- 0.22
12	39	39.3	3075	0.31	1.17 +/- 0.05	0.43 +/- 0.05	0.69 +/- 0.02	0.27 +/- 0.02	6.94 +/- 0.00	9.20 +/- 0.30
19	21	68.6	2783	0.90	1.17 +/- 0.05	0.43 +/- 0.05	0.69 +/- 0.02	0.27 +/- 0.02	6.30 +/- 0.00	8.43 +/- 0.47
19	30	60.9	3131	0.63	1.19 +/- 0.07	0.45 +/- 0.07	0.71 +/- 0.03	0.29 +/- 0.03	6.50 +/- 0.00	8.72 +/- 0.27
19	39	54.9	3478	0.49	1.04 +/- 0.08	0.30 +/- 0.07	0.63 +/- 0.05	0.21 +/- 0.05	6.30 +/- 0.01	8.33 +/- 0.67
28	30	76.9	3647	0.93	1.09 +/- 0.05	0.35 +/- 0.05	0.64 +/- 0.04	0.22 +/- 0.04	5.28 +/- 0.00	7.08 +/- 0.40
36	21	95.6	3749	1.71	1.16 +/- 0.05	0.43 +/- 0.05	0.66 +/- 0.32	0.24 +/- 0.32	5.43 +/- 0.00	7.12 +/- 0.31

Initial average lantern wet weight was 0.74 +/- 0.04 g

Initial average lantern dry weight was 0.64 +/- 0.05 g





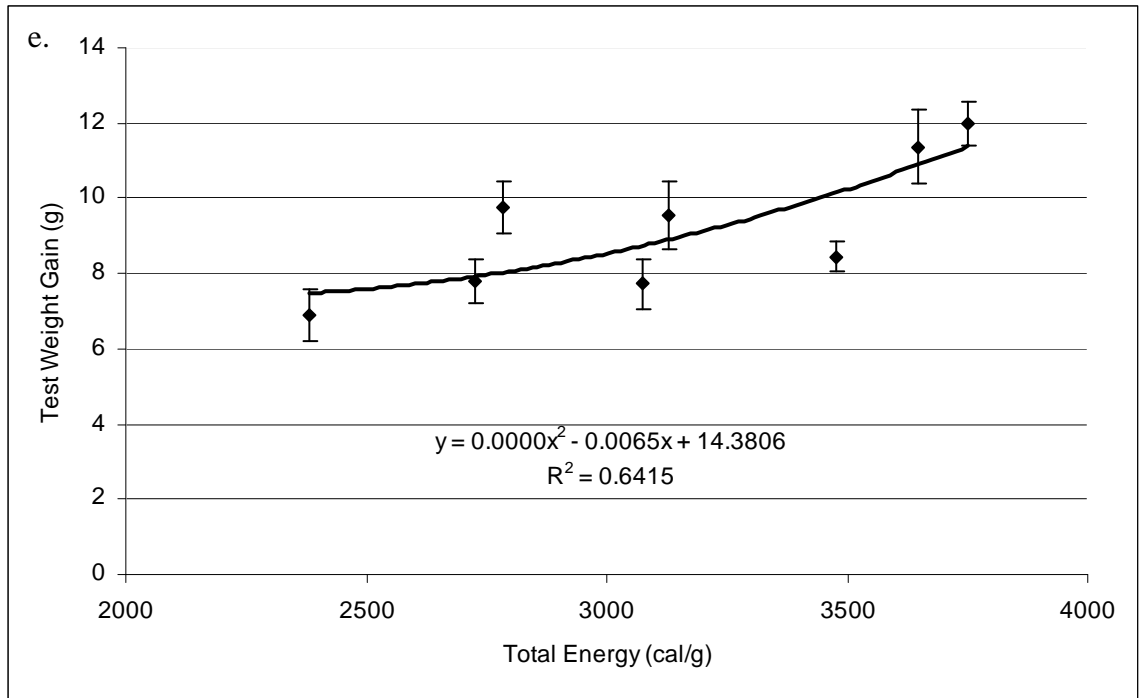
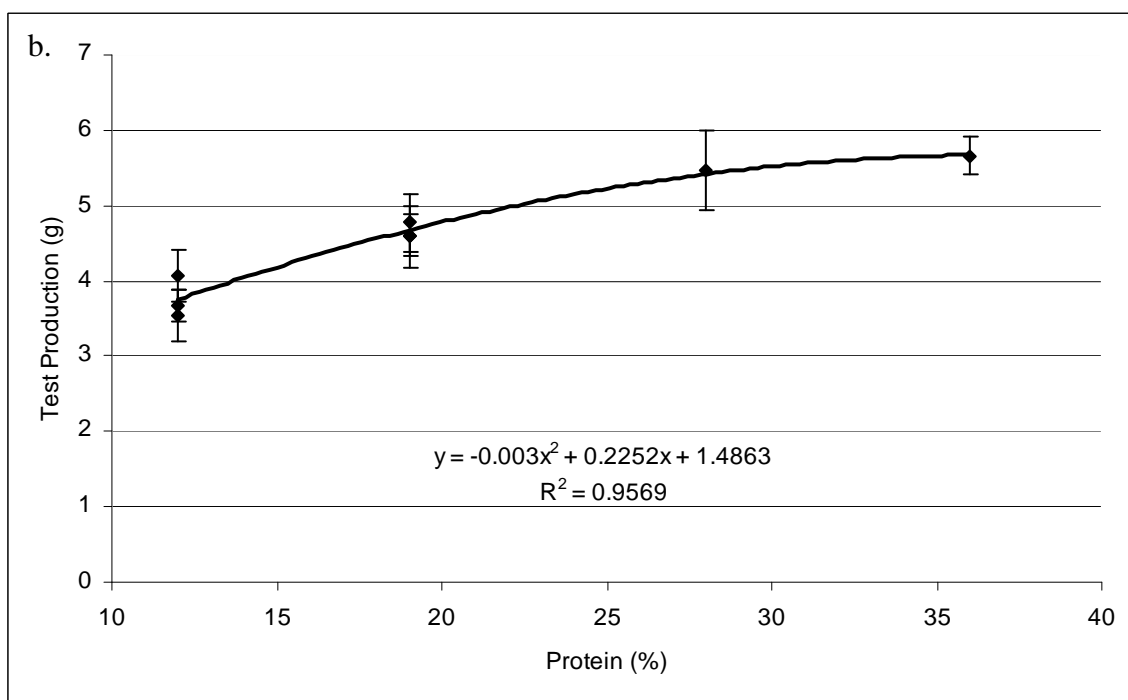
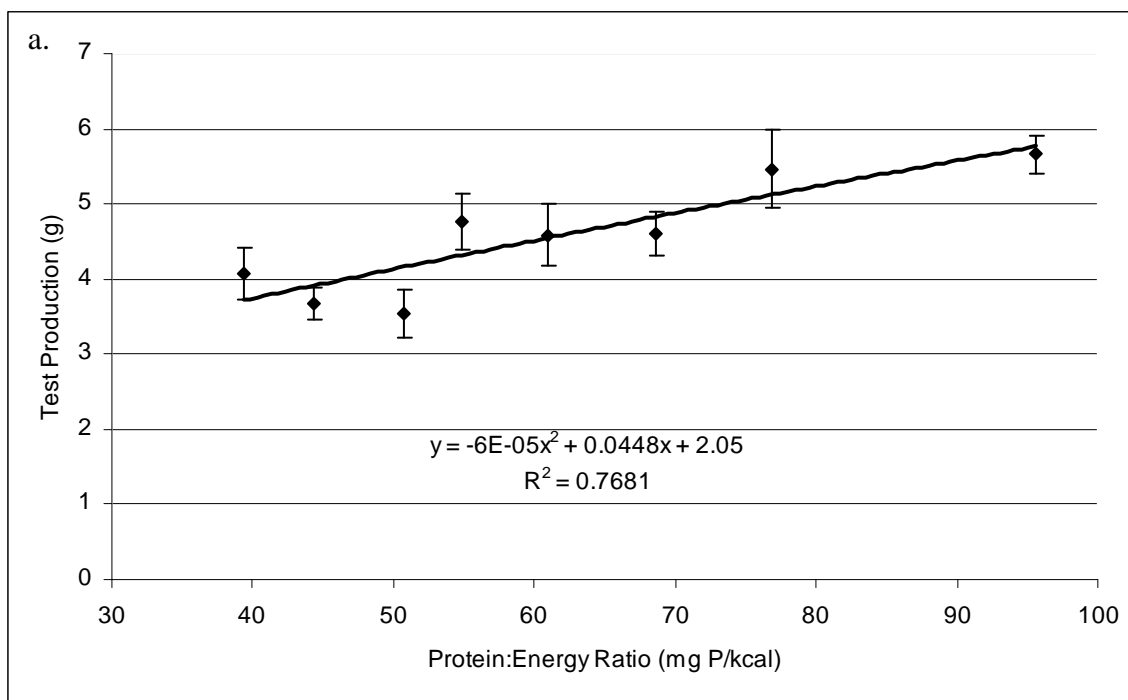
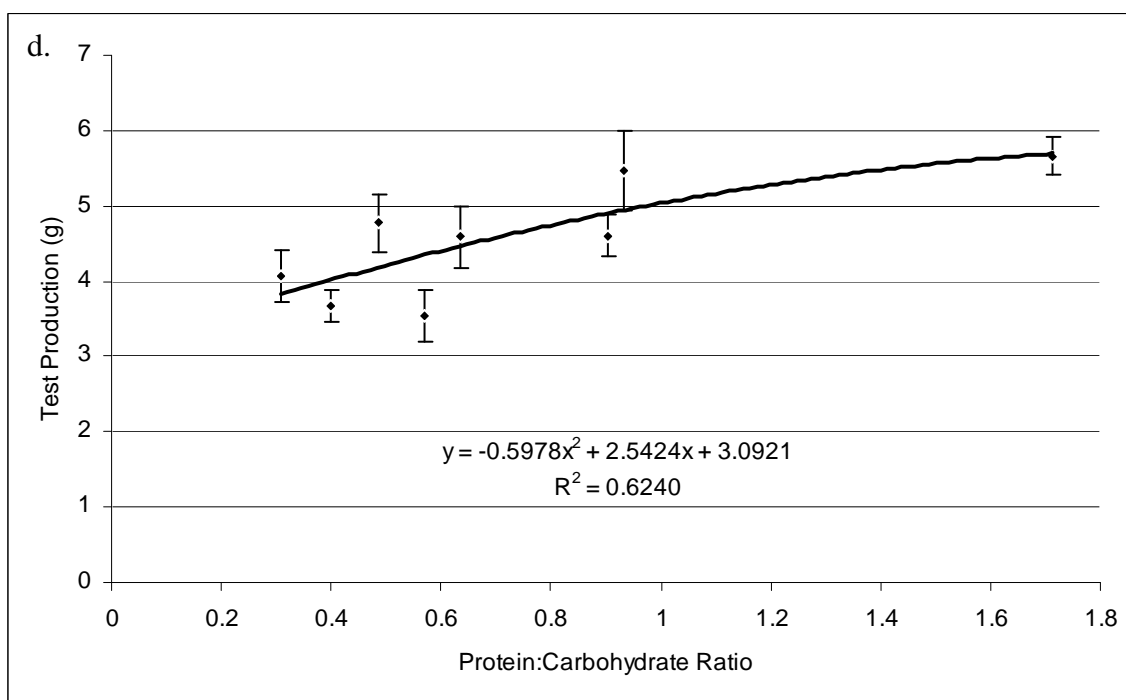
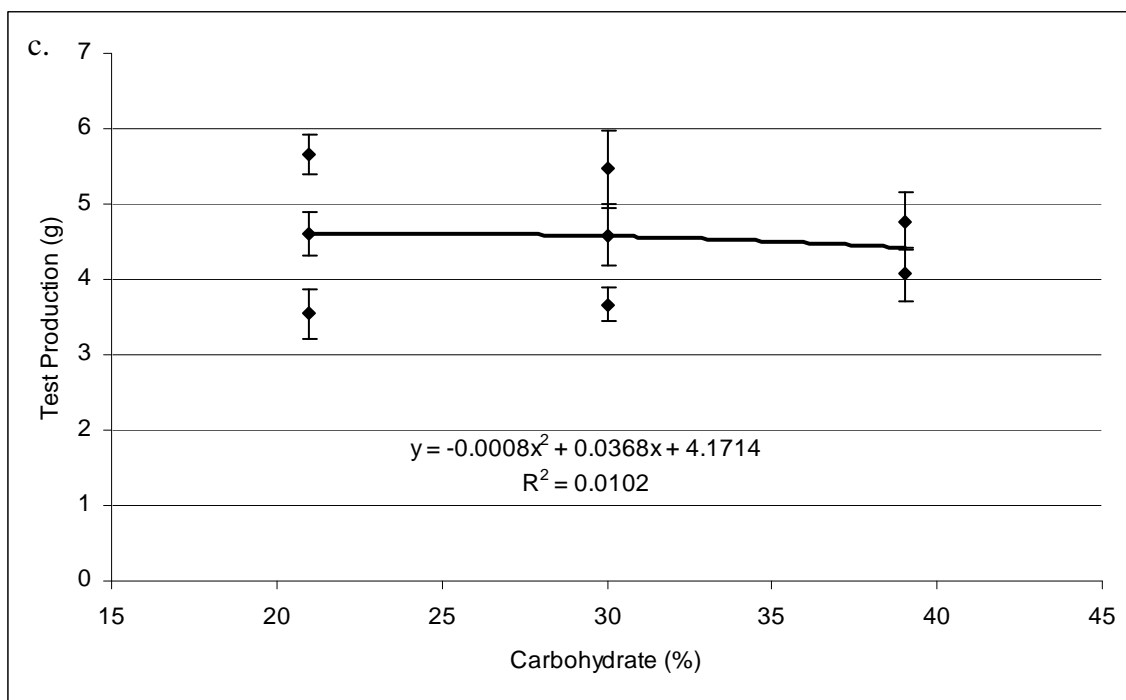


Figure 1. Trend analysis of test wet weight vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).





