

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

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22 **Abstract**

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

39 **Introduction**

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

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

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

73 **Methods**

74 *Diet preparation*

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

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

91

92 *Fish handling*

93 All animal procedures were approved by the CSIRO Animal Ethics Committee (Approval
94 A9/2011). Juvenile barramundi (*Lates calcarifer*) were obtained from a commercial hatchery
95 (BettaBarra, Walkamin, QLD, Australia), and on-grown to 69.6 ± 0.75 g (mean ± SD, n=480) in
96 preparation for the experiment. During the on-growing period all fish were fed the same diet (Marine
97 Float; Ridley Aquafeeds, QLD, Australia) and kept in 2 x 5000L seawater tanks. At the initiation of
98 the trial 40 fish were weighed on an electronic top-loading balance to 0.1 g accuracy to determine the
99 mean and standard deviation of the population. Following this, 20 fish were allocated to each of 24 x
100 300L tanks based on having to be within the mean ± 1 x S.D. The experiment was conducted at the
101 Bribie Island Research Centre at Woorim, in a flow-through (3L min⁻¹), aerated, heated seawater tank
102 array. Water temperature was maintained at 29.9 ± 0.12 °C (mean ± S.D.) and dissolved oxygen 5.5 ±
103 0.56 mg L⁻¹ for the 42-day duration of the experiment.

104 Each diet was manually fed to each tank. Three ration levels were used; a satiety, 80% and
105 60% of the initial satiety levels. The satiety rations were fed twice daily, with AM (0900 – 0930) and
106 PM (1630 - 1700) feeds. The satietal rations were determined by feeding to slight excess, with all feed
107 fed and all uneaten feed was accounted for and correction factors applied to allow for the
108 determination of solubilisation losses and pellet dry matters and therefore of actual feed consumption
109 within each tank based on methods reported by Helland et al., (18). The two restricted rations used in

110 this study were based on 80% and 60% of the measured initial demand which was also consistent with
111 the model of Glencross (4). These rations were not adjusted over time. Each treatment was duplicated
112 within the 24-tank array, based on the plan for using regression analysis in this experiment it was
113 proposed that a 3 rations x 2 replicates design was stronger than a 2 rations x 3 replicates approach.
114

115 *Sample preparation and chemical analysis*

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

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

135

136 *Diet digestibility analysis*

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

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

$$150 \quad AD_{diet} = \left(1 - \frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}} \right) \times 100$$

152
153 where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and
154 $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (dry matter, protein or
155 energy) content of the diet and faeces respectively.

156

157 *Protein and energy utilisation analysis*

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

165

166 *Nutrient and energy balance and deposition assessment*

167 The net balance for protein (P), lipid (L) and energy (E) were calculated based on the data
168 derived in this study. The methods used for these calculations were based on those reported by
169 Saravanan et al (11). Gross intake levels of each nutrient were determined based on total feed intake
170 for each tank multiplied by the percent composition of the feed being fed. Digestible intake levels
171 were measured similarly based on the digestibility of P, L and E from each diet. Faecal losses were
172 determined as the reciprocal of the digestible levels. Retained nutrients and energy were determined
173 based the net gain in nutrients and energy between the fish at the end of the trial and those from the
174 initial sample. Branchial and urinary nitrogen (BUN) were determined based on the difference
175 between digestible nitrogen intake and retained nitrogen with energy values defined based on 24.85 kJ
176 \times branchial and urinary nitrogen using values reported by Saravanan et al (11). The metabolisable
177 energy intake (MEI) was determined based on the digestible energy intake minus the branchial and
178 urinary energy losses. Heat production (HP) was determined based on the difference between
179 metabolisable energy and retained energy (RE). Basal metabolism (HeE) was calculated based on the
180 reported fasting energy losses of $34.4 \text{ kJ/ kg}^{0.8} / \text{d}$ (4). The Heat increment (HiE) was determined based
181 on the MEI minus the RE and the HeE. Net energy (NE) was determined based on MEI minus HiE
182 (23).

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Statistical analysis

All figures are mean \pm 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).

196 **Results**

197 *Effect of macronutrient energy bias on growth and body composition*

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

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

214

215 *Effect of macronutrient energy bias on energy utilisation*

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

220 (Eq. 1) $y_S = 0.508(\pm 0.010)x - 8.859(\pm 2.496)$, $R^2 = 0.998$

221 (Eq. 2) $y_L = 0.730(\pm 0.023)x - 29.821(\pm 5.461)$, $R^2 = 0.996$

222 (Eq. 3) $y_P = 0.715(\pm 0.012)x - 26.324(\pm 2.774)$, $R^2 = 0.999$

223 (Eq. 4) $y_C = 0.607(\pm 0.015)x - 8.686(\pm 3.717)$, $R^2 = 0.997$

224 The coefficient of utilisation (k_E) was significantly lower for Diet S relative to each of the
225 other diets. Similarly, the utilisation coefficient for Diet C was also significantly lower than that of
226 Diets P and L. There was no difference in the energy utilisation coefficient between Diets P and L.
227 Maintenance digestible energy intake (HEm) was calculated by extrapolation of the linear regression
228 to the intercept of the X-axis. From this the following HEm values were derived; Diet S : 17.4(±0.81)
229 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)
230 kJ/kg^{0.8}/d. There were significant differences in the HEm values between Diets L and P relative to
231 Diets S and C, but not within those pairings.

