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9 **Replacement of fishmeal with commercial soybean meal and EnzoMeal in**10 **juvenile barramundi *Lates calcarifer***

11

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22

23 Running title: Fishmeal replacement in barramundi

24

25

26 **Abstract**

27 This study investigates the impact of fishmeal replacement by soybean meal (SM) and  
28 improved SM known as EnzoMeal (EZ) on fish growth performance. The SM and EZ  
29 were used in five experimental diets: fishmeal (600 g kg<sup>-1</sup>) as the control diet, 50%  
30 fishmeal replaced with soybean meal (SM50), 50% fishmeal replaced with EnzoMeal  
31 (EZ50), 100% fishmeal replaced with soybean meal (SM100), and 100% fishmeal  
32 replaced with EnzoMeal (EZ100). Fish in the control group gained the most weight,  
33 followed by those under the SM50 and EZ50 diets, while the least weight gain was  
34 observed in fish fed the SM100 and EZ100 diets. The specific growth rate (SGR) was  
35 higher in the control, but was not different between fish fed SM50 and EZ50, or  
36 between SM100 and EZ100. The highest feed intake occurred in the control but  
37 decreased sequentially from fish fed SM50 to fish fed EZ50, EZ100 and SM100.  
38 Protein digestibility was best in SM50, poorest in EZ100, but no difference between the  
39 control, EZ50 and SM100 diets. This study indicates that EnzoMeal, containing high  
40 crude protein (56%), is a potential source of plant meal to replace fishmeal in the  
41 barramundi diet, but low feed intake is a challenge when EnzoMeal is >300 g kg<sup>-1</sup>.

42

43 Keywords: barramundi *Lates calcarifer*, fishmeal replacement, soybean meal,  
44 EnzoMeal, rearing performance.

45

46

## 47 **Introduction**

48 In finfish aquaculture, feed cost is the main expenditure as fishmeal is used as the  
49 protein source for the diet. Most carnivorous fish species usually require 30-55% crude  
50 protein in the diet to provide a suite of amino acids essential for growth and health  
51 (Hepher 1988). Fishmeal has been traditionally the main protein ingredient in fish feed  
52 due to its excellent palatability, balanced amino acid profile and good digestibility  
53 (Alexis & Nengas 2001). Fishmeal is usually sourced from small, bony fish species  
54 including herring, menhaden, capelin, anchovy, pilchard, sardines, and mackerel that  
55 are not generally used for human consumption (Halver & Hardy 2002). However,  
56 catches of these fish species specifically used for fishmeal production have declined in  
57 the past few decades, although global demand for fishmeal is increasing (Watanabe  
58 2002 ).

59

60 Over the last 20 years, fish nutrition research has focused on the replacement of  
61 fishmeal with more sustainable alternative protein ingredients (Tacon & Metian 2008;  
62 Bowyer, Qin & Stone 2013). It was identified that alternative protein ingredients  
63 should possess adequate nutritional properties, i.e., a high level of protein with a  
64 favorable amino acid profile, high nutrient digestibility and acceptable palatability, and

65 be relatively inexpensive compared to fishmeal (Gatlin, Barrows, Brown, Dabrowski,  
66 Gaylord, Hardy, Hermen, Hu, Krogdahl, Nelson, Overturf, Rust, Sealey, Skonberg,  
67 Souza, Stone, Wilson & Wurtele 2007). Soybean meal is currently the most common  
68 plant protein used as a fishmeal replacer and has been used as a feed ingredient for a  
69 variety fish species due to its high content of available essential fatty acids and  
70 unsaturated fatty acids, and for its favorable amino acid profile (Hertrampf &  
71 Piedad-Pascual 2000). However, to date, soybean meal usually makes up  
72 approximately 20% of the fish feed ingredient. A higher level of soybean meal could  
73 reduce fish growth, feed intake and digestibility because of the presence of  
74 anti-nutritional factors such as protease, phytates, lectins, and saponins in the soybean  
75 meal (NRC 2011). Recently, the Ohio Soybean Council has developed an improved  
76 soybean meal, known as EnzoMeal™, with enhanced protein content (56%) and with  
77 reduced carbohydrates, oligosaccharides, and phytic acid content compared to  
78 commercial soybean meal (Kumar 2016). Previous studies in rainbow trout  
79 (*Oncorhynchus mykiss*), yellow perch (*Perca flavescens*) and Pacific white shrimp  
80 (*Litopenaeus vannamei*) have suggested that there is potential for partial to total  
81 replacement of fishmeal with EnzoMeal™ in the feed used in aquaculture (Craig,  
82 Harris & Betka 2015; Kumar 2016; Hulefeld, Kumar, Tidwell, Lalgudi, Cain &  
83 McGraw 2017).

84

85 Barramundi (*Lates calcarifer*), also commonly known as Asian seabass, is  
86 geographically distributed throughout the northern Indian and tropical western Indian

87 Oceans, from Iran to northern Australia, including China and Papua New Guinea  
88 (Tucker 2002). Since the 1980s, barramundi has become an important fish species for  
89 aquaculture in Australasia (Tucker 2002) due to its market demand, fast growth, and  
90 adaptation to grow in either a freshwater or marine environment for aquaculture  
91 (Schipf, Bosmans & Humphrey 2007). Barramundi aquaculture is a fast-growing  
92 industry and its annual production has exceeded 3,500 tonnes, generating \$25 million  
93 in Australia (Skirtun & Vieira 2013). Barramundi requires 400-600 g kg<sup>-1</sup> crude protein  
94 (Glencross 2006) and can tolerate up to 300 g kg<sup>-1</sup> of solvent-extracted soybean meal to  
95 support their growth in the feed (Williams 1998).

96  
97 The aim of this study was to investigate the impact of fishmeal replacement using  
98 EnzoMeal<sup>TM</sup> versus commercial soybean meal on barramundi growth performance, gut  
99 histology and digestibility. Our approach was to use a similar feed formulation as the  
100 one developed for yellow perch using EnzoMeal<sup>TM</sup> and to test its suitability as a protein  
101 source for a carnivorous warm-water fish species. The significance of this study is to  
102 widen our choice for plant protein as a replacement for fishmeal in aquafeed fish and to  
103 improve aquaculture sustainability.

104

## 105 **Materials and methods**

106

107 *Experiment design and system*

108

109 In this study, conventional soybean meal and enzyme treated soybean meal  
110 (EnzoMeal™) were supplied by the Ohio Soybean Council (USA). The EnzoMeal™  
111 was prepared using a proprietary technology and obtained from Ohio Soybean Council.  
112 The main features of EnzoMeal™ are as follows: protein  $56.0 \pm 1.5\%$ , 1100 trypsin  
113 inhibitor activity unit (TIU)  $\text{g}^{-1}$ , and oligosaccharides  $<0.05\%$  (Lalgudi, Cain &  
114 McGraw 2015). The proximate composition of the major experimental ingredients is  
115 presented in Table 1. In this study, five experimental diets were formulated including (1)  
116 a full fishmeal diet ( $600 \text{ g kg}^{-1}$ , i.e., 0% fishmeal replacement) as control, (2) 50%  
117 fishmeal replaced with commercial soybean meal (SM50), (3) 50% fishmeal replaced  
118 with EnzoMeal™ (EZ50), (4) 100% fishmeal replaced with conventional soybean meal  
119 (SM100), and (5) 100% fishmeal replaced with EnzoMeal™ (EZ100). Three replicates  
120 were used for each diet treatment and a total of 15 tanks (135 L) were used. The  
121 formulation and nutrient profiles of the five diets are presented in Table 2. During diet  
122 preparation, feed ingredients and 1.5 L of distilled water were homogenized in a 60 L  
123 food mixer for 20 min. Pellets (4-mm diameter) were then produced using a meat  
124 grinder, air-dried at room temperature and stored at  $-20 \text{ }^\circ\text{C}$  until use.

