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Article type : Original Article

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

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](https://doi.org/10.1111/are.13790). Please cite this article as [doi: 10.1111/are.13790](https://doi.org/10.1111/are.13790)

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Running title: Fishmeal replacement in barramundi

Abstract

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

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

Introduction

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

Over the last 20 years, fish nutrition research has focused on the replacement of fishmeal with more sustainable alternative protein ingredients (Tacon & Metian 2008; Bowyer, Qin & Stone 2013). It was identified that alternative protein ingredients should possess adequate nutritional properties, i.e., a high level of protein with a 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

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

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

Materials and methods

Experiment design and system

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

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

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

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 μ 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

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

Enzymatic assays

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

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

ethylidene-pNP-G7 was used as the substrate to cleave α -amylase. The smaller fragments produced by this procedure could react with α -glucosidase to release chromophore that could then be measured at 405 nm wavelength.

Lipase (E.C. 3.1.1.3) activity was assayed via a lipase assay kit (catalog no. K723-100, BioVision Incorporated, Milpitas, CA, USA). In this assay, lipase hydrolyzed the substrate to generate a product which reacted with the 2-nitrobenzoic acid probe to produce a color that was measured at 412 nm wavelength. Alkaline phosphatase (E.C. 3.1.3.1) was assayed by the phospholipase D activity colorimetric assay kit (catalog no. K725-100, BioVision Incorporated, Milpitas, CA, USA). In this assay, phospholipase cleaved choline from phosphatidylcholine. The free choline was then oxidized by the phospholipase enzyme mix to generate an intermediate which reacted with the phospholipase probe to generate a color with an optical peak density at 570 nm wavelength. Enzymatic activities were expressed as specific activity (mU mg^{-1} protein or U mg^{-1} protein). Soluble protein of crude enzyme extracts was quantified by the bicinchoninic acid protein assay kit (catalog no. BCA1 & B9643; Sigma-Aldrich, St. Louis, MO, USA).

Calculations and statistical analysis

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

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

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

Results

In this study, fish survival was not significantly affected by soybean type and fishmeal level ($P > 0.05$, Table 3). Furthermore, there was no interaction between soybean type and fish level ($P = 0.08$). The survival ranged from $80.00 \pm 10.00\%$ to $93.33 \pm 11.55\%$. At the end of the feeding trial, weight gain was significantly affected by the fishmeal

level ($P = 0.001$). The highest body weight of 126.63 ± 13.88 g was observed on fish in the control group, and lowest body weight was found in the SM100 and EZ100 groups. The specific growth rate (SGR) was significantly affected by the fishmeal level ($P = 0.001$). The SGR was highest in the control group and lowest in the SM100 and EZ100 groups, but SGR was not significantly different between SM50 and EZ50 ($P > 0.05$), or between SM100 and EZ100 ($P < 0.05$). The feed conversion ratio (FCR) was significantly affected by soybean type and fishmeal level ($P < 0.05$). Furthermore, there was a significant interaction effect between the soybean type and the fishmeal level on the FCR ($P = 0.001$). The FCR in the control was not significantly different from SM50 and EZ50 ($P > 0.05$), but was significantly lower than that in SM100 and EZ100 ($P < 0.05$). The poorest FCR was observed in fish fed EZ100. The feed intake was significantly affected by the fishmeal level ($P = 0.001$), and an interaction effect between the soybean type and the fishmeal level was observed ($P = 0.001$). The highest feed intake was 1.87 ± 0.01 g fish⁻¹ d⁻¹ in the control fish (Table 3), and the lowest feed intake (0.49 ± 0.05 g fish⁻¹ d⁻¹) was observed in the fish fed SM100. The highest body protein content was observed in fish fed SM100 or EZ100. The lipid content of fish was highest in the control group, and lowest in the SM100 and EZ100 treatments (Table 3).

