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Effect of dietary non-protein energy source on growth, nutrient retention and circulating insulin-like growth factor I and triiodothyronine levels in juvenile barramundi, *Lates calcarifer*

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Abstract

In this study, the effect of the ratio of lipid to carbohydrate non-protein energy sources on the growth and circulating insulin-like growth factor (IGF)-I and triiodothyronine (T3) levels of juvenile barramundi was examined using a 2×3 factorial design experiment. Isonitrogenous diets (50% crude protein) were formulated at two gross energy levels (18 and 21 MJ kg^{-1}) and three ratios of lipid to carbohydrate non-protein energy (60:40, 70:30 and 80:20). Animals were held in 70 l aquaria receiving recirculated seawater (28 ‰) at 25°C. Animals were fed daily to satiety for 70 days. T3 and IGF-I levels were measured using radioimmunoassays validated for use for this species. Lipid and carbohydrate energy were supplied as marine fish oil and gelatinized corn starch, respectively. Gelatinized starch was generally well utilized by juvenile barramundi, though its utilization may be limited above the 17% inclusion level. Increases in dietary energy resulted in higher growth rates and feed conversion as well as elevated protein and lipid gain. A significant protein sparing effect of both carbohydrate and lipid was demonstrated for juvenile barramundi. Circulating levels of T3 and IGF-I responded to dietary treatment, however they did not relate directly to any measured growth parameter. The relationships between nutrition, growth and the underlying mechanisms controlling growth are discussed.

Keywords: Lipid; Carbohydrate; Growth; Barramundi; IGF-I; Triiodothyronine

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1. Introduction

Much research have been directed toward finfish nutrition and resultant growth, however the mechanisms by which the quantity and quality of feed influences growth and nutrient utilization have received limited attention (MacKenzie et al., 1998). By investigating the effect of nutrition on growth, nutrient partitioning, and hormonal status, a better understanding of the growth process may be achieved. However, attaining this understanding is made difficult by the multiple points for regulation (Eales, 1995) and multiple nutrient sensitive locations in endocrine pathways (MacKenzie et al., 1998)

The retention of dietary protein for growth is the aim of finfish nutritionists in the development of cost effective, environmentally sustainable diets. Maximizing the utilization of dietary protein for growth is related to both the dietary inclusion level of protein and the availability of non-protein energy sources, namely lipid and carbohydrate. Inclusion of non-protein energy has been shown to spare dietary protein from catabolism to provide energy and enhance its utilization for growth, a process known as "protein sparing" (Millikin, 1983; Dias et al., 1998; Grisdale-Helland and Helland, 1998; Helland and Grisdale-Helland, 1998). Although lipid is recognized as the major non-protein energy yielding molecule for fish, the low cost and ready availability of carbohydrate make its inclusion in diets beneficial (Millikin, 1983). However, carbohydrates are generally poorly utilized by fish, particularly carnivorous species (Perez-Sanchez et al., 1995; Fernandez et al., 1998). Cooking or gelatinization of starch has been demonstrated to increase carbohydrate utilization for fish (Catacutan and Coloso, 1995, 1997; Dias et al., 1998; Fernandez et al., 1998).

A variety of hormone axes have been implicated in the control of growth in vertebrates. Recent studies have documented the interaction between the endocrine control of somatic growth and metabolism in teleost fish (Perez-Sanchez and LeBail, 1999). In fish, the growth hormone:insulin-like growth factor (IGF) and the thyroid hormone axes have received considerable attention (Funkenstein et al., 1989; Gray and Kelley, 1991; Duan and Plisetskaya, 1993; Eales, 1995). IGF-I is a peptide hormone thought to mediate many of the growth promoting activities of GH (Gray and Kelley, 1991; Duan and Plisetskaya, 1993; Peter and Marchant, 1995; Plisetyskaya, 1995). In fish, circulating IGF-I levels have been found to be regulated by ration size and dietary protein and energy levels (Perez-Sanchez et al., 1995; Plisetyskaya, 1995; Matthews, 1997). Thyroid hormones are thought to act synergistically with GH to regulate growth and nutrient partitioning in teleosts, with triiodothyronine (T3) directly stimulating production of growth hormone releasing hormone receptor in the pituitary (Peter and Marchant, 1995; Korytko and Cuttler, 1997). Nutrition, in terms of both quality and quantity of diet, has been found to influence thyroid hormone levels (Leatherland et al., 1977; Leatherland and Farbridge, 1992; Eales, 1988; Himick et al., 1991). Starvation decreases circulating levels of T3 and T4 and hepatic 5′ outer ring deiodinase (ORD) in salmonids (Leatherland et al., 1977; Leatherland and Farbridge, 1992; Eales, 1988). Dietary protein was found to regulate 5′ORD activity and circulating T3 levels in rainbow trout (Eales et al., 1992), although Himick et al. (1991) found dietary carbohydrate to be the most effective dietary factor in stimulating circulating T3 and T4

levels. Therefore, investigation of the GH-IGF and the thyroid hormone axes may provide a greater understanding of the endocrine control of growth in teleost fish.

