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1 Comparison of faecal collection methods, and diet acclimation times for the measurement of
2 digestibility coefficients in barramundi (*Lates calcarifer*)

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4 David Blyth^{1,3}, Simon Tabrett^{1,2}, Nicholas Bourne^{1,2}, Brett Glencross^{1,2*}

5

6 ¹CSIRO Food Futures Flagship, GPO Box 2583 Brisbane, QLD 4001, Australia

7 ²CSIRO Marine and Atmospheric Research, GPO Box 2583 Brisbane, QLD 4001, Australia

8 ³CSIRO Marine and Atmospheric Research, Bribie Island Research Centre, Woorim,
9 Australia

10

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13 *Correspondence to: (p) 61-7-3833-5926

14 (e) Brett.Glencross@csiro.au

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17 Key words: Asian seabass, Stripping collection, Settlement collection, Diet
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24

25 **Abstract**

26

27 This study aimed to investigate the effects of two faecal collection methods (stripping and
28 settlement) on the apparent digestibility coefficients (ADC) of dry matter, protein and energy
29 of three different diets fed to barramundi. In a second experiment, the effect of acclimation
30 time (i.e. number of days fed the diet) on the calculation of ADCs was also investigated. Each
31 tank of fish was fed one of three diets for 12 days. Faeces were collected by both stripping
32 and settlement, though only settlement was used prior to day seven of the acclimation period.
33 Faeces were collected using the settlement method at regular intervals from day one to day
34 12. Comparisons between faecal collection methods were only made based on faecal material
35 collected over a similar acclimation period. The collection of faeces by stripping produced
36 more conservative ADCs, which were also more consistent than those obtained using the
37 settlement technique. The calculated ADCs typically fluctuated for the first three days of
38 collection before the variability diminished. Barramundi should be acclimated to diets for a
39 minimum of four days before collection of faecal material, and collection by stripping is
40 recommended to obtain the most reliable digestibility data.

41

42 **Introduction**

43 The basis for sound diet formulation depends on having accurate and reliable data on
44 the digestible nutrient and energy value of raw materials that are used to make those diets
45 (reviewed by Glencross et al., 2007). The determination of the digestible nutrient and energy
46 value of raw materials depends on having a viable method to measure the digestibility of
47 these parameters from the diets (Choubert et al., 1982; Suigura et al., 1998; Weatherup &
48 McCracken, 1998). However, the assessment of the digestibility of aquaculture diets can be
49 highly variable and the digestibility values are known to vary significantly depending on the
50 different methods used (reviewed by Glencross et al., 2007). It is well recognised that faecal
51 collection is an integral part of the process for calculating digestibility values, and the
52 collection process can have a significant effect on the determination of the digestibility values
53 of diets (Windell et al., 1978; Weatherup & McCracken, 1998; Vandenberg & de la Noue,
54 2001; Glencross et al., 2005).

55 Faecal collection methods can be grouped under two main methods; collection of un-
56 defecated digesta, and collection of faeces settled from the water column. The three most
57 common techniques to collect un-defecated digesta are intestinal dissection, suction, and
58 stripping (Austreng et al., 1978; Vandenberg & de la Noue, 2001; Glencross et al., 2005;
59 Aslaksen et al., 2007). Collection of faeces from the water column involves either syphoning
60 faeces from the bottom of the tank, collection of decanted faeces, or continuous collection
61 (Choubert et al., 1982; Cho & Kaushik, 1990; Vandenberg & de la Noue, 2001; Glencross et
62 al., 2005).

63 Collection of un-defecated digesta is generally more labour intensive than collecting
64 faeces from the water column and is also restricted by fish size (i.e. fish can be too small or
65 large to handle). Moreover, samples are collected at one point in time providing a snapshot of
66 the ADC and the amount of sample collected can be limiting. In contrast, the collection of
67 faeces from the water column is typically less labour intensive, and can be applied to fish of
68 any size, and does not inflict stress on the animals (reviewed by Glencross et al., 2007).
69 However, owing to passive nature of this collection method, there is a risk of the sample
70 being contaminated by scales, mucous and other exogenous material as well as leaching of
71 nutrients into the water column (reviewed by Glencross et al., 2007). While each method has
72 advantages and disadvantages, it has been suggested that the collection of un-defecated
73 digesta results in a reduced Apparent Digestibility Coefficient (ADC) values (Vandenberg &
74 de la Noue, 2001; Glencross et al., 2005). Although there have been comparisons of methods
75 for other species, there have been no direct comparisons for barramundi when faeces have

76 been collected by stripping or settlement methods (Vandenberg & de la Noue, 2001;
77 Glencross et al., 2005; Glencross, 2006).