The apparent feed digestibility coefficient was not significantly different between commercial soybean meal and EnzoMealTM ($P > 0.05$). The apparent digestibility coefficient (ADC) of protein was significantly affected by the soybean type and the fishmeal level ($P < 0.05$), and no interaction effects were found between the soybean

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

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

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

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

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

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

Discussion

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

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

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

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

±0.04 % day⁻¹ in the control to 1.81 ± 0.20 % day⁻¹ (SM50) and 1.65 ± 0.28 % day⁻¹ EZ50 when 50% fishmeal was replaced by either SM50 or EZ50, respectively. When fishmeal was completely replaced in the feed, the SGR further decreased to 0.42 ± 0.11% day⁻¹ in the SM100 group and 0.54 ± 0.28% day⁻¹ in EZ100 group. These results are consistent to the results of previous studies in barramundi (Glencross, Rutherford & Jones 2011; Glencross, Blyth, Irvin, Bourne, Campet, Boisot & Wadw 2016), suggesting that the increase of dietary plant protein source in the feed hinders the growth performance of juvenile barramundi, despite significant removal of carbohydrates, oligosaccharides and phytic acid from EnzoMealTM.

A major cause of poor growth performance of fish is poor voluntary feed intake after fishmeal replacement by plant protein in the diet (Gomes, Rema & Kaushik 1995; Dias, Alvarez, Diez, Arzel, Corraze, Bautista & Kaushik 1998). Glencross *et al.* (2016) demonstrated that the primary response of barramundi to the replacement of fishmeal was reduction of feed intake, presumably for a palatability reason, directly leading to poor growth performance (Glencross *et al.* 2016). When the level of dietary fishmeal reduces to 150 g kg⁻¹, feed intake of barramundi declines dramatically (Glencross *et al.*, 2011). Here, feed intake was significantly affected by the fishmeal replacement level. When dietary fishmeal inclusion was reduced from 600 g kg⁻¹ in the control to 300 g kg⁻¹ in the SM50 and EZ50 diets, feed intake dropped from 1.87 ± 0.01 g fish⁻¹ day⁻¹ to 1.37 ± 0.01 g fish⁻¹ day⁻¹ in the SM50 diet or to 1.12 ± 0.07 g fish⁻¹ day⁻¹ in fish fed with the EZ50 diet. When dietary fishmeal was completely replaced, feed intake

373 further reduced to 0.49 ± 0.05 g fish⁻¹ day⁻¹ in fish fed with SM100 and 0.77 ± 0.02 g
374 fish⁻¹ day⁻¹ 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⁻¹ day⁻¹ rather than g feed kg⁻¹ body weight day⁻¹. 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

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

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

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

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

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

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

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

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

Acknowledgments

This study was financially supported by the Ohio Soybean Council (USA). We wish to thank Leslie Morrison and Sarah Churcher in the Animal House for checking fish health and activity, Yvette DeGraaf at Flinders Microscopy Biomedical Services and Daniel Jardine at Flinders Analytical for technical support.

References

- Aksnes, A., Hjertnes, T. & Opstvedt, J. (1996) Comparison of two assay methods for determination of nutrient and energy digestibility in fish. *Aquaculture*, **140**, 343-359
- Alexis, M.N. & Nengas, I. (2001) Current state of knowledge concerning the use of soy products in diets for feeding sea bass and sea bream needs for future research, pp. 1-32. Brussels, Belgium.
- Ayadi, F.Y., Rosentrater, K.A. & Muthukumarappan, K. (2012) Alternative protein sources for aquaculture feeds. *J Aquacult Feed Sci Nutr*, **4**, 1-26.
- Bakke-McKellep, A.M., Press, C.M., Baeverfjord, G., Krogdahl, A. & Landsverk, T.

(2000) Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. *J Fish Dis*, **23**, 115-127.

Bjerkeng, B., Refstie, S., Fjalestad, K.T., Storebakken, T., Rodbotten, M. & Roem, A.J. (1997) Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. *Aquaculture*, **157**, 297-309.

