An analysis of the partial efficiencies of energy utilisation of different macronutrients by barramundi (*Lates calcarifer)* shows that starch restricts protein utilisation in a carnivorous fish

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Abstract

This study examined the effect of including different dietary proportions of starch, protein and lipid, in diets balanced for digestible energy, on the utilisation efficiencies of dietary energy by barramundi (*Lates calcarifer*). Each diet was fed at one of three ration levels (satiety, 80% of initial satiety and 60% of initial satiety) for a 42-day period. Fish performance measures (weight gain, feed intake, and feed conversion ratio) were all affected by dietary energy source. The efficiency of energy utilisation was significantly reduced in fish fed the starch diet relative to the other diets, but there were no significant effects between the other macronutrients. This reduction in the efficiency of utilisation was derived from a multifactorial change in both protein and lipid utilisation. The rate of protein utilisation deteriorated as the amount of starch included in the diet increased. Lipid utilisation was most dramatically affected by inclusion level of lipid in the diet, with those diets low in lipid producing component lipid utilisation rates well above 1.3 which indicates substantial lipid synthesis from other energy sources. However, the energetic cost of lipid gain was as low as 0.65 kJ per kJ of lipid deposited, indicating that barramundi very efficiently store energy in the form of lipid, in particular from dietary starch energy. This study defines how the utilisation efficiency of dietary digestible energy by barramundi is influenced by the macronutrient source providing that energy, and that the inclusion of starch causes problems with protein utilisation in this species.

Introduction

Barramundi are an obligate carnivorous fish species that is the basis of a significant aquaculture industry in Southeast Asia and Australia (1). The development of high-nutrient density formulated extruded feeds has been underpinned by the development of both a series of factorial bioenergetic nutritional models and foundation empirical studies (1, 2, 3, 4, 5). These nutritional models have so far relied on the assumption that the dietary digestible energy (DE) source is irrelevant; that is that the dietary DE derived from protein, lipid and starch is utilised with equal efficiency, subject to key nutrients (e.g. protein) being provided at/or above minimum critical ratios to energy supply (4, 5, 6, 7, 8, 9, 10).

Each of the different macronutrients (starch, protein and lipid) supplies energy by distinct metabolic pathways. In aquatic animals it is recognised that there are different levels of efficiency in the utilisation of each these macronutrients for energy (11, 12). It is now recognised that this difference requires an amendment of the digestible nutritional values of each macronutrient to those of metabolisable nutritional values and/or net energy nutritional values (9, 12, 13, 14). Recent work by Schrama et al. (14) examined the utilisation of both starch and lipid for growth by the omnivorous fish Nile tilapia (*Oreochromis niloticus*). These authors observed that each macronutrient had a different effect on the partial efficiencies of utilisation of digestible energy (k_{DE}) by the fish, with dietary utilisation coefficients of 0.561 and 0.663 being observed for the starch and lipid biased diets respectively. These observations clearly indicated that this fish species used lipid as an energy source for growth more efficiently. However, the third key macronutrient, protein, was not considered in this study. In that same study, Schrama et al. (14) in reviewing the literature identified that there was a wide variability (0.31 to 0.82) in the k_gDE of different studies. It was suggested that the three primary reasons for this variability were: different dietary macronutrient compositions; trophic level of the fish species; and the composition of the growth. In addition, there is increasing evidence that the roles of gluconeogenesis, glycolysis and β-oxidation play substantially different relative roles in energy provision in fish compared to other vertebrates (11, 14, 15, 16, 17).

The objective of this study was to determine the partial efficiencies of utilisation of each of the different diets based on equivalent digestible energy densities, but differing in the ratio of each of the macronutrient energy substrates. By using a diet by ration factorial study it was proposed that it would be possible to not only derive the partial efficiencies for each diet, but by overlaying a multiple regression analysis of the responses, to derive the discrete partial energetic efficiencies for each of the macronutrients. By determining these responses it will help provide the evidence for the true energetic role that each of the three macronutrients (protein, lipid and starch) play as energy sources in diets when fed to barramundi.

Methods

Diet preparation

Each of the diets used in this study were based on equivalent digestible energy densities, but differed in the ratio of each of the macronutrient energy substrates. From this design it will be possible to not only derive the partial efficiencies for each diet, but by overlaying a multiple regression analysis of the responses, to derive the discrete partial energetic efficiencies for each of the macronutrients used within each diet. The diets used in this study are based on those diets used in the earlier study by Glencross et al (12). In this experiment each of the diets were formulated to be isoenergetic (15.3 MJ-DE kg^{-1}) on a digestible nutrient basis based on the ingredient digestibility values determined in Glencross et al. (12). Most diets were also isoproteic (475 g kg^{-1}) on a digestible basis, with the exception of the 'P' diet in which the digestible protein was 562 g kg^{-1}. An additional diet (C) was used to provide a reference to diet specifications typically used in commercial diets.

Diets were made by mixing all the dry ingredients and then processed by the addition of the oil component and water (about 30 % of mash dry weight) to all ingredients while mixing to form a dough. The dough was then screw-pressed through a 4 mm diameter die using a pasta maker (Dolly, La Monferrina, Castell'Alfero, Italy). The resultant moist pellets were oven dried at 65 ºC for 12 h before being air-cooled, bagged and stored at –20 ºC. Formulations and composition of the diets are presented in Table 1.

Fish handling

All animal procedures were approved by the CSIRO Animal Ethics Committee (Approval A9/2011). Juvenile barramundi (*Lates calcarifer*) were obtained from a commercial hatchery (BettaBarra, Walkamin, QLD, Australia), and on-grown to 69.6 ± 0.75 g (mean ± SD, n=480) in preparation for the experiment. During the on-growing period all fish were fed the same diet (Marine Float; Ridley Aquafeeds, QLD, Australia) and kept in 2 x 5000L seawater tanks. At the initiation of the trial 40 fish were weighed on an electronic top-loading balance to 0.1 g accuracy to determine the mean and standard deviation of the population. Following this, 20 fish were allocated to each of 24 x 300L tanks based on having to be within the mean ± 1 x S.D. The experiment was conducted at the Bribie Island Research Centre at Woorim, in a flow-through (3L min^{-1}), aerated, heated seawater tank array. Water temperature was maintained at 29.9 ± 0.12 ºC (mean ± S.D.) and dissolved oxygen 5.5 ± 0.56 mg L^{-1} for the 42-day duration of the experiment.

Each diet was manually fed to each tank. Three ration levels were used; a satiety, 80% and 60% of the initial satiety levels. The satiety rations were fed twice daily, with AM (0900 – 0930) and PM (1630 - 1700) feeds. The satietal rations were determined by feeding to slight excess, with all feed fed and all uneaten feed was accounted for and correction factors applied to allow for the determination of solubilisation losses and pellet dry matters and therefore of actual feed consumption within each tank based on methods reported by Helland et al., (18). The two restricted rations used in

this study were based on 80% and 60% of the measured initial demand which was also consistent with the model of Glencross (4). These rations were not adjusted over time. Each treatment was duplicated within the 24-tank array, based on the plan for using regression analysis in this experiment it was proposed that a 3 rations x 2 replicates design was stronger than a 2 rations x 3 replicates approach.

Sample preparation and chemical analysis

Five fish were euthanized from the population at the beginning of the experiment as a representative initial sample. At the end of experiment, five whole fish from each tank were euthanized by immersion in an overdose of AQUI-S™ before then being placed in an iced-seawater slurry. Following sample collection, each whole fish sample was frozen prior to being minced by two passes through an industrial food processor to ensure sample homogeneity. Samples were then collected and their moisture content determined by oven drying at 105 ºC for 24 h and a second sample freeze-dried for chemical analysis. Freeze-dried fish samples were milled prior to analysis for dry matter, ash, fat, nitrogen and gross energy content. Diet and faecal samples were analysed for dry matter, yttrium, nitrogen, lipid, starch and gross energy content.

Dry matter was calculated by gravimetric analysis following oven drying at 105 ºC for 24 h. Total yttrium concentrations were determined after mixed acid digestion using inductively coupled plasma mass spectrometry (ICP-MS). Protein levels were calculated from the determination of total nitrogen by CHNOS auto-analyser, based on N x 6.25. Total starch content of the diets was measured using an enzymatic method with the Megazyme Total Starch Kit, K-TSTA, following a modified AOAC Method 996.11. Total lipid content of the diets was determined gravimetrically following extraction of the lipids using chloroform:methanol (2:1). Gross ash content was determined gravimetrically following the loss of mass after combustion of a sample in a muffle furnace at 550 °C for 12 h. Gross energy was determined by ballistic bomb calorimetry. All methods were conducted in accordance with the specifications of AOAC (19).