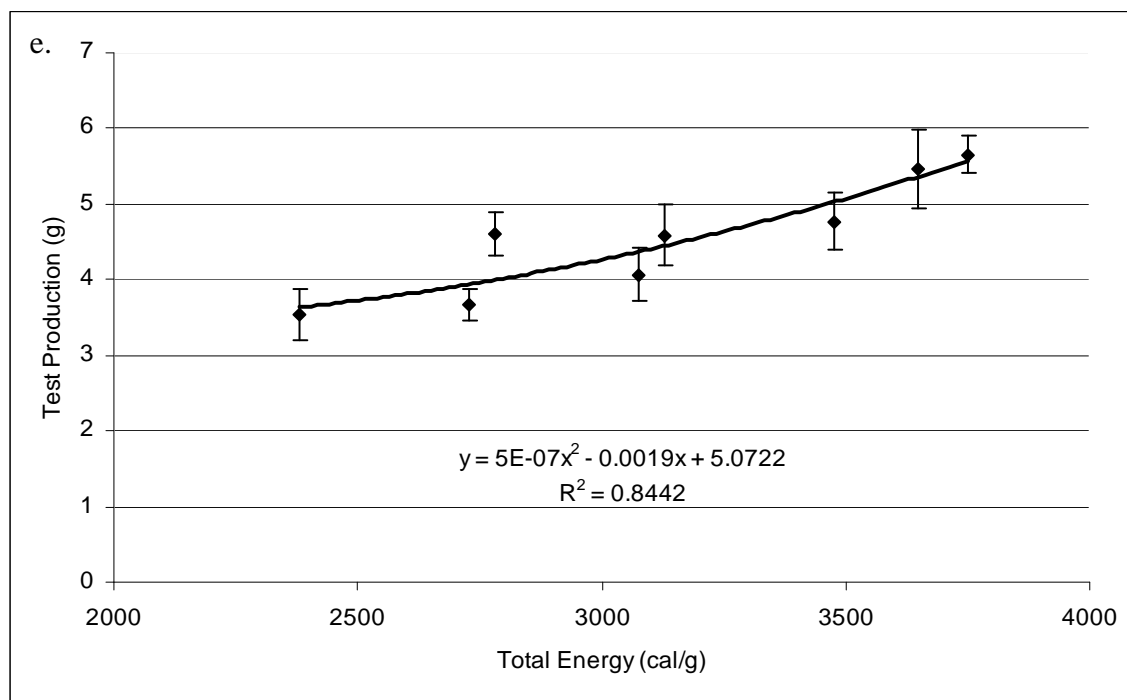
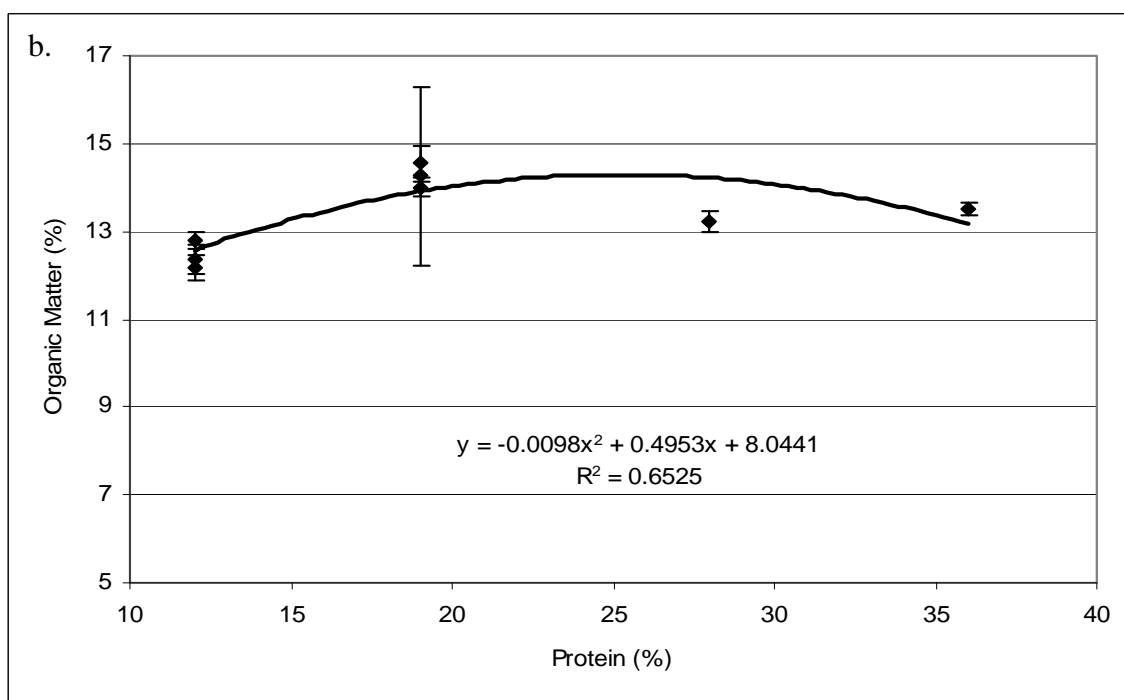
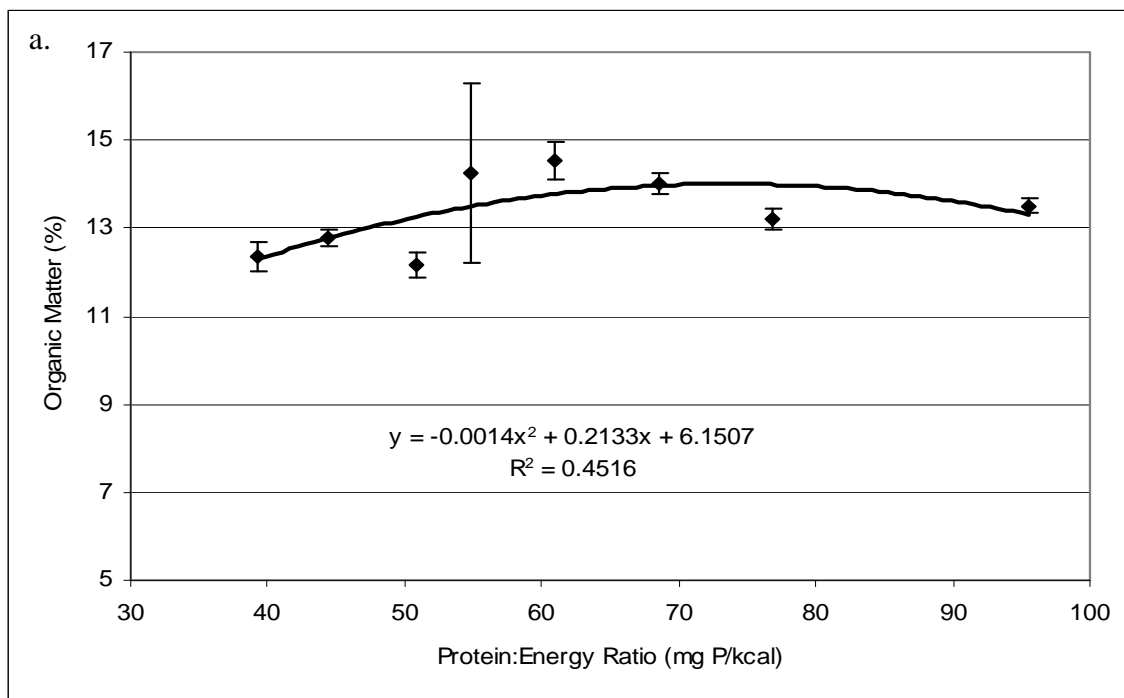
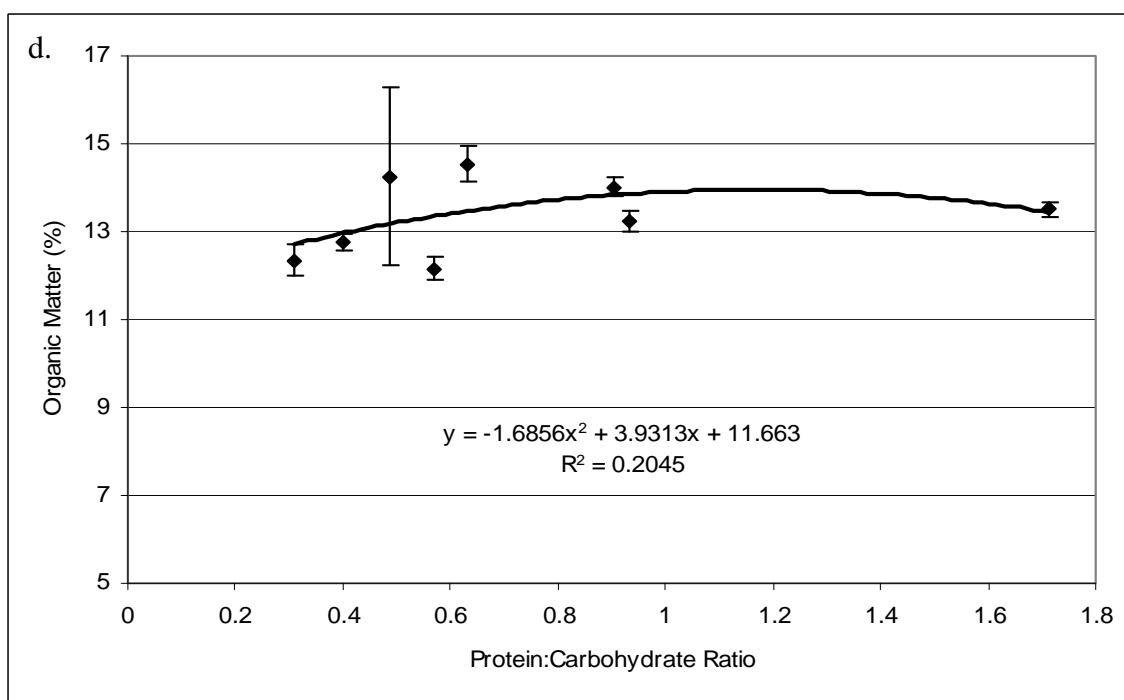
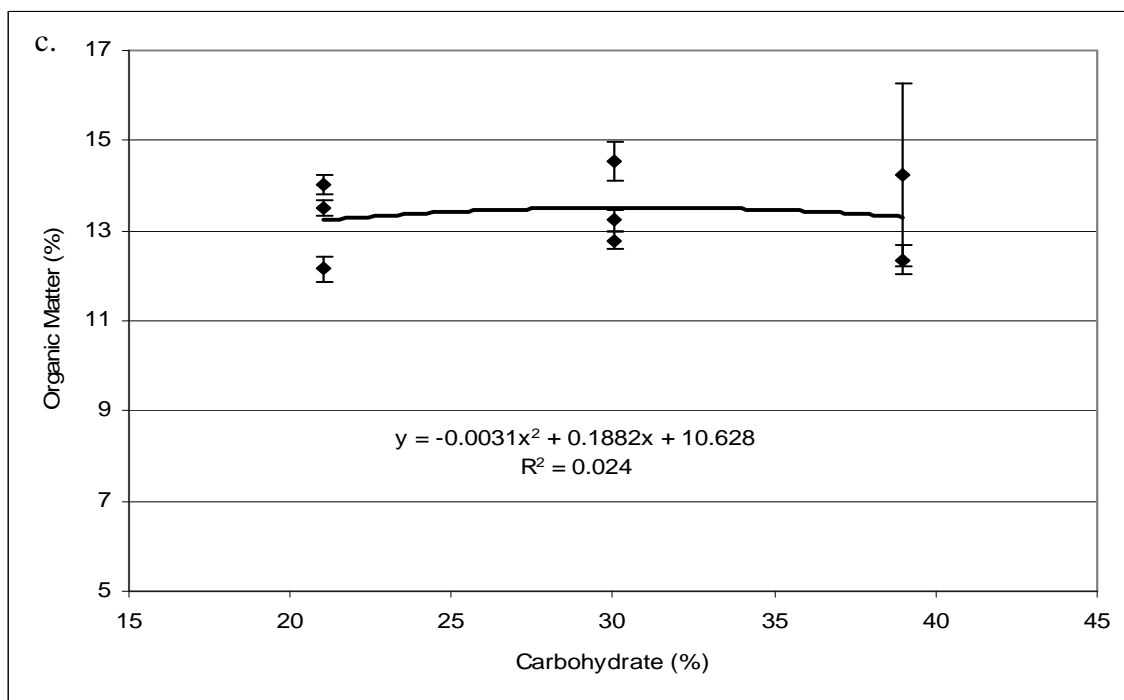


Figure 2 Trend analysis of test dry matter production (g) vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).