Studies investigating nutritional requirements and the nutritional regulation of growth regulating hormones in fish often include elevated levels of carbohydrate to manipulate the dietary energy level (Perez-Sanchez et al., 1995; Matthews, 1997). However, if the dietary carbohydrate is not well utilized, the gross dietary energy does not relate to the energy available for metabolism. Thus, the present study aims to determine the effect of replacing dietary lipid energy with dietary carbohydrate energy on growth, nutrient partitioning and serum T3 and IGF-I for juvenile barramundi.

2. Materials and methods

2.1. Experimental animals

One hundred and seventy barramundi, *Lates calcarifer* (43.7 g ± 2.7, mean ± SEM), were transported to a controlled environment aquarium system (25 ± 1°C, 12 h light/12 h dark) at James Cook University and allowed to acclimate to experimental conditions for 1 week. Experimental animals were distributed into 32 experimental aquaria (70 l) where they were fed a commercial fish diet (Ridley Barrastock, Australia) until all animals were feeding. Animals were either held at a density of five fish per aquaria (30 treatment aquaria) or at 10 fish per aquaria (10 fish for initial promiate analysis and 10 replacement fish). Replacement fish were fed the commercial diet for the duration of the experiment. All aquaria received a continuous flow of recirculated seawater (28 ‰, 1 l min^{-1}) and aeration.

2.2. Experimental diets

Six isonitrogenous experimental diets were formulated at 50% crude protein and two gross energy levels, 18 or 21 MJ kg^{-1} (Table 1). Within each energy level, the non-protein energy was provided at three ratios of lipid: carbohydrate non-protein energy; 60:40, 70:30 and 80:20. Diets were extruded using a Hobart #12 chopper attachment with a 1/8″ die plate, powered by a Model A120 Hobart mixer (Hobart, Troy, Ohio, USA). All diets were dried at 50°C for 12 h and stored at −20°C until use.

2.3. Experimental protocol

Each experimental diet was fed to fish in five replicate tanks. Animals were fed a satiety ration. Uneaten diet was siphoned from the tank, isolated on a sieve (250 μm mesh size) and dried overnight at 50°C before being weighed and deducted from the amount offered to determine feed intake. The growth experiment was conducted for 70 days. At the commencement of the experiment, all fish were anaesthetized in a 1 g l^{-1} solution of benzocaine (ethyl-ρ-amino benzoate, Sigma, USA), identified by fin clipping and their weight measured. Time zero control fish were sacrificed via cervical dislocation for initial body proximate composition analysis. At the completion of the trial, all

Table 1
Diet formulation (g kg^{-1}) and proximate composition of experimental diets

Ingredients	Diet					
	18 60:40[a]	18 70:30[a]	18 80:20[a]	21 60:40[a]	21 70:30[a]	21 80:20[a]
Fish meal[b]	414	414	414	414	414	414
Casein[c]	204	204	204	204	204	204
Gelatin[d]	40	40	40	40	40	40
Starch[e]	101	76	51	169	127	84
Cellulose[f]	146	160	173	30	53	76
Fish oil[g]	70	82	94	118	138	157
Vitamin mix[h]	30	30	30	30	30	30
Mineral mix[i]	5	5	5	5	5	5
Chemical analyses (g kg^{-1} or MJ kg^{-1})						
Dry matter	2.2	3.6	2.0	3.5	4.3	0.7
Composition in dry matter						
Crude protein	53.6	52.6	51.7	52.9	52.2	52.1
Crude lipid	10.0	11.1	11.9	14.3	16.6	18.5
Gross energy	19.5	19.6	19.5	21.6	21.5	21.5

[a] Gross energy MJ kg^{-1}: (lipid energy/carbohydrate energy).

[b] Peruvian fish meal. Chemical composition: dry matter, 92.7%, in dry matter/protein, 66.8 %, energy 19.8 MJ kg^{-1}.

[c] Bonlac Food, Melbourne, Victoria, Australia.

[d] Wyandra Australia, Queensland, Australia.

[e] Goodman Fielder Mills, NSW, Australia. Autoclaved 125°C for 25 min prior to incorporation into diets.

[f] BW40 Solka floc, James River, Berlin, New Hampshire.

[g] Gibson's, Tasmania, Australia.

[h] Vitamin premix, g/kg of premix: retinyl acetate (500 000IU g^{-1}), 0.4; ascorbic acid, 75; cholecalciferol, 42.5; menadione, 1.1; alpha-tocopherol, 12.5; choline chloride, 40.0; myo-inositol, 8.5; PABA, 3.5; thiamin, 0.6; riboflavin, 0.7; pyridoxine HCl, 0.65; Ca–D–pantothenate acid, 1.85; nicotinic acid, 2.5; biotin, 0.02; cyanocobalamin, 0.002; folic acid, 0.15; ethoxyquin, 4.25; citric acid, 200; cellulose, 605.8.

[i] Mineral premix, g/kg of premix: $AlCl_3 \cdot 6H_20$, 0.45; $CoCl_2 \cdot 6H_20$, 0.2; $CuSO_4 \cdot 5H_20$, 2.0; $FeSO_4 \cdot 7H_20$, 19.5; KI, 0.5; $KCr(SO_4)_2$, 0.3; $MgSO_4 \cdot 7H_20$, 300.0; $MnSO_4 \cdot H_20$, 7.5; $NaSeO_3$, 0.02; $ZnSO_4 \cdot 7H_20$, 37; cellulose 632.5.

treatment fish were anaesthetized and their weight measured. A blood sample was taken from the caudal sinus allowed to clot on ice for 2 h and serum produced by centrifugation at 14 000 × g for 10 min. Fish were subsequently sacrificed via cervical dislocation and stored at −20°C until analyzed. The livers were removed and weighed for determination of hepatosomatic indices (HSIs).