Bonaldo, A., Parma, L., Mandrioli, L., Sirri, R., Fontanillas, R., Badiani, A. & Gatta, P.P. (2011) Increasing dietary plant proteins affects growth performance and ammonia excretion but not digestibility and gut histology in turbot (*Psetta maxima*) juveniles. *Aquaculture*, **318**, 101-108.

Bonaldo, A., Roem, A.J., Fagioli, P., Pecchini, A., Cipollini, I. & Gatta, P.P. (2008) Influence of dietary levels of soybean meal on the performance and gut histology of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). *Aquacult Res*, **39**, 970-978.

Bonaldo, A., Roem, A.J., Pecchini, A., Grilli, E. & Gatta, P.P. (2006) Influence of dietary soybean meal levels on growth, feed utilization and gut histology of Egyptian sole (*Solea aegyptiaca*) juveniles. *Aquaculture*, **261**, 580-586.

Boonyaratpalin, M. (1997) Nutrient requirements of marine food fish cultured in Southeast Asia. *Aquaculture*, **151**, 283-313.

Bowyer, J.N., Qin, J.G. & Stone, D.A.J. (2013) Protein, lipid and energy requirements of cultured marine fish in cold, temperate and warm water. *Rev Aquacult*, **5**,

527 10-32.

528 Bureau, D.P., Kaushik, S.J. & Cho, C.Y. (2002) Bioenergetics In *Fish Nutrition*

529 (Halver, J.E. & Hardy, R.W. eds.), pp. 1-59. Academic Press, San Diego.

530 Caballero, M.J., Izquierdo, M.S., Kjorsvik, E., Montero, D., Socorro, J., Fernandez,

531 A.J. & Rosenlund, G. (2003) Morphological aspects of intestinal cells from

532 gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources.

533 *Aquaculture*, **225**, 325-340.

534 Catacutan, M.R. & Coloso, R.M. (1995) Effect of dietary protein to energy ratios on

535 growth, survival, and body composition of juvenile Asian sea bass, *Lates*

536 *calcarifer*. *Aquaculture*, **131**, 125-133.

537 Chen, N.S., Ai, Q.H. & Wang, D.Z. (1998) Studies on soybean protein as a substitute

538 for fish meal in formulated diets for *Anguilla anguilla*. *J Fish China*, **22**, 283-287.

539 Chou, R.L., Su, M.S. & Chen, H.Y. (2001) Optimal dietary protein and lipid levels for

540 juvenile cobia (*Rachycentron canadum*). *Aquaculture*, **193**, 81-89.

541 Craig, S.R., Harris, H.W. & Betka, M. (2015) Evaluation of EnzoMeal (EZM) in

542 commercial production diets for rainbow trout (*Oncorhynchus mykiss*), pp. 24.

543 Final Report to Ohio Soybean Council.

544 Day, O.J. & Plascencia-Gonzalez, H.G. (2000) Soybean protein concentrate as a

545 protein source for turbot *Scophthalmus maximus* L. *Aquacult Nutr*, **6**, 221-228.

546 Dias, J., Alvarez, M.J., Diez, A., Arzel, J., Corraze, G., Bautista, J.M. & Kaushik, S.J.

547 (1998) Regulation of hepatic lipogenesis by dietary protein/energy in juvenile