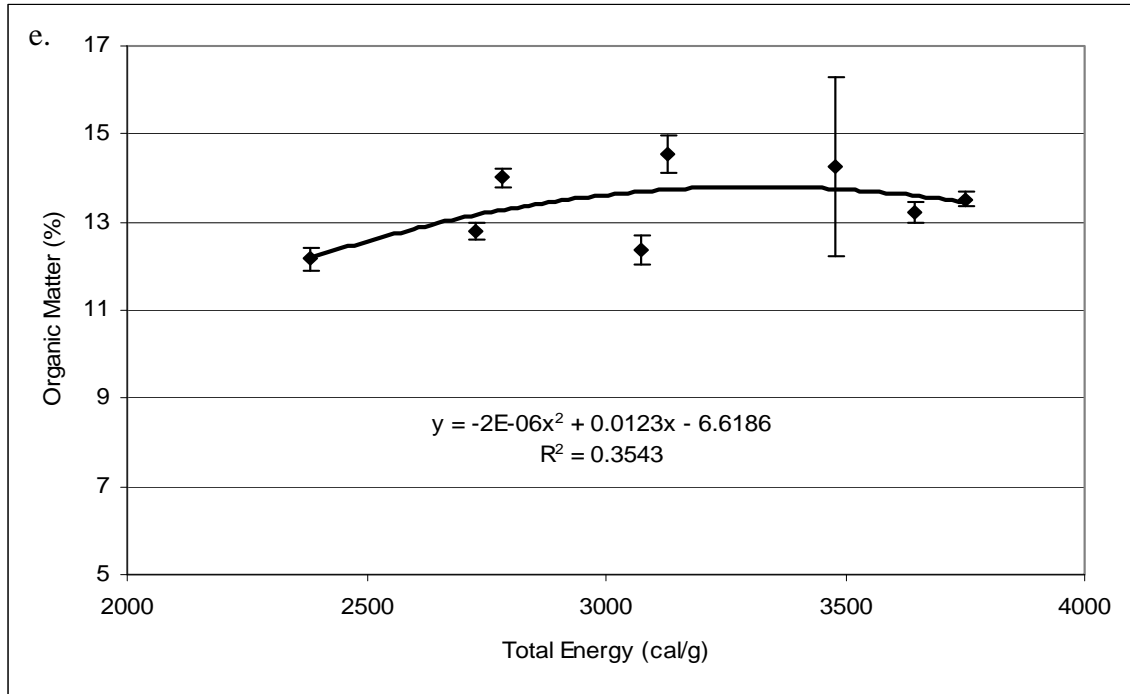
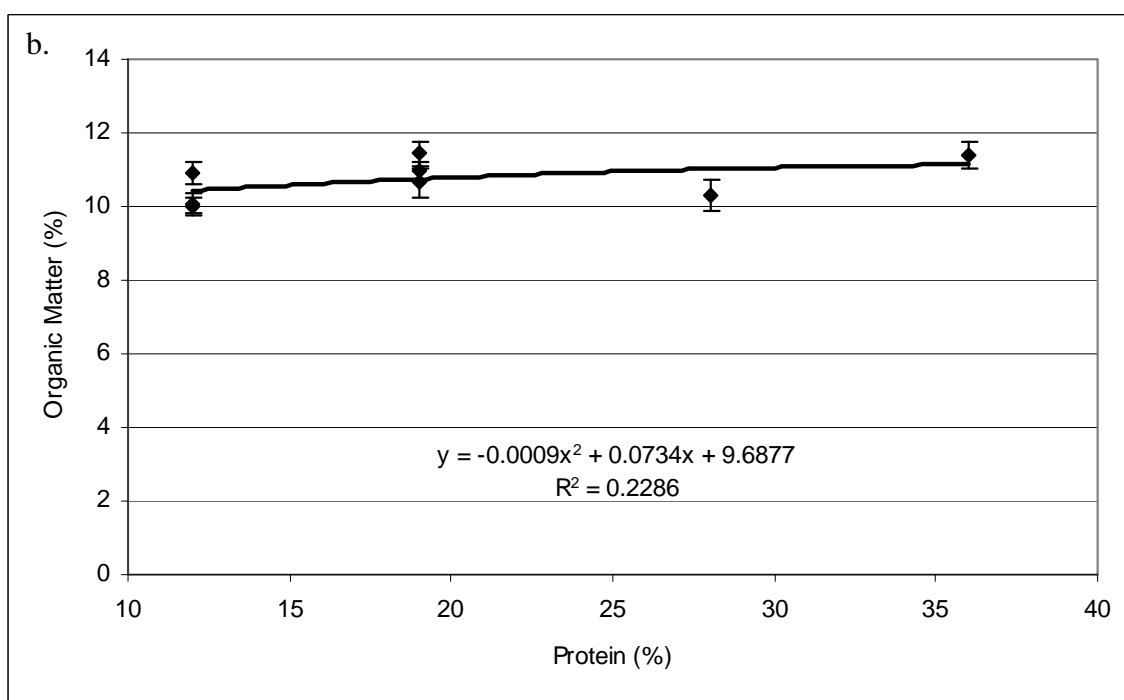
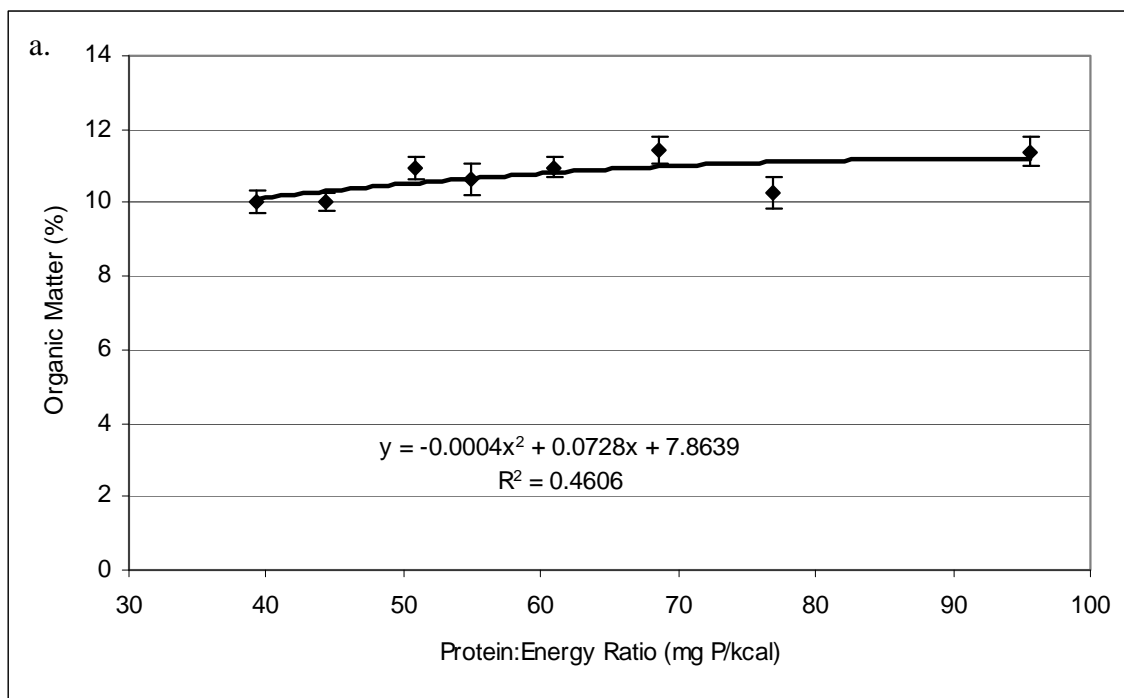
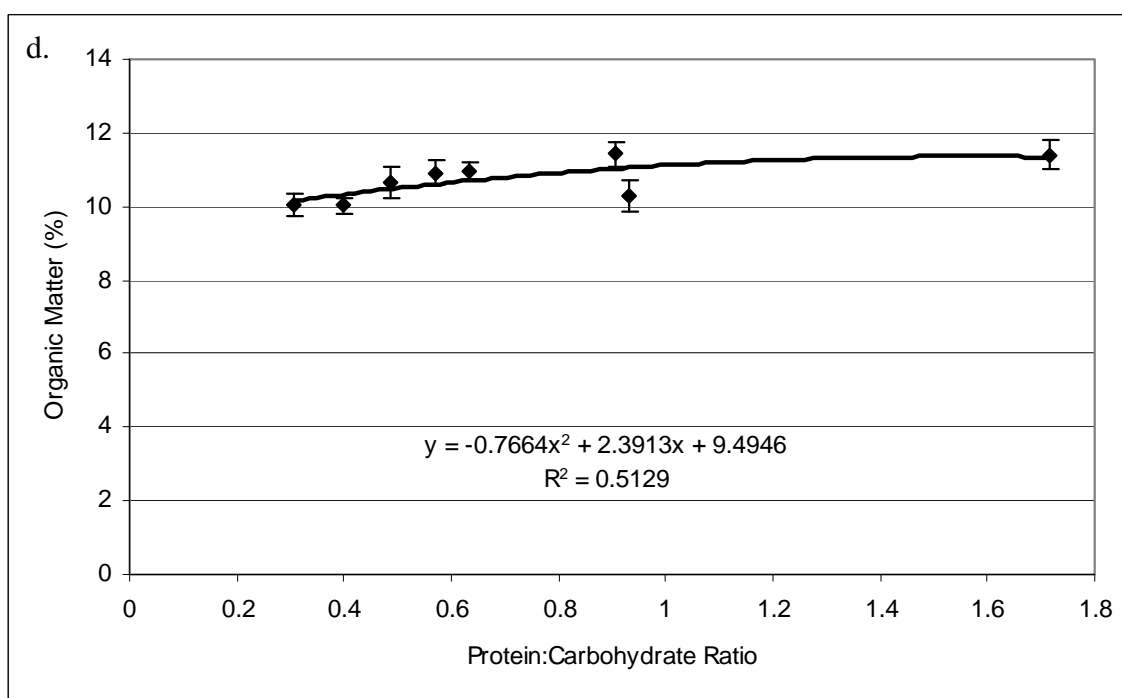
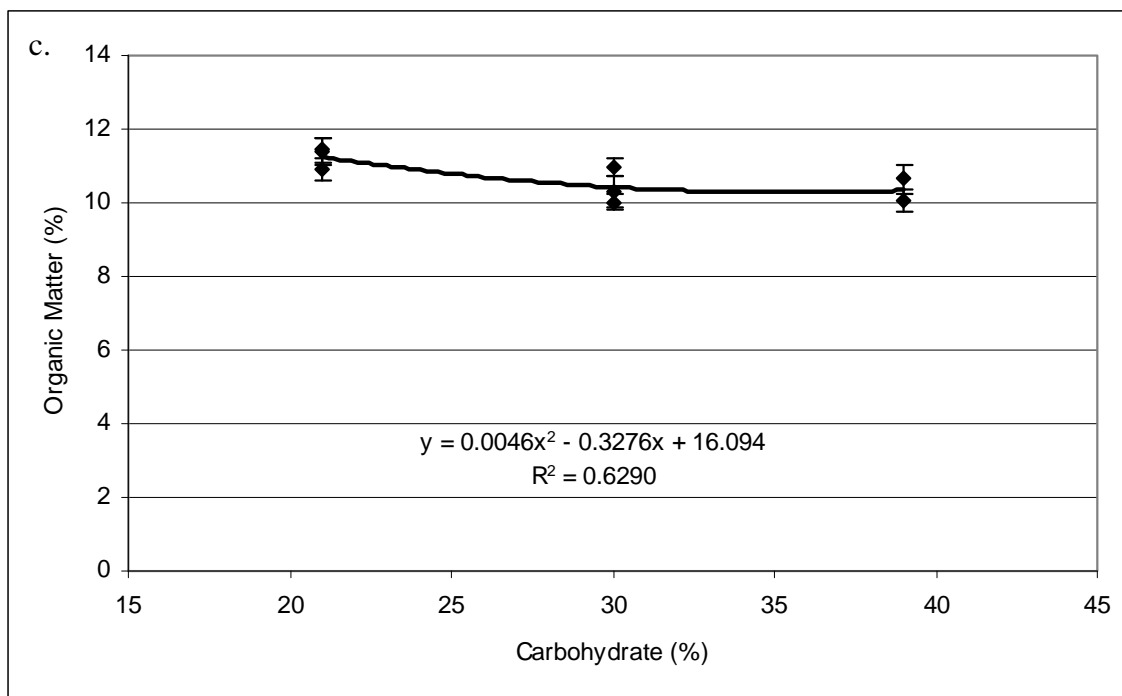


Figure 3. Trend analysis of percent organic matter of the test (% dry weight) vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).