Diet digestibility analysis

At the end of the growth experiment and following sample collection, the remaining fish in each of the eight satiety fed tanks were used for faecal collection. The fish were stripped of their faeces once daily about 6h post feeding. Faecal stripping was based on the methods reported by Blyth et al. (20). This involved the netting of fish into a separate tank and the rapid sedation of the fish to induce muscle relaxation. Once muscle relaxation had occurred, the fish were removed from the anaesthetic containing water, stripped with gentle manual abdominal pressure and the faeces expelled into a collection jar. Each fish was then returned to their original tank for recovery. Faeces were collected over a minimum of three stripping events, pooled within each tank and kept frozen pending analysis.

Differences in the ratios of dry matter, protein, lipid (insufficient faecal sample was available for starch analysis) or gross energy to yttrium, in the feed and faeces in each treatment were calculated to determine the apparent digestibility (AD_{diet}) for each of the nutritional parameters examined in each diet based on the following formula:

$$AD_{diet} = \left(1 - \left(\frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}} \right) \right) \times 100$$

where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (dry matter, protein or energy) content of the diet and faeces respectively.

Protein and energy utilisation analysis

Protein (N x 6.25) and energy (E) utilisation were determined based on the gain in both N and E over the period of the experiment, against the respective consumption of digestible N and E over the period of the experiment. Both gain and intake values were calculated based on a daily gain amount per unit body weight. To provide some independence of size effects, modelling of the protein, lipid and energy utilisation data was done with respect to known protein, lipid and energy body-weight exponents for barramundi of $x^{0.7}$, $x^{0.9}$ and $x^{0.8}$ respectively (21, 22). Both protein-energy and lipid-energy utilisation was transformed to the energy body-weight exponent value of $x^{0.8}$.

Nutrient and energy balance and deposition assessment

The net balance for protein (P), lipid (L) and energy (E) were calculated based on the data derived in this study. The methods used for these calculations were based on those reported by Saravanan et al (11). Gross intake levels of each nutrient were determined based on total feed intake for each tank multiplied by the percent composition of the feed being fed. Digestible intake levels were measured similarly based on the digestibility of P, L and E from each diet. Faecal losses were determined as the reciprocal of the digestible levels. Retained nutrients and energy were determined based the net gain in nutrients and energy between the fish at the end of the trial and those from the initial sample. Branchial and urinary nitrogen (BUN) were determined based on the difference between digestible nitrogen intake and retained nitrogen with energy values defined based on 24.85 kJ x branchial and urinary nitrogen using values reported by Saravanan et al (11). The metabolisable energy intake (MEI) was determined based on the digestible energy intake minus the branchial and urinary energy losses. Heat production (HP) was determined based on the difference between metabolisable energy and retained energy (RE). Basal metabolism (HeE) was calculated based on the reported fasting energy losses of 34.4 kJ/ $kg^{0.8}$ /d (4). The Heat increment (HiE) was determined based on the MEI minus the RE and the HeE. Net energy (NE) was determined based on MEI minus HiE (23).

Statistical analysis

All figures are mean ± SEM unless otherwise specified. Effects of diet treatment and ration levels were examined by MANOVA using the software package Statistica (Statsoft®, Tulsa, OA, USA). Levels of significance were determined using Fishers LSD test for planned comparisons, with critical limits being set at $P < 0.05$. Regression figures presented were constructed using Microsoft Excel. Error terms for linear functions were determined using the regression feature of the Data Analysis package within Microsoft Excel. Multiple regression analysis was used to determine the component energy utilisation parameters based on having definitive assessments of the protein energy utilisation efficiencies for each diet which then enabled the derivation, by multiple regression, of the contribution of both lipid energy and starch energy to the partial efficiency of energy utilisation in each diet (24).

Results

Effect of macronutrient energy bias on growth and body composition

There were significant differences between each of the diets and feed ration levels on the final weight, weight gain, feed intake and feed conversion ratio (FCR) (Table 2). Significant interaction terms between diet and ration level were also observed on feed intake, but none of the other performance parameters. There were no significant effects on survival attributable to diet, ration or the interaction term. Among those fish fed to satiety, weight gain was greatest in those fish fed Diet L and worst in those fish fed Diet S. However, among those fish fed to satiety, feed conversion was best in those fish fed Diet P and worst in those fish fed Diet S. Of those treatments fed to satiety there were some significant differences in feed intake, with intake highest by those fish fed the Diet S and lowest by those fed Diet P (Table 2).

There was a significant effect of both feed ration level and diet on final live-weight protein concentration, lipid concentration and energy content (Table 2). No significant differences observed of diet on final live-weight dry matter composition (Table 2). There were also significant interaction terms between diet and ration level on each of the parameters of final live-weight dry matter, protein, lipid and energy concentrations. Key compositional differences of note included those fish fed Diet P, which had less lipid than those fish fed the Diet L. This effect was most notable at the lower fixed ration levels (Table 2).

Effect of macronutrient energy bias on energy utilisation

The pair-wise comparison within feed ration levels between each dietary treatment showed significantly different levels of energy retention between the starch diet and every other treatment (Table 3). The energy utilisation efficiencies (kJ/kg$^{0.8}$/d) for each diet were described by the following linear equations (Figure 1);

(Eq. 1) $y_S = 0.508(\pm0.010)x - 8.859(\pm2.496)$, $R^2 = 0.998$

(Eq. 2) $y_L = 0.730(\pm0.023)x - 29.821(\pm5.461)$, $R^2 = 0.996$

(Eq. 3) $y_P = 0.715(\pm0.012)x - 26.324(\pm2.774)$, $R^2 = 0.999$

(Eq. 4) $y_C = 0.607(\pm0.015)x - 8.686(\pm3.717)$, $R^2 = 0.997$

The coefficient of utilisation (k_E) was significantly lower for Diet S relative to each of the other diets. Similarly, the utilisation coefficient for Diet C was also significantly lower than that of Diets P and L. There was no difference in the energy utilisation coefficient between Diets P and L. Maintenance digestible energy intake (*HEm*) was calculated by extrapolation of the linear regression to the intercept of the X-axis. From this the following *HEm* values were derived; Diet S : 17.4(±0.81) kJ/kg$^{0.8}$/d, Diet L : 40.8(±0.98) kJ/kg$^{0.8}$/d, Diet P : 36.8(±0.59) kJ/kg$^{0.8}$/d, Diet C : 14.3(±1.14) kJ/kg$^{0.8}$/d. There were significant differences in the *HEm* values between Diets L and P relative to Diets S and C, but not within those pairings.

Effect of macronutrient energy bias on protein and lipid energy utilisation

The pair-wise comparison within feed ration levels between each dietary treatment also showed significantly different levels of protein energy retention between the starch diet and every other treatment (Table 3). The protein energy utilisation efficiencies ($g/kg^{0.8}/d$) for each diet were described by the following linear equations (Figure 2):

(Eq. 5) $y_S = 0.412(\pm0.003)x - 1.302(\pm0.417\ R^2 = 0.994$

(Eq. 6) $y_L = 0.582(\pm0.006)x - 8.094(\pm0.572),\ R^2 = 0.995$

(Eq. 7) $y_P = 0.556(\pm0.005)x - 7.637(\pm0.527),\ R^2 = 0.996$

(Eq. 8) $y_C = 0.534(\pm0.004)x - 0.088(\pm0.588),\ R^2 = 0.986$

The coefficient of utilisation was significantly lower for Diet S relative to each of the other diets. There was no difference in the protein energy utilisation coefficient (k_{PE}) between Diets P, L and C.

There were also different levels of lipid energy retention between the starch diet and every other treatment (Table 3). This resulted in the coefficient of utilisation being significantly higher for Diet S relative to each of the other diets. However, Diet P also had a significantly higher level of lipid energy utilisation relative to the lipid and control diets. The lipid energy utilisation efficiencies ($kJ/kg^{0.8}/d$) for each diet were described by the following linear equations (Figure 3):

(Eq. 9) $y_S = 1.5478(\pm0.015)x - 7.332(\pm0.500),\ R^2 = 0.991$

(Eq. 10) $y_L = 1.070(\pm0.002)x - 19.619(\pm1.469),\ R^2 = 0.998$

(Eq. 11) $y_P = 1.387(\pm0.006)x - 17.558(\pm0.456),\ R^2 = 0.994$

(Eq. 12) $y_C = 1.081(\pm0.002)x - 8.375(\pm0.183),\ R^2 = 0.999$

When the lipid energy utilisation coefficients (k_{LE}) were examined relative to the dietary concentration of lipid a strong, but non-significant (p=0.127) linear relationship was observed (Figure 4).

Determination of macronutrient component contributions to energy utilisation

The different combinations of protein, lipid and starch among the diets in the present study allow for the analysis of the component contributions of each macronutrient to energy retention (Table 4). This assumes that each macronutrient is contributing part of the dietary energy proportional to its content in the diet, its energetic value and a component utilisation value.