232 *Effect of macronutrient energy bias on protein and lipid energy utilisation*

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

237 (Eq. 5) $y_S = 0.412(\pm 0.003)x - 1.302(\pm 0.417)$, $R^2 = 0.994$

238 (Eq. 6) $y_L = 0.582(\pm 0.006)x - 8.094(\pm 0.572)$, $R^2 = 0.995$

239 (Eq. 7) $y_P = 0.556(\pm 0.005)x - 7.637(\pm 0.527)$, $R^2 = 0.996$

240 (Eq. 8) $y_C = 0.534(\pm 0.004)x - 0.088(\pm 0.588)$, $R^2 = 0.986$

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

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

248 (Eq. 9) $y_S = 1.5478(\pm 0.015)x - 7.332(\pm 0.500)$, $R^2 = 0.991$

249 (Eq. 10) $y_L = 1.070(\pm 0.002)x - 19.619(\pm 1.469)$, $R^2 = 0.998$

250 (Eq. 11) $y_P = 1.387(\pm 0.006)x - 17.558(\pm 0.456)$, $R^2 = 0.994$

251 (Eq. 12) $y_C = 1.081(\pm 0.002)x - 8.375(\pm 0.183)$, $R^2 = 0.999$

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

254

255 *Determination of macronutrient component contributions to energy utilisation*

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

260 Based on the prior mentioned assumptions, each of the component energy utilisation values
261 was derived using multiple regression analysis. For each of the diets the protein contribution can be
262 defined by converting the protein utilisation to protein energy utilisation and defining from that the
263 component protein energy utilisation (Figure 2). Therefore, because we have a definitive assessment
264 of the protein energy utilisation efficiencies (see equations 5 to 8) we can also derive by multiple
265 regression the remaining unknown variables, which constitute the contribution of both lipid energy
266 and starch energy to the partial efficiency of energy utilisation in each diet (Table 1 and Table 3).
267 Although we have an assessment of the partial efficiency of lipid energy utilisation (Figure 3), the fact
268 that lipid energy gain in this representation also includes lipid deposited from non-lipid origins (i.e.

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

271

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

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

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

300 Gross Energy Intake (GEI) was highest by those fish fed Diet S at ration level H with the
301 corresponding lowest GEI at the same ration level being from Diet P (Table 3). Among the lower
302 ration levels there was no significant differences in GEI. These differences were also reflected in the
303 DEI across the treatments. Faecal Energy (FE) was highest by those fish fed both Diet C and S and
304 lowest from those fish fed Diet P. BUE losses were highest from fish fed Diet S at ration level H and
305 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).
323

324 **Discussion**

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

330

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

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

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

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

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

376

377 *Effects of macronutrient bias on energy utilisation*

378 The efficiency of energy utilisation (i.e. the ratio of gross energy gain as a function of
379 digestible energy intake over a range of intake levels, expressed as k_E) differed among each of the
380 treatments. In this study, the relationship between energy intake and gain was observed to be linear,
381 with a calculated energy utilisation constant value that varied between $k_E = 0.507$ and $k_E = 0.730$,
382 subject to diet. For Diet C (the most analogous to a commercial diet) the $k_E = 0.607$, which is
383 generally consistent with other k_E values that have been determined for this species (4, 21). In earlier
384 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
385 and shown to be marginally affected by fish size. In subsequent work the k_E values have also shown to
386 be influenced by temperature, with k_E values ranging from 0.42 to 0.59 and being lower outside
387 optimal thermal regimes (29).

388 In the present study, a range of k_E values was observed and clearly related to the variation in
389 macronutrients used to supply equivalent levels of digestible energy in each of the diets. Those diets
390 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
391 starch) $k_E = 0.507$, compared to Diet P (17 g/kg starch) $k_E = 0.715$ and Diet L (29 g/kg starch) $k_E =$
392 0.730. A clear negative relationship between the k_E values and diet starch concentration is seen
393 (Figure 5). Our findings in the present study are similar to those reported by Schrama et al., (14), who
394 also reported a range in k_E values when diets were biased to either starch ($k_E = 0.561$) or lipid ($k_E =$
395 0.663). A key difference between these studies was that in the present one we can isolate this effect
396 from differences in digestible energy concentration of the diets, and clearly ascribe the effects solely
397 to macronutrient supply differences. Some significant differences in maintenance energy demands

398 (HEm) were observed among the different diets. For those diets largely devoid of starch the HEm was
399 estimated to be 36.8 to 40.8 kJ/kg^{0.8}/d, where as those diets with starch had HEm values estimated at
400 14.3 to 17.4 kJ/kg^{0.8}/d. However, an important constraint is that these are estimated values derived
401 from extension of the linear regression functions to their intercept of the X-axis, and given that there
402 were no ration levels below the HEm values these estimations are beyond the bounds of the data. As
403 such we suggest that these differences may be an artefact of the extrapolation of the data set.

404

405 *Effects of macronutrient bias on protein and lipid utilisation*

406 The protein utilisation efficiency was determined as the amount of dietary digestible protein
407 (g /kg^{0.7}/d) required to deposit a gram of protein in the body of the animal. In the present study values
408 (k_P) determined in the present study ranged from $k_P = 0.412$ to 0.580 (data not shown). This compares
409 well with values ($k_P = 0.49$ to 0.54) determined by Glencross (4) and Glencross & Bermudes (29) for
410 barramundi of different sizes and at different temperatures. The values also compare well to other
411 carnivorous marine species like the European seabass (*Dicentrarchus labrax*) for which a value of k_P
412 = 0.52 was reported (30).