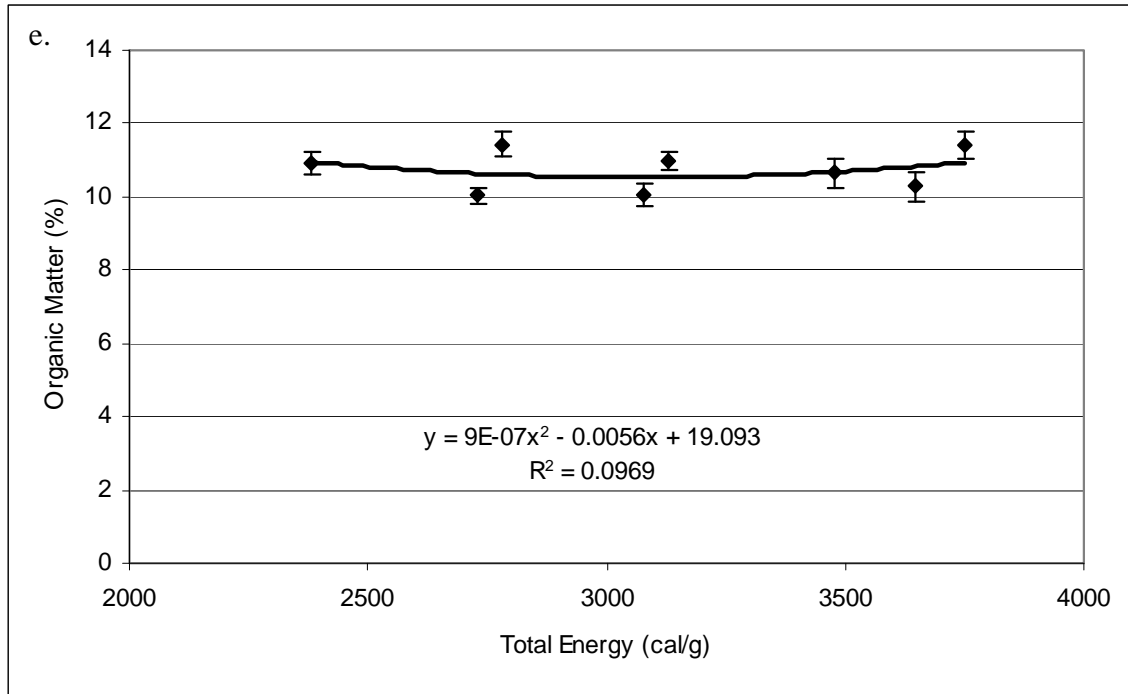
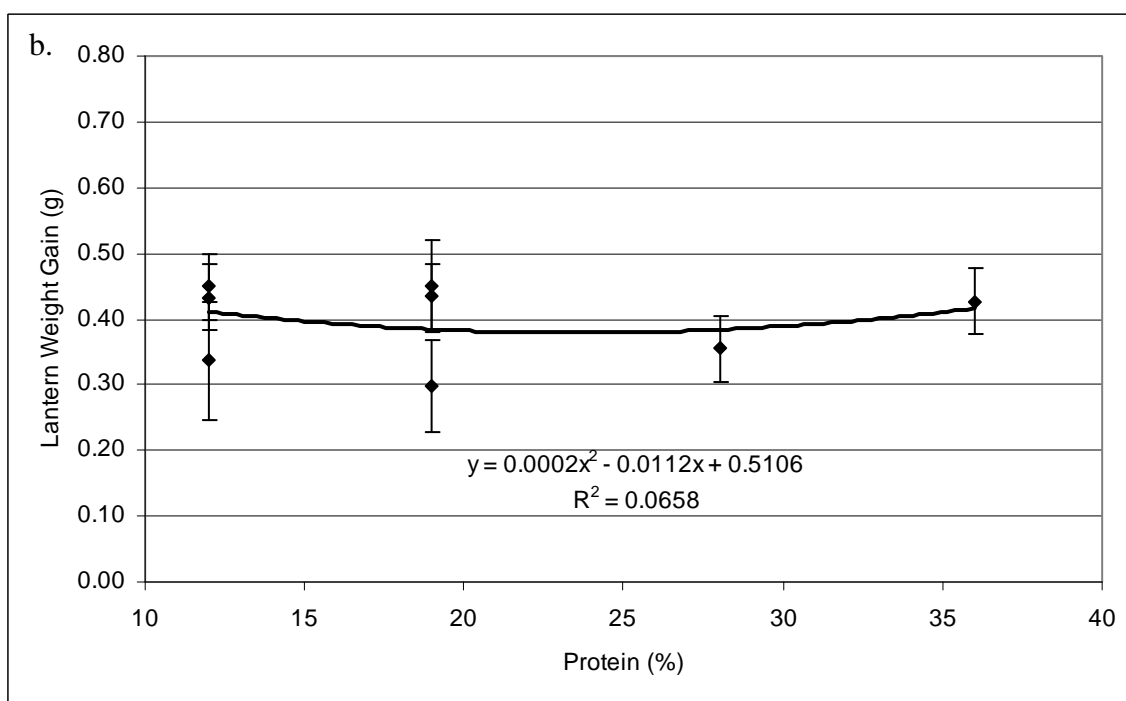
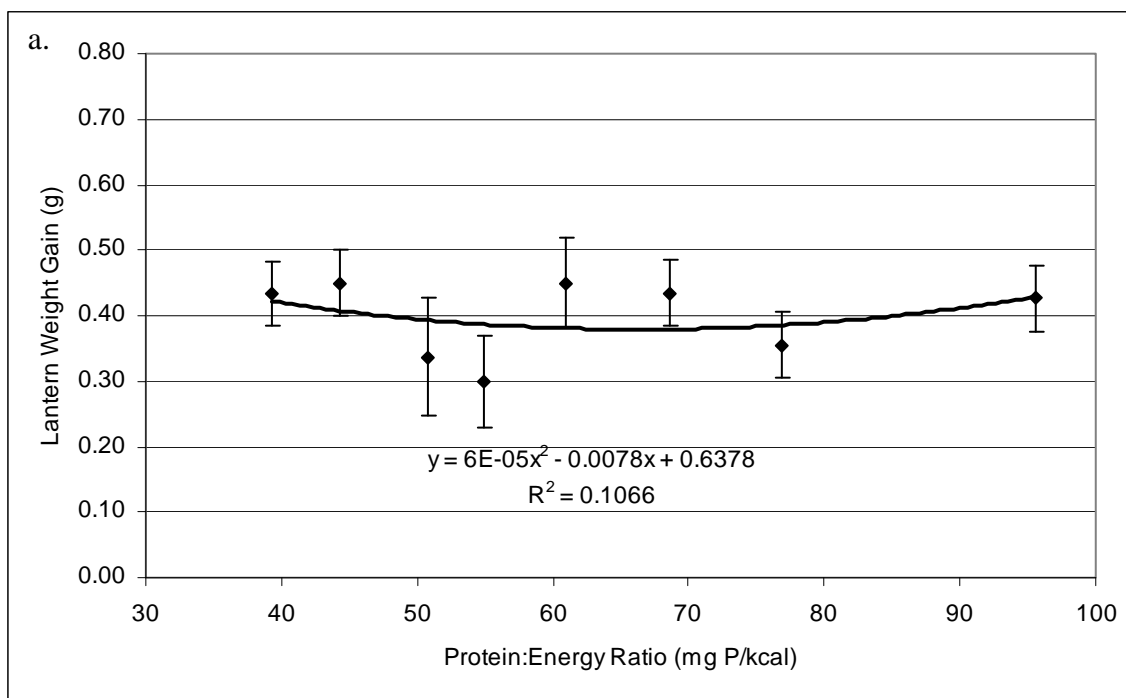
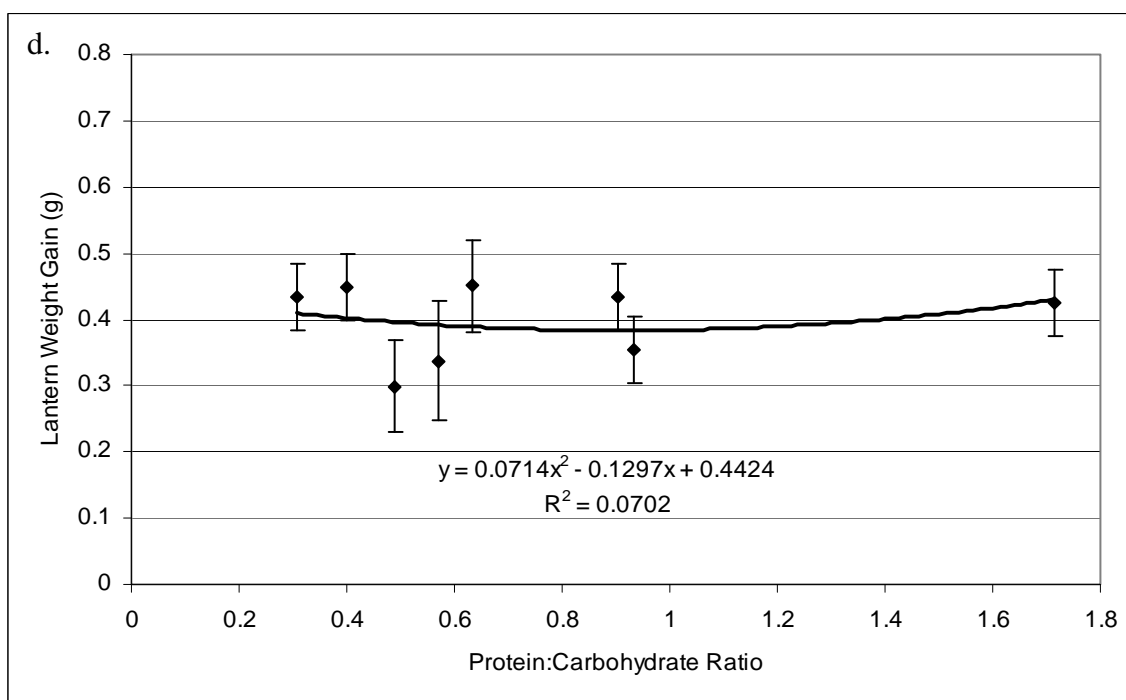
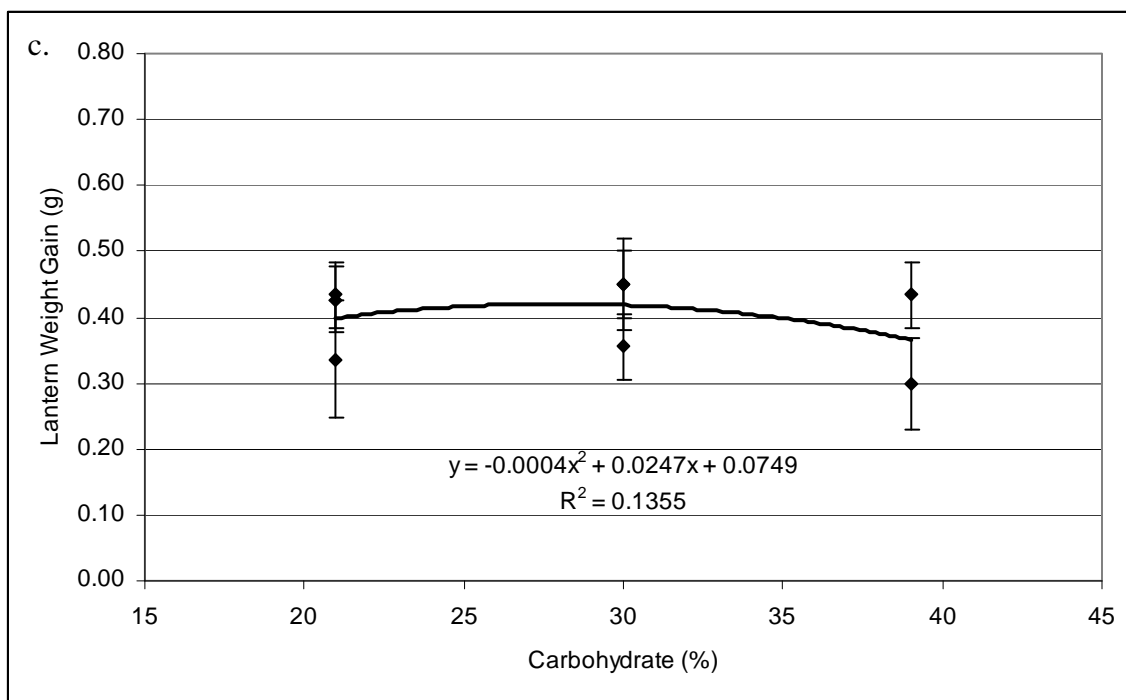


Figure 4. Trend analysis of percent organic matter of spines (% dry weight) vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).