Based on the prior mentioned assumptions, each of the component energy utilisation values was derived using multiple regression analysis. For each of the diets the protein contribution can be defined by converting the protein utilisation to protein energy utilisation and defining from that the component protein energy utilisation (Figure 2). Therefore, because we have a definitive assessment of the protein energy utilisation efficiencies (see equations 5 to 8) we can also derive by multiple regression the remaining unknown variables, which constitute the contribution of both lipid energy and starch energy to the partial efficiency of energy utilisation in each diet (Table1 and Table 3). Although we have an assessment of the partial efficiency of lipid energy utilisation (Figure 3), the fact that lipid energy gain in this representation also includes lipid deposited from non-lipid origins (i.e.

starch and/or protein energy), it was necessary to derived the component lipid energy utilisation using multiple regression methods.

Effect of macronutrient energy bias on protein, lipid and energy budgets

There were a range of significant effects attributable to diet, feed ration level and the interaction term on the protein, lipid and energy budget parameters (Table 3). Exceptions to this were for the Digestible Protein Intake (DPI), for which there were no significant interactions between diet and ration level. Gross Protein Intake (GPI) was highest by those fish fed Diet P at ration level H with the corresponding lowest GPI at the same ration level being from Diet L (Table 3). Faecal Protein (FP) was also highest by those fish fed Diet P and this was consistent across each of the ration levels. The lowest FP, again across each of the ration levels was also from Diet L. Digestible Protein Intake (DPI) was highest by those fish fed Diet P at ration level H, and although these differences were significant, they were much smaller than those seen on GPI. Protein losses through branchial and urinary equivalents (BUN Peq) were highest by those fish fed Diet S at ration level H, though differences at the lower ration levels were less obvious. Retained Protein (RP) at the highest ration levels was similar from each of diet C, P and L, but significantly poorer from Diet S. The ratio of RP/DPI was highest from those fish fed Diet C at ration level M. At ration level H there was no significant difference among the RP/DPI for Diets C, P and L, but Diet S was significantly lower (Table 3).

Gross Lipid Intake (GLI) was highest by those fish fed Diet L at ration level H with the corresponding lowest GLI at the same ration level being from Diet S (Table 3). Faecal Lipid (FL) was highest by those fish fed Diet P and this was consistent across each of the ration levels. The lowest FL, across each of the ration levels was also from both Diets C and S. Digestible Lipid Intake (DLI) was highest by those fish fed Diet L at ration level H, and for the other ration levels DLI was also significantly higher from Diet L. Retained Lipid (RL) at the highest ration levels was similar from each of diet C, P and S, but significantly higher from Diet L. The ratio of RL to DLI was highest from those fish fed Diet S and this was consistent across each of the ration levels. The ratio of RL/DLI was lowest from those fish fed Diet L and this too was consistent across each of the ration levels. The ratio between RL/RP for Diets L and S were similar and significantly higher than those from fish fed Diets C and P. In most cases this ration declined with declining ration, though no such effect was observed with Diet C (Table 3).

Gross Energy Intake (GEI) was highest by those fish fed Diet S at ration level H with the corresponding lowest GEI at the same ration level being from Diet P (Table 3). Among the lower ration levels there was no significant differences in GEI. These differences were also reflected in the DEI across the treatments. Faecal Energy (FE) was highest by those fish fed both Diet C and S and lowest from those fish fed Diet P. BUE losses were highest from fish fed Diet S at ration level H and M, though at the lowest ration level BUE was highest from Diet P. The highest metabolisable energy

306 intake (MEI) at ration level H was from Diet S, but at the two lower ration levels it was higher from
307 Diet C. Lowest MEI were from Diet P and the highest ration level (H), but at the two lower ration
308 levels the MEI intake was lower from Diet S. Retained Energy (RE) was highest by those fish fed
309 Diet L at ration level H, and poorest by fish fed Diet S at the lowest ration, although RE by fish fed
310 Diet S was poorest within each of the ration levels. Heat Production (HP) was highest, and
311 substantially so, in those fish fed Diet S at ration level H, though differences at the lower ration levels
312 were less obvious. Basal metabolism (HeE) had significant effects attributable to both diet and ration,
313 but not the interaction. The Heat increment energy (HiE) was highest by those fish fed Diet S at ration
314 level H, which was more than twice that of fish fed the same ration from Diet P. This effect was
315 reversed at the lower ration levels with higher HiE values observed from Diet S at the two lowest
316 ration levels. Net Energy intake (NEI) was highest by this fish fed Diet L and poorest by those fish
317 fed Diet S. Ration also had a clear effect on NEI, though differences between fish fed Diets C, P and
318 L at each of the ration levels were nominal. The NEI by fish fed Diet S were significantly lower at
319 each ration level. The ratio of RE/DEI typically declined with declining ration. The RE/DEI values
320 were similar between Diets P and L at reach of the ration levels, but significantly poorer by Diet S at
321 each ration level except the lowest one. Diet C was a little different to the other diets and showed a
322 largely consistent RE/DEI across the ration levels and at a high level (>50%) (Table 3).
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Discussion

The present study sought to define the relative contributions of each of the three macronutrients (protein, lipid and starch) in suppling digestible energy in diets fed to juvenile barramundi. This has enabled an insight into the roles that these macronutrients play in contributing to energy provision in this species. Understanding this relationship is critical to fish nutrition due to the strong intrinsic link between fish growth, energy demand and diet energy density.

Effect of macronutrient energy bias on growth, feed utilisation and body composition

Using diets with equivalent levels of digestible energy but differences in the proportions of protein, lipid or starch providing that energy, clear effects were seen in this experiment. For each of these treatments, the strategy of feeding each diet at specific ration levels has allowed us to build substantially on earlier findings from using these same diets, that were previously fed over a much longer term basis (12). Therefore, in the present study we focus our discussion on the effects within ration levels to allow us to examine the diet specific effects. At the highest ration level, the responses of growth were generally consistent with the earlier study (12). In that earlier study the best growth was seen with Diet P, where as in the present study the best growth was seen with Diet L. However, in both studies the poorest growth was seen with Diet S. At the lower ration levels (M and L) the growth was not consistent with the pattern seen at the H ration level. At the lower ration levels, the best growth was seen from Diet P, followed by Diet L and fish fed Diet S still performed the poorest. These results are directly comparable to those from our earlier study and suggest that at the highest ration level, which was fed to apparent satiety, that feed intake variability may have altered the responses. In another similar study by Saravanan et al. (11) with rainbow trout fed either high or low protein diets with energy biased towards either starch or lipid, the fish down regulated their feed intake when fed the starch biased diets. This observation was a direct contrast to the present study where barramundi increased their satietal intakes of the starch biased diets. Differing again were the observations of Schrama et al (14), who observed in the omnivorous species tilapia that growth was not compromised with the use of starch as an energy source relative to that growth seen when lipid was used instead. We suggest that these differences are directly linked to the ability of tilapia to digest and utilise glucose from starch, whereas starch digestion by barramundi is comparatively poorer and its ability to regulate blood glucose questionable (25, 26, 27). Clearly there appears to be different nutritional capacity among different fish species to utilise starch as an energy source.

The responses of feed efficiency (FCR) to ration within each diet are consistent with observations of most studies on restricting nutrient/energy supply to fish, and the present findings are consistent in this regard with other findings from this species (4, 28). An advantage of using this pair-feeding regime is that it allows for a very clear examination of the effect of the diet composition on performance criteria independent from feed intake variability. However, we do acknowledge that this does potentially cause complications in the application of digestibility values across variable feed

intake levels. Some of the clearest implications from the variation in energy supply by different macronutrients can be seen by the cross-diet comparison of FCR at each of the two lower ration levels in the present study.

Effects of each of the diets on fish body composition were noted primarily in terms of the whole-body lipid, dry matter and protein concentrations. One of the most notable compositional effects at the highest ration level (H) was the difference in lipid concentrations of those fish fed Diet L relative to the other treatments, and that Diet P had the lowest lipid concentrations. These observations from the present study contrast those from an earlier study using these same diets, in that the lipid concentration in the fish fed Diet S are considerably lower and those of Diet L are higher (12). At lower ration levels in the present study this effect of the diets with considerable starch content (Diet C and S) on the lipid concentration in the body is more consistent with our earlier work. Reasons for this discrepancy at the satiety (H) ration level is unclear. These present results (from the H ration) are however consistent with those of Schrama et al. (14), who also noted higher levels of lipid in the whole body of fish (Tilapia) fed diets high in lipid, but less so in fish fed diets high in starch.