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

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

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

448

449 *Effects of macronutrient bias on component energy utilisation*

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

456 When examining the components of energy utilisation, we have worked on the premise that it
457 is the sum of the digestible value of protein, lipid and starch, their relative energetic proportions (%)
458 in the diet and a discrete component utilisation ($\theta_{k_{PE}}$, $\theta_{k_{LE}}$ or $\theta_{k_{SE}}$) of each macronutrient that
459 combines to provide the overall k_E value for any particular diet (Table 4). Using this premise, we
460 observed that the component protein energy utilisation value ($\theta_{k_{PE}}$) was significantly impaired with
461 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
462 lower levels of digestible starch (e.g. Diet C $\theta_{k_{PE}} = 0.534$; 111 g/kg), although a numerically lower
463 $\theta_{k_{PE}}$ was observed, it was not significantly reduced relative to those diets with nominal levels of
464 starch (e.g. Diet P $\theta_{k_{PE}} = 0.557$).

465 The component lipid energy utilisation value ($\theta_{k_{LE}}$) was highly variable compared to the other
466 component energy utilisation values ($\theta_{k_{PE}}$ or $\theta_{k_{SE}}$) for the other macronutrients, with $\theta_{k_{LE}}$ values
467 ranging from 0.821 to 1.345 (Table 4). These determined values appear to reflect both the inclusion of
468 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
469 on the component lipid energy utilisation (e.g. Diet P $\theta_{k_{LE}} = 1.345$ cf. Diet L $\theta_{k_{LE}} = 1.036$). We
470 suspect that the variability in this component utilisation value reflects the responsive nature of the

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

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

481

482 *Conclusions*

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

492

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497

498 **Author Roles**

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

503

504 **References**

505

506 1. Glencross BD (2006) Nutritional management of barramundi, *Lates calcarifer* – A review.
507 *Aquacult Nutr* **12**, 291-309.

508

509 2. Williams KC, Barlow CG, Rodgers L *et al.* (2003). Asian seabass *Lates calcarifer* perform
510 well when fed pellet diets high in protein and lipid. *Aquaculture* **225**, 191-206.

511

512 3. Williams KC, Barlow C, Rodgers L *et al.* (2006) Dietary composition manipulation to
513 enhance the performance of juvenile barramundi (*Lates calcarifer* Bloch) reared in cool
514 water. *Aquacult Res* **37**, 914-927.

515

516 4. Glencross BD (2008) A factorial growth and feed utilisation model for barramundi, *Lates*
517 *calcarifer* based on Australian production conditions. *Aquacult Nutr* **14**, 360-373.

518

519 5. Glencross BD & Bermudes M (2012) Using a bioenergetic modelling approach to understand
520 the implications of heat stress on barramundi (*Lates calcarifer*) growth, feed utilisation and
521 optimal protein and energy requirements – Options for adapting to climate change? *Aquacult*
522 *Nutr* **18**, 411-422.

523

524 6. Boujard T & Medale F (1994) Regulation of voluntary feed intake in juvenile rainbow trout
525 fed by hand or by self-feeders with diets containing two different protein/energy ratios.
526 *Aquatic Liv Res* **7**, 211-215.

527

528 7. Catacutan MR & Coloso RM (1995) Effect of dietary protein to energy ratios on growth,
529 survival, and body composition of juvenile Asian seabass, *Lates calcarifer*. *Aquaculture* **131**,
530 125-133.

531

532 8. Lupatsch I, Kissil GW & Sklan D (2003) Comparison of energy and protein efficiency among
533 three fish species *Sparus aurata*, *Dicentrarchus labrax* and *Epinephelus aeneus*: energy
534 expenditure for protein and lipid deposition. *Aquaculture* **225**, 175-189.

535

536 9. Hua K, Birkett S, De Lange CFM *et al.* (2010) Adaptation of a non-ruminant nutrient-based
537 growth model to rainbow trout (*Oncorhynchus mykiss* Walbaum). *J Agric Sci* **148**, 17-29.