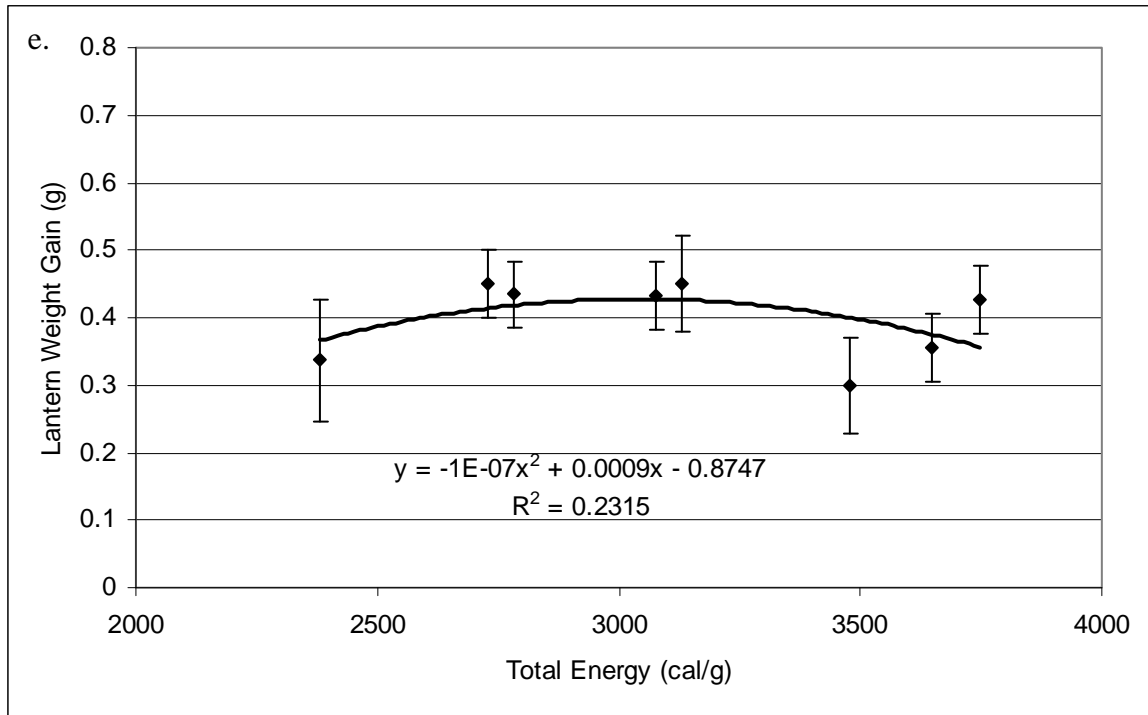
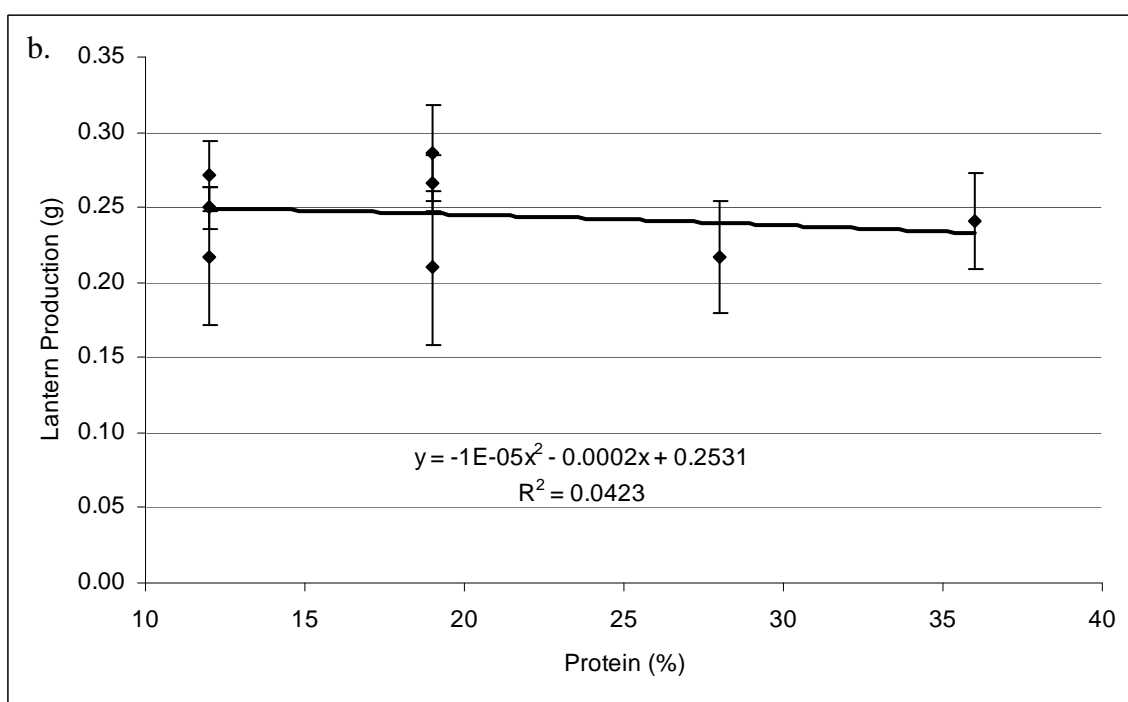
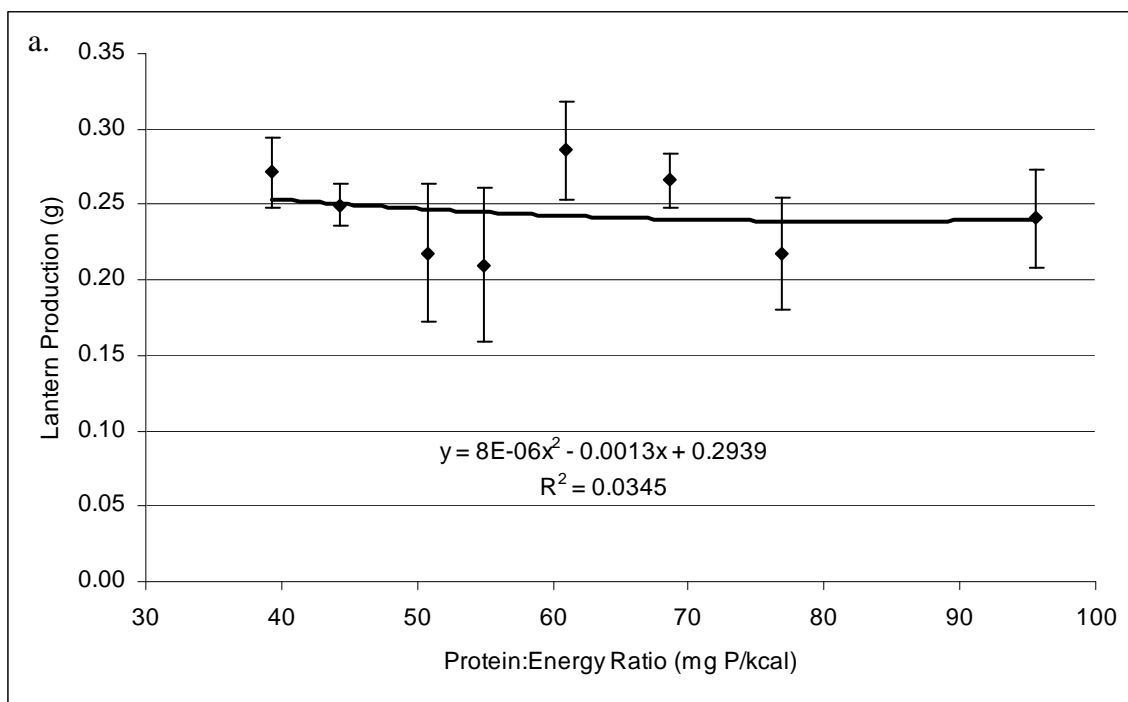
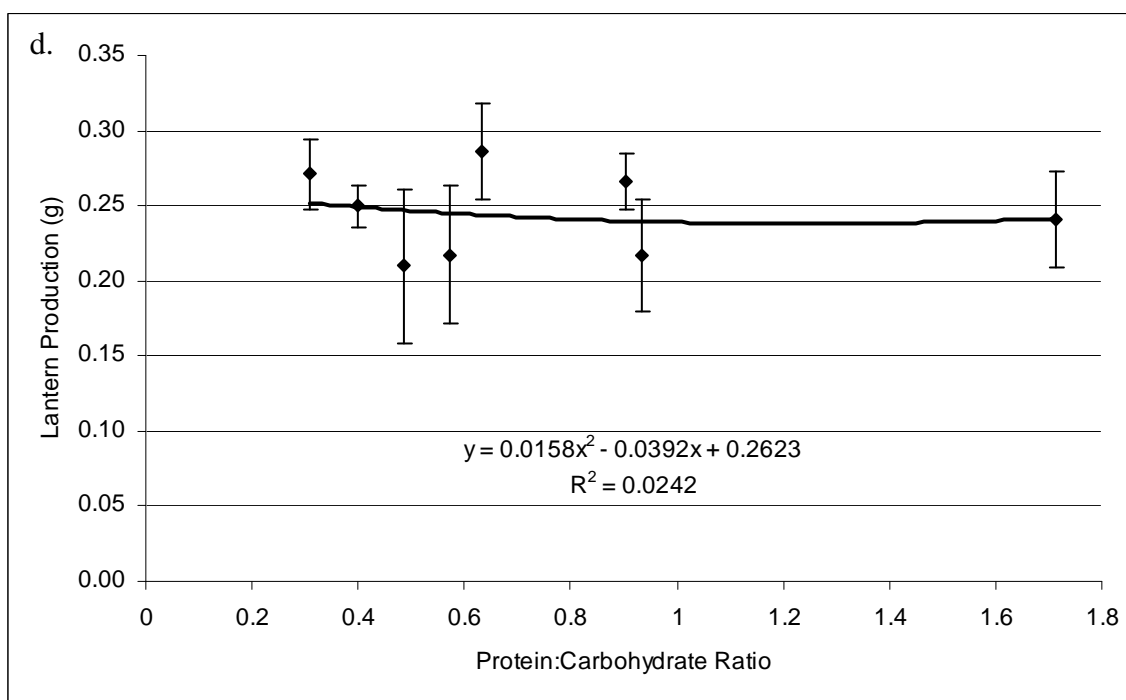
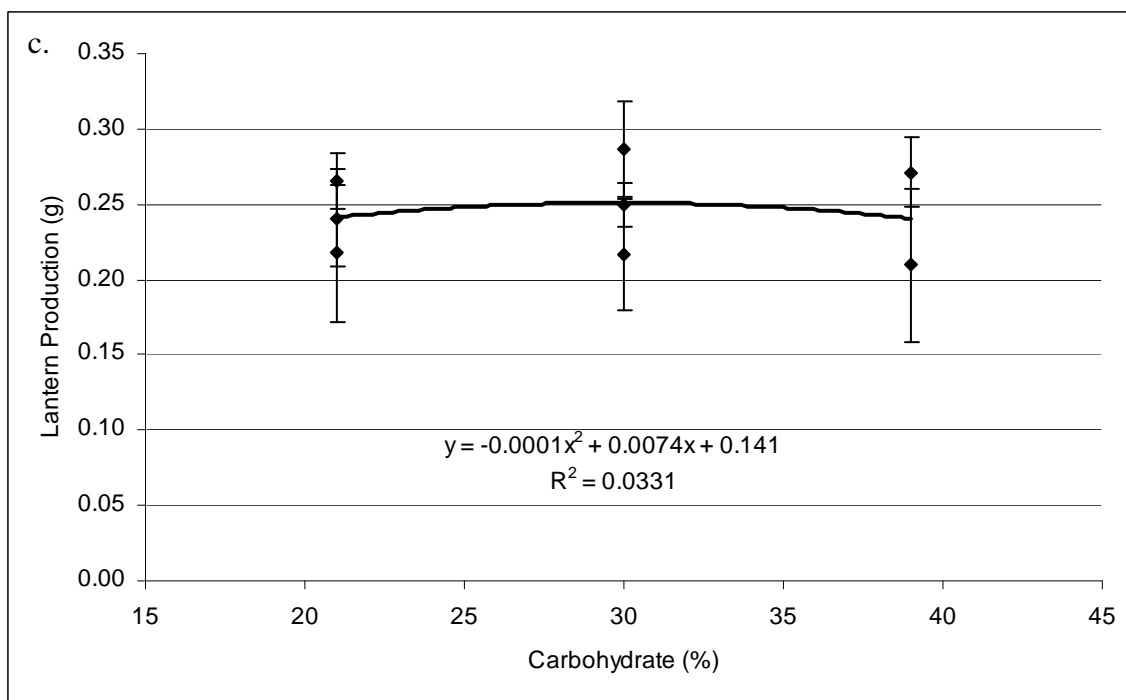


Figure 5. Trend analysis of lantern wet weight gain (g) vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).