548 European seabass (*Dicentrarchus labrax*). *Aquaculture*, **161**, 169-186.

- 549 Evans, J.J., Pasnik, D.J., Peres, H., Lim, C. & Klesius, P.H. (2005) No apparent
550 differences in intestinal histology of channel catfish (*Ictalurus punctatus*) fed
551 heat-treated and nonheat- treated raw soybean meal. *Aquacult Nutr*, **11**, 123-129.
- 552 Folch J., Lees M. & Sloane Stanley G.H. (1957) A simple method for the isolation
553 and purification of total lipides from animal tissues. *J Bio Chem*, **226**, 497-509.
- 554 Gatlin, D.M., Barrows, T.F., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W.,
555 Hermen, E., Hu, G., Kroghdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W.,
556 Skonberg, D., Souza, E.J., Stone, D.A.J., Wilson, R. & Wurtele, E. (2007)
557 Expanding the utilization of sustainable plant products in aquafeeds: a review.
558 *Aquacult Res*, **38**, 551-579.
- 559 Glencross, B. (2006) The nutritional management of barramundi, *Lates calcarifer* - a
560 review. *Aquacult Nutr*, **12**, 291-309.
- 561 Glencross, B., Blyth, D., Irvin, S., Bourne, N., Campet, M., Boisot, P. & Wade, N.M.
562 (2016) An evaluation of the complete replacement of both fishmeal and fish oil in
563 diets for juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, **451**, 298-309.
- 564 Glencross, B., Rutherford, N. & Jones, B.B. (2011) Evaluating options for fishmeal
565 replacement in diets for juvenile barramundi (*Lates calcarifer*). *Aquacult Nutr*, **17**,
566 e722-e732.
- 567 Gomes, E.F., Rema, P. & Kaushik, S.J. (1995) Replacement of fish meal by plant
568 proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and
569 growth performance. *Aquaculture*, **130**, 177-186.
- 570 Grisdale-Helland, B., Helland, S.J., Baeverfjord, G. & Berge, G.M. (2002) Full-fat

571 soybean meal in diets for Atlantic halibut: growth, metabolism and intestinal
 572 histology. *Aquacult Nutr*, **8**, 265-270.

573 Halver, J.E. & Hardy, R.W. (2002) Nutrient flow and retention. In *Fish Nutrition*
 574 (Halver, J.E. & Hardy, R.W. eds.). Academic Press, California, CA, USA.

575 Heikkinen, J., Vielma, J., Kemilainen, O., Tirola, M., Eskelinen, P., Kiuru, T.,
 576 Navia-Paldanius, D. & von Wright, A. (2006) Effects of soybean meal based diet
 577 on growth performance, gut histopathology and intestinal microbiota of juvenile
 578 rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **261**, 259-268.

579 Hephher, B. (1988) *Nutrition of Pond Fishes*, Cambridge University Press, Cambridge.

580 Hernández, M.D., Martínez, F.J., Jover, M. & García García, B. (2007) Effects of
 581 partial replacement of fish meal by soybean meal in sharpsnout seabream
 582 (*Diplodus puntazzo*) diet. *Aquaculture*, **263**, 159-167.

583 Hertrampf, J.W. & Piedad-Pascual, F. (2000) *Handbook on Ingredients for Aquaculture*
 584 *Feeds*, Kluwer Academic Publishers, Dordrecht, the Netherlands.

585 Hulefeld, R., Kumar, V., Tidwell, J.H., Lalgudi, R.S., Cain, R. & McGraw, B. (2017)
 586 Improved soybean meal (enzomeal) alternative of fishmeal for sustainable
 587 production of pacific white shrimp *Litopenaeus vannamei*. In *Aquaculture*
 588 *America 2017*. Meeting Abstract, San Antonio, Texas.

589 Ilham, I., Siddik, M.A.B. & Fotedar, R. (2016) Effects of organic selenium
 590 supplementation on growth, accumulation, haematology and histopathology of
 591 juvenile barramundi (*Lates calcarifer*) fed high soybean meal diets. *Biol Trace Ele*
 592 *Res*, **174**, 436-447.