There was a single mortality during the trial experiment and this fish was replaced by a fish of a similar size and not included in analyses.

2.4. Sample analysis

Carcass homogenates were prepared using the autoclave method of Williams et al. (1995). Experimental diets and fish homogenates were analyzed for dry weight (gravi-

metrically following drying to constant weight at 50°C), ash (combustion at 550°C), crude lipid (gravimetrically after Folch et al., 1957) and crude protein (Kjehldahl nitrogen × 6.25 after the method of Baethgen and Alley, 1989). Diets were also analyzed for crude energy (bomb calorimetry). All samples were assayed in triplicate.

Serum T3 levels were determined by radioimmunoassay with a free T3 solid phase component system (ICN Pharmaceuticals, Costa Mesa, CA, USA). All samples were measured in duplicate. Inter- and intra-assay coefficients of variation were 10.43% and 5.74%, respectively. Serum IGF-I activity was measured in acid ethanol extracts using protocol and reagents of a "generic" fish IGF-I RIA kit (GroPep, Australia) validated for use in this species (Quinn et al., 1999). Assay sensitivity was 2.0 ng ml^{-1} IGF-I. Acid ethanol extraction of the serum prior to assay has been shown to be sufficient for removing interference by IGF-I binding proteins in this species (Quinn et al., 1999). Extraction efficiencies from samples spiked with recombinant barramundi IGF-I, 2–10 ng ml^{-1}, were 100% or greater. Inter- and intra-assay coefficients of variation were 16.0% and 3.0%, respectively.

2.5. Calculations

Specific growth rate (SGR), %/day: ((ln final weight − ln initial weight)/days) × 100
Feed intake, % bw/day feed: (100 × (total dry feed intake per fish, g)/(initial fish weight − final fish weight × 0.5))/(days fed)
Feed conversion ratio (FCR): dry feed intake, g/wet weight gain, g
Hepatosomatic index (HSI), %: (liver weight, g/whole weight, g) × 100
Nutrient gain = final weight nutrient, g − initial weight nutrient, g
Protein efficiency ratio (PER): wet weight gain, g/protein intake, g
Protein retention efficiency (PRE): (protein gain/protein intake) × 100

2.6. Statistical analysis

Dietary effects on growth, feed utilization and nutrient retention were determined using two-way (dietary gross energy and energy source) and one-way analysis of variance (ANOVA) using the tank as the replicate. Two-way ANOVA found no significant affect of lipid:carbohydrate energy ratio and there was no significant interaction between dietary gross energy and lipid:carbohydrate energy ratio for the parameters measured. Post hoc analysis was conducted using Tukey's HSD test. Pearson correlation analysis was performed to examine the relationships between serum T3 and IGF-I levels and growth. Differences for all analyses were considered significant at $P < 0.05$. All analyses were conducted using SPSS Version 8.

3. Results

3.1. Growth and nutrient retention

Dietary energy significantly affected growth, feed conversion and feed intake of juvenile barramundi, with no effect of non-protein energy source (Table 2). An increase

Table 2
Specific growth rate (SGR), feed conversion ratio (FCR), hepatosomatic index (HSI), feed intake, carcass composition, nutrient gain and protein retention of juvenile barramundi fed isonitrogenous diets (50% protein) at two gross energy levels (18 and 21 MJ kg^{-1}) and three lipid to carbohydrate non-protein energy ratios (60:40, 70:30 and 80:20). Values are expressed as means ± SEM. Values in the same row with the same superscript ($^{a-b}$) are not significantly ($P > 0.05$) different

	Diet[1]					
	18:(60:40)	18:(70:30)	18:(80:20)	21:(60:40)	21:(70:30)	21:(80:20)
SGR	1.04 ± 0.04^{a}	1.00 ± 0.04^{a}	1.01 ± 0.02^{a}	1.21 ± 0.04^{b}	1.30 ± 0.07^{b}	1.31 ± 0.05^{b}
FCR	2.57 ± 0.15^{a}	2.89 ± 0.18^{a}	2.70 ± 0.11^{a}	1.57 ± 0.04^{b}	1.48 ± 0.16^{b}	1.54 ± 0.06^{b}
Feed intake[2]	2.92 ± 0.10^{a}	2.79 ± 0.02^{a}	2.76 ± 0.09^{a}	1.82 ± 0.07^{b}	1.86 ± 0.14^{b}	1.90 ± 0.06^{b}
HSI	1.46 ± 0.05^{ab}	$1.39 \pm .07^{a}$	1.47 ± 0.06^{ab}	1.59 ± 0.04^{ab}	1.65 ± 0.06^{b}	1.67 ± 0.07^{b}
Carcass protein[3]	59.4 ± 0.6^{a}	61.0 ± 0.6^{a}	59.5 ± 0.7^{a}	56.7 ± 0.6^{b}	54.7 ± 0.9^{b}	55.2 ± 1.0^{b}
Carcass lipid[3]	23.8 ± 0.5^{a}	23.7 ± 0.6^{a}	22.1 ± 0.8^{a}	28.4 ± 0.7^{b}	30.5 ± 0.4^{c}	30.5 ± 1.3^{c}
Carcass ash[3]	17.2 ± 0.4^{a}	17.0 ± 0.2^{a}	17.3 ± 0.3^{a}	15.7 ± 0.2^{b}	15.3 ± 0.3^{b}	15.2 ± 0.2^{b}
Carcass moisture[4]	68.7 ± 0.2	69.4 ± 0.2	69.3 ± 0.4	68.0 ± 0.6	67.0 ± 0.3	67.4 ± 0.4
Protein gain[5]	169.7 ± 10.0	145.5 ± 17.2	139.5 ± 10.0	168.0 ± 6.2	181.8 ± 11.8	173.8 ± 10.1
Lipid gain[5]	17.7 ± 1.7^{a}	17.2 ± 1.7^{a}	16.7 ± 2.2^{a}	25.6 ± 1.7^{b}	27.9 ± 2.6^{b}	30.3 ± 3.0^{b}
PER	0.72 ± 0.03^{a}	0.67 ± 0.03^{a}	0.75 ± 0.03^{a}	1.20 ± 0.03^{b}	1.45 ± 0.09^{c}	1.26 ± 0.08^{bc}
PRE	0.40 ± 0.02^{a}	0.38 ± 0.02^{a}	0.42 ± 0.03^{a}	0.64 ± 0.03^{b}	0.76 ± 0.04^{b}	0.64 ± 0.06^{b}