Effects of macronutrient bias on energy utilisation

The efficiency of energy utilisation (i.e. the ratio of gross energy gain as a function of digestible energy intake over a range of intake levels, expressed as k_E) differed among each of the treatments. In this study, the relationship between energy intake and gain was observed to be linear, with a calculated energy utilisation constant value that varied between $k_E = 0.507$ and $k_E = 0.730$, subject to diet. For Diet C (the most analogous to a commercial diet) the $k_E = 0.607$, which is generally consistent with other k_E values that have been determined for this species (4, 21). In earlier work (4), a range in the values of k_E of 0.61 to 0.76, with an average of 0.68 have been determined and shown to be marginally affected by fish size. In subsequent work the k_E values have also shown to be influenced by temperature, with k_E values ranging from 0.42 to 0.59 and being lower outside optimal thermal regimes (29).

In the present study, a range of k_E values was observed and clearly related to the variation in macronutrients used to supply equivalent levels of digestible energy in each of the diets. Those diets higher in starch had poorer k_E values, with Diet C (135 g/kg starch) $k_E = 0.607$ and Diet S (225 g/kg starch) $k_E = 0.507$, compared to Diet P (17 g/kg starch) $k_E = 0.715$ and Diet L (29 g/kg starch) $k_E = 0.730$. A clear negative relationship between the k_E values and diet starch concentration is seen (Figure 5). Our findings in the present study are similar to those reported by Schrama et al., (14), who also reported a range in k_E values when diets were biased to either starch ($k_E = 0.561$) or lipid ($k_E = 0.663$). A key difference between these studies was that in the present one we can isolate this effect from differences in digestible energy concentration of the diets, and clearly ascribe the effects solely to macronutrient supply differences. Some significant differences in maintenance energy demands

(HEm) were observed among the different diets. For those diets largely devoid of starch the HEm was estimated to be 36.8 to 40.8 kJ/kg$^{0.8}$/d, where as those diets with starch had HEm values estimated at 14.3 to 17.4 kJ/kg$^{0.8}$/d. However, an important constraint is that these are estimated values derived from extension of the linear regression functions to their intercept of the X-axis, and given that there were no ration levels below the HEm values these estimations are beyond the bounds of the data. As such we suggest that these differences may be an artefact of the extrapolation of the data set.

Effects of macronutrient bias on protein and lipid utilisation

The protein utilisation efficiency was determined as the amount of dietary digestible protein (g /kg$^{0.7}$/d) required to deposit a gram of protein in the body of the animal. In the present study values (k_P) determined in the present study ranged from k_P = 0.412 to 0.580 (data not shown). This compares well with values (k_P = 0.49 to 0.54) determined by Glencross (4) and Glencross & Bermudes (29) for barramundi of different sizes and at different temperatures. The values also compare well to other carnivorous marine species like the European seabass (*Dicentrarchus labrax*) for which a value of k_P = 0.52 was reported (30).

In the present study, a focus was made on the energy retention as protein energy retention. This was estimated based on its energy equivalent, in this case 23.6 kJ/g protein, and expressed relative to the metabolic body weight (W$^{0.8}$) of the animal rather than its protein body weight (W$^{0.7}$) (8). The calculated energy cost as DE (kJ) for deposition of protein from each diet varied and was shown to be significantly higher with the inclusion of starch in the diet. The energy cost values ($1/k_{PE}$) determined in the present study for protein deposition ranged from = 1.72 to 2.43 kJ per kJ of protein energy deposited, with the higher cost values of 1.87 to 2.43 being from those diets higher in starch. This further supports that protein synthesis in the presence of higher dietary starch levels is more energetically expensive. In comparison to other marine fish species (e.g. *Sparus aurata*, *Dicentrarchus labrax* and *Epinephelus aeneus*) which had $1/k_{PE}$ values ranging 1.79 to 1.90 and in carp (*Cyprius carpio*) a $1/k_{PE}$ was estimated at 1.78 (8, 31).

The lipid utilisation efficiency (data not shown) was determined as the amount of digestible dietary lipid (g /kg$^{0.9}$/d) required to deposit a gram of lipid in the body of the animal (21). In the present study the lipid utilisation efficiency values (k_L) determined ranged from k_L = 1.07 to 1.55. The utilisation of dietary lipid energy for lipid energy deposition to determine the partial efficiencies of lipid energy utilisation (k_{LE}) was also examined. What appeared unusual about these values is that they were all greater than one. This implied that there was greater lipid energy deposition than lipid energy intake resulting in a net energy gain from this macronutrient and clearly indicating synthetic activity. While a similar scenario for protein would be impossible, for lipid it demonstrates that there is lipid being synthesised from other macronutrient substrates (e.g. starch or protein). From those diets low in lipid it can be noted that the relative contribution to lipid synthesis from these other macronutrients is enhanced.

The energy cost ($1/k_{LE}$) for lipid gain in the present study ranged from 0.65 to 0.93 kJ per kJ of lipid deposited. This was similar to the range of values (0.83 to 0.86) reported by Glencross et al. (32) with rainbow trout (*Oncorhynchus mykiss*), but was substantially lower than that the 1.10, 1.11 and 1.31 reported by Lupatsch et al. (8) for three marine species (*Sparus aurata, Dicentrarchus labrax* and *Epinephelus aeneus*). In carp the efficiency was estimated at 1.39 (31), demonstrating that lipid accumulation from lipid energy intake was a highly efficient process in barramundi, similar to other carnivorous species (32). That the energy cost of lipid gain is below one also demonstrates that this is an energetically efficient process in terms of energy storage. In contrast with the values of the energy cost of protein deposition, which showed that the energetic cost of protein deposition was almost twice that of the energetic value of what was being synthesised support the reason why lipid is so much more useful in terms of its storage mechanisms, because it uses less energy for storage than its own energetic value. One observation of note was the differences in the $1/k_{LE}$ values, with Diet S having the lowest value of $1/k_{LE} = 0.65$ showing that lipid storage from starch to be very efficient.

Effects of macronutrient bias on component energy utilisation

Energy retention in fish consists almost exclusively of protein or lipid deposition, therefore the efficiency of energy gain in terms of protein and lipid gain can be considered separately using multiple regression analysis as described first by Kielanowski (33). The comparison of the four diets in this study showed that the inclusion of starch in the diet had a significant effect on the gain of either protein or lipid relative to digestible energy intake, and a clear reduction of protein synthesis with the inclusion of this macronutrient in the diets.

When examining the components of energy utilisation, we have worked on the premise that it is the sum of the digestible value of protein, lipid and starch, their relative energetic proportions (%) in the diet and a discrete component utilisation (θk_{PE}, θk_{LE} or θk_{SE}) of each macronutrient that combines to provide the overall k_E value for any particular diet (Table 4). Using this premise, we observed that the component protein energy utilisation value (θk_{PE}) was significantly impaired with the higher inclusion levels of dietary starch (Diet S $\theta k_{PE} = 0.412$ cf. Diet L $\theta k_{PE} = 0.582$). In diets with lower levels of digestible starch (e.g. Diet C $\theta k_{PE} = 0.534$; 111 g/kg), although a numerically lower θk_{PE} was observed, it was not significantly reduced relative to those diets with nominal levels of starch (e.g. Diet P $\theta k_{PE} = 0.557$).

The component lipid energy utilisation value (θk_{LE}) was highly variable compared to the other component energy utilisation values (θk_{PE} or θk_{SE}) for the other macronutrients, with θk_{LE} values ranging from 0.821 to 1.345 (Table 4). These determined values appear to reflect both the inclusion of dietary starch (e.g. Diet S $\theta k_{LE} = 0.821$ cf. Diet P $\theta k_{LE} = 1.345$), and influences of dietary lipid level on the component lipid energy utilisation (e.g. Diet P $\theta k_{LE} = 1.345$ cf. Diet L $\theta k_{LE} = 1.036$). We suspect that the variability in this component utilisation value reflects the responsive nature of the

metabolism of lipids by this animal in response to variable nutrient supply. In effect, what we are observing is an enhanced capacity of the animal to produce lipid from protein energy sources. Although it is less efficient than that from lipid or protein, there is still substantial lipid synthesis from starch energy occurring.

The component starch energy utilisation values (θk_{SE}) determined from using the multiple regression approach were determined to be the same across all diets (θk_{SE} = 0.438). Energy deposition from starch was clearly the least efficient of all the macronutrients (although a poorer θk_{PE} was noted for Diet S). We suggest that barramundi has limited metabolic capacity to utilise starch derived energy. While it can produce lipids from glucose precursors, it clearly does so at a less efficient rate than that seen from either protein or lipid directly.