- Johnston, I.A. & Goldspink, G. (1973) Some effects of prolonged starvation on the metabolism of the red and white myotomal muscles of the plaice *Pleuronectes platessa*. *Mar Bio*, **19**, 348-358.
- Kaushik, S.J., Coves, D., Dutto, G. & Blanc, D. (2004) Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture*, **230**, 391-404.
- Kiernan, J.A. (2003) *Histological and Histochemical Methods: Theory and Practice*. 3rd ed. London.
- Krogdahl, A., Bakke-McKellep, A.M. & Bæverfjord, G. (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquacult Nutr*, **9**, 361-371.
- Kumar, V. (2016) EnzoMeal as a fish meal replacer: effects on growth, digestive and oxidative status of yellow perch (*Perca flavescens*). pp. 49. Final Report, Ohio Soybean Council.
- Lalgudi, R.S, Cain, R.J. & McGraw, B.L. (2015) Mitigation of anti-nutritional substances in plant meal, *US Patent No.* 20150342221.
- Leenhouwers, J.I., Adjei-Boateng, D., Verreth, J.A.J. & Schrama, J.W. (2006) Digesta viscosity, nutrient digestibility and organ weights in African catfish (*Clarias gariepinus*) fed diets supplemented with different levels of a soluble non-starch polysaccharide. *Aquacult Nutr*, **12**, 111-116.
- Li, Y., Ai, Q., Mai, K., Xu, W., Deng, J. & Cheng, Z. (2014) Comparison of

high-protein soybean meal and commercial soybean meal partly replacing fish meal on the activities of digestive enzymes and aminotransferases in juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier, 1828). *Aquacult Res*, **45**, 1051-1060.

Li, Y., Ai, Q.H., Mai K.S., Xu, W. & Cheng, Z.Y. (2012) Effects of the partial substitution of dietary fish meal by two types of soybean meals on the growth performance of juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier, 1828). *Aquacult Res*, **43**, 458-466.

Mundheim, H., Aksnes, A. & Hope, B. (2004) Growth, feed efficiency and digestibility in salmon (*Salmo salar* L.) fed different dietary proportions of vegetable protein sources in combination with two fish meal qualities. *Aquaculture*, **1-4**, 315-331

Monzer, S., Nasser, N., Babikian, J. & Saoud, I.P. (2017) Substitution of fish meal by soybean meal in diets for juvenile marbled spinefoot, *Signaus rivulatus*. *J App Aquacult*, **29**, 101-116.

NRC (2011) Nutrient Requirements of Fish and Shrimp, National Academies Press, Washington D.C.

Oliva-Teles, A., Gouveia, A., Gonmes, E. & Rema, P. (1994) The effect of different processing treatment on soybean meal utilization by rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **124**, 343-349.

Perera, E. & Yufera, M. (2017) Effects of soybean meal on digestive enzymes activity, expression of inflammation-related genes, and chromatin modification in marine

- 637 fish (*Sparus aurata* L.) larvae. *Fish Physiol Biochem*, **43**, 563-578.
- 638 Pongmaneerat, J. & Watanabe, T. (1992) Utilisation of soybean meal as protein source
639 in diets for rainbow trout. *Nippon Suisan Gakk*, **58**, 1983-1990.
- 640 Rachmansyah, U., Laining, A., Ahmad, T. & William, K.C. (2005) Optimum dietary
641 protein and lipid specifications for grow-out of humpback grouper *Cromileptes*
642 *Altivelis* (Valenciennes). *Aquacult Res*, **36**, 1285-1292.
- 643 Rodríguez, M.A.S.D., Díaz-Rosales, P., Chabrilón, M., Smidt, H., Arijo, S.,
644 León-Rubio, J.M., Alarcón, F.J., Balebona, M.C., Morinigo, M.A. & Cara, J.B.
645 (2008) Effect of dietary administration of probiotics on growth and intestine
646 functionality of juvenile senegale sole (*Solea senegalensis*, Kaup 1858). *Aquacult*
647 *Nutr*, **15** 177–185.
- 648 Schipp, G., Bosmans, J. & Humphrey, J. (2007) Barramundi Farming Handbook.
649 Department of Primary Industries, Fisheries and Mines, Darwin, NT.
- 650 Shiao, S.Y. & Lan, C.W. (1996) Optimum dietary protein level and protein to energy
651 ratio for growth of grouper (*Epinephelus malabaricus*). *Aquaculture*, **145**,
652 259-266.
- 653 Sitjà-Bobadilla, A., Pena-Llopis, S., Gómez-Requeni, P., Medale, F., Kaushik, S. &
654 Perez-Sanchez, J. (2005) Effect of fish meal replacement by plant protein sources
655 on non-specific defence mechanisms and oxidative stress in gilthead sea bream
656 (*Sparus aurata*). *Aquaculture*, **249**, 387-400.
- 657 Skirtun, M.S. & Vieira, S. (2013) Australian fisheries statistics 2012 (2010/208), Final
658 Report. ABARES, Canberra.

- Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* **285**, 146-158.
- Touying, A. (2011) Using exogenous enzymes to increase the nutritional value of soybean meal in poultry diet. In *Soybean and Nutrition* (El-Shemy, H. ed.), pp. 201-230. InTech, Croatia.
- Trejo-Escamilla, I., Galaviz, M.A., Flores-Ibarra, M., Gonzalez, C.A.A. & Lopez, L.M. (2017) Replacement of fishmeal by soya protein concentrate in the diets of *Totoaba macdonaldi* (Gilbert, 1890) juveniles: effect on the growth performance, in vitro digestibility, digestive enzymes and the haematological and biochemistry parameters. *Aquacult Res*, **48**, 4038-4057.
- Trushenski, J., Laporte, J., Lewis, H., Schwarz, M., Delbos, B., Takeuchi, R. & Sampaio, L.A. (2011) Fish meal replacement with soy-derived protein in feeds for juvenile cobia: influence of replacement level and attractant supplementation. *J World Aquacult Soc*, **42**, 435-443.
- Tucker, J.W. (2002) Barramundi culture: A success story for aquaculture in Asia and Australia. *World Aquaculture Mag*, **33**, 53-59.
- Wang, C., Xie, S., Zhu, X., Lei, W., Yang, Y. & Liu, J. (2006) Effects of age and dietary protein level on digestive enzyme activity and gene expression of *Pelteobagrus fulvidraco* larvae. *Aquaculture*, **254**, 554-562.
- Wang, P., Zhu, J., Feng, J., He, J., Lou, Y. & Zhou, Q. (2017a) Effects of dietary soy protein concentrate meal on growth, immunity, enzyme activity and protein

metabolism in relation to gene expression in large yellow croaker *Larimichthys*
crocea. *Aquaculture*, **477**, 15-22.

Wang, Y., Wang, L., Zhang, C. & Song, K. (2017b) Effects of substituting fishmeal
with soybean meal on growth performance and intestinal morphology in
orange-spotted grouper (*Epinephelus coioides*). *Aquacult Rep*, **5**, 52-57.

Watanabe, T. (2002) Strategies for further development of aquatic feeds. *Fish Sci*, **68**,
242-252.

Windell, J.T., Foltz, J.W. & Sarokok (1978) Effect of fish size, temperature and
amount fed on nutrient digestibility of a pelleted diet by rainbow trout (*Salmo*
gairdneri). *T. Am. Fish. Soc.* **107**, 613-616.

Williams, K.C. (1998) Fishmeal replacement in Aquaculture Feeds for Barramundi,
pp. 101. Final Report to Fisheries R&D Corporation, Canberra, Australia.

Williams, K.C. & Barlow, C.G. (1999) Dietary requirement and optimal feeding
practices for barramundi (*Lates calcarifer*), pp. 95. Final Report to Fisheries R&D
Corporation, Canberra, Australia.

Williams, K.C., Barlow, C.G., Rodgers, L., Hockings, I., Agcopra, C. & Ruscoe, I.
(2003) Asian seabass *Lates calcarifer* perform well when fed pellet diets high in
protein and lipid. *Aquaculture*, **225**, 191-206.

Yang, Y., Wang, Y., Lu, Y. & Li, Q. (2011) Effect of replacing fish meal with soybean
meal on growth, feed utilization and nitrogen and phosphorus excretion on
rainbow trout (*Oncorhynchus mykiss*). *Aquacult Int*, **19**, 405-419.