78 Most studies allow fish to adapt to new diets before commencement of faecal
79 sampling; with times varying between five days and 14 days for a range of temperate and
80 tropical species (Glencross et al., 2005; Barrows et al., 2007; Glencross et al., 2012). This is
81 done supposedly to allow the fish to adapt to the chemical composition of a new diet and
82 establish an equilibrium within the animals gut in terms of the absorption efficiencies from
83 that new diet before any sampling is initiated. However, although it is widely accepted that
84 fish require a period of time to acclimate to new diets, there have been limited studies
85 published that actually investigate the time that it actually take to adapt to introduction of a
86 new a diet or indeed variable levels of feed intake (reviewed by Glencross et al., 2007).
87 Given the importance of accurately determining the digestibility of diets and raw ingredients,
88 this is an area which requires further attention.

89 Therefore, the present study was conducted to examine two key methodological issues
90 for digestibility assessment with barramundi (*Lates calcarifer*). In the first experiment,
91 differences in the digestibilities of dry matter, protein and energy of three diets (basal, starch
92 and lupin-meal based) were evaluated after faeces were collected by stripping or settlement
93 methods. In the second experiment, the variability of ADCs were evaluated over the first 14
94 days when barramundi were introduced to a new diet, using faeces collected by settlement
95 collection methods.

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99 **Methods**

100

101 *Ingredient preparation and diet formulation*

102 The experiment design was based on a diet formulation strategy that allowed for the
103 diet-substitution digestibility method to be used (Aksnes et al., 1996). For this, a basal diet
104 was formulated and prepared as one large batch (60 kg) to include approximately 540 g/kg
105 DM protein, 120 g/kg DM fat and an inert marker (yttrium oxide at 1 g/kg) (Table 1). This
106 basal mash was prepared and thoroughly mixed, forming the basis of the experimental diets
107 in this study. Each of the test diets were made by the inclusion of 30% of the test ingredient
108 to a sub-sample of the basal mash.

109 Two test ingredients were used in this study, pre-gelatinised wheat starch, and
110 *Lupinus angustifolius* cv. Myallie (MKM) (Table 2). The fishmeal was ground using a
111 Mikro-Pulveriser hammer mill through a 500 µm screen (Hosokawa Micron Powder
112 Systems, Summit, New Jersey, USA). The lupin meal was ground using a Retsch™ ZM200
113 rotor mill (Retsch Pty Ltd, North Ryde, NSW, Australia) such that it passed through a 750
114 µm screen. The other ingredients were supplied in fine flour (< 500 µm) forms and required
115 no further milling. The composition and source of all of the ingredients used are presented in
116 Table 2.

117 Each of the diets were processed by addition of water (about 30% of mash dry
118 weight) to the mash whilst mixing to form a dough, which was subsequently screw pressed
119 using a Dolly Pasta Extruder (La-Monferrina, Sant'Ambrogio di Torino, Italy) through a 5
120 mm diameter die. The moist pellets were then oven dried at 60°C for approximately 24 h and
121 then allowed to cool to ambient temperature in the oven. The basal diet was prepared in a
122 similar manner, but without the addition of any test ingredient.

123

124 *Fish Handling and Faecal Collection*

125 Juvenile barramundi were kept in an experimental tank array (6 x 300 L) supplied
126 with flow-through seawater (salinity =35 PSU) at a rate of about 4 L min⁻¹ and maintained
127 with a dissolved oxygen content of 6.4 ± 0.2 mg L⁻¹ at 28.8 ± 0.2°C. Each of the tanks were
128 stocked with 10 fish of an initial weight of 398 ± 69 g (mean ± S.D.; n = 40 from a
129 representative sample of the population). Treatments were randomly assigned amongst the 6
130 tanks, with each treatment having four replicates, but the experiment being conducted over
131 two block events to achieve this level of replication. The same batch of fish was used for both

132 blocks, but a complete randomised design applied to each block to ensure experimental
133 validity. The fish were allowed to acclimate to their allocated dietary treatment for at least
134 seven days before stripping faecal collection commenced.

135 All fish were manually fed the basal diet for 1 week prior to the commencement of the
136 trial. On commencement, the fish were fed their respective diets to apparent satiety as
137 determined by the loss of feeding activity after being offered food on three independent
138 feeding episodes over a ninety-minute period once daily (1530 to 1700), seven days a week.
139 Faeces were then collected the following morning (0830 – 1030) from each fish within each
140 tank using stripping techniques based on those reported by Glencross (2011). Fish were
141 anaesthetised using AQUI-S™ (0.02 mL L⁻¹). Once loss of equilibrium by the fish was
142 observed, close attention was then paid to the relaxation of the ventral abdominal muscles of
143 the fish to enable the fish to be removed from the water prior to the faecal pellet being
144 expelled. The faeces were then removed from the distal intestine using gentle abdominal
145 pressure during this muscle relaxation. Hands were rinsed between handling each fish to
146 ensure that the faeces were not contaminated by urine or mucous. Fish were also not stripped
147 on consecutive days in order to minimise stress on the animal (as determined by loss of
148 appetite and physical damage, of which none was observed) and maximise feed intake prior
149 to faecal collection. Faecal samples from different days were pooled within tank, and kept
150 frozen at –20°C before being freeze-dried in preparation for analysis. Faeces were collected
151 from three separate stripping events within one week.