Conclusions

The results from this study show that barramundi have clear metabolic inefficiencies associated with the inclusion of starch in their diet. With the increasing inclusion of starch in the diet of this species there was a reduction in the efficiency of protein (protein energy) utilisation and this contributed to an overall decline in the efficiency of energy utilisation. In the absence of starch, protein utilisation was constant and it was unaffected by its concentration in the diet. Collectively, the findings of this study support the notion that the concentration and type of macronutrient mix in a diet for barramundi has a significant effect on the ability of the fish to use those nutrients for energy. This finding suggests the existence of a metabolic mechanism that influences the ability of fish to utilise discrete nutrients for energy, independent of total energy intake.

Acknowledgements and Statement of Conflict of Interest

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Author Roles

BG, DB, SI and NW all had input into the experiment design. BG, DB, SC, NB and SI all contributed to the conduct of the experiment. DB manufactured the diets, NB and SC undertook most of the sample analysis. BG undertook the analysis of the data. BG, SI and NW all contributed to the interpretation of the data and the writing of the manuscript.

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Table and Figure Legends

Table 1. Formulation, composition and relative digestible contributions of the energy of each macronutrient in each of the experimental diets

Table 2. Growth and feed utilisation responses for each treatment.

Table 3. Nitrogen, lipid and energy balance analysis

Table 4. Component energetic contributions from each macronutrient in each diet and the calculated and measured energetic parameters

Figure 1. Energy gain ($kJ/kg^{0.8}/d$) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There was no significant difference in the linear regressions between the control, protein and lipid diet treatments. The regression equation of the fish fed the starch diet was significantly different from each of the other treatments.

Figure 2. Protein energy gain ($kJ/kg^{0.8}/d$) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There was no significant difference in the linear regressions between the control, protein and lipid diet treatments. The regression equation of the fish fed the starch diet was significantly different from each of the other treatments.

Figure 3. Lipid energy gain ($kJ/kg^{0.8}/d$) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There was no significant differences in the linear regressions among each of the control, protein, lipid and starch diet treatments.

Figure 4. Lipid energy utilisation coefficients relative to the dietary concentration of lipid. Data is means ± SEM.

Figure 5. Relationship between diet starch concentration and energy utilisation coefficient (kE) values. Equation for the relationship was $y = -0.001x + 0.747$, $R^2 = 0.987$.

661 **Tables and Figures**

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663 Table 1. Formulation, composition and relative digestible contributions of the energy
664 of each macronutrient in each of the experimental diets
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	C	P	L	S
Diet Formulations				
Fishmeal (Anchovetta)	560	640	560	560
Wheat Gluten	100	100	100	100
Casein	50	100	50	50
Fish oil (Anchovetta)	50	40	100	0
Pregelatinised Wheat Starch	120	0	0	240
Yttrium Oxide	2	2	2	2
Vitamin-mineral premix	5	5	5	5
Cellulose	113	113	183	43
Diet Composition				
Dry Matter	974	975	945	909
Crude Protein	505	603	483	493
Digestible Protein	448	545	455	441
Total Lipid	107	107	148	68
Digestible Lipid	107	94	148	67
Ash	108	122	104	104
Total Carbohydrates	280	169	264	336
Total Starch	135	17	29	225
Digestible Starch	111	13	29	214
Gross Energy (kJ /g DM)	21.39	20.24	20.69	20.71
Digestible Energy (kJ/ g DM)	16.61	16.70	16.91	16.69
Digestible Energy as Protein (%)	63.6	76.5	63.4	62.4
Digestible Energy as Lipid (%)	24.8	21.5	33.6	15.5
Digestible Energy as Starch (%)	11.6	2.0	3.0	22.2

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Table 2. Growth and feed utilisation responses for each treatment.

	Diet C			Diet P			Diet L			Diet S			Pooled	P-values		
Ration	H	M	L	H	M	L	H	M	L	H	M	L	SEM	D	R	D x R
Performance Parameters																
Initial weight (g/fish)	68.9	69.3	69.7	69.9	69.7	70.2	70.4	68.9	69.5	68.8	70.1	70.2	0.03	0.576	0.429	0.212
Final weight (g/fish)	273.6 ^{b}	138.9 ^{d}	116.6 ^{ef}	275.0 ^{ab}	152.8 ^{c}	125.9 ^{de}	285.6 ^{a}	143.2 ^{cd}	117.9 ^{ef}	271.2 ^{b}	133.1 ^{d}	111.5 ^{f}	4.30	0.003	0.000	0.149
Weight gain (g/fish)	204.6 ^{b}	69.6 ^{d}	46.9 ^{ef}	205.1 ^{ab}	83.1 ^{c}	55.7 ^{de}	215.2 ^{a}	74.3 ^{cd}	48.4 ^{ef}	202.4 ^{b}	62.9 ^{d}	41.3 ^{f}	4.31	0.002	0.000	0.133
Intake (g/fish)	190.4 ^{b}	59.7 ^{d}	40.3 ^{e}	176.2 ^{c}	58.7 ^{d}	40.4 ^{e}	190.2 ^{b}	59.0 ^{d}	39.4 ^{e}	205.1 ^{a}	58.7 ^{d}	40.4 ^{e}	4.13	0.005	0.000	0.002
FCR (intake/gain)	0.93 ^{b}	0.86 ^{c}	0.86 ^{c}	0.86 ^{c}	0.71 ^{e}	0.73 ^{e}	0.88 ^{bc}	0.79 ^{d}	0.81 ^{cd}	1.01 ^{a}	0.93 ^{b}	0.98 ^{ab}	0.01	0.000	0.000	0.269
Survival (%)	97.5	97.5	97.5	100.0	100.0	97.5	100.0	100.0	100.0	100.0	100.0	97.5	0.4	0.330	0.472	0.942
Final Live-weight Composition																
Dry matter (%)	31.2 ^{a}	26.5 ^{cd}	26.9 ^{c}	30.4 ^{a}	26.7 ^{cd}	25.7 ^{d}	31.7 ^{a}	27.1 ^{c}	27.1 ^{c}	28.3 ^{b}	26.9 ^{c}	27.2 ^{c}	0.41	0.095	0.000	0.049
Protein (%)	21.0 ^{ab}	20.3 ^{b}	17.9 ^{cd}	22.2 ^{a}	17.6 ^{cd}	17.7 ^{cd}	21.0 ^{ab}	17.6 ^{cd}	17.9 ^{cd}	18.2 ^{c}	16.7 ^{e}	18.0 ^{c}	0.36	0.000	0.000	0.000
Lipid (%)	8.6 ^{b}	6.2 ^{c}	5.9 ^{c}	8.1 ^{b}	5.5 ^{c}	4.3 ^{d}	10.0 ^{a}	6.6 ^{c}	5.2 ^{a}	8.4 ^{b}	6.4 ^{c}	5.5 ^{c}	0.35	0.000	0.000	0.015
Energy (kJ/g)	8.27 ^{ab}	7.16 ^{b}	6.49 ^{bc}	8.37 ^{ab}	6.26 ^{cd}	5.83 ^{d}	8.83 ^{a}	6.70 ^{bc}	6.23 ^{cd}	8.53 ^{a}	6.40 ^{c}	6.38 ^{cd}	0.201	0.001	0.000	0.003

Different superscripts within rows indicate significant differences at $P<0.05$.