125

126 Barramundi (*Lates calcarifer*) juveniles were obtained from the hatchery of Robarra  
127 Pty. Ltd., West Beach, Australia and transferred to the aquaculture laboratory at  
128 Flinders University of South Australia. All the experimental fish were acclimated in 15  
129 recirculated 135 L tanks for two weeks and fed with a commercial feed supplied by  
130 Robarra Pty. Ltd. After acclimation, 10 fish remained in each tank and the diet

131 treatments were randomly assigned into each tank. Fish were then co-fed with the  
132 commercial diet and respective experimental diets for one week. Upon completion of  
133 weaning, the experiment lasted 8 weeks with fish of similar weight (25 - 27 g, Table 3).  
134 Fish were fed to apparent satiation once a day in the morning and the exact amount of  
135 feed delivered to each tank was recorded to calculate fish intake and feed conversion  
136 ratio. Waste feed and fish feces were removed daily with a siphon tube. The feces for  
137 measuring apparent digestibility was collected with a fine-mesh dip net on the bottom  
138 of the tank soon after feeding. During the experimental period the water temperature  
139 was controlled at  $28 \pm 1$  °C. The water quality parameters were measured daily and  
140 maintained at ammonia nitrogen  $<0.05$  mg L<sup>-1</sup>, nitrite nitrogen  $<0.09$  mg L<sup>-1</sup>, pH 7-8,  
141 and dissolved oxygen  $>7.0$  mg L<sup>-1</sup>. Any deviation of water quality parameters beyond  
142 these ranges was treated by increasing water exchange rate. Water was run through a  
143 mechanical filter and biofilters (3-cm diameter bioballs). However, the water was  
144 partially replaced if the water quality parameters exceeded the temperature, ammonia,  
145 nitrite or pH ranges above. The photoperiod was controlled at 14 h light:10 h dark. At  
146 the end of the experiment, all fish were anaesthetized in 7% AQUI-S (AQUI-S New  
147 Zealand Ltd, Lower Hutt, New Zealand) before handling and sampling, in accordance  
148 with the animal ethic protocols approved by Animal Welfare Committee (E437-16).  
149 All fish were bulk-weighed for assessment of growth performance and three fish were  
150 sampled from each tank for histological and biochemical analyses.

151

152 *Proximate composition and biochemical analysis*

153

154 The amino acid profile of all major feed ingredients was analyzed at the Australian  
155 Proteome Analysis Facility. Proximate composition of all experimental diets and body  
156 muscle were analyzed at the National Collaborative Research Infrastructure  
157 Strategy (NCRIS) Algae and Biofuels Facility through standard methods. In brief,  
158 moisture content (%) was calculated from the sample weight before and after  
159 freeze-drying. Total nitrogen (% dry weight) was determined using a Leco CNS  
160 analyzer. Crude protein content was calculated as  $N\% \times 6.25$ . Ash content was  
161 determined gravimetrically at 600 °C for 5 h. Crude lipids were measured  
162 gravimetrically using the Folch technique (Folch, Lees & Sloane Stanley 1957). The  
163 yttrium oxide ( $Y_2O_3$ ) content in fish diets and feces were analyzed using  
164 high-performance liquid chromatography (HPLC) at Flinders Analytical, Flinders  
165 University of South Australia.

166

#### 167 *Histological analysis*

168 The liver, foregut and hindgut were collected from three fish per tank and fixed in  
169 Davidson's Fixative (Kiernan 2003). The fixed tissues were embedded in paraffin  
170 blocks and sliced in a series of transverse sections (5  $\mu$ m thick) using a Leica RM 2135  
171 rotary microtome. The hematoxylin–eosin (HE) stain was used for general histological  
172 analysis. Each slide with tissue sections was mounted permanently using DePex  
173 mounting medium (VWR, Rochester, New York). The sections were randomly  
174 examined under a light microscope. Photographs were taken with an Olympus digital

175 camera attached to the microscope. The number of hepatic sinusoid was analyzed by  
176 counting five random areas ( $2\ 500\ \mu\text{m}^2$ ) per slide and the height of mucosal epithelium  
177 cells of foregut and hindgut were randomly measured on 10 microscopic fields at  $400\times$   
178 magnification.

179

#### 180 *Enzymatic assays*

181

182 At the end of the feeding trial, three fish from each tank were collected and dissected  
183 after anesthesia in a 7% AQUI-S solution. Stomach, foregut and hindgut from each  
184 tank were pooled and immediately preserved into liquid nitrogen. For each assay,  
185 pooled samples from each tank were thawed, weighed and homogenized using a tissue  
186 homogenizer on ice in five volumes of 0.2 M NaCl (w/v). The suspensions were  
187 centrifuged at  $16,000\times g$  for 10 min at  $0\ ^\circ\text{C}$ . Subsequently, the supernatant was  
188 incubated in the enzyme substrate at  $25\ ^\circ\text{C}$  or  $37\ ^\circ\text{C}$  and read on a spectrophotometer  
189 (Fluostar Omega, BMG Labtech, Ortenberg, Germany).

190

191 Pepsin (E.C. 3.4.23.1) activity was analyzed according to the method of  
192 spectrophotometric stop-rate determination (Zaugg & Wood, 1976). One unit of pepsin  
193 activity produced a change of 0.001 in absorbance at 280 nm wavelength per min  
194 measured as trichloroacetic acid soluble products using hemoglobin as a substrate at pH  
195 2.0 and  $37\ ^\circ\text{C}$ . The  $\alpha$ -amylase (E.C. 3.2.1.1) was measured using a colorimetric assay  
196 kit (catalog no. K711-100, BioVision Incorporated, Milpitas, CA, USA). In this assay,

197 ethylidene-pNP-G7 was used as the substrate to cleave  $\alpha$ -amylase. The smaller  
198 fragments produced by this procedure could react with  $\alpha$ -glucosidase to release  
199 chromophore that could then be measured at 405 nm wavelength.  
200  
201 Lipase (E.C. 3.1.1.3) activity was assayed via a lipase assay kit (catalog no. K723-100,  
202 BioVision Incorporated, Milpitas, CA, USA). In this assay, lipase hydrolyzed the  
203 substrate to generate a product which reacted with the 2-nitrobenzoic acid probe to  
204 produce a color that was measured at 412 nm wavelength. Alkaline phosphatase (E.C.  
205 3.1.3.1) was assayed by the phospholipase D activity colorimetric assay kit (catalog no.  
206 K725-100, BioVision Incorporated, Milpitas, CA, USA). In this assay, phospholipase  
207 cleaved choline from phosphatidylcholine. The free choline was then oxidized by the  
208 phospholipase enzyme mix to generate an intermediate which reacted with the  
209 phospholipase probe to generate a color with an optical peak density at 570 nm  
210 wavelength. Enzymatic activities were expressed as specific activity ( $\text{mU mg}^{-1}$  protein  
211 or  $\text{U mg}^{-1}$  protein). Soluble protein of crude enzyme extracts was quantified by the  
212 bicinchoninic acid protein assay kit (catalog no. BCA1 & B9643; Sigma-Aldrich, St.  
213 Louis, MO, USA).

214

215 *Calculations and statistical analysis*

216

217 Specific growth rate (SGR) was calculated as:  $\text{SGR} = 100 \times (\ln(W_f) - \ln(W_i)) / \Delta T$ ,  
218 where  $W_f$  was the final body weight;  $W_i$  was the initial body weight; and  $\Delta T$  was the

219 experimental duration. Feed conversion ratio (FCR) was calculated as  $FCR = \text{dry feed}$   
220  $\text{fed/body wet weight gain}$ . Feed intake (FI,  $\text{g fish}^{-1}\text{days}^{-1}$ ) was calculated as  $FI = (\text{feed}$   
221  $\text{consumed per tank/ fish})/\text{days}$  (Ilham, Siddik & Fotedar 2016). Apparent digestibility  
222 coefficient of dry matter (ADC) =  $1 - (Y_2O_3 \text{ in feed}/Y_2O_3 \text{ in feces})$ . The apparent  
223 digestibility coefficient of protein was calculated as  $ADC_{\text{protein}} = 1 - (Y_2O_3 \text{ in feed}/$   
224  $Y_2O_3 \text{ in feces}) \times (\text{Protein content of feces}/\text{Protein content of feed})$ .