152 Settled faeces were collected overnight from the same tanks and fish using settlement
153 methods based on those reported by Cho & Kaushik (1990) on days 1, 2, 3, 4, 6, 8, 10, and
154 12. The collection chamber was flushed 1 hour after feeding to remove any feed partials
155 before a chiller jacket (tube with a frozen block of water inside and a hole to allow for the
156 faecal collection tube to be inserted) was placed over the collection tube. Faeces were
157 removed from the ice-chilled collection tube at 0830 on each day, prior to the fish being
158 stripped, and transferred into a large vial before being stored at -18°C.

159 For comparison of faecal collection methods, the stripped faecal data was compared against
160 the data from the last four days of settlement collection so as to ensure that the samples were
161 from a similar period of acclimation to the diets.

162

163 *Chemical and digestibility analysis*

164 Faecal, ingredient and diet samples were analysed for dry matter, yttrium, nitrogen
 165 and gross energy content. All methods were done in accordance with AOAC methodology
 166 (2005). In addition, diet and ingredient samples were analysed for ash and total lipids and
 167 carbohydrate content calculated. Dry matter content was calculated following oven drying at
 168 105°C for 24 h. Total yttrium concentrations were determined using inductively coupled
 169 plasma mass spectrophotometry (ICP-MS) after mixed acid digestion based on the method
 170 described by (McQuaker et al., 1979). Protein was determined based on measurement of total
 171 nitrogen by CHNOS auto-analyser, and then multiplied by 6.25. Total lipid content of the
 172 diets was determined gravimetrically following extraction of the lipids using
 173 chloroform:methanol (2:1). Gross ash content was determined gravimetrically following loss
 174 of mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Gross energy
 175 was determined by adiabatic bomb calorimetry. Total carbohydrates were calculated based on
 176 the dry matter content of a sample minus the protein, lipid and ash. Amino acid composition
 177 of samples was determined by an acid hydrolysis prior to separation via HPLC. The acid
 178 hydrolysis destroyed tryptophan making it unable to be determined using this method.

179 The apparent digestibility (AD_{diet}) for each of the nutritional parameters examined in
 180 each diet was calculated based on the following formula (Maynard & Loosli, 1979):

$$181 \quad AD_{diet} = \left(1 - \frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}} \right) \times 100$$

182
 183 where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and
 184 $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (dry matter,
 185 protein or energy) content of the diet and faeces respectively. Ingredient digestibility values
 186 were not determined for the present study.
 187
 188

189 *Statistical analysis*

190 All figures are mean \pm SE unless otherwise specified. Effects of diet and collection
 191 method were examined by two-way ANOVA. Levels of significance were determined using a
 192 Tukey's HSD test, with critical limits being set at $P < 0.05$. Effects of sampling time on the
 193 digestibility parameters were also analysed by two-way ANOVA. All statistical analyses
 194 were done using the software package Statistica™ (Statsoft®, Tulsa, OA, USA) although
 195 graphically presented using Microsoft Excel (Microsoft Corporation, USA).
 196

197 **Results**

198

199 *Faecal collection methods*

200 Faecal collection method (settlement or stripping) affected the digestibility of dry
201 matter, protein and energy ($P < 0.05$; Table 3). When faeces were collected by settlement
202 compared with stripping the dry matter digestibilities were higher, but both protein and
203 energy digestibilities were lower.

204 For faeces collected by stripping, the DM digestibility varied between diets ($P < 0.05$)
205 with the digestible DM of the MKM diet being significantly lower than that of the Starch
206 based diet ($P < 0.05$; Table 4). Protein digestibility was not different between diets when
207 faeces were collected by stripping ($P > 0.05$; Table 4) although energy digestibility differed
208 significantly among each of the diets. The energy digestibility was lowest for the MKM diet
209 compared with the basal and starch diets, and the basal diet energy digestibility was
210 significantly higher than the digestible energy of the starch diet ($P < 0.05$; Table 4).

211 Collection of faeces by settlement displayed similar results, with the digestible DM of
212 the MKM diet being significantly lower than both the basal and starch based diets ($P < 0.05$;
213 Table 4). No differences were observed between protein digestibility ($P > 0.05$; Table 4),
214 whilst energy digestibility was significantly lower for the MKM diet compared with the basal
215 and starch diets, and the digestibility of the basal diet was significantly higher than that of the
216 starch based diet ($P < 0.05$; Table 4).

217 There was good correlation between both the stripping and settlement faecal
218 collection methods and this can be seen by the high R^2 values in Figure 2. Correlation was
219 strongest with energy digestibility ($R^2 = 0.979$), followed by dry matter digestibility
220 ($R^2 = 0.823$) and protein digestibility ($R^2 = 0.655$).

221

222 *Temporal variation in digestibility values*

223 Statistically there was no temporal variation ($P = 0.148$) or interaction effect ($P = 0.517$)
224 with time and diet in the DM digestibility, but it did vary between diets ($P = 0.001$; Table 5).
225 Protein digestibility was also different between diets ($P = 0.003$), but not over time ($P = 0.102$)
226 and again there was no interaction effect ($P = 0.700$; Table 5). Energy digestibility differed
227 significantly with diet ($P < 0.001$), but not with time ($P = 0.346$). In contrast to the other two
228 digestibility parameters the energy digestibility did exhibit an interaction effect between diet
229 and time ($P < 0.001$; Table 5).