538

539

- 540 10. Dumas A, de Lange CFM, France J *et al.* (2007) Quantitative description of body
541 composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss*).
542 *Aquaculture* **273**, 165-181.
543
- 544 11. Saravanan S, Schrama JW, Figueirido-Silva A *et al.* (2012) Constraints on energy intake in
545 fish: The link between diet composition, energy metabolism, and energy intake in rainbow
546 trout. *PlosOne* **7**(4): e34743. doc:10.1371/journal.pone.0034743.
547
- 548 12. Glencross BD, Blyth D, Bourne N, *et al.* (2014) An analysis of the effects of different dietary
549 macronutrient energy sources on the growth and energy partitioning by juvenile barramundi,
550 *Lates calcarifer*, reveal a preference for protein-derived energy. *Aquacult Nutr* **20**, 583-594.
551
- 552 13. Azevedo PA, van Milgen J, Leeson S *et al.* (2005) Comparing efficiency of metabolisable
553 energy utilisation by rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*)
554 using factorial and multivariate approaches. *J Anim Sci* **83**, 1-11.
555
- 556 14. Schrama JW, Saravanan S, Geurden I *et al.* (2012) Dietary nutrient composition affects
557 digestible energy utilisation for growth: a study on Nile tilapia (*Oreochromis niloticus*) and a
558 literature comparison across fish species. *Br J Nutr* **108**, 277-289
559
- 560 15. Sa R, Pousao-Ferreira P & Oliva-Teles A (2007) Growth performance and metabolic
561 utilization of diets with different protein:carbohydrate ratios by white sea bream (*Diplodus*
562 *sargus*, L.) juveniles. *Aquacult Res* **38**, 100-105.
563
- 564 16. Enes P, Panserat S, Kaushik S *et al.* (2009) Nutritional regulation of hepatic glucose
565 metabolism in fish. *Fish Physiol Biochem* **35**, 519-539.
566
- 567 17. Lansard M, Panserat S, Plagnes-Juan E *et al.* (2010) Integration of insulin and amino acid
568 signals that regulate hepatic metabolism-related gene expression in rainbow trout: role of
569 TOR. *Amino Acids* **39**, 801-810.
570
- 571 18. Helland S, Grisdale-Helland B & Nerland S (1996) A simple method for the measurement of
572 daily feed intake of groups of fish in tanks. *Aquaculture* **139**, 156-163
573
- 574 19. AOAC (Association of Official Analytical Chemists). (2005) *Official Methods of Analysis of*
575 *the Association of Official Analytical Chemists*. 15th edition. Association of Official
576 Analytical Chemists. Washington, DC, USA.
577

- 578 20. Blyth D, Tabrett SJ & Glencross BD (2014) Comparison of faecal collection methods and diet
579 acclimation times for the measurement of digestibility coefficients in barramundi (*Lates*
580 *calcarifer*). *Aquacult Nutr* **21**, 248-255.
- 581
- 582 21. Glencross BD & Bermudes M (2011) Effect of high water temperatures on energetic
583 allometric scaling in barramundi (*Lates calcarifer*). *Comp Biochem Physiol – Part A* **159**,
584 167-174.
- 585
- 586 22. Salini MJ, Poppi DA, Turchini GM, Glencross, BD (2016) Defining the allometric
587 relationship between size and nutrient turnover in barramundi *Lates calcarifer*. *Comp.*
588 *Biochem. Physiol. Part A Mol. Integr. Physiol.* **201**, 79-86.
- 589
- 590 23. Bureau DP, Kaushik SJ & Cho CY (2002) Bioenergetics. In: *Fish Nutrition*, Third Edition.
591 Elsevier Science, USA. pp 2-61.
- 592
- 593 24. Steel GD & Torrie JH (1980) Principles and procedures of statistics – A biometrical
594 approach. Second Edition. McGraw Hill Publishing, pp633.
- 595
- 596 25. Stone DAJ (2003) Dietary carbohydrate utilisation by fish. *Rev Fisheries Sci* **11**, 337-369.
- 597
- 598 26. Glencross BD, Blyth D, Tabrett SJ *et al.* (2012) An examination of digestibility and technical
599 qualities of a range of cereal grains when fed to juvenile barramundi (*Lates calcarifer*) in
600 extruded diets. *Aquacult Nutr* **18**, 388-399.
- 601
- 602 27. Wade NM, Skiba-Cassy S, Dias K, *et al.* (2013) Postprandial molecular responses in the liver
603 of the barramundi, *Lates calcarifer*. *Fish Physiol Biochem* **40**, 427-443.
- 604
- 605 28. Bermudes M, Glencross BD, Austen K *et al.* (2010) Effect of high water temperatures on
606 nutrient and energy retention in barramundi (*Lates calcarifer*). *Aquaculture* **306**, 160-166.
- 607
- 608 29. Glencross BD & Bermudes M (2010) Effect of high water temperatures on the utilisation
609 efficiencies of energy and protein by juvenile barramundi, *Lates calcarifer*. *Fisheries*
610 *Aquacult J* **14**, 1-11.
- 611
- 612 30. Lupatsch I, Kissil GW & Sklan D (2001) Optimization of feeding regimes for European sea
613 bass *Dicentrarchus labrax*: a factorial approach. *Aquaculture* **202**, 289-302.
- 614

- 615 31. Schwarz FJ & Kirchgessner M (1995) Effects of different diets and levels of feeding
616 on retention and efficiency of utilization of energy and protein by carp (*Cyprinus*
617 *carpio*). *J App Ichthyol* **11**, 363-366.
618
- 619 32. Glencross BD, Hawkins WE, Evans D *et al.* (2008) Evaluation of the influence of *Lupinus*
620 *angustifolius* kernel meal on dietary nutrient and energy utilisation efficiency by rainbow
621 trout (*Oncorhynchus mykiss*). *Aquacult Nutr* **14**, 129-138.
622
- 623 33. Kielanowski J (1965) Estimates of the energy cost of protein deposition in growing animals.
624 In: Blaxter, K.L. (Ed.) *Proceedings of the 3rd Symposium on Energy Metabolism*. Academic
625 Press, London, pp. 13-20.
626