225  
226 The data were expressed as mean  $\pm$  SD. Statistical analyses were carried out by IBM  
227 SPSS Statistics (version 21 for Mac). The effects of dietary soybean type (commercial  
228 soybean meal vs EnzoMeal<sup>TM</sup>), replacement level (50% and 100% replacements) and  
229 interactions were tested with a two-way ANOVA. When a significant main effect was  
230 detected, then the variable was analyzed using a one-way ANOVA to compare the  
231 difference between 0%, 50% and 100% fishmeal replacement. All percentage data were  
232 log transformed to satisfy the assumptions of ANOVA. Post-Hoc Tukey's test with  $P =$   
233 0.05 was used when a significant effect was found in the ANOVA.

## 235 **Results**

236  
237 In this study, fish survival was not significantly affected by soybean type and fishmeal  
238 level ( $P > 0.05$ , Table 3). Furthermore, there was no interaction between soybean type  
239 and fish level ( $P = 0.08$ ). The survival ranged from  $80.00 \pm 10.00\%$  to  $93.33 \pm 11.55\%$ .

240 At the end of the feeding trial, weight gain was significantly affected by the fishmeal

241 level ( $P = 0.001$ ). The highest body weight of  $126.63 \pm 13.88$  g was observed on fish in  
242 the control group, and lowest body weight was found in the SM100 and EZ100 groups.  
243 The specific growth rate (SGR) was significantly affected by the fishmeal level ( $P =$   
244  $0.001$ ). The SGR was highest in the control group and lowest in the SM100 and EZ100  
245 groups, but SGR was not significantly different between SM50 and EZ50 ( $P > 0.05$ ), or  
246 between SM100 and EZ100 ( $P < 0.05$ ). The feed conversion ratio (FCR) was  
247 significantly affected by soybean type and fishmeal level ( $P < 0.05$ ). Furthermore, there  
248 was a significant interaction effect between the soybean type and the fishmeal level on  
249 the FCR ( $P = 0.001$ ). The FCR in the control was not significantly different from SM50  
250 and EZ50 ( $P > 0.05$ ), but was significantly lower than that in SM100 and EZ100 ( $P <$   
251  $0.05$ ). The poorest FCR was observed in fish fed EZ100. The feed intake was  
252 significantly affected by the fishmeal level ( $P = 0.001$ ), and an interaction effect  
253 between the soybean type and the fishmeal level was observed ( $P = 0.001$ ). The highest  
254 feed intake was  $1.87 \pm 0.01$  g fish<sup>-1</sup> d<sup>-1</sup> in the control fish (Table 3), and the lowest feed  
255 intake ( $0.49 \pm 0.05$  g fish<sup>-1</sup> d<sup>-1</sup>) was observed in the fish fed SM100. The highest body  
256 protein content was observed in fish fed SM100 or EZ100. The lipid content of fish was  
257 highest in the control group, and lowest in the SM100 and EZ100 treatments (Table 3).  
258  
259 The apparent feed digestibility coefficient was not significantly different between  
260 commercial soybean meal and EnzoMeal<sup>TM</sup> ( $P > 0.05$ ). The apparent digestibility  
261 coefficient (ADC) of protein was significantly affected by the soybean type and the  
262 fishmeal level ( $P < 0.05$ ), and no interaction effects were found between the soybean

263 type and the fishmeal level ( $P = 0.79$ , Table 3). The highest ADC of protein was  
264 observed in fish fed SM50, and the lowest protein digestive efficiency was found in fish  
265 fed EZ100 ( $P < 0.05$ ). The ADC of protein was not significantly different between fish  
266 that had been fed the control, EZ50 or SM100 diet ( $P > 0.05$ ), but was significantly  
267 lower than the value in fish fed SM50 ( $P < 0.05$ ).

268

269 In this study, the number of hepatic sinusoids was significantly affected by soybean  
270 type ( $P < 0.05$ ). The number of hepatic sinusoids in the liver increased when fish were  
271 fed with SM50 and SM100 (Fig. 1), while the histological structure of the liver in fish  
272 fed with EZ50 and EZ100 was similar to the control group. No enteritis was observed in  
273 both foregut and hindgut of fish in any feeding group (Fig. 1). The morphological  
274 structure of foregut and hindgut was not significantly different between fish fed with  
275 commercial soybean meal and EnzoMeal™ (Fig. 2). The height of enterocytes in the  
276 foregut was not significantly affected by soybean type and fishmeal level ( $P > 0.05$ ).  
277 The height of enterocytes in the foregut was highest in the control group, but was not  
278 significantly different between the EZ50, SM100 and EZ100 groups ( $P > 0.05$ , Fig. 3).  
279 The enterocyte height in the hindgut was similar to that in the foregut.

280

281 In this study, pepsin activity was significantly affected by soybean type ( $P = 0.02$ ), and  
282 not significantly affected by fishmeal level ( $P = 0.12$ ). No interaction effect on the  
283 pepsin activity was observed between the soybean type and the fishmeal replacement  
284 level ( $P = 0.52$ ). The highest pepsin activity was observed in the control fish, and

285 lowest pepsin activity was found in the fish fed with SM50 and SM100 ( $P < 0.05$ , Fig.  
286 4). The pepsin activity was not significantly different when fish were fed commercial  
287 soybean meal and EnzoMeal™ ( $P > 0.05$ ). Diet types significantly affected the amylase  
288 activity in the foregut and hindgut ( $P < 0.05$ , Fig. 5). The fishmeal level significantly  
289 affected the amylase activity in the foregut ( $P = 0.001$ ). In the foregut, the highest  
290 amylase activity was observed in the control group, and the lowest amylase activity was  
291 found in the SM50, SM100 and EZ100 groups. In the hindgut, amylase activity was not  
292 significantly affected by the soybean type and the fishmeal level ( $P > 0.05$ ). The highest  
293 amylase activity was observed in the control group, followed by the SM50 and EZ50  
294 group. No amylase activity was detected in the SM100 and EZ100 groups.

295  
296 The lipase activity in the foregut was not significantly affected by the soybean type and  
297 the fishmeal level ( $P > 0.05$ , Fig. 5), but was significantly affected by the fishmeal level  
298 in the hindgut ( $P = 0.03$ , Fig. 4). Furthermore, there was no interaction effect between  
299 the soybean type and the fishmeal level on the lipase activity in the hindgut. The highest  
300 lipase activity was observed in the control group, but the lowest activity occurred in  
301 fish fed with SM100 or EZ100 in the hindgut. The activity of phospholipase in fish  
302 foregut and hindgut was significantly affected by the dietary treatments ( $P < 0.05$ , Fig.  
303 5). The fishmeal level only significantly affected the phospholipase activity in the  
304 foregut ( $P = 0.04$ ), and no interaction effect was observed in both foregut and hindgut  
305 ( $P > 0.05$ ). In the foregut, the phospholipase activity in the control, SM50 and EZ50  
306 was significantly higher than that in the SM100 and EZ100 groups ( $P < 0.05$ ). In the

307 hindgut, the highest phospholipase activity was found in the control group, and the  
308 lowest activity was recorded in the SM50, SM100 and EZ100 groups ( $P < 0.05$ , Fig. 5).

309

## 310 **Discussion**

311 This study compared the effect of fishmeal replacement by commercial soybean meal  
312 and EnzoMeal™ on the growth performance of juvenile barramundi. Fish weight gain  
313 decreased with the increasing levels of fishmeal replacement by SM and EZ meals, but  
314 the fish specific growth rate was not significantly different between the SM50 and  
315 EZ50 treatments. The apparent digestibility coefficient of protein in the control, EZ50  
316 and SM100 was not significantly different. Despite the reduction in enzyme activities  
317 with the increase of fishmeal replacement, fish fed with EnzoMeal™ showed slightly  
318 higher pepsin activity than those fed commercial soybean meal. Apparently, low feed  
319 intake in fish fed with the SM or EZ diet may contribute to slow growth.