230 From Figure 3 it can be noted that the DM digestibility values stabilised between days
231 three and four for all diets. Variance within the DM digestibility values was highest on day 1
232 and thereafter subsided and for all samples, except the MKM, was minimal from day two
233 onwards. There was a limited amount of variation during the first four days in the protein
234 digestibility in all diets, before the values stabilised. Notably the variance within the protein
235 digestibility data was the lowest of each of the three digestibility parameters. What variance
236 there was within the protein digestibility values also minimised after two days (Figure 3).
237 Energy digestibility values were variable over time and also took two to four days till the
238 trend in the digestibility value stabilised. Similar to protein digestibility the variance within
239 the energy digestibility values was also nominal and this too diminished within two to four
240 days.

241

242

243

244 **Discussion**

245

246 The key foci of this study were methodological, in that the study sought to define the effects
247 of faecal collection method and also acclimation time to diets, on the digestibility values determined
248 in barramundi. Although studies have been performed comparing the determination of whole diet
249 digestibilities based on faeces collected using either settlement or stripping techniques in salmonids
250 (Windell et al., 1978; Weatherup & McCracken, 1998; Vandenberg & de la Noue, 2001; Glencross et
251 al., 2005), this is the first study to compare the influence of these faecal collection methods with
252 barramundi. Additionally, the study also examines the variation in digestibility over time to establish
253 what is the best acclimation time to diets prior to faecal collection. Similar such data from other
254 species could not be found.

255

256 *Faecal collection method influences*

257 There has been much debate on the positives and negatives associated with either faecal
258 collection method used in digestibility studies (reviewed by Glencross et al., 2007). However, it is
259 widely acknowledged that the two faecal collection methods do result in different diet digestibility
260 value determinations (Windell et al., 1978; Weatherup & McCracken, 1998; Vandenberg & de la
261 Noue, 2001; Glencross et al., 2005). These differences imply that there are compositional differences
262 in the faeces collected which immediately have connotations on the use of each faecal collection
263 method. Despite being more laborious and costly to collect, the data produced from faeces collected
264 using the stripping method was more conservative than the data produced from faeces collected using
265 the settlement method. This factor alone means that when provided with the option to use either data
266 set the rational decision is to use the data from the stripping method because of this conservatism.

267 It was noted in the earlier work of Glencross et al. (2005) that the greatest differences
268 between the nutrient digestibility assessments from the two faecal collection methods were those
269 ingredients with higher levels of carbohydrates. A similar result was also observed in the present
270 study with a greater number of significant differences in the digestibility of the Starch diet than either
271 the Basal or MKM diets. It is likely that this is due to high levels of carbohydrates in the faeces
272 decreasing faecal integrity and as such increases the dissolution of the faecal matter collected using
273 settlement techniques.

274

275 *Temporal variation in digestibility values*

276 One of the key elements of this study was to determine the time period over which the fish
277 should be fed a diet before faecal collection is initiated. Unfortunately there was little literature with
278 which to compare our data in this part of the study. Therefore, in assessing this question the key
279 parameter was considered to be the level of variability (as noted by the magnitude of the standard
280 error) in the data collected and also how the data at any time point compares to that data obtained at

281 the longest acclimation time point. This was based on the assumption that by this time point the fish
282 would have acclimated to the diet. The different digestibility parameters (dry matter, protein, energy)
283 were also subtly different in how they responded over time with respect to the variability and also
284 how they fared compared to the digestibility values from day 12 of the study. Fish fed the MKM diet
285 took the longest to acclimate to it and there was a higher level of data variance within the dry matter
286 digestibilities determined from that diet even up to day 10. However the protein and energy
287 digestibility parameters for that diet showed little variance and were relatively consistent from day
288 four onwards based on Figure 3.

289 An important observation in this study though is the level of variability seen of the data from
290 the Basal diet. As indicated in the methods, the fish were fed this diet for one week before any faecal
291 collection commenced, yet on day one of faecal collection a decline in dry matter digestibility was
292 observed relative to the longer-term mean (Figure 3). In fact throughout the two week study period
293 there was an inconsistency in the digestibility values determined for dry matter from this diet (and the
294 other two) which perhaps indicates that some variation in digestibility might be a natural feature
295 independent of acclimation time.

296

297 *Conclusions*

298 The two faecal collection methods used in this study are the two main methods used by fish
299 nutritionists worldwide and this study provides a good estimate of how well each method compares
300 when used with barramundi. The faecal stripping collection method is the more conservative of the
301 two assessments used in this study and therefore is the one we recommend for use with this species.