627 **Table and Figure Legends**

- 628 Table 1. Formulation, composition and relative digestible contributions of the energy of each
629 macronutrient in each of the experimental diets
630
- 631 Table 2. Growth and feed utilisation responses for each treatment.
632
- 633 Table 3. Nitrogen, lipid and energy balance analysis
634
- 635 Table 4. Component energetic contributions from each macronutrient in each diet and the calculated
636 and measured energetic parameters
637
- 638 Figure 1. Energy gain (kJ/kg^{0.8}/d) by barramundi when fed different rations of each
639 experimental diet. The regression equation of each of the diets is also shown. There
640 was no significant difference in the linear regressions between the control, protein and
641 lipid diet treatments. The regression equation of the fish fed the starch diet was
642 significantly different from each of the other treatments.
643
- 644 Figure 2. Protein energy gain (kJ/kg^{0.8}/d) by barramundi when fed different rations of each
645 experimental diet. The regression equation of each of the diets is also shown. There
646 was no significant difference in the linear regressions between the control, protein and
647 lipid diet treatments. The regression equation of the fish fed the starch diet was
648 significantly different from each of the other treatments.
649
- 650 Figure 3. Lipid energy gain (kJ/kg^{0.8}/d) by barramundi when fed different rations of each
651 experimental diet. The regression equation of each of the diets is also shown. There
652 was no significant differences in the linear regressions among each of the control,
653 protein, lipid and starch diet treatments.
654
- 655 Figure 4. Lipid energy utilisation coefficients relative to the dietary concentration of lipid. Data
656 is means \pm SEM.
657
- 658 Figure 5. Relationship between diet starch concentration and energy utilisation coefficient (kE)
659 values. Equation for the relationship was $y = -0.001x + 0.747$, $R^2 = 0.987$.
660

661 **Tables and Figures**

662

663 Table 1. Formulation, composition and relative digestible contributions of the energy
664 of each macronutrient in each of the experimental diets
665

	C	P	L	S
<i>Diet Formulations</i>				
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
<i>Diet Composition</i>				
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

666

667

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

Ration	Diet C			Diet P			Diet L			Diet S			Pooled SEM	P-values		
	H	M	L	H	M	L	H	M	L	H	M	L		D	R	D x R
<i>Performance Parameters</i>																
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
<i>Final Live-weight Composition</i>																
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

Ration	Diet C			Diet P			Diet L			Diet S			SEM	<i>P-values</i>		
	H	M	L	H	M	L	H	M	L	H	M	L		<i>D</i>	<i>R</i>	<i>D x R</i>
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/FP	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	377 ^{de}	298 ^e	895 ^c	384 ^{de}	324 ^{de}	971 ^{bc}	432 ^d	318 ^{de}	1507 ^a	467 ^d	333 ^{de}	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	151 ^{de}	87 ^e	598 ^c	149 ^{de}	106 ^{de}	668 ^c	204 ^d	106 ^{de}	1213 ^a	244 ^d	126 ^{de}	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).