320

321 Warm water carnivorous fish generally need high dietary protein to sustain growth and  
322 metabolism cost at high temperatures. During the grow-out phase, the level of optimal  
323 protein requirement for warm water fish species such as cobia (*Rachycentron canadum*)  
324 (Chou, Su & Chen 2001), humpback grouper (*Cromileptes altivelis*) (Rachmansyah,  
325 Laining, Ahmad & William 2005), Malabar grouper (*Epinephelus malabaricus*) (Shiau  
326 & Lan 1996) is recommended at 400-500 g kg<sup>-1</sup>, and the protein requirement in the  
327 juvenile stage is >500 g kg<sup>-1</sup> (Boonyaratpalin 1997). In barramundi, the current  
328 literature suggests that the optimal dietary protein requirements are 450-600 g kg<sup>-1</sup> with

329 a SGR of  $\approx 2.2\%$  day<sup>-1</sup> with fish size from 2 g to 70 g (Williams & Barlow 1999;  
330 Williams, Barlow, Rodgers, Hocking, Agcopra & Ruscoe 2003; Catacutan & Coloso  
331 1995). In the present study, the dietary protein level was maintained at 370 g kg<sup>-1</sup>,  
332 which is similar to the dietary protein level in yellow perch, and the SGR in the control  
333 group achieved 2.34% day<sup>-1</sup>. This suggests that 370 g kg<sup>-1</sup> dietary protein is sufficient to  
334 support the growth of barramundi at the initial weight of 25.5 g.

335  
336 To reduce the cost of fish feed ingredients, soybean meal has been intensively studied  
337 to replace fishmeal protein in the past two decades (Trejo-Escamilla, Galaviz,  
338 Flores-Ibarra, Gonzalez & Lopez 2017; Ayadi, Rosentrater & Muthukumarappan  
339 2012; Touying 2011). However, the physiological tolerance of fish to dietary soybean  
340 protein is species specific. For instance, the growth performance of juvenile cobia  
341 *Rachycentron canadum* was not affected when 50% fishmeal protein was replaced by  
342 soybean protein (Trushenski, Laporte, Lewis, Schwarz, Delbos, Takeuchi & Sampaio  
343 2011). When employing phytase-treated soybean meal or a mixture of plant proteins,  
344 60% fishmeal can be replaced without causing significant reduction in growth  
345 performance in species such as rainbow trout *Oncorhynchus mykiss* (Yang, Wang, Lu  
346 & Li 2011; Pongmaneerat & Watanabe 1992), sharpsnout seabream *Diplodus puntazzo*  
347 (Hernández, Martínez, Jover & García García 2007), and gilthead sea bream *Sparus*  
348 *aurata* (Sitjà-Bobadilla, Pena-Llopis, Gomez-Requeni, Medale, Kaushik &  
349 Perez-Sanchez 2005). In the present study, soybean meal replacement significantly  
350 reduced the growth performance of barramundi. The SGR of fish decreased from 2.34

351  $\pm 0.04 \text{ \% day}^{-1}$  in the control to  $1.81 \pm 0.20 \text{ \% day}^{-1}$  (SM50) and  $1.65 \pm 0.28 \text{ \% day}^{-1}$   
352 EZ50 when 50% fishmeal was replaced by either SM50 or EZ50, respectively. When  
353 fishmeal was completely replaced in the feed, the SGR further decreased to  $0.42 \pm 0.11\%$   
354  $\text{day}^{-1}$  in the SM100 group and  $0.54 \pm 0.28\% \text{ day}^{-1}$  in EZ100 group. These results are  
355 consistent to the results of previous studies in barramundi (Glencross, Rutherford &  
356 Jones 2011; Glencross, Blyth, Irvin, Bourne, Campet, Boisot & Wadw 2016),  
357 suggesting that the increase of dietary plant protein source in the feed hinders the  
358 growth performance of juvenile barramundi, despite significant removal of  
359 carbohydrates, oligosaccharides and phytic acid from EnzoMeal™.

360

361 A major cause of poor growth performance of fish is poor voluntary feed intake after  
362 fishmeal replacement by plant protein in the diet (Gomes, Rema & Kaushik 1995; Dias,  
363 Alvarez, Diez, Arzel, Corraze, Bautista & Kaushik 1998). Glencross *et al.* (2016)

364 demonstrated that the primary response of barramundi to the replacement of fishmeal  
365 was reduction of feed intake, presumably for a palatability reason, directly leading to  
366 poor growth performance (Glencross *et al.* 2016). When the level of dietary fishmeal  
367 reduces to  $150 \text{ g kg}^{-1}$ , feed intake of barramundi declines dramatically (Glencross *et*  
368 *al.*, 2011). Here, feed intake was significantly affected by the fishmeal replacement  
369 level. When dietary fishmeal inclusion was reduced from  $600 \text{ g kg}^{-1}$  in the control to  
370  $300 \text{ g kg}^{-1}$  in the SM50 and EZ50 diets, feed intake dropped from  $1.87 \pm 0.01 \text{ g fish}^{-1}$   
371  $\text{day}^{-1}$  to  $1.37 \pm 0.01 \text{ g fish}^{-1} \text{ day}^{-1}$  in the SM50 diet or to  $1.12 \pm 0.07 \text{ g fish}^{-1} \text{ day}^{-1}$  in fish  
372 fed with the EZ50 diet. When dietary fishmeal was completely replaced, feed intake

373 further reduced to  $0.49 \pm 0.05$  g fish<sup>-1</sup> day<sup>-1</sup> in fish fed with SM100 and  $0.77 \pm 0.02$  g  
374 fish<sup>-1</sup> day<sup>-1</sup> in the EZ100 group. Likewise, Glencross *et al.* (2011) found that when  
375 barramundi were fed with equivalent protein and energy diets, feed intake decreased  
376 with the decreasing level of dietary fishmeal. In this study, the unit for the expression of  
377 fish intake was g feed fish<sup>-1</sup> day<sup>-1</sup> rather than g feed kg<sup>-1</sup> body weight day<sup>-1</sup>. This is  
378 because it was noticed that fish fed with the SM100 and EZ100 diets held the feed  
379 pellets in their mouths during feeding, but some feed was spit out afterward. If the  
380 estimate of feed intake had been based on fish body weight, the value would have been  
381 much higher.

382

383 FCR is a common indicator to measure the ratio of feed mass input and mass gain in  
384 fish. In turbot *Psetta maxima* juveniles, FCR becomes worse with increasing levels of  
385 dietary plant protein in the diet (Bonaldo, Parma, Mandrioli, Sirri, Fontanillas, Badiani  
386 & Gatta 2011). In juvenile cobia, FCR significantly increases in fish fed with diets that  
387 have soybean protein over 40% fishmeal replacement (Zhou, Mai, Tan & Liu 2005).  
388 Here, FCR of fish fed with the control, SM50 or EZ50 diets was not significantly  
389 different, but was significantly better than the FCR of fish fed with SM100 or EZ100.  
390 Similar to rainbow trout (Oliva-Teles, Gouveia, Gonmes & Rema 1994), European eel  
391 *Anguilla anguilla* (Chen, Ai & Wang 1998) and turbot *Scophthalmus maximus* (Day &  
392 Plascencia-Gonzalez 2000), the decline in feed utilization and increase of FCR are a  
393 result of low feed intake due to poor diet palatability. In the present study, the feed  
394 intake of fish in the control group was higher than that that in the SM50 group, but

395 protein digestibility in the control was lower than in the SM50 group, suggesting that  
396 the inclusion of 600 g fishmeal per kg diet (365 g protein/kg diet) may exceed the  
397 optimal level of fishmeal the barramundi diet.