302 When assessing the variability in digestibility over time, it was observed that in the first three
303 days after a new diet is introduced, that the digestibility data obtained using the faecal settlement
304 methods, was particularly variable. After this time this variability diminished and values became more
305 uniform. We therefore recommend at least four days acclimation to new diets for barramundi before
306 any faeces are collected for digestibility studies.

307

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309 **References**

310

311 Aksnes, A., Hjertnes, T. & Opstvedt, J. (1996) Comparison of two assay methods for determination of
312 nutrient and energy digestibility in fish. *Aquaculture* 140, 343-359.

313

314 AOAC (Association of Official Analytical Chemists). (2005) *Official Methods of Analysis of the*
315 *Association of Official Analytical Chemists*. 16th edition. Association of Official Analytical Chemists.
316 Washington, DC, USA.

317

318 Austreng, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of
319 different segments of the gastrointestinal tract. *Aquaculture* 13, 265-272.

320

321 Burel, C., Boujard, T., Tulli, F. & Kaushik, S., (2000) Digestibility of extruded peas, extruded lupin,
322 and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). *Aquaculture*
323 188, 285-298.

324

325 Cho, C.Y. & Kaushik, S.J. (1990) Nutritional energetics in fish: Energy and protein utilisation in
326 rainbow trout (*Salmo gairdnerii*). *World Review of Nutrition and Dietetics* 61, 132-172.

327

328 Choubert, G., De la Noue, J. & Luquet, P. (1982) Digestibility in fish: improved device for the
329 automatic collection of feces. *Aquaculture* 29, 185-189.

330

331 Cheng, Z.J. & Hardy, R.W., (2003) Effects of extrusion processing of feed ingredients on apparent
332 digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*) *Aquaculture Nutrition*
333 9, 77-87.

334

335 Glencross, B.D., (2011) A comparison of the diet and raw material digestibilities between rainbow
336 trout (*Oncorhynchus mykiss*) and barramundi (*Lates calcarifer*) – Implications for inferences of
337 digestibility among species. *Aquaculture Nutrition* 17, e207-e215.

338

339 Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R. & Sipsas, S. (2005)
340 Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to
341 rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods.
342 *Aquaculture* 245, 211-220.

343

344 Glencross, B.D., Booth, M. & Allan, G.L. (2007) A feed is only as good as its ingredients – A review
345 of ingredient evaluation for aquaculture feeds. *Aquaculture Nutrition* 13, 17 – 34.

346
347 Glencross, B.D., Rutherford, N.R. & Jones, J.B., (2011) Fishmeal replacement options for juvenile
348 barramundi (*Lates calcarifer*). *Aquaculture Nutrition* 17; e722–e732.
349
350 Glencross, B.D., Blyth D., Tabrett, S.J., Bourne, N., Irvin, S., Fox-Smith, T. & Smullen, R.P., (2012).
351 An examination of digestibility and technical qualities of a range of cereal grains when fed to juvenile
352 barramundi (*Lates calcarifer*) in extruded diets. *Aquaculture Nutrition* 18, 388-399.
353
354 Hillebrand, W.F., Lundell, G.E.F., Bright, H.A. & Hoffman, J.I., (1953) *Applied Inorganic Analysis*.
355 Wiley, New York, USA.
356
357 Maynard, L.A., Loosli, J.K. (1979) *Animal Nutrition*, 6th Edition. New York, NY: McGraw-Hill Book
358 Co.
359
360 Sugiura, S.H., Dong, F.M., Rathbone, C.K. & Hardy, R.W. (1998) Apparent protein digestibility and
361 mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 159, 177-202.
362
363 Vandenberg, G.W. & de la Noue, J. (2001) Apparent digestibility comparison in rainbow trout
364 (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility
365 markers. *Aquaculture Nutrition* 7, 237-245.
366
367 Weatherup, R.N. & McCracken, K.J., (1998) Comparison of the estimation of digestibility of two
368 diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum), using two markers and two methods of
369 faeces collection. *Aquaculture Research* 29, 527-533.
370
371 Windell, J.T., Foltz, J.W. & Sarokon, J.A., (1978) Methods of fecal collection and nutrient leaching in
372 digestibility studies. *Progressive Fish Culturist* 40, 51-55.
373

374 Table 1. Formulations and composition diets (all values are g kg⁻¹ DM unless otherwise
 375 indicated) of the experimental diets
 376

	Basal Diet	Starch Diet	Lupin Diet
Fishmeal	640	448	448
Fish oil ^a	100	70	70
Cellulose	124	86.8	86.8
Wheat gluten	130	91	91
Pregelld Starch	-	300	-
<i>L. angustifolius</i> kernel meal	-	-	300
Vitamin and mineral premix*	5	3.5	3.5
Yttrium oxide ^b	1	0.7	0.7
Dry matter	959	924	960
Protein	546	396	502
Lipid	129	85	108
Ash	106	75	82
Gross energy (MJ kg ⁻¹ DM)	22.0	21.0	21.0

377 * Vitamin and mineral premix includes (IU/kg or g/kg of premix): Vitamin A,
 378 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K,3, 1.7 g; Vitamin B1,
 379 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g;
 380 Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline,
 381 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g;
 382 Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g. ^a Sourced from Skretting
 383 Australia, Cambridge, TAS, Australia. ^b Sourced from SIGMA, St Louis, Missouri,
 384 United States.