Table 3. Protein (g/fish), lipid (g/fish) and energy (kJ/fish) balance analysis

	Diet C			Diet P			Diet L			Diet S			Pooled	*P-values*		
Ration	H	M	L	H	M	L	H	M	L	H	M	L	SEM	*D*	*R*	*D x R*
GPI	96.2^{a}	30.2^{b}	20.3^{c}	106.3^{a}	35.4^{b}	24.4^{c}	91.9^{a}	28.5^{b}	19.0^{c}	101.1^{a}	28.9^{b}	19.9^{c}	7.27	0.000	0.000	0.032
FP	10.8^{b}	3.4^{d}	2.3^{e}	14.2^{a}	4.7^{c}	3.3^{d}	5.4^{c}	1.7^{e}	1.1^{e}	10.6^{b}	3.0^{d}	2.1^{e}	0.86	0.000	0.000	0.000
DPI	85.4^{a}	26.8^{b}	18.1^{bc}	92.0^{a}	30.7^{b}	21.1^{bc}	86.5^{a}	26.8^{b}	17.9^{c}	90.5^{a}	25.9^{b}	17.8^{c}	6.50	0.002	0.000	0.246
BUN(Peq)	40.2^{b}	11.0^{d}	9.6^{e}	43.5^{ab}	16.3^{c}	11.0^{d}	39.1^{b}	13.9^{cd}	9.3^{e}	53.5^{a}	16.3^{c}	10.3^{de}	3.25	0.000	0.000	0.001
RP	45.2^{ab}	15.8^{c}	8.4^{d}	48.5^{ab}	14.3^{c}	10.1^{cd}	47.4^{a}	12.9^{a}	8.6^{cd}	37.0^{b}	9.6^{cd}	7.5^{d}	3.38	0.000	0.000	0.000
RP/DPI	53%^{b}	59%^{a}	47%^{c}	53%^{b}	47%^{c}	48%^{bc}	55%^{ab}	48%^{bc}	48%^{bc}	41%^{d}	37%^{d}	42%^{d}	1.3%	0.000	0.016	0.005
GLI	20.4^{b}	6.4^{de}	4.3^{ef}	18.7^{b}	6.2^{e}	4.3^{ef}	28.1^{a}	8.7^{d}	5.8^{e}	13.7^{c}	3.9^{f}	2.7^{f}	1.62	0.000	0.000	0.000
FL	0.1^{c}	0.0^{c}	0.0^{c}	2.1^{a}	0.7^{b}	0.5^{b}	0.3^{bc}	0.1^{c}	0.1^{c}	0.1^{c}	0.0^{c}	0.0^{c}	0.12	0.000	0.000	0.000
DLI	20.3^{b}	6.4^{d}	4.3^{d}	16.6^{bc}	5.5^{d}	3.8^{d}	27.9^{a}	8.6^{cd}	5.8^{d}	13.7^{c}	3.9^{d}	2.7^{d}	1.59	0.000	0.000	0.000
RL	20.2^{ab}	5.2^{c}	3.5^{c}	19.0^{b}	5.0^{c}	2.0^{c}	25.3^{a}	6.1^{c}	2.8^{c}	19.5^{b}	5.2^{c}	2.8^{c}	1.71	0.000	0.000	0.000
RL/DLI	99%^{b}	82%^{c}	81%^{c}	114%^{b}	91%^{bc}	53%^{e}	91%^{bc}	71%^{cd}	49%^{e}	142%^{a}	132%^{a}	103%^{b}	5.7%	0.000	0.000	0.018
RL/RP	45%^{b}	33%^{c}	42%^{b}	39%^{bc}	35%^{c}	20%^{d}	53%^{a}	47%^{ab}	33%^{c}	53%^{a}	53%^{a}	37%^{c}	2.1%	0.000	0.000	0.001
GEI	4074^{a}	1278^{b}	862^{b}	3566^{a}	1188^{b}	818^{b}	3935^{a}	1221^{b}	814^{b}	4249^{a}	1216^{b}	837^{b}	291.4	0.000	0.000	0.001
FE	910^{a}	285^{c}	193^{cd}	624^{b}	208^{c}	143^{d}	718^{b}	193^{cd}	149^{d}	908^{a}	260^{c}	179^{cd}	60.4	0.000	0.000	0.000
DEI	3164^{a}	992^{b}	669^{c}	2942^{a}	980^{b}	674^{c}	3217^{a}	1027^{b}	666^{c}	3341^{a}	956^{b}	658^{c}	232.3	0.023	0.000	0.006
BUE	160^{b}	44^{cd}	38^{d}	173^{ab}	65^{c}	44^{a}	156^{b}	55^{cd}	37^{d}	213^{a}	65^{c}	41^{d}	12.9	0.000	0.000	0.001
MEI	3004^{a}	948^{b}	631^{b}	2769^{a}	915^{b}	631^{b}	3061^{a}	972^{a}	629^{b}	3128^{a}	891^{b}	617^{b}	219.7	0.011	0.000	0.005
RE	1841^{ab}	572^{c}	333^{cd}	1875^{ab}	531^{c}	306^{d}	2090^{a}	540^{c}	311^{d}	1621^{b}	424^{cd}	284^{d}	144.7	0.000	0.000	0.000
HP	1163^{b}	377de	298^{e}	895^{c}	384de	324de	971^{bc}	432^{d}	318de	1507^{a}	467^{d}	333de	81.4	0.000	0.000	0.000
HeE	295^{a}	226^{bc}	211^{c}	297^{a}	235^{b}	218^{bc}	303^{a}	228^{bc}	211^{c}	294^{a}	223^{bc}	208^{c}	7.8	0.001	0.000	0.117
HiE	868^{b}	151de	87^{e}	598^{c}	149de	106de	668^{c}	204^{d}	106de	1213^{a}	244^{d}	126de	74.4	0.000	0.000	0.000
NEI	2136^{ab}	797^{c}	544^{c}	2172^{ab}	766^{c}	524^{c}	2393^{a}	768^{c}	523^{c}	1915^{b}	647^{c}	491^{c}	152.4	0.000	0.000	0.000
RE/DEI	58%^{b}	58%^{b}	50%^{c}	64%^{a}	54%^{bc}	45%^{de}	65%^{a}	53%^{c}	47%^{de}	49%^{cd}	44%^{de}	43%^{e}	1.5%	0.000	0.000	0.014

GPI: Gross Protein Intake. FP : Faecal Protein. DPI : Digestible Protein Intake. BUN(Peq) : Brachial and Urinary Nitrogen (Protein equivalent). RP: Retained Protein. GLI : Gross Lipid Intake. FL : Faecal Lipid. DLI : Digestible Lipid Intake. RL : Retained Lipid. GEI : Gross Energy Intake. FE : Faecal Energy. DEI : Digestible Energy Intake. BUE : Brachial and Urinary Energy. MEI : Metabolisable Energy Intake. RE : Retained Energy. HP : Heat Production. HeE : Basal Metabolism. HiE : Heat Increment Energy. NEI : Net Energy Intake. *D, R* and *D x R* are the *P-values* for effects of Diet, Ration or the Interaction respectively. Different superscripts within rows indicate significant differences at $P<0.05$.

Table 4. Component energetic contributions from each macronutrient in each diet and the calculated and measured energetic parameters

Diet	Parameter	Protein	Lipid	Starch	Energy	
					Calculated	Measured
	Assumed energetic value (MJ/kg)	23.6	38.5	17.3		
Control	Digestible nutrient (g/kg)	448	107	111		
	Digestible energy (MJ/kg)	10.57	4.12	1.92	16.61	16.61
	Proportion of total energy (%)	63.6	24.8	11.6		
	Utilisation Coefficients	0.534	0.821	0.438	0.594	0.607
Protein	Digestible nutrient (g/kg)	545	94	19		
	Digestible energy (MJ/kg)	12.86	3.62	0.33	16.81	16.70
	Proportion of total energy (%)	76.5	21.5	2.0		
	Utilisation Coefficients	0.557	1.345	0.438	0.715	0.715
Lipid	Digestible nutrient (g/kg)	455	148	29		
	Digestible energy (MJ/kg)	10.74	5.70	0.50	16.94	16.91
	Proportion of total energy (%)	63.4	33.6	3.0		
	Utilisation Coefficients	0.582	1.036	0.438	0.730	0.730
Starch	Digestible nutrient (g/kg)	441	67	214		
	Digestible energy (MJ/kg)	10.41	2.58	3.70	16.69	16.69
	Proportion of total energy (%)	62.4	15.5	22.2		
	Utilisation Coefficients	0.412	0.821	0.438	0.481	0.507

Digestible energy value is derived from assumed energetic value of the digestible nutrient concentration in each diet. The calculated energy value of each diet is the sum of the component macronutrient digestible energy values. The measured energy value is the digestible energy measured from *in vivo* studies. Protein utilisation coefficients are derived from equations 5 to 8. Lipid utilisation for diets P and L, where starch was absent, are derived from equations 10 and 11. Component lipid utilisation coefficients for each of the diets were derived from multiple regression of energy utilisation equations (1 and 4). Similarly, component starch utilisation coefficients were derived by multiple regression of energy utilisation equations (1 and 4).