398

399 Digestibility is primarily a measure of the acceptance of nutrients in a given feed to fish.  
400 Parameters such as feed intake, fish size, and water temperature can affect the  
401 digestibility of fish during the feeding experiment (Aksnes, Hjertnes & Opstvedt 1996;  
402 NRC 2011). In the present study, the ADC in the SM50 was significantly higher than  
403 those observed in the control. High feed intake in the control compared to the data  
404 observed in SM50 may suggests that fishmeal in the control was not fully digested  
405 (Windell, Foltz & Sarokok 1978; Mundheim, Aksnes & Hope 2004), and causes a  
406 relatively low digestibility. In the present study, the low ADC observed in EZ100 was  
407 consistent with low feed intake and poor feces production. This may cause by the low  
408 feed intake due to poor diet palatability and feces production (NRC 2011).

409

410 Fishmeal replacement by soybean meal in fish diets may sometime change the  
411 proximate composition in fish muscle. In cobia and rainbow trout, replacement of  
412 fishmeal with soybean meal shows no obvious effect on the proximate composition in  
413 the muscle in terms of moisture, protein, lipid and ash (Yang *et al.* 2011; Zhou *et al.*  
414 2005). On the contrary, replacement of fishmeal with soybean meal significantly  
415 affects the body proximate composition of Atlantic salmon *Salmo salar* (Bjerkeng,  
416 Refstie, Fjalestad, Storebakken, Rodbotten & Roem 1997), European seabass

417 *Dicentrarchus labrax* (Kaushik, Coves, Dutto & Blanc 2004), and yellowhead catfish  
418 *Pelteobagrus fulvidraco* (Wang, Xie, Zhu, Lei, Yang & Liu 2006). In the present study,  
419 the proximate composition of fish muscle was significantly affected by the levels of  
420 soybean protein inclusion. Muscle protein increased with the increasing levels of  
421 dietary soybean protein, while muscle lipid decreased with the increase of dietary  
422 soybean protein level. Similarly, marbled spinefoot *Siganus rivulatus* showed reduced  
423 muscle protein when soybean protein totally replaced fishmeal in the diet (Monzer,  
424 Nasser, Babikian & Saoud 2017). The reduction of lipid in fish muscle indicates that  
425 energy intake from the diet may not meet the requirement of energy consumption  
426 (Bureau, Kaushik & Cho 2002), and results in catabolism of muscle lipid for energy  
427 supply (Johnston & Goldspink 1973). Liver histological analysis indicates no obvious  
428 morphological change in hepatic cells despite the numerical increase of hepatic  
429 sinusoids in the liver of fish fed with SM50 and SM100. However, it is not clear if  
430 replacement of fishmeal with soybean meal has caused any functional change in the  
431 liver.

432  
433 Digestive function is supported by the structure of the intestinal tract, which is sensitive  
434 to the change of diet composition (Bonaldo *et al.* 2011; Bonaldo, Roem, Fagioli,  
435 Pecchini, Cipollini & Gatta 2008; Wang, Wang, Zhang & Song 2017b). The use of  
436 soybean in feed can cause morphological and histological changes of intestinal tissue in  
437 fish (Rodrig nez, D az-Rosales, Chabril n, Smidt, Arijo, Le n-Rubio, Alarc n,  
438 Balebona, Morinigo & Cara 2008; Caballero, Izquierdo, Kjorsvik, Montero, Socorro,

439 Fernandez & Rosenlund 2003; Bakke-McKellep, Press, Baeverfjord, Krogdahl &  
440 Landsverk 2000). In Atlantic salmon and rainbow trout, dietary soybean meal can  
441 cause distinct morphological alteration in intestine and enteritis (Krogdahl,  
442 Bakke-McKellep & Baeverfjord 2003; Heikkinen, Vielma, Kemilainen, Tirola,  
443 Eskelinen, Kiurur, Navia-Paldanius & von Wright 2006). Some fish species such as  
444 Atlantic halibut *Hippoglossus hippoglossus*, channel catfish *Ictalurus punctatus*,  
445 gilthead sea bream *Sparus aurata*, European sea bass *Dicentrarchus labrax* and  
446 Egyptian sole *Solea aegyptiaca* are less sensitive to the inclusion of dietary soybean  
447 meal, and do not usually exhibit inflammatory response in intestinal mucosa  
448 (Grisdale-Helland, Helland, Baeverfjord & Berge 2002; Evans, Pasnik, Peres, Lim &  
449 Klesius 2005; Bonaldo, Rome, Pecchini, Grilli & Gatta 2006; Bonaldo *et al.*, 2008). In  
450 the present study, no histological lesion was observed in the digestive tract of fish  
451 regardless of diet types. When dietary fishmeal replacement was over 50%, the  
452 enterocyte height reduced significantly, suggesting that there is an adaptive response of  
453 intestinal tissues to the inclusion of soybean protein in barramundi diet.

454  
455 The activity of digestive enzymes in fish is related to the digestive function and  
456 capacity to process feed ingredients during feed digestion (Wang, Zhu, Feng, He, Lou  
457 & Zhou 2017a; Perera & Yufera, 2017; Zhao, Song, Xie, Ge, Liu, Xia & Zhu 2016). In  
458 this study, the activity of digestive enzymes in juvenile barramundi decreased with the  
459 increasing levels of dietary replacement of fishmeal with soybean meal, which agrees  
460 with previously published results in feed digestibility in Japanese seabass *Lateolabrax*

461 *japonicus* (Li, Ai, Mai, Xu, Deng & Cheng 2014; Li, Ai, Mai, Xu & Cheng 2012). The  
462 level of dietary soybean meal has impacted the digestive function of barramundi. The  
463 presence of anti-nutritional factors in soybean meal can reduce the digestibility of feed  
464 ingredients especially for proteins (NRC, 2011; Leenhouwers, Adjei-Boateng, Verreth  
465 & Schrama 2006). In this study, we compared the digestive enzyme activities of fish  
466 fed commercial soybean meal and EnzoMeal™ where some anti-nutritional factors  
467 were reduced or removed. Taking pepsin activity for instance, fish showed slightly  
468 higher pepsin activity to digest EnzoMeal™ than commercial soybean meal at the same  
469 level of inclusion (both 50% and 100% fishmeal replacement), suggesting that the  
470 improved EnzoMeal™ could be a better source of plant protein to replace fishmeal in  
471 fish feed, given its higher protein content (56%) compared to the low protein content  
472 (47%) in commercial SBM.

473

474 In summary, the present study compared the impact of commercial soybean meal and  
475 EnzoMeal™ as a fishmeal replacement on the growth performance of juvenile  
476 barramundi. Although the apparent feed digestibility coefficients of commercial  
477 soybean meal and EnzoMeal™ were not significantly different, enzymatic evidence  
478 suggests a better digestive efficiency in fish fed with EnzoMeal™ compared to fish fed  
479 with commercial soybean. Replacement of fishmeal with either 50% soybean meal or  
480 50% EnzoMeal™ resulted in poor growth compared with the diet with sole fishmeal  
481 protein. However, fish weight gain was not significantly different between fish fed the  
482 diet with 50% fishmeal replacement by soybean protein (300 g fishmeal kg<sup>-1</sup>) and 50%

483 fishmeal replacement by EnzoMeal™ (300 g EnzoMeal™ kg<sup>-1</sup>). Palatability is a major  
484 limiting factor to further increase the level of EnzoMeal™ in the barramundi diet.  
485 Further studies should focus on improvement of the feed palatability of EnzoMeal™ by  
486 including more animal source proteins (e.g. meat meal, blood meal) and feed stimulant  
487 in the diet while replacing the fishmeal component.