[1] Gross energy MJ kg^{-1}: (lipid energy:carbohydrate energy).
[2] Percent body weight/day.
[3] % dry weight.
[4] % wet weight.
[5] g dry weight/tank.

in dietary energy from 18 to 21 MJ kg^{-1}, resulted in an increase in SGR and a decrease in both FCR and feed intake values. HSI values for fish fed the 21 MJ kg^{-1} diets were generally greater than for fish fed the 18 MJ kg^{-1} diets (Table 2). At the 21 MJ kg^{-1} dietary energy level, fish fed diets containing 70:30 and 80:20 lipid:carbohydrate non-protein energy ratios demonstrated significantly higher HSI values than those fed the 18 MJ kg^{-1}, 70:30 lipid:carbohydrate non-protein energy. Animals fed the 60:40 lipid:carbohydrate non-protein energy ratios at both dietary energy levels and the 80:20 lipid:carbohydrate non-protein energy ratio at 18 MJ kg^{-1} displayed intermediate HSI values.

The proximate composition of juvenile barramundi was significantly affected by diet and non-protein energy source (Table 2). Carcass lipid content increased significantly with increases in dietary energy. At the 21 MJ kg^{-1} dietary energy level, animals fed the high carbohydrate (60:40 lipid:carbohydrate non-protein energy) diet had significantly lower carcass lipid than the fish fed the other two diets. Carcass protein and ash decreased significantly with increasing dietary energy, with no influence of non-protein energy source. Carcass moisture tended to be lower for animals fed the 21 MJ kg^{-1} diets than those fed the 18 MJ kg^{-1} energy diets, however changes were not significant.

Lipid gain significantly increased with increased dietary energy, with no effect of dietary non-protein energy source. Protein gain for animals fed the 21 MJ kg^{-1} diets tended to be higher than those fed the 18 MJ kg^{-1} diets for the 70:30 and 80:20

lipid/carbohydrate non-protein energy ratios, however large variance prevented these differences from being significant. Both PER and PRE increased significantly with increased dietary energy (Table 2). At the 21 MJ kg^{-1} dietary energy level, PER values for fish fed diets containing the 70:30 lipid:carbohydrate non-protein energy ratio were also significantly higher than those fed the 60:40 treatment.

3.2. Serum T3 and IGF-I levels

Serum T3 levels were significantly affected by dietary treatment (Fig. 1). For animals fed diets containing 21 MJ kg^{-1} dietary energy, there was no affect of lipid: carbohydrate non-protein energy ratio on serum T3 levels, with values ranging from 13.22 ± 0.49 to 13.42 ± 0.44 pg ml^{-1}. The serum T3 level for animals fed diets containing 80:20 lipid:carbohydrate non-protein energy ratio at the 18 MJ kg^{-1} dietary energy level (13.20 ± 0.64 pg ml^{-1}) was similar to those for animals fed the 21 MJ kg^{-1} diets. Fish fed the 60:40 lipid:carbohydrate non-protein energy ratio had significantly lower serum T3 levels, 10.31 ± 0.58 pg ml^{-1} (Fig. 1). Fish fed the 18 MJ kg^{-1}, 70:30 lipid:carbohydrate treatment were found to have an intermediate T3 level (11.91 ± 0.76 pg ml^{-1}). There was no significant correlation between serum T3 level and any if the growth or nutrient retention parameters measured (data not shown).