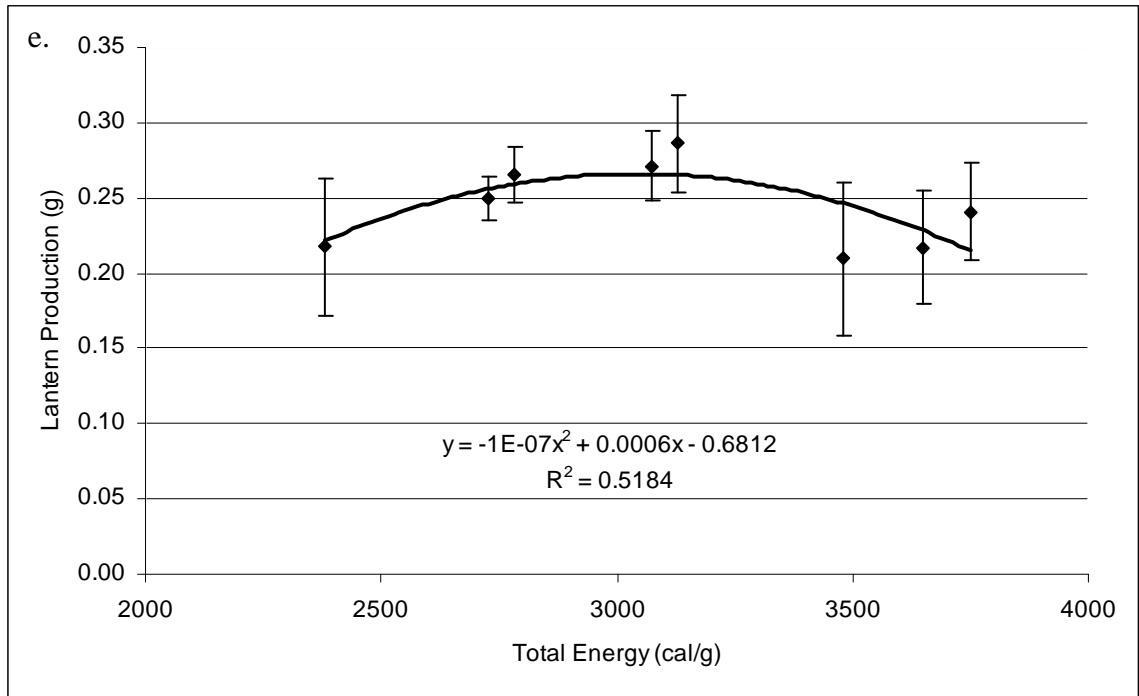
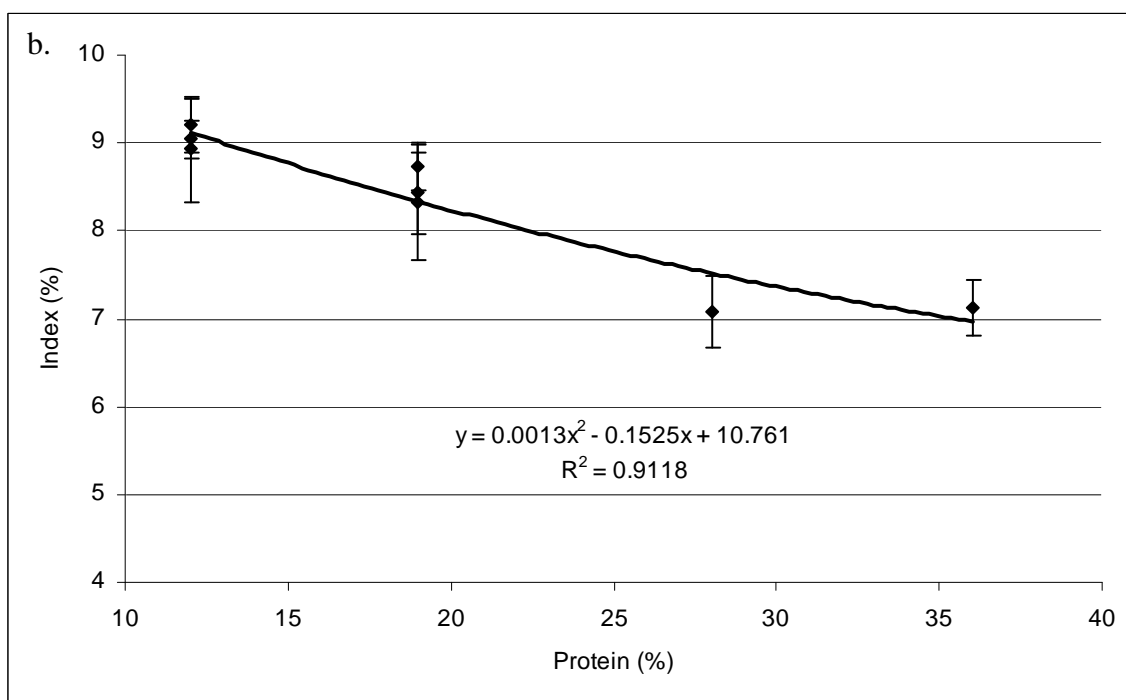
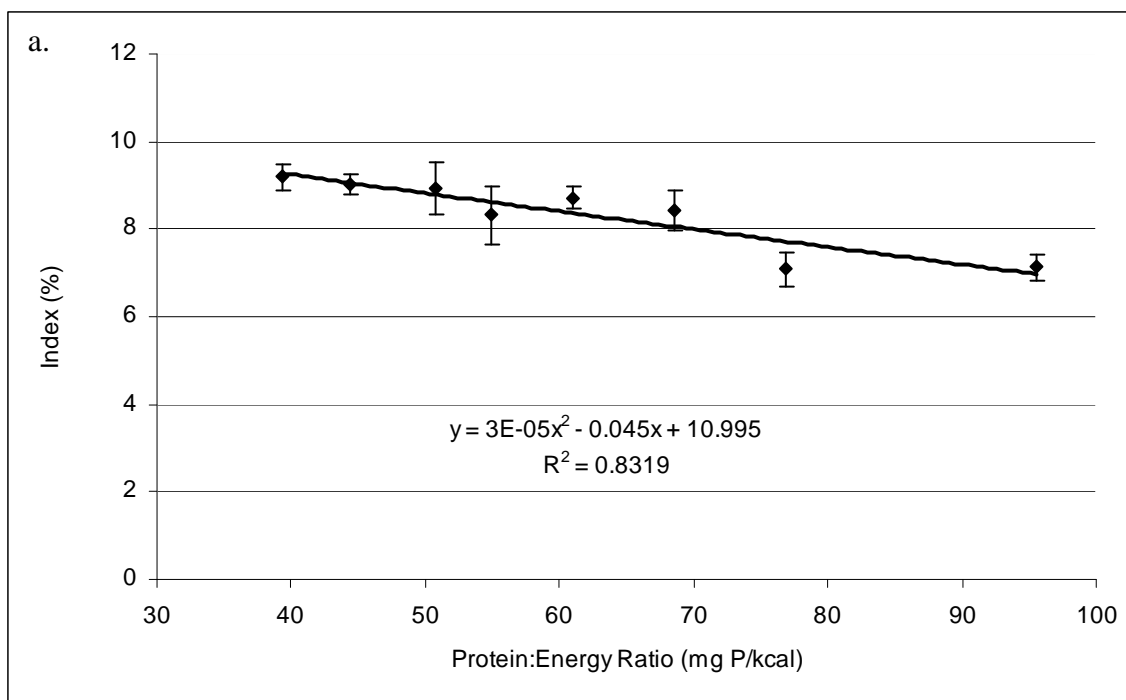
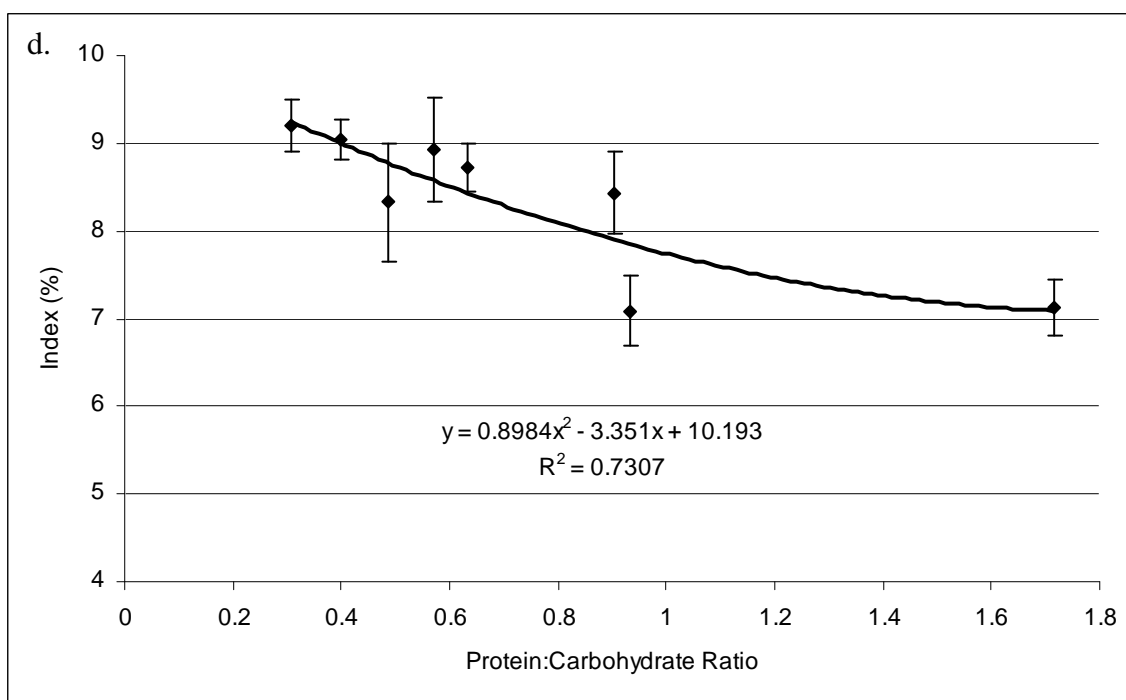
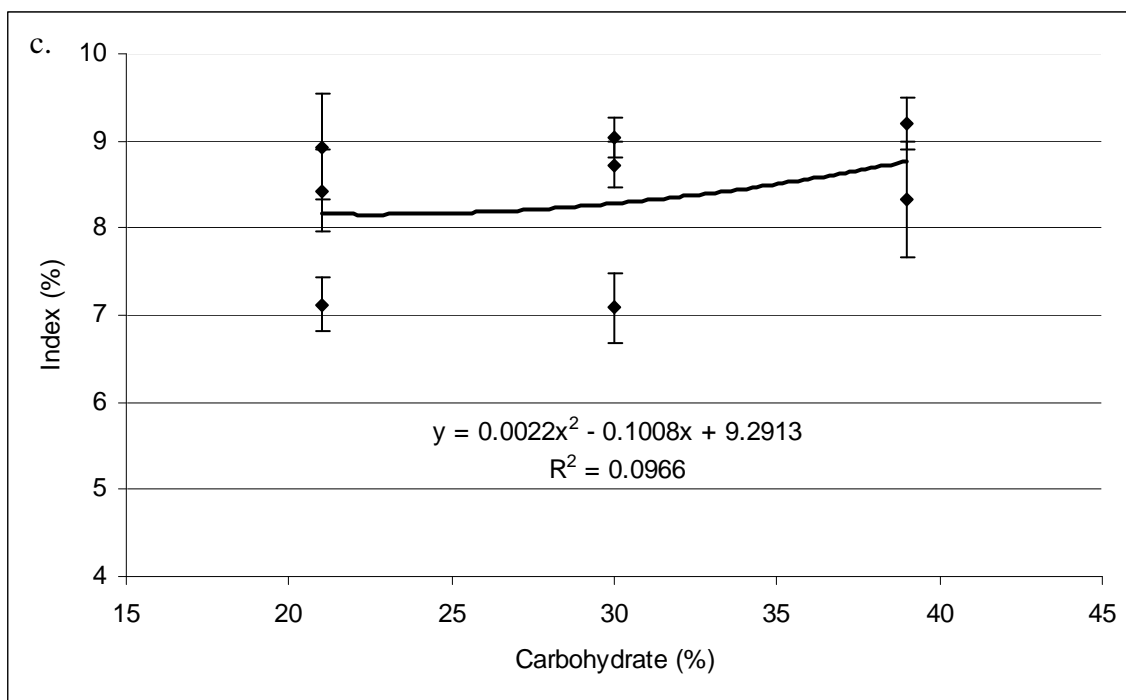


Figure 6. Trend analysis of lantern dry matter production (g) vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).