Zaugg, W.S. & Wood, C.M. (1976) Influence of water temperature on gill sodium,

potassium-stimulated ATPase activity in juvenile coho salmon (*Oncorhynchus kisutch*). *Comp Biochem Physiol*, **54A**, 419-412.

Zhao, Z.X., Song, C.Y., Xie, J., Ge, X.P., Liu, B., Xia, S.L. & Zhu, S.H. (2016) Effects of fish meal replacement by soybean peptide on growth performance, digestive enzyme activities, and immune responses of yellow catfish *Pelteobagrus fulvidraco*. *Fish Sci*, **82**, 665-673.

Zhou, Q.C., Mai, K.S., Tan, B.P. & Liu, Y.J. (2005) Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). *Aquacult Nutr*, **11**, 175-182.

Table 1 Nutrient composition of the key experimental ingredients (g kg⁻¹)

	Soybean			Soybean	Wheat
	meal	EnzoMeal TM	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⁻¹), proximate composition of the diets (air-dry basis,
 724 g kg⁻¹) and gross energy content (MJ kg⁻¹) in five experimental diets

Ingredients	Control	SM50	EZ50	SM100	EZ100
Fishmeal ^a	600	300	300	0	0
Wheat flour ^b	258	203	250	140	240
Conventional soybean meal ^c	0.0	300	0.0	600	0.0
EnzolMeal ^{TMc}	0.0	0.0	300	0.0	600
Soybean concentrate ^a	10	57	10	100	0.0
Meanhaden oil ^d	121	129	129	149	149
Vitamin and mineral premix ^a	10	10	10	10	10
Yttrium oxide ^e	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 ^a Supplied by Lienert Australia, Adelaide, Australia. Vitamin and mineral premix (g kg⁻¹):
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 ^b Wheatfields Plain Flour, Manildra Group of Companies, Auburn, Australia.

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

736 ^d Supplied by Adam & Amos Abalone Foods Pty. Ltd.