There was a trend for serum IGF-I levels to decrease with dietary energy and with non-protein energy source at the 18 MJ kg^{-1} dietary energy level, however differences were not significant (Fig. 2). Fish fed the 21 MJ kg^{-1} diet had a slightly higher, but not significant, IGF-I level, 31 ± 2.56 ng ml^{-1}, than animals fed all other diets. The serum IGF-I level of animals fed the 80:20 lipid:carbohydrate non-protein energy ratio at the 18 MJ kg^{-1} dietary energy level (27.60 ± 2.92 ng ml^{-1}) and fed the 60:40 (27.87 ± 3.06 ng ml^{-1}) and 70:30 (28.64 ± 3.94 ng ml^{-1}) lipid:carbohydrate non-protein energy ratio

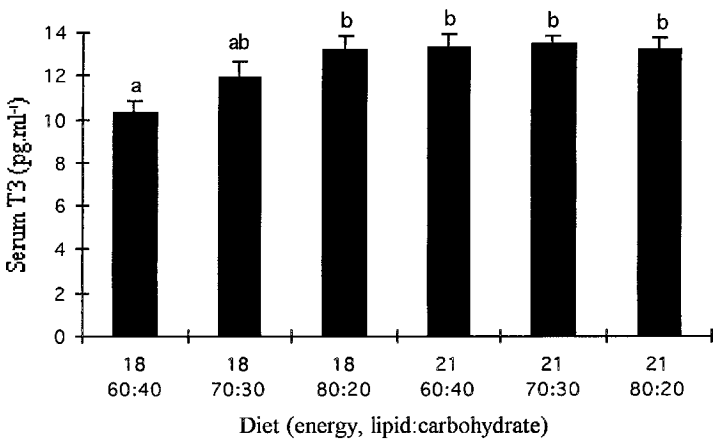

Fig. 1. Circulating T3 levels in barramundi serum after 70 days of being fed isonitrogenous diets (50% protein) at two energy levels (18 and 21 MJ kg^{-1}), and three lipid:carbohydrate ratios (60:40, 70:30 and 80:20). Values are means ± SEM. Values with the same superscript ($^{a-b}$) are not significantly different.

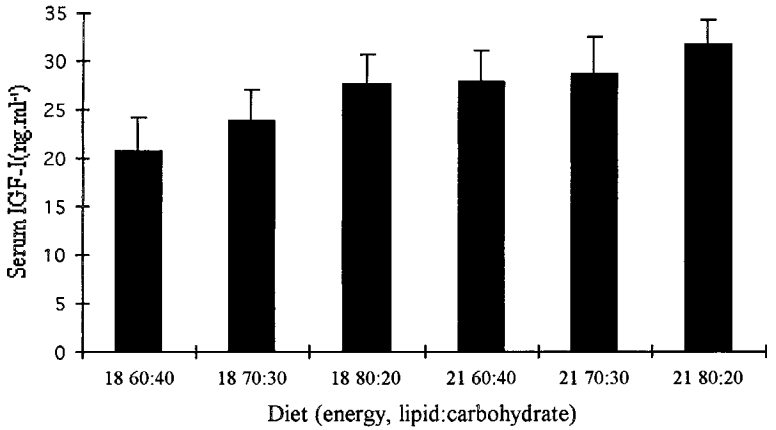

Fig. 2. Circulating IGF-I levels in barramundi serum after 70 days of being fed isonitrogenous diets (50% protein) at two energy levels (18 and 21 MJ kg^{-1}), and three lipid: carbohydrate non-protein energy ratios (60:40, 70:30 and 80:20). Values are means ± SEM.

at the 21 MJ kg^{-1} energy level were similar. At the 18 MJ kg^{-1} dietary energy level, 70:30 and 60:40 lipid:carbohydrate non-protein energy ratio diets resulted in decreases in serum IGF-I levels, to 23.76 ± 3.27 and 20.85 ± 3.53 ng ml^{-1}, respectively. There was no significant correlation between serum IGF-I level and any of the growth or nutrient retention parameters measured (data not shown).

4. Discussion

Growth parameters measured in the present study were generally affected by gross dietary energy levels, with no influence of non-protein energy source. Increases in dietary energy resulted in higher growth rate and improvement of feed conversion. SGR values from the present study are lower than previously found in our laboratory (unpublished data) and values found by Catacutan and Coloso (1995, 1997). FCR values in the present study were slightly higher than the FCR values reported by Catacutan and Coloso (1995, 1997) and Tucker et al. (1988) for barramundi. Although gross SGR and FCR levels differed, the changes in SGR and FCR in those studies in response to dietary energy were similar to those found in the present study.

Increased dietary energy resulted in increased carcass lipid, decreased carcass protein content, and increased lipid and protein gain. Increased carcass lipid content and lipid gain suggest that the additional dietary energy is at least partially stored as body lipid. Such trends have been reported previously for many finfish species (Hillestad and Johnsen, 1994; Dias et al., 1998; Company et al., 1999; Vergara et al., 1999). A significant increase in carcass lipid with increasing dietary lipid at 21 MJ kg^{-1} dietary energy suggests that at this energy level, dietary lipid energy is more available than dietary carbohydrate energy, although no effect on SGR was observed. Diets containing 21 MJ kg^{-1} energy had the highest total carbohydrate level and higher inclusion levels

of carbohydrate may have restricted energy availability. Greater HSI values for diets containing higher levels of dietary lipid also suggest that lipid was more readily utilized by juvenile barramundi than carbohydrate. When studying lipid:carbohydrate manipulations in hybrid striped bass, Nematipour et al. (1992) also found no differences in weight gain or nutrient efficiency, but found significant increases in intra-peritoneal fat with increased lipid and decreased carbohydrate intake. The data indicates that available energy was sufficient so as not to limit growth, but there was an increase in excess energy deposition with increased dietary lipid energy. From a product quality viewpoint, increases in carcass lipid content may be deemed as undesirable (Millikin, 1983). The carcass lipid levels found in the present study, 22–30%, are lower than those found for striped bass, 35–45% (Millikin, 1983), but higher than those reported by Catacutan and Coloso (1995) for fingerling barramundi. While these values may seem high, it is the fillet lipid composition and not whole body lipid which is of interest in commercial aquaculture practice (Jobling et al., 1991; Helland and Grisdale-Helland, 1998).