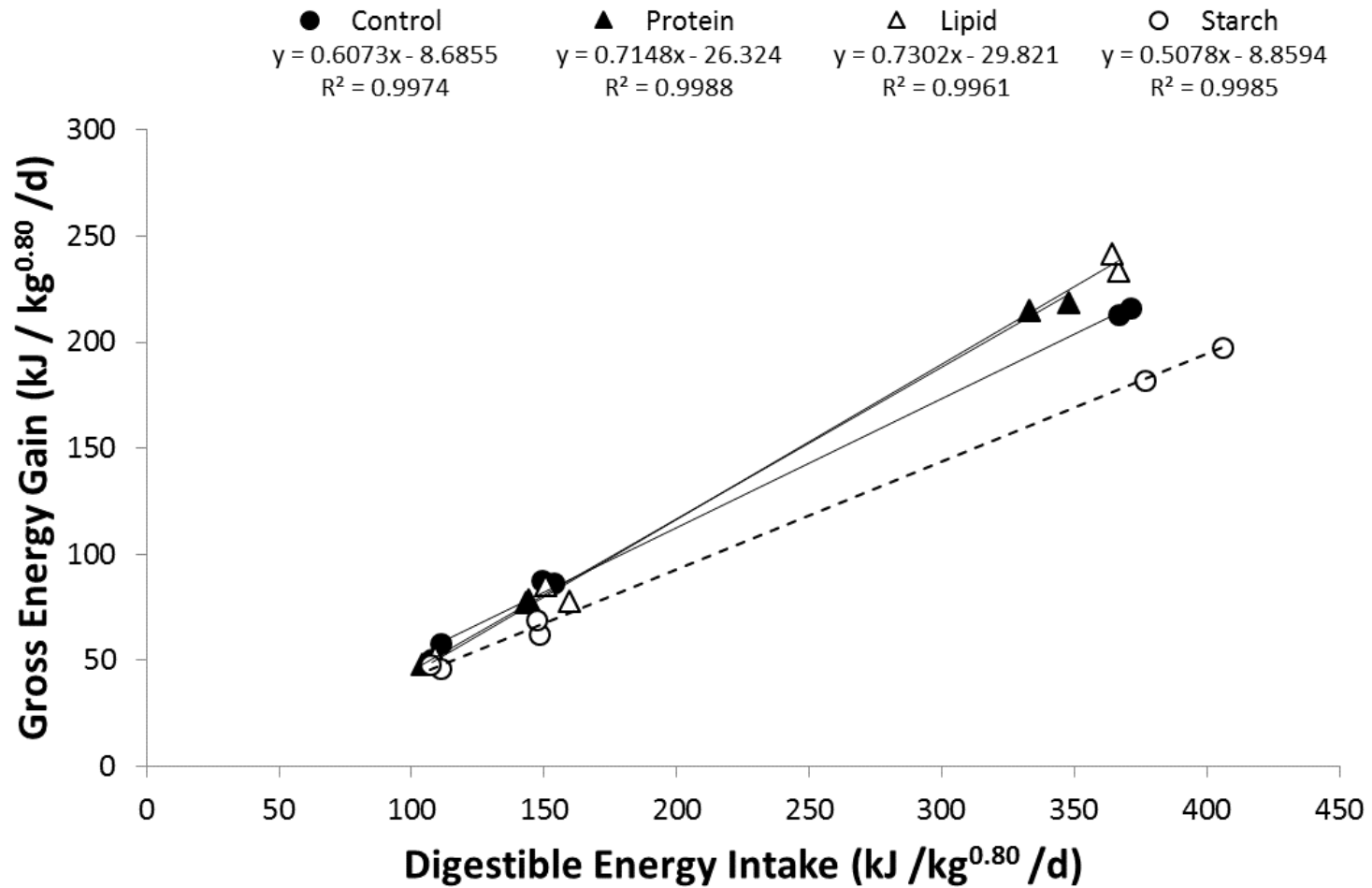


Figure 1. Energy gain (kJ/kg^{0.8}/d) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There was no significant difference in the linear regressions between the control, protein and lipid diet treatments. The regression equation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

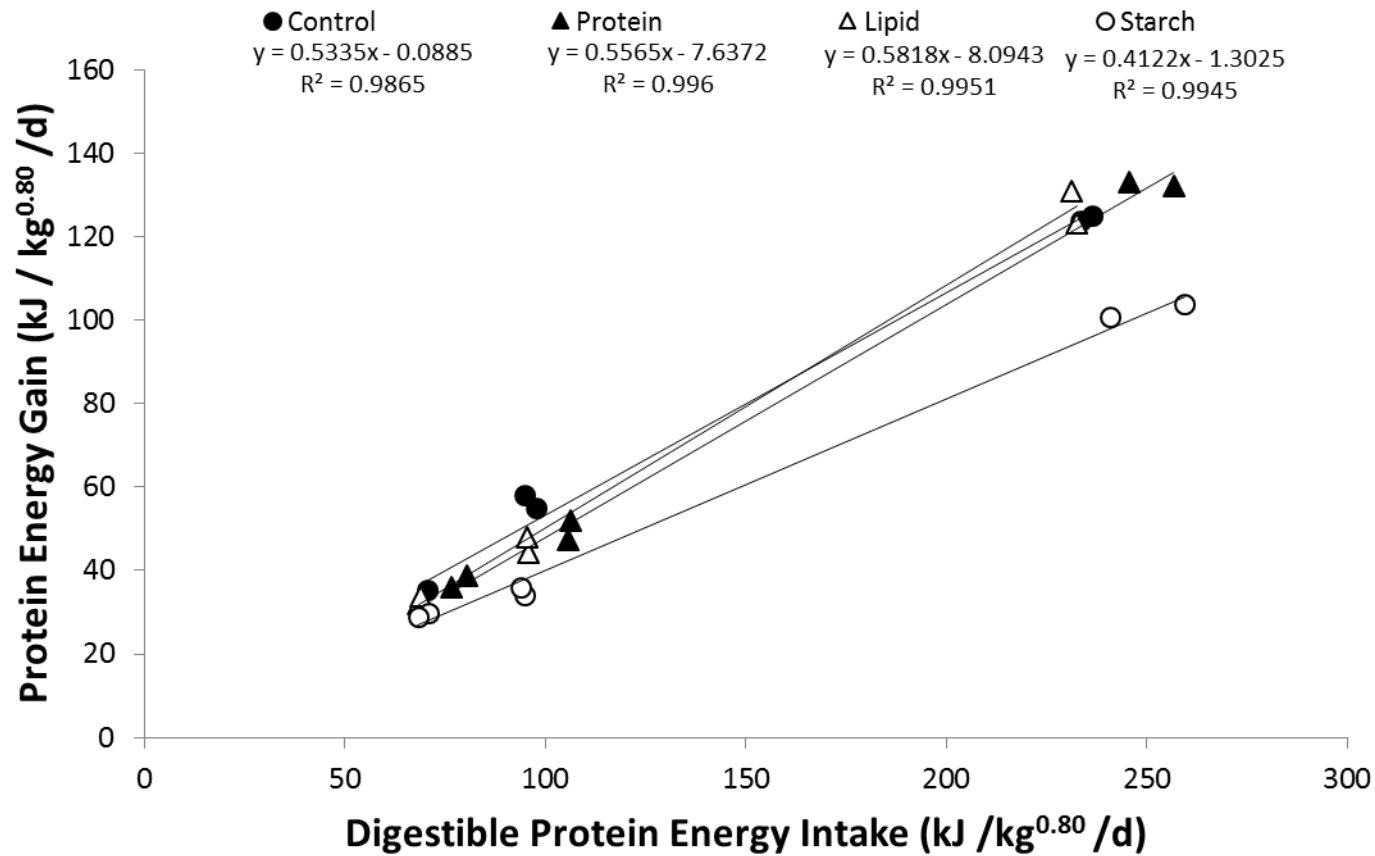


Figure 2. Protein energy gain (kJ/kg^{0.8}/d) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There was no significant difference in the linear regressions between the control, protein and lipid diet treatments. The regression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