737 ^e 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 $\times$ Fishmeal
	Fishmeal replacement level	#0%	50%	100%	50%	100%		
Initial weight (α fish ⁻¹)		27.02 $\pm$ 1.11	26.47 $\pm$ 0.86	25.22 $\pm$ 0.22	26.42 $\pm$ 1.20	25.57 $\pm$ 0.2	N/A	N/A
Final weight (α fish ⁻¹)		126.63 $\pm$ 13.88 ^a	91.15 $\pm$ 11.50 ^b	30.61 $\pm$ 2.45 ^c	70.07 $\pm$ 7.57 ^b	38.18 $\pm$ 5.15 ^c	0.15	0.01
Weight gain (α fish ⁻¹)		97.51 $\pm$ 10.10 ^a	58.20 $\pm$ 11.04 ^b	6.30 $\pm$ 1.07 ^c	42.27 $\pm$ 8.51 ^b	10.14 $\pm$ 5.65 ^c	0.20	0.01
SGR (% d ⁻¹)		2.34 $\pm$ 0.04 ^a	1.81 $\pm$ 0.20 ^b	0.42 $\pm$ 0.11 ^c	1.65 $\pm$ 0.28 ^b	0.54 $\pm$ 0.28 ^c	0.88	0.30
Survival (%)		93.33 $\pm$ 11.55 ^a	80.00 $\pm$ 10.00 ^a	93.33 $\pm$ 11.55 ^a	93.33 $\pm$ 11.55 ^a	83.33 $\pm$ 5.77 ^a	0.78	0.08
Feed intake (α fish ⁻¹ d ⁻¹)		1.87 $\pm$ 0.04 ^a	1.37 $\pm$ 0.01 ^b	0.40 $\pm$ 0.05 ^c	1.12 $\pm$ 0.07 ^b	0.77 $\pm$ 0.07 ^c	0.68	0.001
FCR		1.27 $\pm$ 0.18 ^a	2.12 $\pm$ 0.07 ^a	4.00 $\pm$ 0.06 ^b	1.80 $\pm$ 0.75 ^a	*8.80 $\pm$ 0.58 ^b	0.001	0.001
ADC dry matter (%)		41.11 $\pm$ 3.04 ^b	70.13 $\pm$ 0.63 ^a	38.20 $\pm$ 5.25 ^b	40.50 $\pm$ 3.14 ^a	20.74 $\pm$ 4.61 ^c	0.001	0.001
ADC protein (%)		48.57 $\pm$ 5.11 ^{b, a}	85.32 $\pm$ 0.07 ^a	50.27 $\pm$ 12.00 ^b	56.10 $\pm$ 4.65 ^a	22.74 $\pm$ 2.21 ^b	0.001	0.70
Moisture content %		44.47 $\pm$ 14.18 ^a	30.04 $\pm$ 2.36 ^a	25.02 $\pm$ 0.80 ^a	20.12 $\pm$ 1.53 ^a	25.40 $\pm$ 1.30 ^a	0.40	0.25
Crude protein (N% $\times$ 6.25)		53.74 $\pm$ 4.02 ^{c, b}	68.40 $\pm$ 4.67 ^b	80.70 $\pm$ 0.78 ^a	72.31 $\pm$ 4.03 ^a	80.25 $\pm$ 1.60 ^a	0.43	0.32
Ash content % DW		7.70 $\pm$ 2.56 ^a	8.07 $\pm$ 5.53 ^a	4.63 $\pm$ 2.20 ^a	4.81 $\pm$ 1.51 ^a	7.60 $\pm$ 1.84 ^a	0.76	0.60
Crude lipid % DW		37.54 $\pm$ 5.12 ^a	24.88 $\pm$ 4.50 ^b	8.14 $\pm$ 2.14 ^c	18.58 $\pm$ 1.57 ^b	0.22 $\pm$ 2.22 ^c	0.15	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.

744

745 Fig. 1 Liver histological sections (magnification ×400) of barramundi fed five
 746 experimental diets (control; fishmeal replaced by 50% soybean meal, SM50; fishmeal
 747 replaced by 50% EnzoMealTM, EZ50; fishmeal replaced by 100% soybean meal,
 748 SM100; fishmeal replaced by 100% EnzoMealTM, EZ100) and the average number of
 749 hepatic sinusoid (mean ± 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 ($P < 0.05$)

752

Fig. 2 The transverse section (magnification $\times 400$) of foregut (left panels: A-E) and hindgut (right panels: F-J) of barramundi fed with experimental diets. A: control; B: SM50; C: EZ50; D: SM100; E: EZ100; F: control; G: SM50; H: EZ50; I: SM100; J:

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

Fig. 3 Average enterocyte height of foregut and hindgut of barramundi fed experimental diets: control, 50% soybean meal (SM50), 50% EnzoMealTM (EZ50), 100% soybean meal (SM100), and 100% EnzoMealTM (EZ100). Error bars represent standard error.

Fig. 4 Average activity of pepsin in barramundi fed different diets: control, 50% soybean meal (SM50), 50% EnzoMealTM (EZ50), 100% soybean meal (SM100), and 100% EnzoMealTM (EZ100). Asterisk (*) indicates significant feed type effect at each fishmeal level. Different letters indicate significantly different ($P < 0.05$). Error bars represent standard error.

771

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

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Title:

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

Date:

2018-10-01

Citation:

Ma, Z., Hassan, M. M., Allais, L., He, T., Leterme, S., Ellis, A. V., McGraw, B. & Qin, J. G. (2018). Replacement of fishmeal with commercial soybean meal and EnzoMeal in juvenile barramundi Lates calcarifer. AQUACULTURE RESEARCH, 49 (10), pp.3258-3269. <https://doi.org/10.1111/are.13790>.

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