In the present study, increased dietary energy resulted in increased PER, indicating greater growth per unit protein consumed, a trend common to a variety of teleost fish species (Hillestad and Johnsen, 1994; Catacutan and Coloso, 1995; Dias et al., 1998; Company et al., 1999; Vergara et al., 1999). Although Catacutan and Coloso (1995) have previously reported no protein sparing effect in barramundi, a significant protein sparing effect of both lipid and carbohydrate energy was found in the present study. Increase dietary energy, irrespective of lipid:carbohydrate energy ratio, resulted in significantly higher PRE and higher protein gain.

The growth and nutrient retention data from the present study suggest that dietary carbohydrate is well utilized as a dietary energy source by juvenile barramundi up to an inclusion level of approximately 17%. Although carcass lipid levels would suggest that at this inclusion level dietary carbohydrate utilization is becoming limited. These data are surprising given the poor utilization of carbohydrates by juvenile (50–200 g) barramundi at similar inclusion levels in previous growth trials in our laboratory (unpublished data). However, Catacutan and Coloso (1997) found that fingerling barramundi could effectively utilize carbohydrate up to a 20% inclusion level. The lipid:carbohydrate non-protein energy ratios used in the present study are also within the ranges which have been reported to be well utilized by channel catfish (Garling and Wilson, 1977), striped bass (Millikin, 1983) and sunshine bass (Rawles and Gatlin, 1998).

The mechanisms by which nutrients influence endocrine function in fish are poorly understood (MacKenzie et al., 1998). The present study presents some data investigating the mechanisms underlying nutritional regulation of growth in the barramundi, a fast growing carnivorous teleost, by examining serum T3 and IGF-I levels in response to dietary manipulations. Dietary protein, carbohydrate and lipid have all been proposed to influence fish thyroid hormone production (Leatherland et al., 1984; Eales et al., 1990, 1992). Interestingly, circulating T3 decreased with increased dietary carbohydrate and decreased dietary lipid inclusion at the 18 MJ kg^{-1} dietary energy level, but was not affected by dietary energy source at the 21 MJ kg^{-1} dietary energy level. Circulating T3 levels were also similar in response to the highest dietary lipid inclusion level at 18 MJ kg^{-1} dietary energy and all three 21 MJ kg^{-1} diets. No effect of dietary lipid or

carbohydrate level on 5′ORD activity or circulating T3 levels was reported for rainbow trout, although dietary carbohydrate level did influence circulating T4 levels (Eales et al., 1990, 1992). Eales et al. (1992) did find an increase in 5′ORD activity with increased caloric intake and suggested that changes in circulating T3 levels due to altered caloric intake reflected protein metabolism. Therefore, energy affects on thyroid hormone levels may be due to an indirect influence on protein metabolism, and dietary protein and not energy may influence circulating T3, as found by Eales et al. (1990). However neither protein deposition nor protein retention reflected circulating T3 levels in the present study. It would appear from the present study that at a restricted dietary non-protein energy level carbohydrate inclusion was negatively affecting T3 production, perhaps indicating carbohydrate as a non-preferred energy source.

Growth parameters and circulating T3 levels both have a variety of regulating factors, with some of these factors common to both. In the present study there was no correlation between growth parameters and circulating T3 levels, indicating that the dietary regulation of growth and thyroid status are at least partially independent. This is supported by Eales et al. (1992), finding only modest correlation between 5′ORD activity and body weight in rainbow trout. Furthermore, when studying ration size manipulations, Farbridge et al. (1992) found no alteration to circulating T3 and T4 levels with increased growth in rainbow trout. Although, Eales and Shostak (1985) found a strong correlation ($r^2 = 0.81$) between SGR and plasma T3 levels in Arctic char. It may also be that the differences in SGR provided in the present experiment were insufficient to cause substantial changes in serum T3 levels. Circulating T4 levels and T4:T3 ratio have also been used as measures of thyroid status (Eales et al., 1992). However, T4 is generally regarded as a relatively inactive precursor of T3 (Eales et al., 1992), and was not found to correlate with growth or nutrition in barramundi (unpublished data).