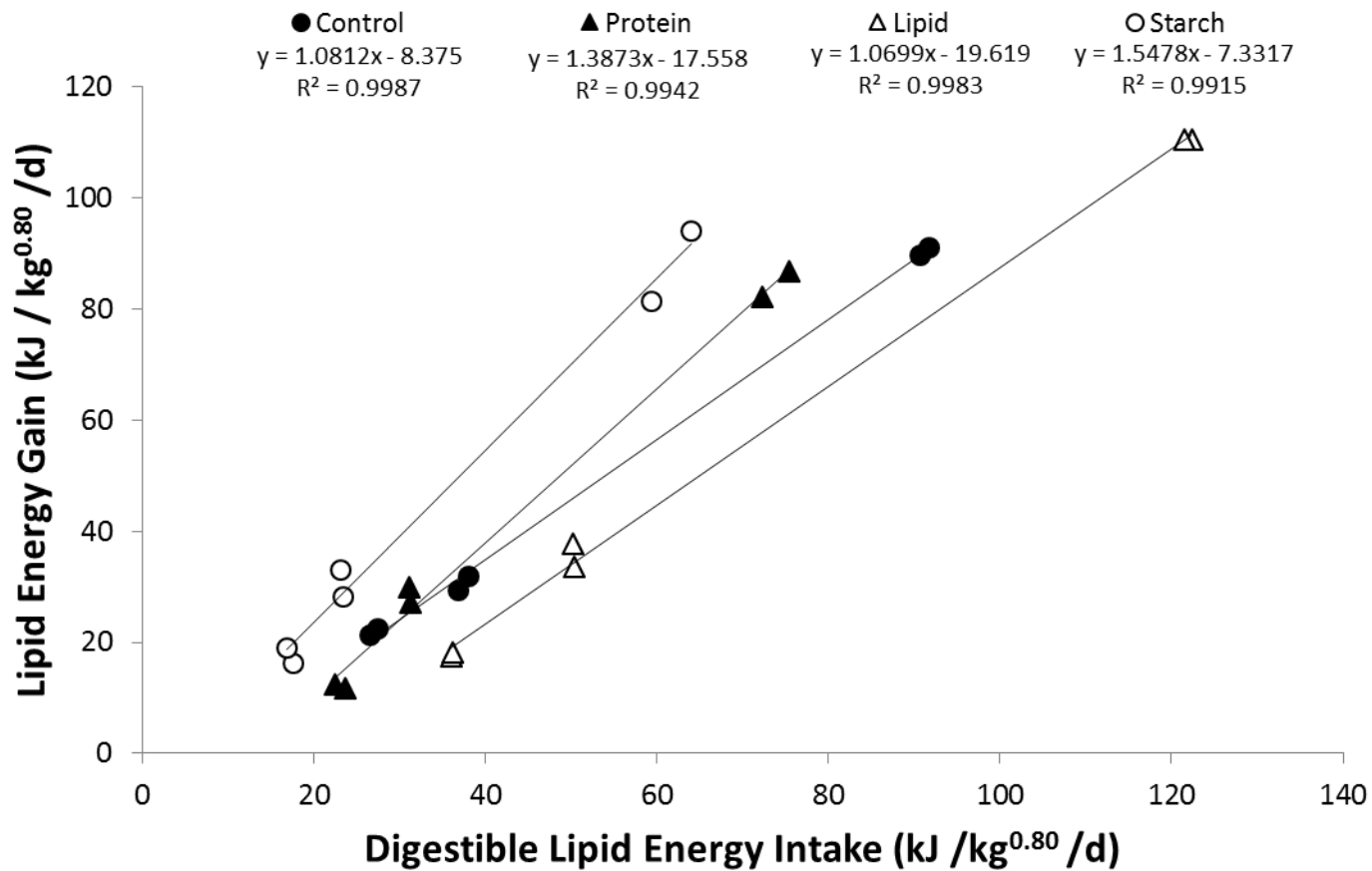


Figure 3. Lipid energy gain (kJ/kg^{0.8}/d) by barramundi when fed different rations of each experimental diet. The regression equation of each of the diets is also shown. There were no significant differences in the linear regressions among each of the control, protein, lipid and starch diet treatments.