385
 386
 387

388 Table 2. Chemical characterisation of the key raw materials used in this study. All values are g
 389 kg⁻¹ DM unless otherwise detailed.
 390

Nutrient	^a Fishmeal	^d Lupin meal	^e Gluten	^f Cellulose	^e Starch
Dry matter (g/kg)	907	902	924	927	907
Protein	744	383	710	7	10
Total lipid	75	54	46	1	1
Ash	162	34	8	2	3
Carbohydrates	19	530	236	991	986
Gross Energy (MJ/kg DM)	20.9	20.6	22.9	17.0	17.1
Alanine	47	13	20	0	0
Arginine	42	44	27	0	0
Aspartate	70	41	27	0	0
Cysteine	8	8	22	0	0
Glutamate	93	87	289	0	0
Glycine	43	16	26	0	0
Histidine	23	7	12	0	0
Isoleucine	31	16	28	0	0
Leucine	56	27	54	0	0
Lysine	55	14	10	0	0
Methionine	24	3	12	0	0
Phenylalanine	30	16	41	0	0
Proline	36	22	84	0	0
Serine	30	22	40	0	0
Taurine	7	0	0	0	0
Threonine	32	15	22	0	0
Tyrosine	24	16	28	0	0
Valine	36	15	29	0	0

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 392 Ingredient origins are as follows: ^a Fishmeal (Anchovetta meal of Peruvian origin): Ridley Aquafeeds, Narangba, QLD, Australia. ^d
 393 *L. angustifolius* cv. Myallie Kernel Meal: Coorow Seed Cleaners, Coorow, WA, Australia. ^e Wheat gluten and prelatinised wheat
 394 starch :Manildra, , Auburn, NSW, Australia. ^f Sourced from SIGMA, St Louis, Missouri, United States.
 395

396 Table 3. Univariate MANOVA analysis with fixed effects of faecal collection method, diet
 397 and method (M) x diet (D)
 398

Variate	Parameter	Sum of Squares	Degrees of Freedom	Mean Square	F value	P value
Method	Dry matter	0.017	1	0.017	12.48	0.002
Diet	Dry matter	0.029	2	0.015	10.51	< 0.001
M x D	Dry matter	0.003	2	0.002	1.07	0.363
Method	Protein	0.003	1	0.003	5.83	0.027
Diet	Protein	0.001	2	0.001	1.55	0.238
M x D	Protein	0.000	2	0.000	0.19	0.830
Method	Energy	0.004	1	0.004	13.84	0.002
Diet	Energy	0.025	2	0.013	41.66	< 0.001
M x D	Energy	0.000	2	0.000	0.45	0.647

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400

401 Table 4. Digestibility (%) specifications of diets as determined using either stripping or
 402 settlement faecal collection methods. Data are mean with pooled SEM. Values within a row
 403 (a,b) or between collection methods (x,y) with a different superscript are significantly
 404 different (P<0.05).

Nutrient	Basal	Starch	MKM	Pooled SEM
<i>Stripping</i>				
Dry matter	66.7 ^{ab, x}	69.8 ^{a,x}	59.3 ^{b,x}	1.60
Protein	92.6 ^{a,x}	91.2 ^{a,x}	92.7 ^{a,x}	0.77
Energy	82.7 ^{a,x}	80.5 ^{b,x}	74.5 ^{c,x}	1.20
<i>Settlement</i>				
Dry matter	62.3 ^{a,x}	61.3 ^{ab,y}	56.0 ^{b,x}	1.35
Protein	94.1 ^{a,x}	93.3 ^{a,x}	95.5 ^{a,x}	0.43
Energy	85.3 ^{a,y}	82.3 ^{b,y}	78.0 ^{c,y}	0.94