Few studies are available on the effects of diet composition on GH and IGF-I in fish. Previous work in our laboratory, using a non-homologous radioreceptor assay, found an effect of both dietary protein and energy on circulating IGF-I levels, but not IGF-II levels (Matthews, 1997). In the present study, circulating IGF-I levels at the 18 MJ kg^{-1} dietary energy level were depressed by increased dietary carbohydrate and decreased dietary lipid inclusion. Natural variation in the individual response of animals to nutritional manipulation and the degree of the hormonal response to nutrition resulted in large variances, preventing these differences from being statistically significant. As with the serum T3 data, serum IGF-I levels did not relate to any of the growth parameters measured in this study. This is in contrast to previous studies in our laboratory and may result from the differences in assay method used. There has been only one previous study investigating the influence of dietary carbohydrate in the regulation of IGF-I in fish. Banos et al. (1998) found the inclusion of highly digestible carbohydrates, 22–37%, to increase the number of IGF-I receptors in brown trout. Thus increasing available energy through increasing the availability of carbohydrate energy sources may lead to an enhanced IGF-I status. An increase in IGF-I receptors may increase the physiological response to similar levels of circulating IGF-I. Therefore regulation of growth in response to dietary carbohydrate may occur at the receptor level and as such would not have been observed in the present study. Perez-Sanchez et al. (1995) identified variable dietary carbohydrate as a potential confounding factor in studies

concerning nutritional regulation of IGF-I. While the present study did not find any significant decrease in available energy with increased carbohydrate levels, depressed serum IGF-I levels resulted from an increased carbohydrate proportion of non-protein energy at the 18 MJ kg^{-1} dietary energy level. Therefore, a constant ratio of lipid/carbohydrate non-protein energy is recommended when investigating dietary protein and energy influences on growth regulating hormones.

The present study determined carbohydrate to be effectively utilized as a dietary energy source for juvenile barramundi up to an inclusion level of approximately 17%. A significant protein sparing effect of dietary energy was demonstrated for juvenile barramundi, irrespective of non-protein energy source. Circulating IGF-I and T3 levels followed similar trends and may be depressed by elevated dietary carbohydrate levels when dietary energy is limiting. While circulating levels of IGF-I and T3 responded to dietary treatment, neither hormone related directly to any growth parameter measured, emphasizing the complexity of hormonal regulation of growth and nutrient utilization. This complexity is highlighted in the mammalian GH:IGF-I axis where 10 nutrient sensitive locations have been identified (MacKenzie et al., 1998). Similarly, there are multiple levels at which regulation of the thyroid axis occurs (Eales, 1988). The results of this study suggest that the impact of dietary manipulations on the regulation of IGF-I and T3 in barramundi may also be complicated and certainly warrants further investigation. Thus, further studies are required on the macronutrient and energetic regulation of IGF-I and T3 in teleost fish, as the processes determining the circulating levels of these hormones remain open to conjecture.

References

Baethgen, W.E., Alley, M.M., 1989. A manual for colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. Comm. Soil Sci. Plant Anal. 20, 961–969.

Banos, N., Baro, J., Castejon, C., Navarro, I., Gutierrez, J., 1998. Influences of high-carbohydrate enriched diets on plasma insulin levels and IGF-I receptors in trout. Regul. Pept. 77, 55–62.

Catacutan, M.R., Coloso, R.M., 1995. Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian sea bass, *Lates calcarifer*. Aquaculture 131, 125–133.

Catacutan, M.R., Coloso, R.M., 1997. Growth of juvenile Asian seabass. *Lates calcarifer*, fed varying carbohydrate and lipid levels. Aquaculture 149, 137–144.

Company, R., Calduch-Giner, J.A., Kaushik, S., Perez-Sanchez, J., 1999. Growth performance and adiposity in gilthead sea bream (*Sparus aurata*): risks and benefits of high energy diets. Aquaculture 171, 279–292.

Dias, J., Alvarez, M.J., Diez, A., Arzel, J., Gorraze, G., Bautista, J.M., Kaushik, S.J., 1998. Regulation of hepatic lipogenesis by dietary protein energy in juvenile European seabass (*Dicentrachus labrax*). Aquaculture 161, 169–186.

Duan, C., Plisetskaya, E.M., 1993. Nutritional regulation of insulin-like growth factor-I mRNA expression in salmon tissues. J. Endocrinol. 139, 243–252.

Eales, J.G., 1988. The influence of nutritional state on thyroid function in various vertebrates. Am. Zool. 28, 351–362.

Eales, J.G., 1995. Regulation and measurement of thyroidal status in fish. Neth. J. Zool. 45, 175–180.

Eales, J.G., Shostak, S., 1985. Correlations between food ration, somatic growth parameters and thyroid function in Arctic charr, *Salvelinus alpinus* L. Comp. Biochem. Physiol. 80A, 553–558.

Eales, J.G., Higgs, D.A., Uin, L.M., MacLatchy, D.L., Bres, O., McBride, J.R., Dosanjh, B.S., 1990. Influence of dietary lipid and carbohydrate levels and chronic 3,5,3′-triiodo-L-thyronine treatment on thyroid function in immature rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 80, 146–154.

Eales, J.G., MacLatchy, D.L., Higgs, D.A., Dosanjh, B.S., 1992. The influence of dietary protein and caloric content on thyroid function and hepatic thyroxine 5′ monodeiodinase activity in rainbow trout, *Oncorhynchys mykiss*. Can. J. Zool. 70, 1526–1535.

Farbridge, K.J., Flett, P.A., Leatherland, J.F., 1992. Temporal effects of restricted diet and compensatory increased dietary intake on thyroid function, plasma growth hormone levels and tissue lipid reserves of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 104, 157–174.

Fernandez, F., Miquel, A.G., Guinea, J., Martinez, R., 1998. Digestion and digestibility in gilthead sea bream (*Sparus aurata*): the effect of diet composition and ration size. Aquaculture 166, 67–84.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226, 497–509.