488

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494

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710 soybean meal in diets for juvenile cobia (*Rachycentron canadum*). *Aquacult Nutr*,  
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714 Table 1 Nutrient composition of the key experimental ingredients (g kg<sup>-1</sup>)

	Soybean			Soybean	Wheat
	meal	EnzoMeal <sup>TM</sup>	Fishmeal	concentrate	flour
Dry matter	884.0	925.0	950.2	964.3	880.04
Crude protein	473.1	560.0	550.0	650.0	109.0
Crude fat	11.0	28.0	90.0	10.0	13.0
Crude fiber	31.8	27.0	0.00	0.00	48.0
Ash	64.5	60.0	200	50.0	43.0
Tryptophan	7.1	8.4	5.0	8.5	1.4
Cystine	6.2	7.2	7.5	7.9	2.5

Methionine	6.4	8.3	13.3	6.0	1.7
Aspartic acid	53.0	63.9	46.6	70.4	5.0
Threonine	18.4	22.5	23.5	25.4	3.3
Serine	23.0	28.6	28.3	32.7	5.6
Glutamic acid	86.6	99.4	65.6	115.6	39.9
Proline	24.1	27.7	32.0	32.3	13.3
Glycine	19.8	24.0	43.6	27.2	4.5
Alanine	20.3	24.8	32.9	26.5	3.5
Valine	23.4	27.7	29.5	31.9	5.0
Isoleucine	21.9	26.5	24.0	31.9	4.3
Leucine	36.9	43.2	40.9	51.2	8.2
Tyrosine	15.6	18.9	17.1	19.5	2.4
Phenylalanine	24.1	28.1	23.4	33.2	5.7
Lysine	30.7	24.0	34.3	39.4	2.7
Histidine	12.2	14.1	15.6	17.0	2.8
Arginine	33.6	36.4	35.9	45.8	4.5

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723 Table 2 Feed composition (g kg<sup>-1</sup>), proximate composition of the diets (air-dry basis,  
 724 g kg<sup>-1</sup>) and gross energy content (MJ kg<sup>-1</sup>) in five experimental diets

Ingredients	Control	SM50	EZ50	SM100	EZ100
Fishmeal <sup>a</sup>	600	300	300	0	0
Wheat flour <sup>b</sup>	258	203	250	140	240
Conventional soybean meal <sup>c</sup>	0.0	300	0.0	600	0.0
EnzolMeal <sup>TMc</sup>	0.0	0.0	300	0.0	600
Soybean concentrate <sup>a</sup>	10	57	10	100	0.0
Meanhaden oil <sup>d</sup>	121	129	129	149	149
Vitamin and mineral premix <sup>a</sup>	10	10	10	10	10
Yttrium oxide <sup>e</sup>	1	1	1	1	1
Total	1000	1000	1000	1000	1000
Proximate composition					
Dry matter	885	877	878	859	855
Crude protein	365	366	367	364	362
Lipid	176	160	165	155	166
Nitrogen-free extract	239	276	284	306	325
Ash	132	90	89	50	46
Gross energy	19.7	19.7	20.1	20.0	20.7

725 <sup>a</sup> Supplied by Lienert Australia, Adelaide, Australia. Vitamin and mineral premix (g kg<sup>-1</sup>):  
726 vitamin A 0.15, VIT C 1.0, VIT D3 0.06, VIT E 1.6, VIT K 0.09, VIT B1 0.02, VIT B2 0.12,  
727 VIT B3 0.37, VIT B5 0.3, VIT B6 0.04, VIT B9 0.03, VIT B12 0.03, VIT Biotin 0.18, choline  
728 chloride 2.0, inositol 0.8, cobalt carbonate 0.05, copper sulphate 0.4, ferrous sulphate 3.0,  
729 potassium iodide 0.03, manganous oxide 0.98, sodium selenite 0.02, zinc sulphate 3.14, borax  
730 pentahydrate 0.4, bioplex copper 0.83, bioplex manganese 0.5, selplex 0.74, bioplex-zinc 2.67,  
731 chromium picolinate 0.03, keyshure iron proteinate 4.0, limestone 1.95, ebanox E 1.25, cereal  
732 carrier 1.75, and white oil 0.25.

733 <sup>b</sup> Wheatfields Plain Flour, Manildra Group of Companies, Auburn, Australia.

734 <sup>c</sup> Supplied by Ohio Soybean Council, Worthington, USA.; conventional soybean meal  
735 was dehulled and defatted meal.

736 <sup>d</sup> Supplied by Adam & Amos Abalone Foods Pty. Ltd.

737 <sup>e</sup> Sigma-Aldrich (catalog no. 205168-250G, Lot # STBG1022V, USA

738 Table 3 Body weights, survival, specific growth rate, feed conversion ratio, proximate composition of muscle of fish fed with five experimental diets.

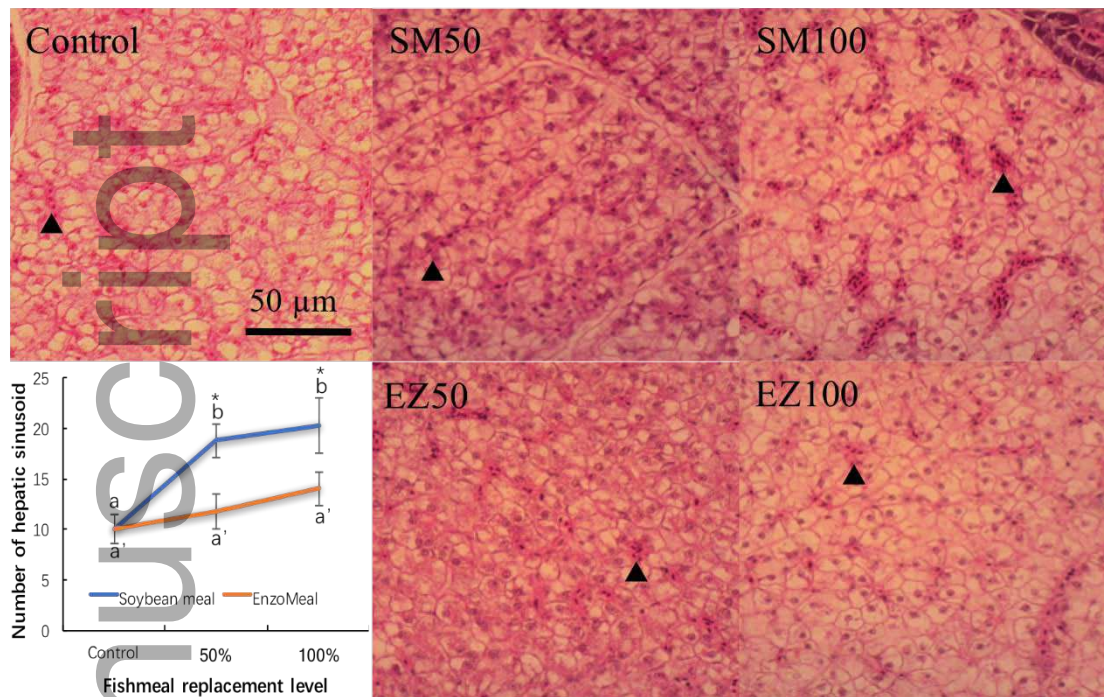
739 Different superscript letters in the same row represent significant difference ( $P < 0.05$ )