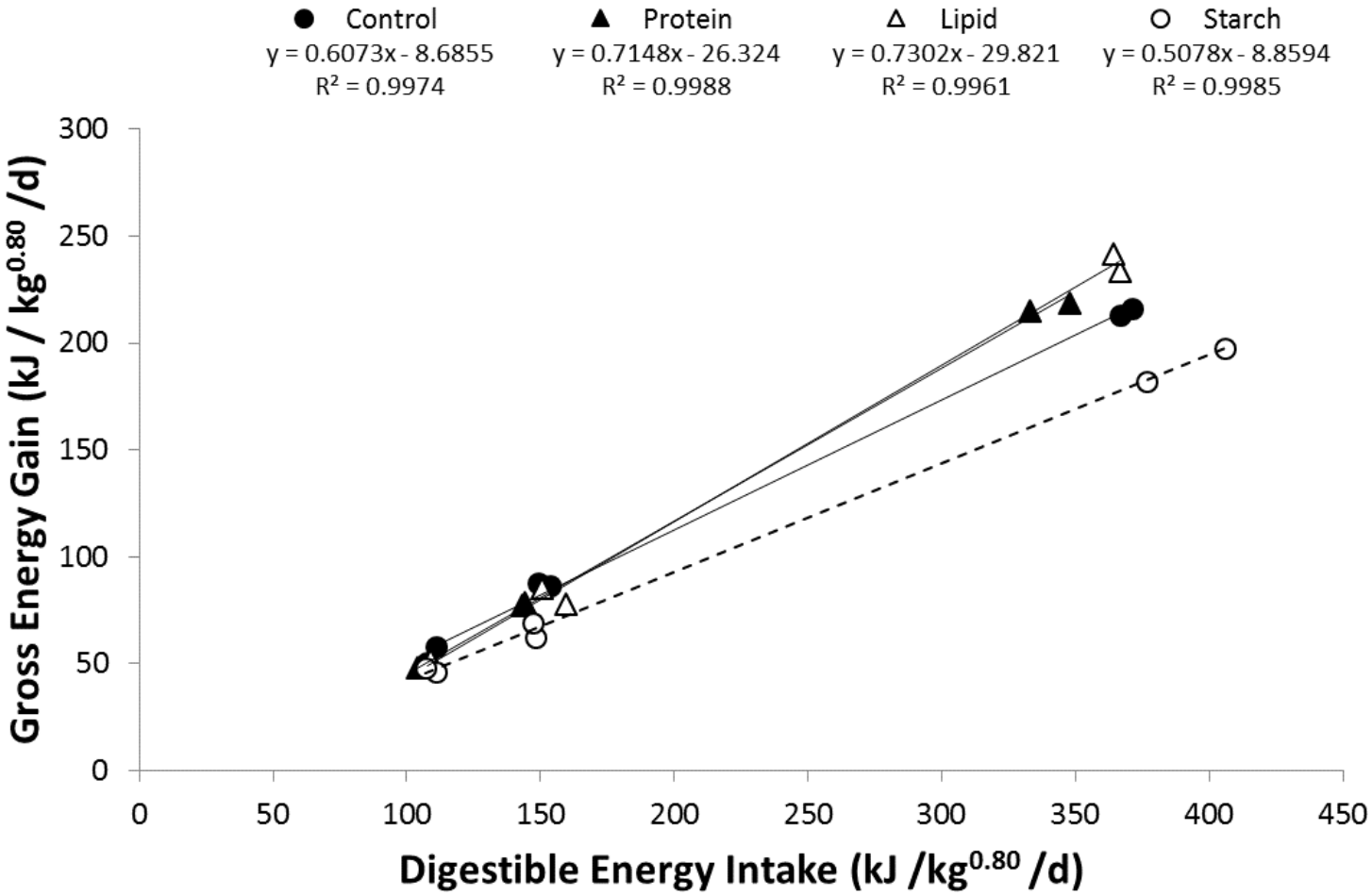

Figure 1. Energy gain (kJ/kg $^{0.8}$/d) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There was no significant difference in the linear regressions between the control, protein and lipid diet treatments. The regression equation of the fish fed the starch diet was significantly different from each of the other treatments.

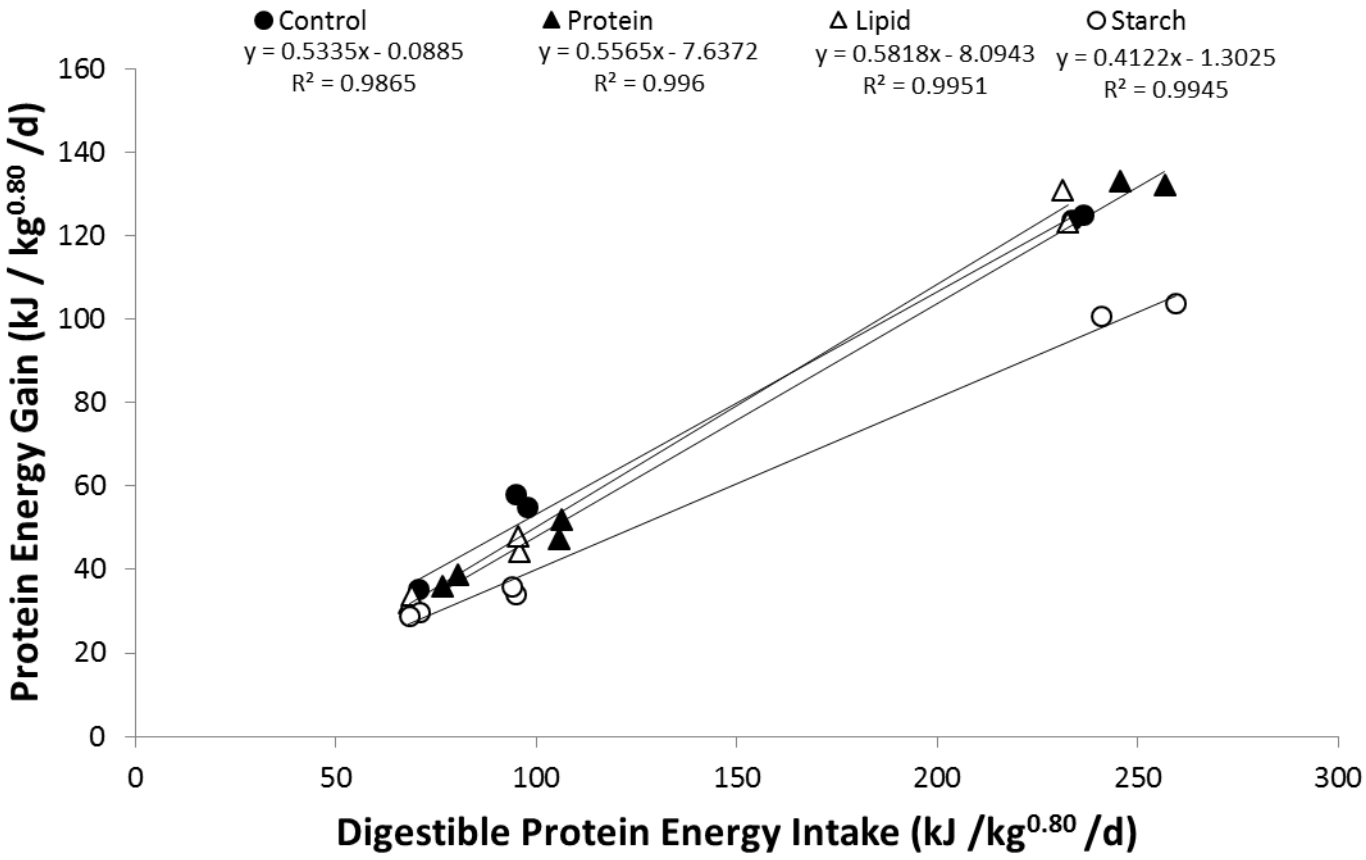

Figure 2. Protein energy gain (kJ/kg $^{0.8}$/d) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There was no significant difference in the linear regressions between the control, protein and lipid diet treatments. The regression equation of the fish fed the starch diet was significantly different from each of the other treatments.