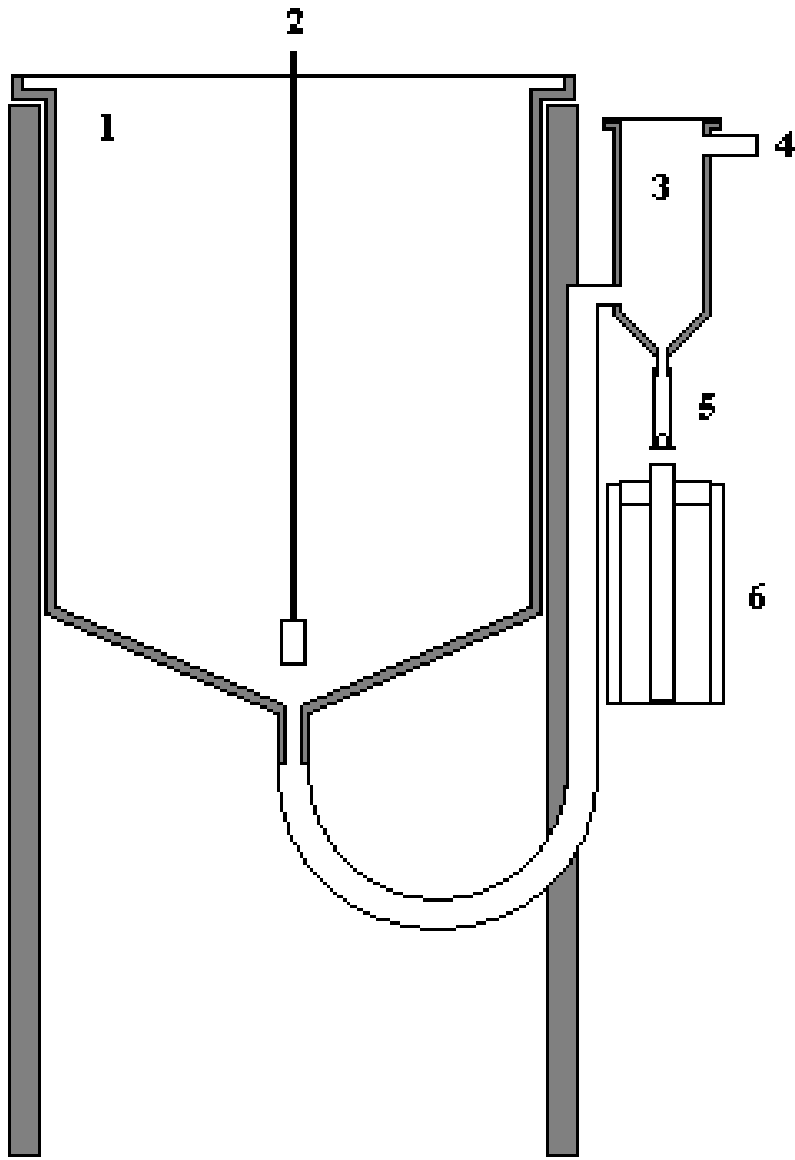
421 MKM : Lupin kernel meal cv. Myallie.

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423 Table 5. Univariate MANOVA analysis with fixed effects of faecal collection time (T), diet
 424 (D) and time x diet
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Variate	Parameter	Sum of Squares	Degrees of Freedom	Mean Square	F value	P value
Diet	Dry matter	0.114	2	0.057	8.0	0.001
Time	Dry matter	0.081	7	0.012	1.6	0.148
D x T	Dry matter	0.094	14	0.007	0.9	0.517
Diet	Protein	0.005	2	0.003	6.4	0.003
Time	Protein	0.005	7	0.001	1.8	0.102
D x T	Protein	0.004	14	0.000	0.8	0.700
Diet	Energy	0.085	2	0.043	59.5	< 0.001
Time	Energy	0.006	7	0.001	1.1	0.346
D x T	Energy	0.048	14	0.003	4.8	< 0.001

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Figure 1. Design of aquaria system used to undertake the experiments from which faeces were collected by both settlement and stripping methods. Features are; 1. Conical Tank, 2. Air supply, 3. Swirl separator, 4. Waste water, 5. Silicon rubber collection tube, 6. Chiller jacket.