Soybean type	Soybean meal			EnzoMeal		Soybean type	Fish	Soybean type × fishmeal
	Fishmeal replacement level	#0%	50%	100%	50%			
Initial weight ( $\mu$ fish <sup>-1</sup> )	27.02 ± 1.11	26.47 ± 0.86	25.22 ± 0.22	26.42 ± 1.20	25.57 ± 0.2	N/A	N/A	N/A
Final weight ( $\mu$ fish <sup>-1</sup> )	126.63 ± 12.88 <sup>a</sup>	91.15 ± 11.50 <sup>b</sup>	30.61 ± 2.45 <sup>c</sup>	70.07 ± 7.57 <sup>b</sup>	38.18 ± 5.15 <sup>c</sup>	0.15	0.001	0.01
Weight gain ( $\mu$ fish <sup>-1</sup> )	99.51 ± 10.10 <sup>a</sup>	58.70 ± 11.04 <sup>b</sup>	6.30 ± 1.07 <sup>c</sup>	42.77 ± 8.51 <sup>b</sup>	10.14 ± 5.65 <sup>c</sup>	0.20	0.001	0.05
SGR ( $\%$ d <sup>-1</sup> )	2.34 ± 0.04 <sup>a</sup>	1.81 ± 0.20 <sup>b</sup>	0.42 ± 0.11 <sup>c</sup>	1.65 ± 0.28 <sup>b</sup>	0.54 ± 0.28 <sup>c</sup>	0.88	0.001	0.30
Survival (%)	93.33 ± 11.55 <sup>a</sup>	80.00 ± 10.00 <sup>a</sup>	93.33 ± 11.55 <sup>a</sup>	93.33 ± 11.55 <sup>a</sup>	83.33 ± 5.77 <sup>a</sup>	0.78	0.78	0.08
Feed intake ( $\mu$ fish <sup>-1</sup> d <sup>-1</sup> )	1.87 ± 0.04 <sup>a</sup>	1.37 ± 0.01 <sup>b</sup>	0.40 ± 0.05 <sup>c</sup>	1.12 ± 0.07 <sup>b</sup>	0.77 ± 0.07 <sup>c</sup>	0.68	0.001	0.001
FCR	1.27 ± 0.18 <sup>a</sup>	2.12 ± 0.07 <sup>a</sup>	4.00 ± 0.06 <sup>b</sup>	1.80 ± 0.75 <sup>a</sup>	*8.80 ± 0.58 <sup>b</sup>	0.001	0.001	0.001
ADC dry matter (%)	41.11 ± 3.04 <sup>b</sup>	70.13 ± 0.63 <sup>a</sup>	38.20 ± 5.25 <sup>b</sup>	40.50 ± 3.14 <sup>a</sup>	20.74 ± 4.61 <sup>c</sup>	0.001	0.001	0.001
ADC protein (%)	48.57 ± 5.11 <sup>b, a</sup>	85.32 ± 0.07 <sup>a</sup>	50.27 ± 12.00 <sup>b</sup>	56.10 ± 4.65 <sup>a</sup>	22.74 ± 2.21 <sup>b</sup>	0.001	0.001	0.70
Moisture content %	44.47 ± 14.18 <sup>a</sup>	30.04 ± 2.36 <sup>a</sup>	25.02 ± 0.80 <sup>a</sup>	29.12 ± 1.53 <sup>a</sup>	25.40 ± 1.30 <sup>a</sup>	0.40	0.01	0.25
Crude protein (N% × 6.25)	53.74 ± 4.02 <sup>c, b</sup>	68.40 ± 4.67 <sup>b</sup>	80.70 ± 0.78 <sup>a</sup>	72.31 ± 4.03 <sup>a</sup>	80.25 ± 1.60 <sup>a</sup>	0.43	0.001	0.32
Ash content % DW	7.70 ± 2.56 <sup>a</sup>	8.07 ± 5.53 <sup>a</sup>	4.63 ± 2.20 <sup>a</sup>	4.81 ± 1.51 <sup>a</sup>	7.60 ± 1.84 <sup>a</sup>	0.76	0.60	0.00
Crude lipid % DW	37.54 ± 5.12 <sup>a</sup>	24.88 ± 4.50 <sup>b</sup>	8.14 ± 2.14 <sup>c</sup>	18.58 ± 1.57 <sup>b</sup>	9.22 ± 2.22 <sup>c</sup>	0.15	0.001	0.05

740 SGR: specific growth rate, FCR: feed conversion ratio, ADC: apparent digestibility coefficient for overall feed, ADC protein: apparent digestibility coefficient for protein.

741 #The 0% control group is shared between soybean meal group and EnzoMeal group for statistical analysis.

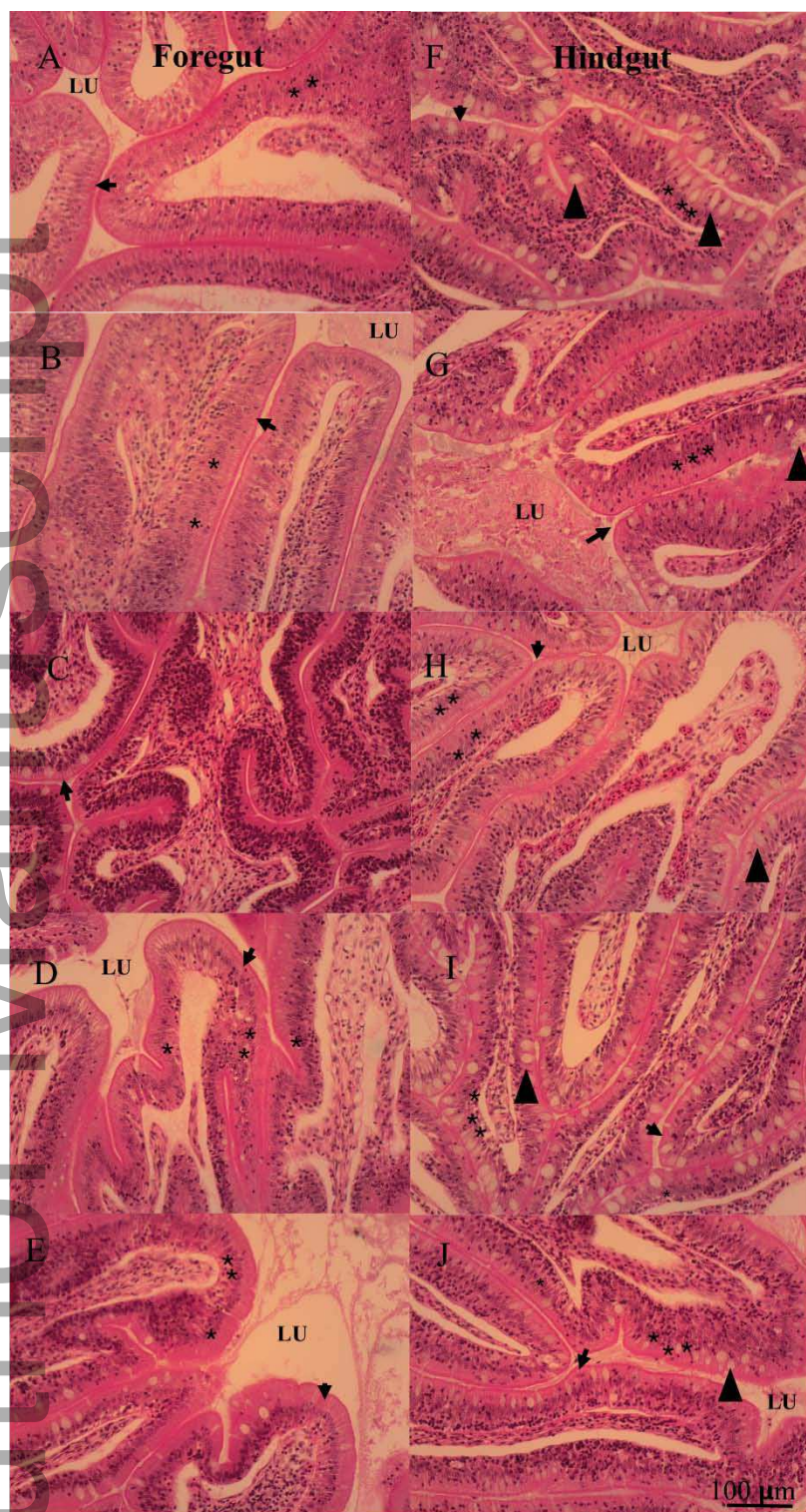
742 \*FCR might be escalated as feed was often spitted out after holding for a while.