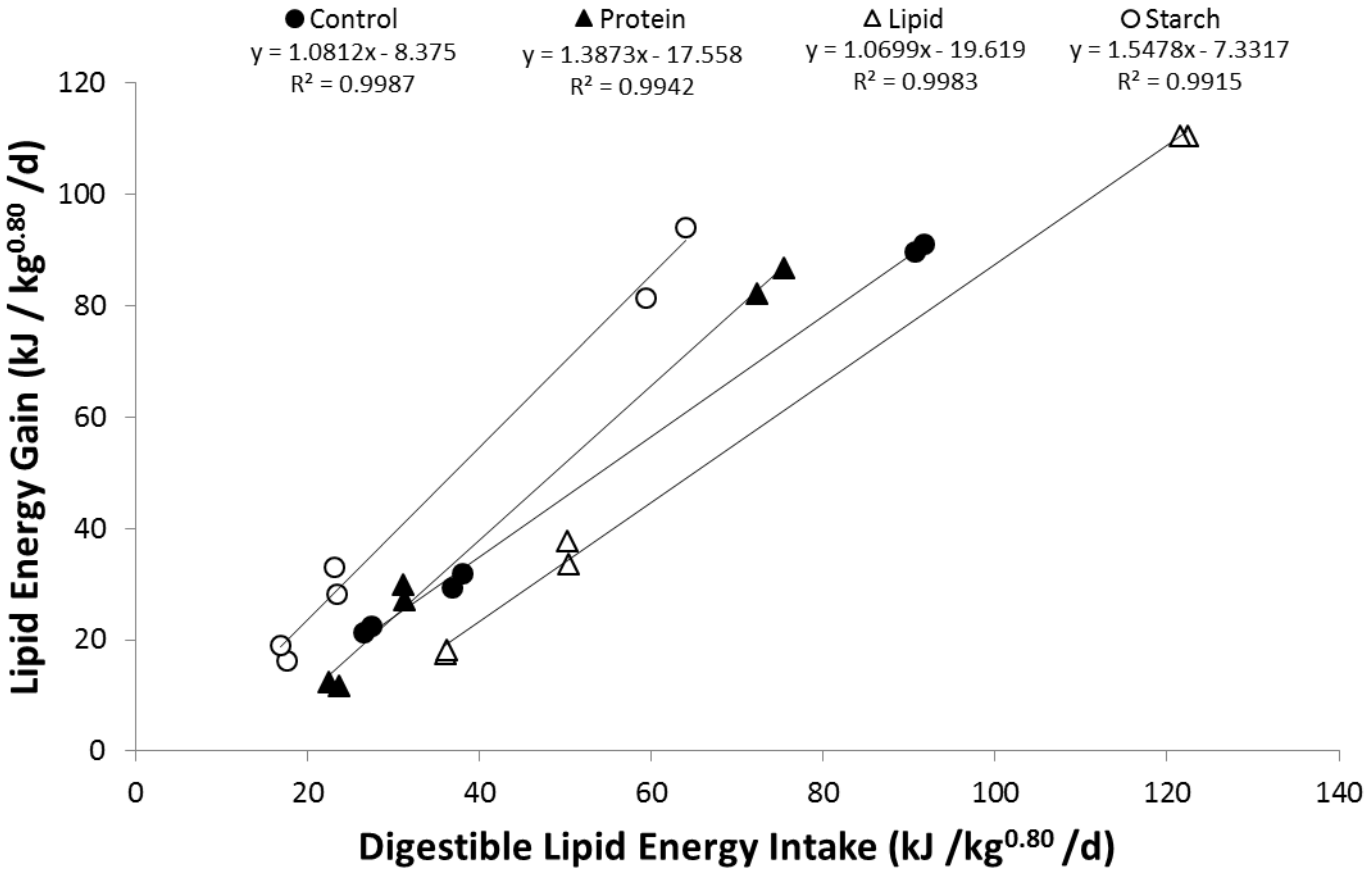

Figure 3. Lipid energy gain (kJ/kg $^{0.8}$/d) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There were no significant differences in the linear regressions among each of the control, protein, lipid and starch diet treatments.

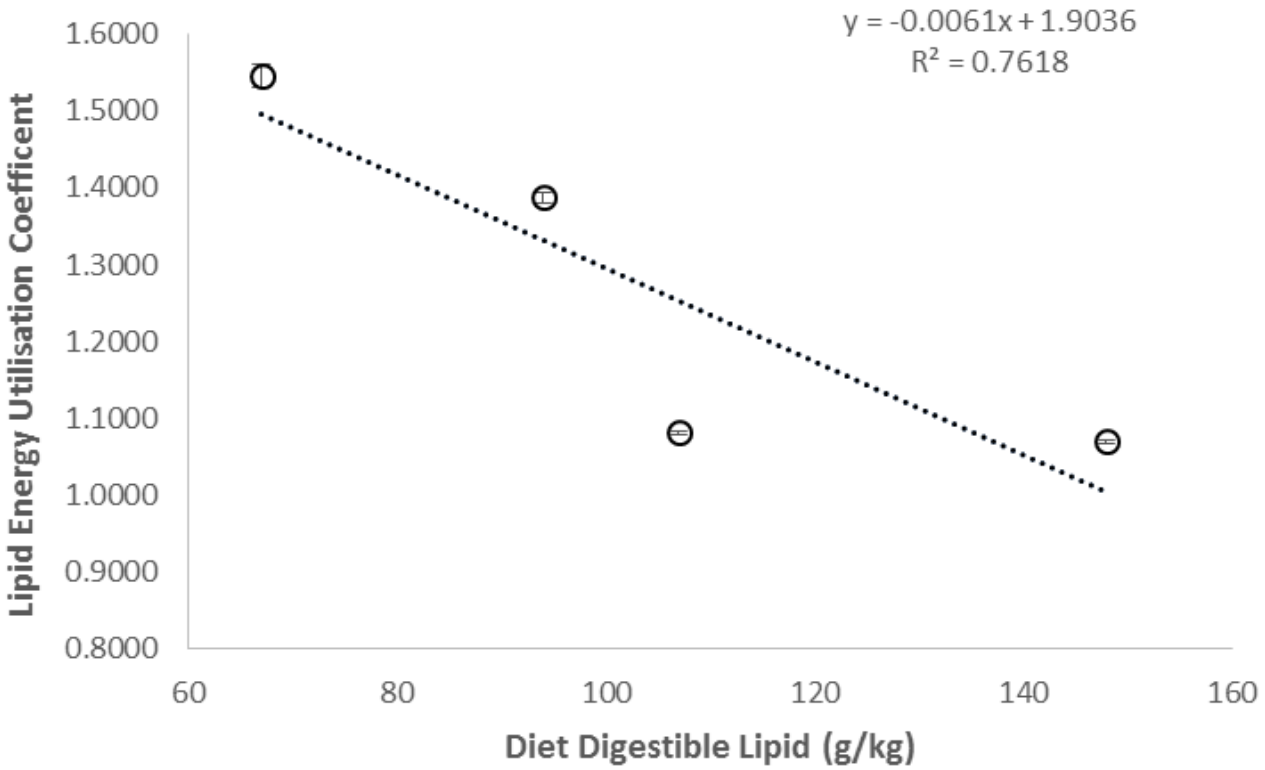

Figure 4. Lipid energy utilisation coefficients relative to the dietary concentration of lipid. Data is means ± SEM.

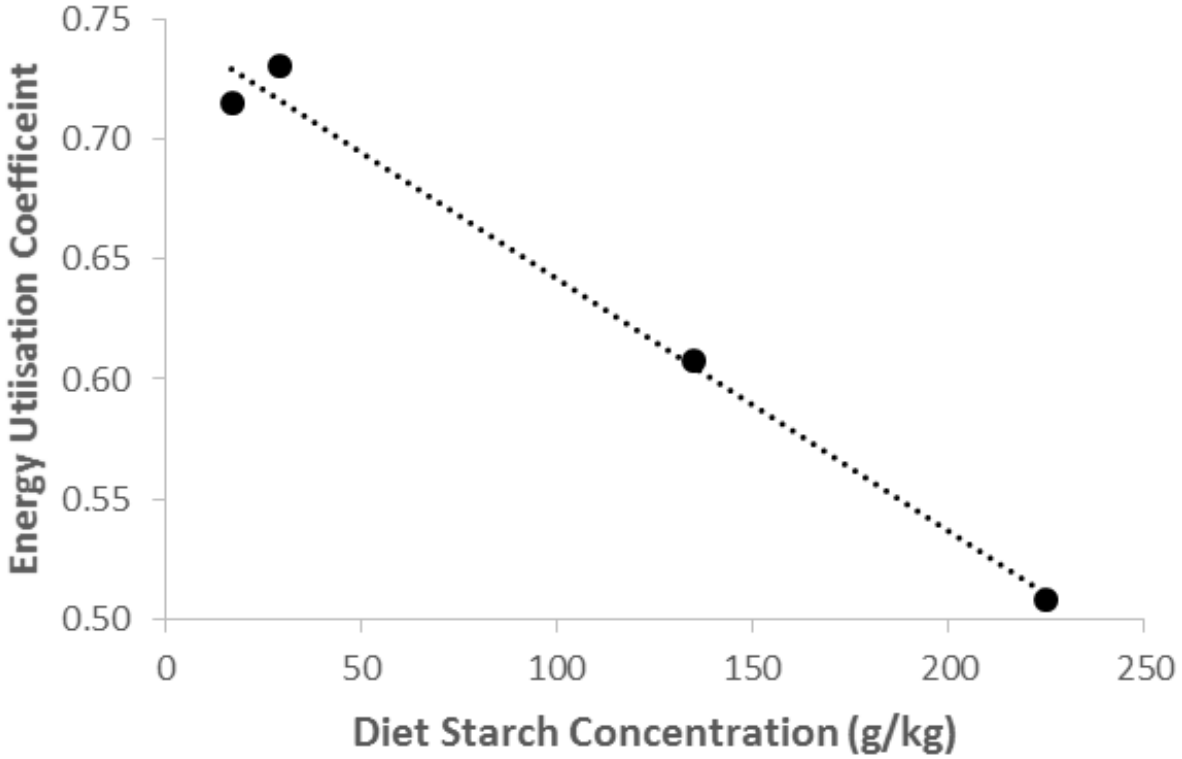

Figure 5. Relationship between diet starch concentration and energy utilisation coefficient (k_E) values. Equation for the relationship was y = -0.001x + 0.747, R^2 = 0.987.