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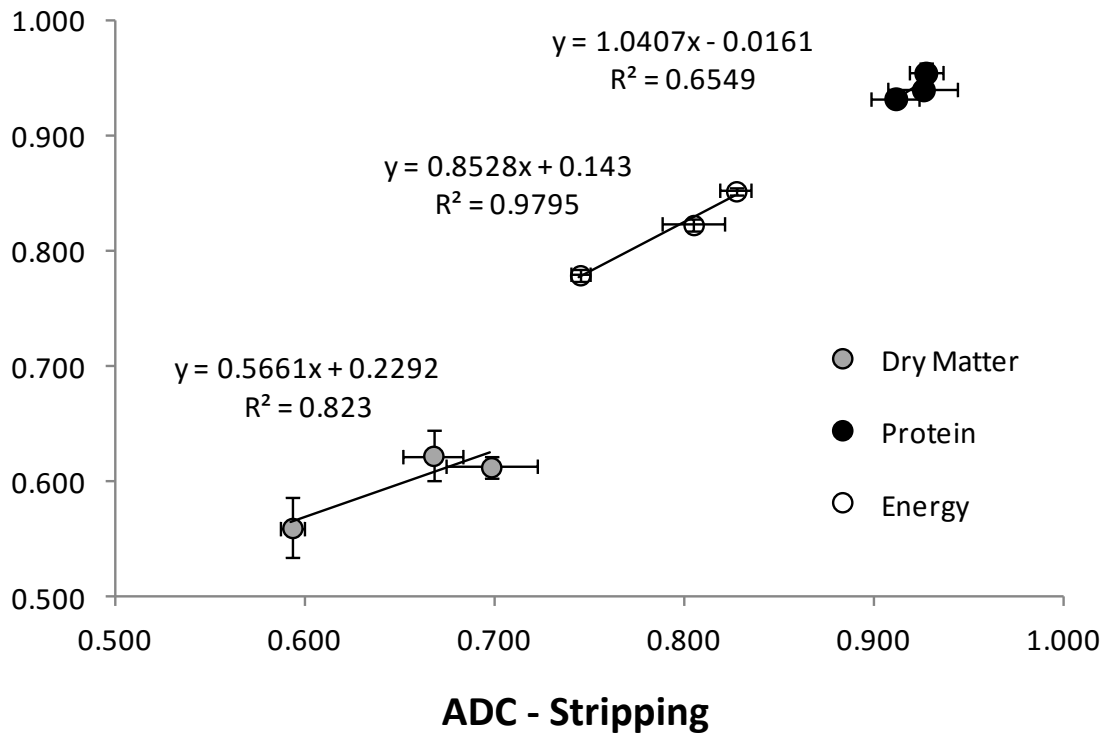
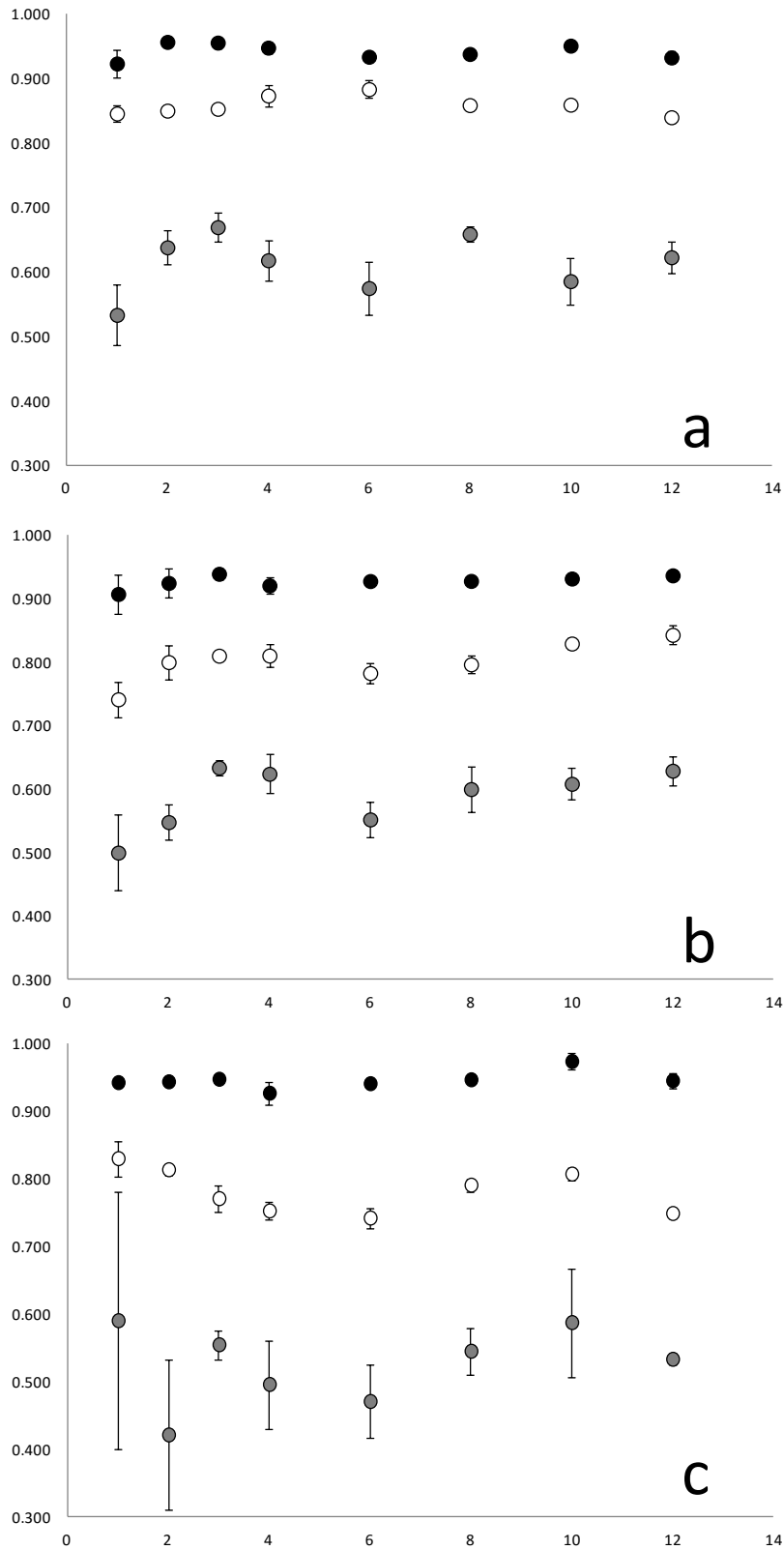


Figure 2. Correlations between apparent digestibility coefficient (ADC) values from each of the different faecal collection methods.

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Figures 3a-c Temporal variation in digestibility values determined for energy (○), protein (●) and dry matter (●) for each diet (basal : a, starch : b, MKM : c) over a 13 day period.