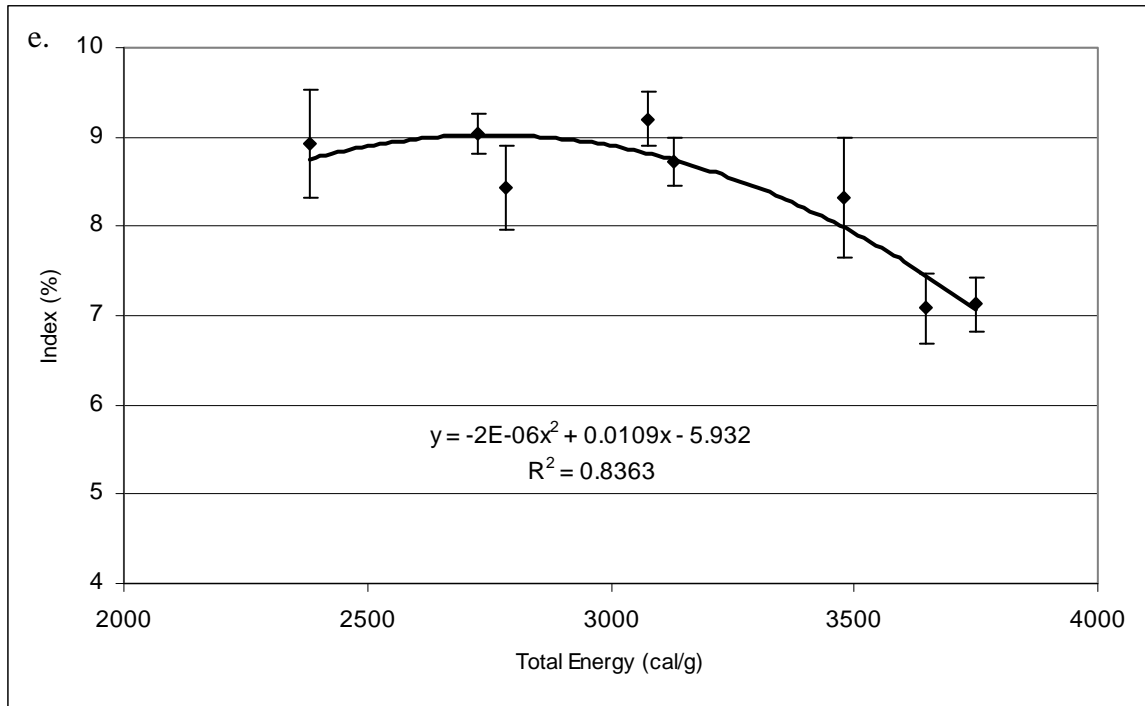
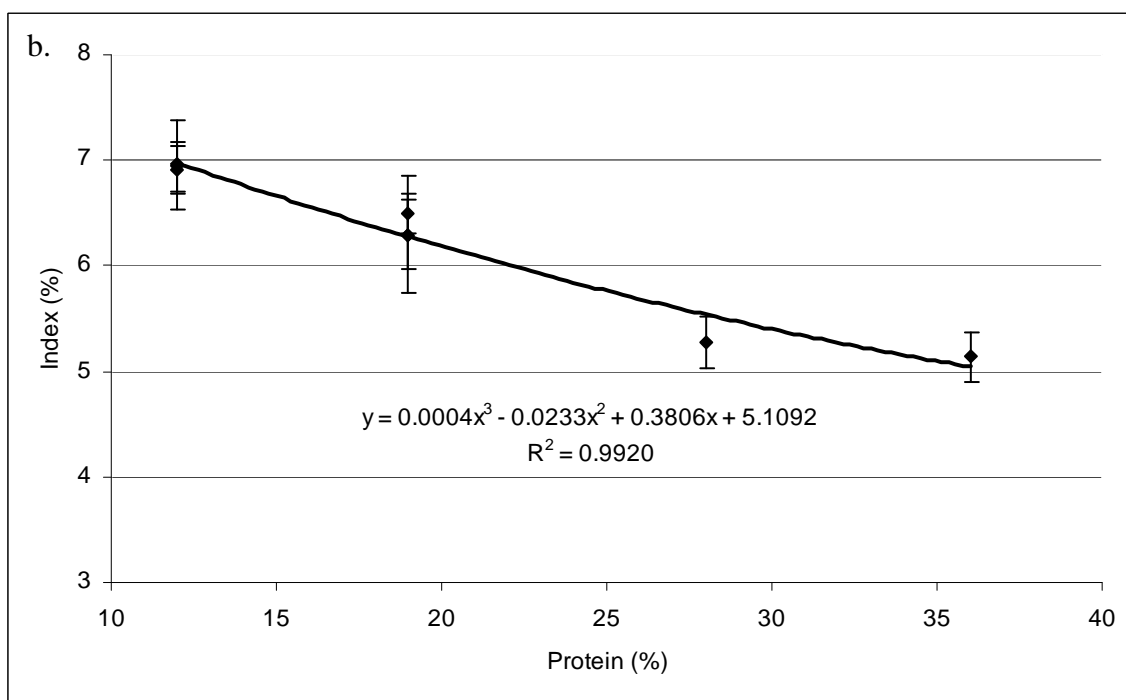
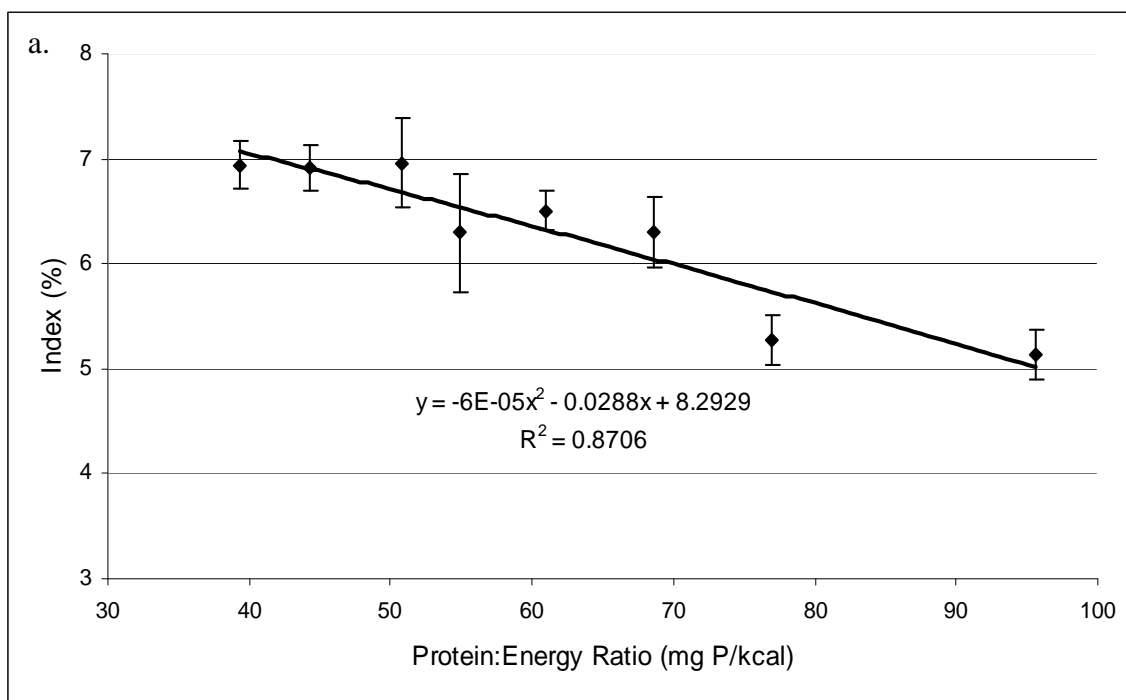
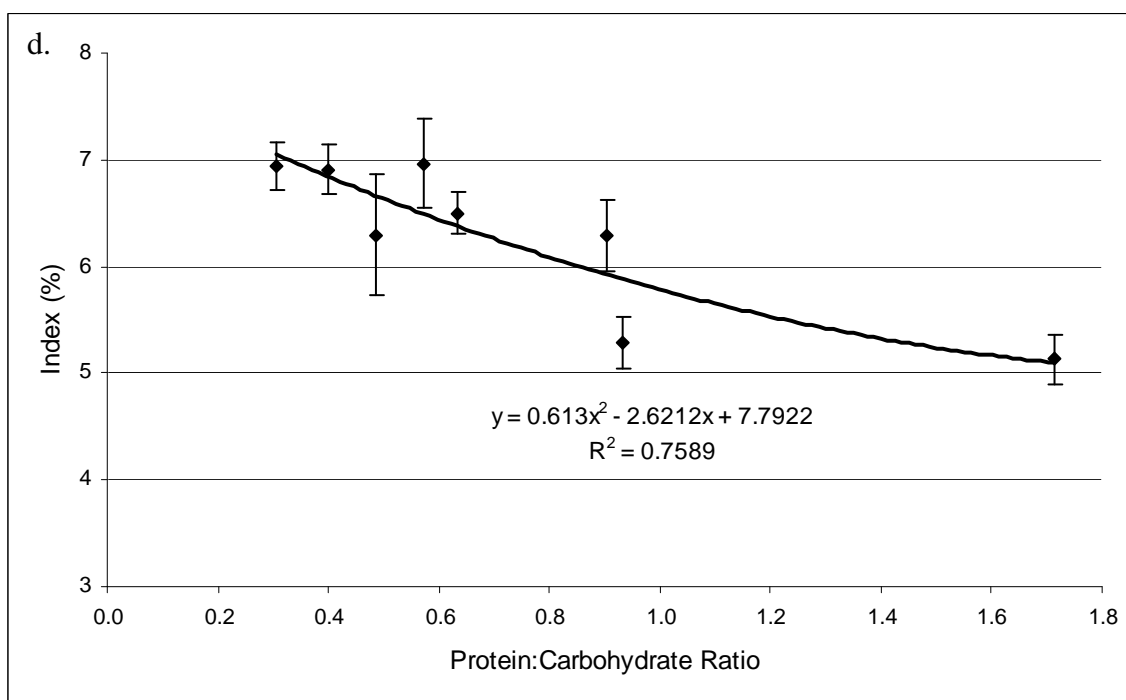
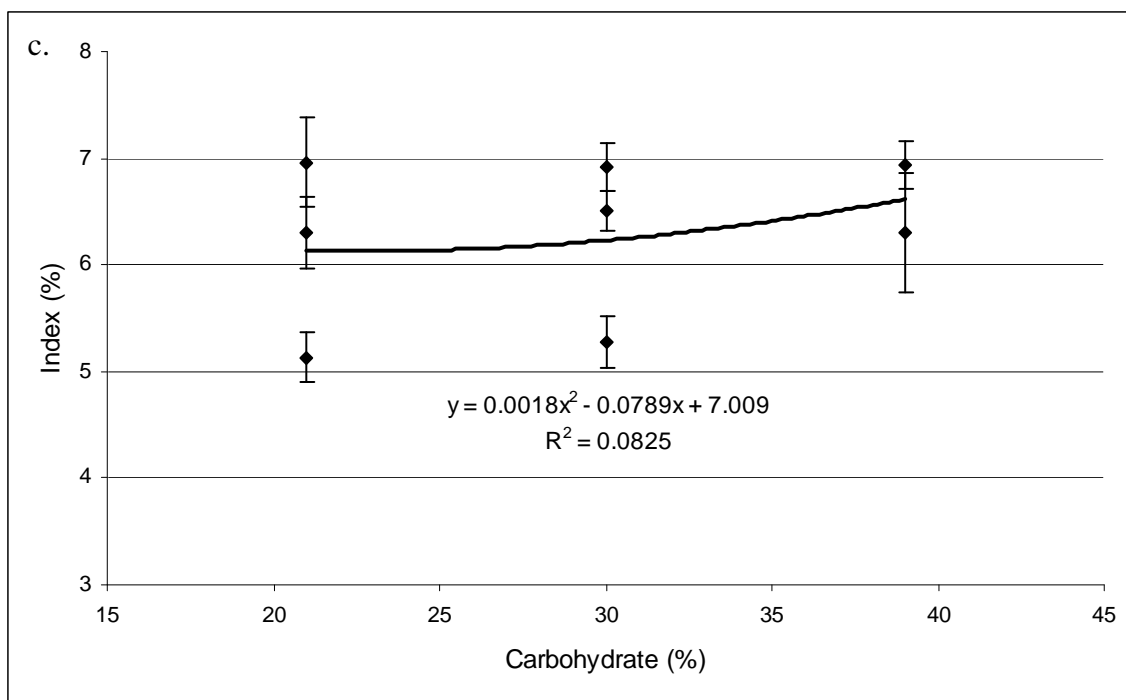


Figure 7. Trend analysis of dry lantern indexed to dry test (L/T) vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).