Garling, D.L., Wilson, R.P., 1977. Effects of dietary carbohydrate-to-lipid ratios on growth and body composition of fingerling channel catfish. Prog. Fish-Cult. 39, 43–47.

Gray, E.S., Kelley, K.M., 1991. Growth regulation in the gobiid teleost, *Gillichthys mirabilis*: roles of growth hormone, hepatic growth hormone receptors and insulin-like growth factor-I. J. Endocrinol. 131, 57–66.

Grisdale-Helland, B., Helland, S.J., 1998. Macronutrient utilization by Atlantic halibut (*Hippoglossus hippoglossus*): diet digestibility and growth of 1 kg fish. Aquaculture 166, 57–65.

Helland, S.J., Grisdale-Helland, B., 1998. Growth, feed utilization and body composition of juvenile Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in the ratio between the macronutrients. Aquaculture 166, 49–56.

Hillestad, M., Johnsen, F., 1994. High energy/low protein diets for Atlantic salmon: effects on growth, nutrient partitioning and slaughter quality. Aquaculture 124, 109–116.

Himick, B.A., Higgs, D.A., Eales, J.G., 1991. The acute affects of alterations in the dietary concentrations of carbohydrate, protein, and lipid levels in rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 82, 451–458.

Jobling, M., Knudsen, R., Pedersen, P.S., DosSantos, J., 1991. Effects of diet composition and energy content on the nutritional energetics of cod, *Gadus morhua*. Aquaculture 92, 243–257.

Korytko, A.I., Cuttler, L., 1997. Thyroid hormone and glucocorticoid regulation of pituitary growth hormone-releasing hormone receptor gene expression. J. Endocrinol. 152, R13–R17.

Leatherland, J.F., Farbridge, K.J., 1992. Chronic fasting reduces the response of the thyroid to growth hormone and TSH and alters growth hormone related chanbges in hepatic 5′-monodeiodinase activity in rainbow trout, *Oncorhbynchus mykiss*. Gen. Comp. Endocrinol. 87, 342–353.

Leatherland, J.F., Cho, C.Y., Slinger, S.J., 1977. Effects of diet, ambient temperature, and holding conditions on plasma thyroxine levels in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34, 677–682.

Leatherland, J.F., Cho, C.Y., Hilton, J., 1984. Effects of diet on serum throid hormone levels in rainbow trout (*Salmo gairdneri*, Richardson). Comp. Biochem. Physiol. 78A, 601–605.

Matthews, S.J., 1997. The regulation of insulin-like growth factors in barramundi, *Lates calcarifer*. Ph.D. Thesis, James Cook University, Townsville, Australia.

MacKenzie, D.S., van Putte, C.M., Leiner, K.A., 1998. Nutrient regulation of endocrine function in fish. Aquaculture 161, 3–25.

Millikin, M.R., 1983. Interactive effects of dietary protein on growth and utilization of age-0 striped bass. Trans. Am. Fish. Soc. 112, 185–193.

Nematipour, G.R., Brown, M.L., Gatlin, D.M. III, 1992. Effects of carbohydrate:lipid ratio on growth and body composition of hybrid striped bass. J. World Aquacult. Soc. 23, 128–132.

Perez-Sanchez, J., LeBail, P.-Y., 1999. Growth hormone axis as a marker of nutritional status and growth performance in fish. Aquaculture 177, 117–128.

Perez-Sanchez, J., Marti-Palanca, H., Kaushik, S.J., 1995. Ration size and protein intake affect circulating growth hormone concentration, hepatic growth hormone binding and plasma insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead sea bream (*Sparus aurata*). J. Nutr. 125, 546–552.

Peter, R.E., Marchant, T.A., 1995. The endocrinology of growth in carp and related species. Aquaculture 129, 299–321.

Plisetyskaya, E.M., 1995. Peptides of insulin and glucogon superfamilies in fish. Neth. J. Zool. 45, 181–188.

Quinn, K., Carragher, J., Lucas, A., Ward, H., Degger, B., Takos, A., Dyer, A., Milner, S., Soole, K., Upton, Z., 1999. Applications of IGFs in aquaculture. Growth Horm. IGF Res. 9, 358–359, (abstract).

Rawles, S.D., Gatlin, D.M., 1998. Carbohydrate utilization in striped bass (*Morone saxatilis*) and sunshine bass (*M. chrysops* × *M. saxatilis*). Aquaculture 161, 201–212.

Tucker, J.W., MacKinnon, M.R., Russel, D.J., O'Brien, J.J., Cazzola, E., 1988. Growth of juvenile barramundi (*Lates calcarifer*) on dry feeds. Prog. Fish-Cult. 50, 81–85.

Vergara, J.M., Lopez-Calero, G., Robaina, L., Caballero, M.J., Montero, D., Izquierdo, M.S., Aksnes, A., 1999. Growth, feed utilization and body lipid content of gilthead seabream (*Sparus aurata*) fed increasing lipid levels and fish meals of different quality. Aquaculture 179, 35–44.

Williams, K.C., Barlow, C.G., Brock, I., Rodgers, L.J., 1995. Use of autoclaving in the preparation of homogenates for determining the proximate chemical and fatty acid composition of fish. J. Sci. Food Agric. 69, 451–456.