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745 Fig. 1 Liver histological sections (magnification  $\times 400$ ) of barramundi fed five  
 746 experimental diets (control; fishmeal replaced by 50% soybean meal, SM50; fishmeal  
 747 replaced by 50% EnzoMeal<sup>TM</sup>, EZ50; fishmeal replaced by 100% soybean meal,  
 748 SM100; fishmeal replaced by 100% EnzoMeal<sup>TM</sup>, EZ100) and the average number of  
 749 hepatic sinusoid (mean  $\pm$  SD). The dark triangles indicate the location of hepatic  
 750 sinusoids. Asterisk (\*) indicates significant feed type effect at each fishmeal level.  
 751 Different letters indicate significantly difference ( $P < 0.05$ )

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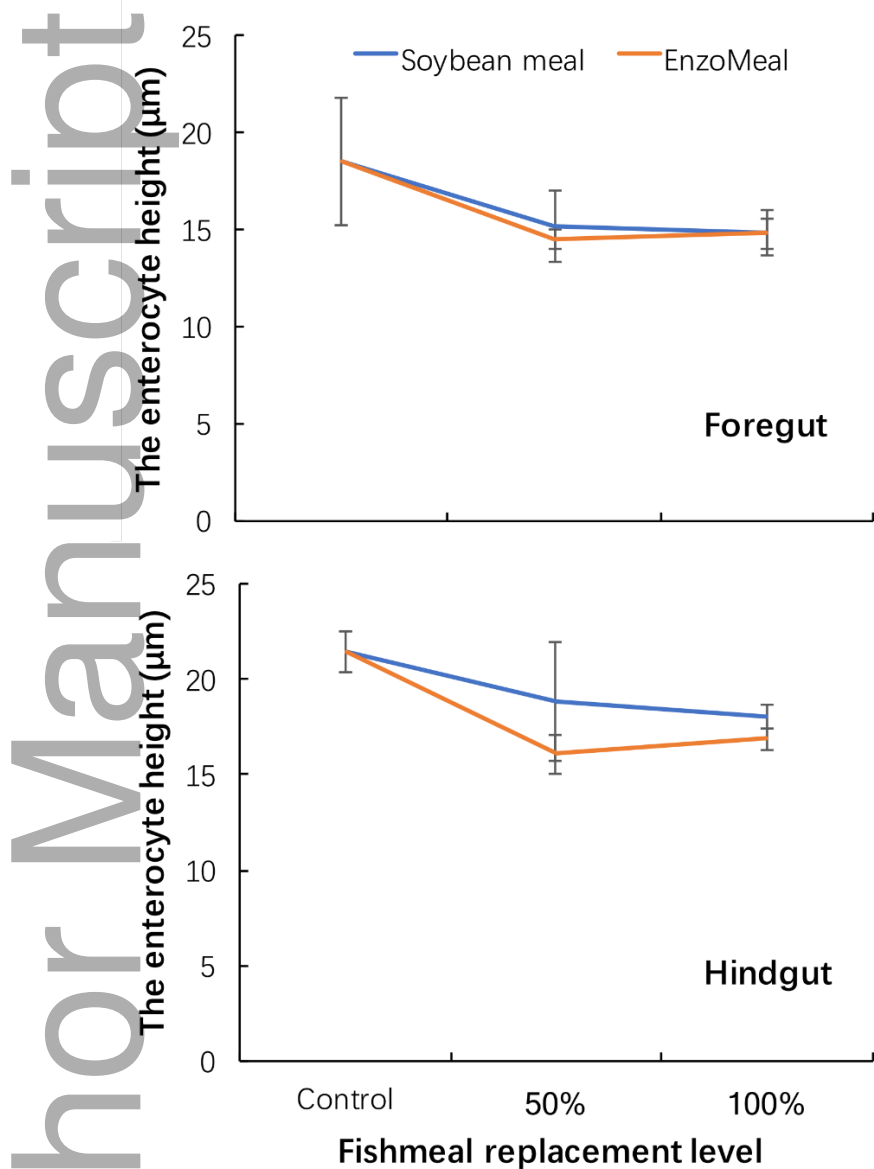
753

754 Fig. 2 The transverse section (magnification  $\times 400$ ) of foregut (left panels: A-E) and

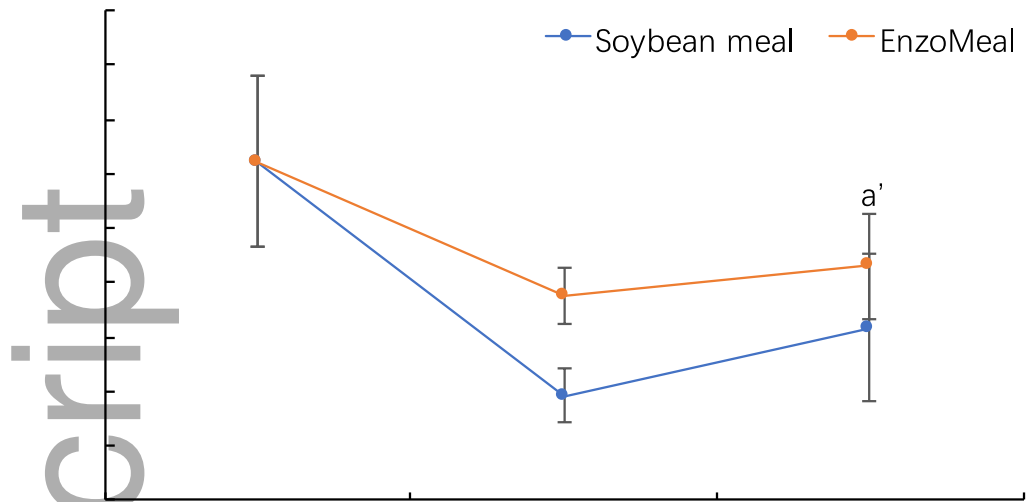
755 hindgut (right panels: F-J) of barramundi fed with experimental diets. A: control; B:

756 SM50; C: EZ50; D: SM100; E: EZ100; F: control; G: SM50; H: EZ50; I: SM100; J:

757 EZ100. Dark triangles indicate the location of goblet cells; small arrows indicate the  
758 location of enterocytes; asterisk (\*) indicates the location of granulocytes; LU: lumen.



759  
760 Fig. 3 Average enterocyte height of foregut and hindgut of barramundi fed  
761 experimental diets: control, 50% soybean meal (SM50), 50% EnzoMeal<sup>TM</sup> (EZ50),  
762 100% soybean meal (SM100), and 100% EnzoMeal<sup>TM</sup> (EZ100). Error bars represent  
763 standard error.



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765 Fig. 4 Average activity of pepsin in barramundi fed different diets: control, 50%

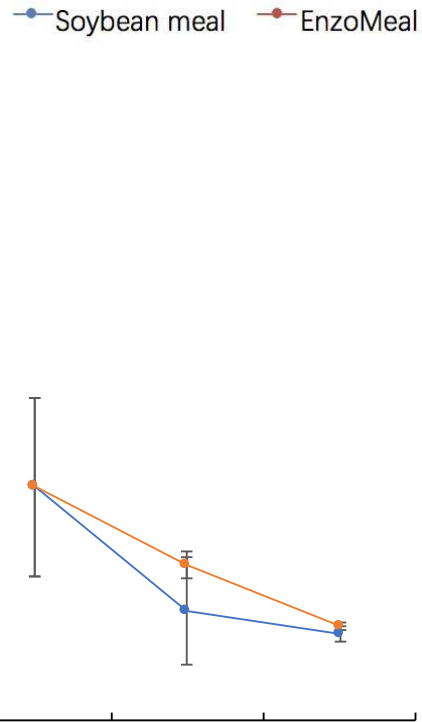
766 soybean meal (SM50), 50% EnzoMeal<sup>TM</sup> (EZ50), 100% soybean meal (SM100), and

767 100% EnzoMeal<sup>TM</sup> (EZ100). Asterisk (\*) indicates significant feed type effect at each

768 fishmeal level. Different letters indicate significantly different ( $P < 0.05$ ). Error bars

769 represent standard error.

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772 Fig. 5 Average activity of amylase, lipase and phospholipase in barramundi fed  
773 different diets: control, 50% soybean meal (SM50), 50% EnzoMeal<sup>TM</sup> (EZ50), 100%  
774 soybean meal (SM100), and 100% EnzoMeal<sup>TM</sup> (EZ100). Different letters indicate  
775 significantly different ( $P < 0.05$ ). Error bars represent standard error.