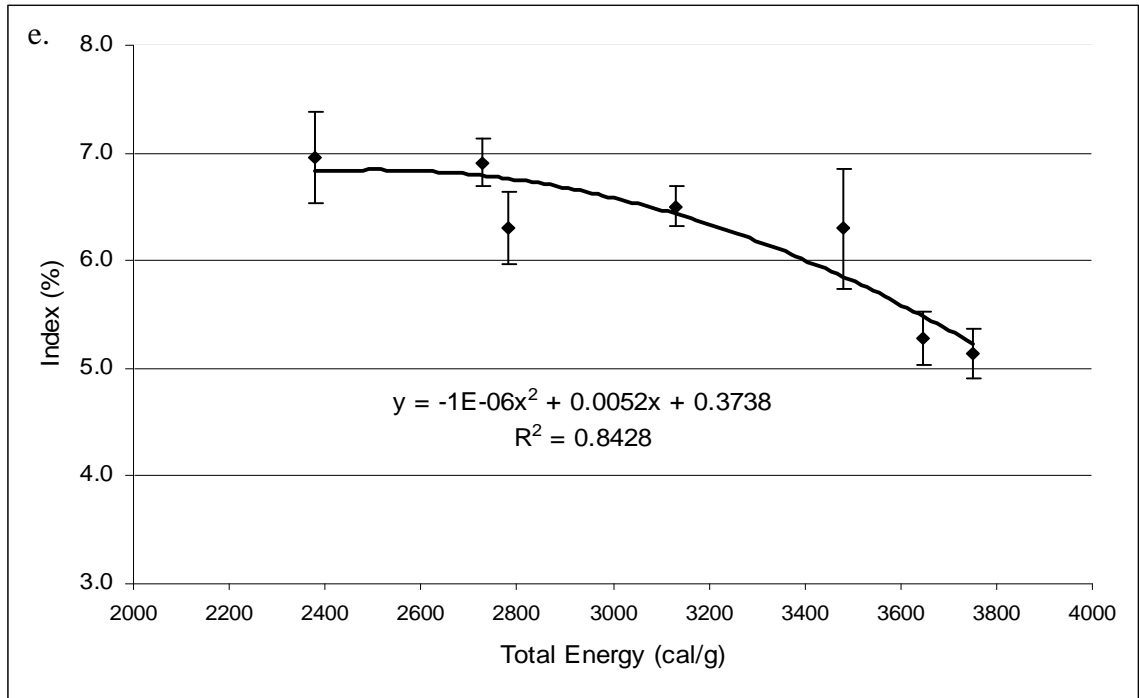


Figure 8. Trend analysis of dry lantern indexed to total dry weight (L/DWt) vs. dietary (a) protein:energy ratio, (b) protein, (c) carbohydrate, (d) protein:carbohydrate ratio, and (e) total energy of *L. variegatus* fed one of eight semi-purified diets for 9 weeks (n = 5-8 individuals per treatment). Values represent means \pm SE. (n = 5-8 individuals per treatment).

DISCUSSION

Water Quality

Water quality parameters maintained in this study were within the ranges suitable for sea urchins (Basuyaux and Mathieu, 1998).

Growth

Like most organisms, the growth rate of sea urchins is greatly influenced by the quality and quantity of food available (Lawrence and Lane, 1982); urchins will increase growth rate with an increase in intake of nutrients. This response is not plasticity but is simply a response to availability of resources (Lawrence and Lane, 1982, Chapter 2). As feed rations in this study were below satiation, any variations in the size of the test and the relative size of the Aristotle's lantern can be directly attributed to variations in the quality of the diets (i.e. for this study the quality varied with dietary protein, carbohydrate, essential amino acids, energy and total ash) instead of the quantity of food consumed.

During food limitation, sea urchins may exhibit plasticity of the test, remodeling the body wall to resorb stored nutrients for metabolism, which results in a decrease in test mass (Ebert, 1968, 1980; Levitan, 1991; Guillou, 2000). This process may be slow to occur (Lares, 1998) and, while it may increase fitness of the individual in that a smaller body size takes fewer resources to maintain (Ebert, 1996), test remodeling is a costly undertaking and probably only occurs under conditions of extreme food restriction. Lares (1998) starved *L. variegatus* for 2.5 months and did not observe changes in test diameter; however, nutrient stores in the test of starved individuals decreased, suggesting that the test diameter of *L. variegatus* may shrink under conditions of extreme food restriction. Individuals in this study were not starved or subjected to significant food restriction and there was no evidence of test shrinkage. All diets used in this study supported weight gain (although weight gain was not maximal at dietary protein levels of 12%, Chapter 2), indicating that the quality and quantity of all diets was sufficient.

Dietary protein levels directly influence growth (test weight and diameter) in sea urchins (reviewed by McBride et al., 1998). Hammer (2006) reported larger diameters and higher wet and dry test weights in *L. variegatus* fed 20% protein as compared to individuals fed 9% protein. McBride et al (1998) found no differences in test diameter among *S. franciscanus* fed prepared diets with protein levels ranging from 30-50%. However, diets used in these studies were not isocaloric and observed differences could be the result of differences in dietary energy. We suggest that dietary protein levels in excess of 20% are not limiting in sea urchins under the conditions of this study. Consequently, increases in protein levels above 30% should not affect test diameter or growth. Under the conditions of this study, test growth (diameter and weight gain) increased with increasing dietary protein level. The 12% protein diets, while adequate for maintenance, do not appear to provide enough protein for maximal test growth.

Feeding at levels below satiation level removed the potential effect of feed consumption. As a result, the data indicate that dietary protein levels were directly correlated with nutrient storage in the body wall. The concentration (%) of organic matter in the tests of urchins fed diets with 12% protein was lower than that of urchins fed diets with 19% protein. Because protein is the major proximate component of the sea urchin test (Lawrence and Lane, 1982), we believe that this difference in organic matter concentration most likely indicates that little protein was allocated to storage in the test of the individuals fed 12% protein. The further decrease in percent organic matter in those fed higher levels of protein suggests that another factor also influenced the storage of organic matter in the tests of these individuals. We hypothesize that the high metabolic cost of processing high levels of dietary protein may decrease the deposition of organic

matter to the test. A similar trend was observed by Hammer et al. (2006) in *L. variegatus* fed at 9%, 20%, and 31% protein. Percent organic matter of the spines was lower than that of the test in all treatments, most likely reflecting structural and functional differences in these tissues. Percent organic matter of the spines varied among dietary treatments but, unlike the test, biological significance of these differences could not be ascertained.

As indicated previously, there is considerable controversy as to whether the Aristotle's lantern shows plasticity in response to nutritional deprivation. There are numerous reports of differential resource allocation in sea urchins (Ebert, 1968, 1980; Lawrence and Lane, 1982; Black et al., 1984; Levitan, 1991; reviewed by Chia and Walker, 1991; Hendler, 1991; Fernandez and Boudouresque, 1997; Lawrence et al. 1997; McShane and Anderson, 1997; Minor and Scheibling, 1997; George, 1999; Guillou, 1999; Meidel and Scheibling, 1999; Olave et al., 2001; Spirlet et al., 2001; Pearce et al., 2002a, 2002b; Otero-Villanueva et al., 2003; Senaratna et al., 2005; Hagen, 2007; Martínez-Pita, 2008; Woods et al., 2008; Lau et al., 2009), the majority of which involve plasticity of the feeding apparatus, the Aristotle's lantern (Ebert, 1968, 1980; Black et al., 1984; Levitan, 1991; Fernandez and Boudouresque, 1997; McShane and Anderson, 1997; Hagen, 2007; Lau et al., 2009). In the current study, final wet weight and dry matter production of the Aristotle's lantern did not vary among treatments, indicating little or no plasticity in the growth of the lantern in response to significant changes in protein and carbohydrate. We suggest that lantern growth is relatively fixed in response to changes in these proximate nutrients. Although the size of the lantern did not vary among treatments, the relative size (index) of the lantern did vary significantly because of differences in test

growth. The Aristotle's lantern index was inversely correlated with dietary protein level, suggesting that, for these urchins, dietary protein levels of 12% were limiting enough to cause individuals to allocate more resources towards growth of the Aristotle's lantern than growth of the test as compared to other diet treatments. Remodeling of the lantern has been reported in some species (Ebert, 1980; Levitan, 1991), but it is probable that the duration of the current study was too short and the nutritional stress, if any, was not high enough to initiate remodeling of the test or lantern.

Of further interest, is the relationship between Aristotle's lantern index and total dietary energy. Lantern indices decreased significantly at energy levels ≥ 3478 cal/g, suggesting that, for individuals in this study, diets with energy levels ≥ 3478 cal/g at the amount fed provided adequate nutrients for maximal test growth. Thus, the Aristotle's lantern indices were comparatively smaller in individuals fed these diets. These data suggest that total energy availability is a physiological trigger for initiating differential nutrient allocation to the test and lantern.

There are multiple hypotheses as to the selective advantage(s) of a comparatively large lantern in an individual (Black et al., 1984; Levitan, 1991). Food proffered to individuals in the current study did not vary in shape, distribution, size, or hardness, and relative feed intake was the same in all individuals. The only differences were the proximate composition and energy content of the diet. As such, increased lantern size would not be advantageous for sea urchins in this study. Observed changes in lantern indices might be interpreted as plasticity, but we would disagree with this interpretation. We suggest that the differences among lantern indices are attributed to differential resource allocation to the test and lantern, induced by changes in diet composition and/or

energy content. Under the conditions of this study, a smaller test size was advantageous to individuals in nutrient-limited diets, as sea urchins with a smaller test (and thus, a smaller body) need fewer resources for maintenance and repair of tissues (Ebert, 1996).

There are many unanswered questions concerning plasticity and remodeling of tissues in sea urchins. If there are benefits to the individual, the benefits must outweigh the costs. As ruderal species, sea urchins must conserve their resources and, although plasticity may come at a high genetic cost, survival and reproduction is ensured when living in a changing environment.

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APPENDIX A
IACUC APPROVAL FORM




THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

NOTICE OF APPROVAL

DATE: June 16, 2009

TO: Watts, Stephen A.
CUI 375-1170
934-2045

FROM: 
Judith A. Kapp, Ph.D., Chair
Institutional Animal Care and Use Committee

SUBJECT: Title: Sea Urchin Culture for Improved Biomedical/Ecotoxicological Testing
Sponsor: Mississippi-Alabama Sea Grant Consortium
Animal Project Number: 090508135

On June 16, 2009, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals.

Species	Use Category	Number in Category
Invertebrates	A	1000

Animal use is scheduled for review one year from May 2009. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 090508135 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7692.

Institutional Animal Care and Use Committee
B10 Volker Hall
1670 University Boulevard
205.934.7692
FAX 205.934.1188

Mailing Address:
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1530 3RD AVE S
BIRMINGHAM AL 35294-0019



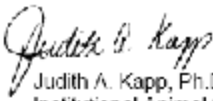
THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

MEMORANDUM

DATE: June 16, 2009

TO: Wallis, Stephen A.
CH-375 1170
934-2045

FROM: 
Judith A. Kapp, Ph.D., Chair
Institutional Animal Care and Use Committee

SUBJECT: **NOTICE OF APPROVAL - Please forward this notice to the appropriate granting agency.**

The following application was reviewed and approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on June 16, 2009.

Title: Sea Urchin Culture for Improved Biomedical/Toxicological Testing
Sponsor: Mississippi-Alabama Sea Grant Consortium

This institution has an Animal Welfare Assurance on file with the Office for Protection from Research Risks (Assurance Number A3255-01) and is registered as a Research Facility with the United States Department of Agriculture. The animal care and use program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

Institutional Animal Care and Use Committee
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