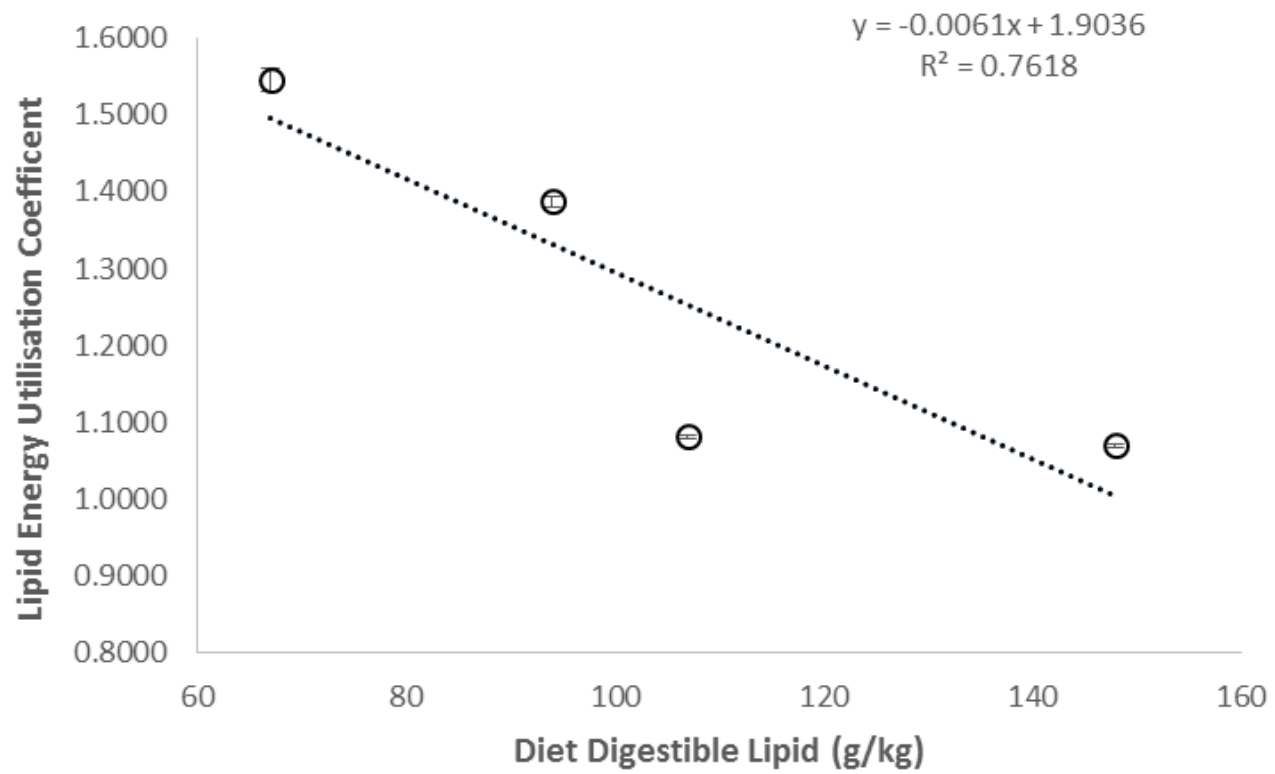


Figure 4. Lipid energy utilisation coefficients relative to the dietary concentration of lipid. Data is means \pm SEM.

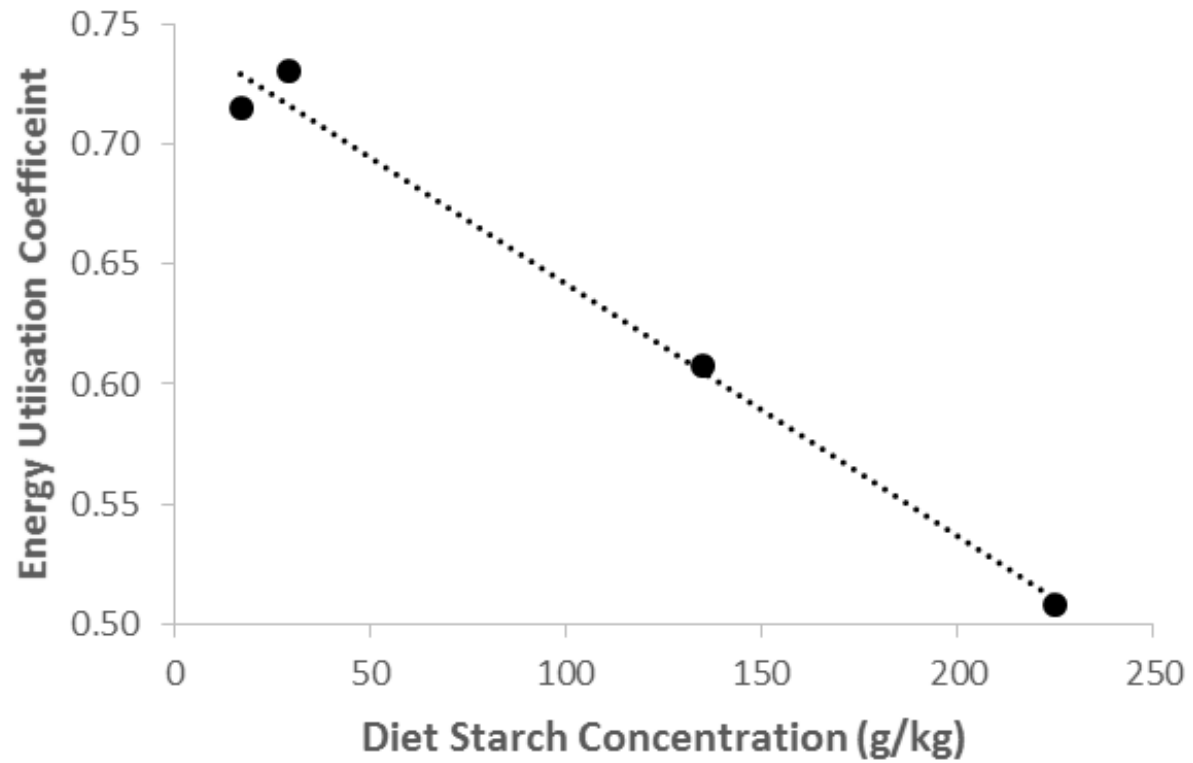


Figure 5. Relationship between diet starch concentration and energy utilisation coefficient (k_E) values. Equation for the relationship was $y = -0.001x + 0.747$, $R^2 = 0.987$.