# Effect of dietary saturated and monounsaturated fatty acids in juvenile barramundi Lates calcarifer

M.J. SALINI<sup>1,2,3</sup>, G.M. TURCHINI<sup>1,2</sup> & B.D. GLENCROSS<sup>4,†</sup>

Deakin University, Geelong, Vic., Australia; <sup>2</sup> School of Life and Environmental Sciences, Deakin University (Warrnambool Campus), Warrnambool, Vic., Australia; <sup>3</sup> CSIRO Agricultural Productivity Flagship, Woorim, Qld, Australia; <sup>4</sup> CSIRO Agricultural Productivity Flagship, Dutton Park, Qld, Australia

## **Abstract**

Barramundi (Lates calcarifer), a catadromous teleost of commercial interest, perform well when fed a wide range of dietary oils. However, the range of alternative oils now being explored is typically rich in saturated and monounsaturated fatty acids (SFA and MUFA). In this study, the response of juvenile barramundi (47.0 g per fish initial weight) fed isolipidic and isoenergetic diets with 82 g  $kg^{-1}$ added oil was tested. The experimental test diets had a 2 : 1 or 1 : 2 ratio of SFA to MUFA (SFA-D and MUFA-D, respectively) compared to a control diet (CTRL-D) fed for 8 weeks. The diets containing mostly olive oil (dietary MUFA-D) and mostly refined palm oil (dietary SFA-D) did not impact the growth performance or feed utilization parameters of the barramundi. The in vivo beta-oxidation activity was consistent with the dietary fatty acid composition, with the most dominant FA being heavily beta-oxidized. Together, the in vivo whole-body mass balance of fatty acids showed that n-3 long-chain polyunsaturated fatty acids (LC-PUFA) were most efficiently utilized in the SFA-D- and MUFA-D-fed fish. This study provides evidence that additional dietary MUFA and SFA are suitable lipid classes for juvenile barramundi and they are both equally efficient at sparing LC-PUFA from an oxidative fate.

KEY WORDS: barramundi, fatty acid, metabolism, monounsaturated fatty acids, saturated fatty acids,  $\beta$ -oxidation

## **Introduction**

In the context of feeding fish with alternative oils, many studies have focused on the essential fatty acids and their bioactive long-chain derivatives, eicosapentaenoic and docosahexaenoic acids (20:5n-3 and 22:6n-3). However, it is suggested that saturated and monounsaturated fatty acids (SFA and MUFA) are preferred substrates for  $\beta$ -oxidation in fish, sparing the more essential fatty acids for their functional and biological roles (review by Henderson 1996). As alternative oils are being increasingly used in aquafeeds, it is important to understand the effect of increasing dietary levels of SFA and MUFA on lipid metabolism in fish.

Carnivorous fish such as barramundi (Lates calcarifer), also known as the Asian sea bass, rely heavily on  $\beta$ -oxidation of lipid for their energetic requirements. Past studies have consistently shown positive responses to the replacement of fish oil (FO) with a range of alternative oils such as soybean, canola, rapeseed and Echium, usually included as blends (Raso & Anderson 2003; Williams et al. 2006; Alhazzaa et al. 2011). Moreover, it is also clear that higher dietary levels of long-chain polyunsaturated fatty acids (LC-PUFA), originating from FO, are either beta-oxidized or potentially retro-converted, rather than being efficiently deposited in the flesh (Salini et al. 2015a).

Atlantic salmon (Salmo salar) also performed well when fed diets with either low n-3 and high monounsaturated fatty acids (MUFA), and in addition, MUFA were found to be good substrates for  $\beta$ -oxidation (Menoyo *et al.*) 2003). In the same species,  $\beta$ -oxidation was found to be dependent on energy demand and that when fatty acids were provided in excess to synthetic demands, they were increasingly b-oxidized for energy production (Torstensen et al. 2004; Stubhaug et al. 2007). Denstadli et al. (2011) found that the medium-chain length decanoic acid (10:0)

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Correspondence: M.J. Salini, School of Life and Environmental Sciences Deakin University (Warrnambool Campus). Princess Hwy, Sherwood Park, PO BOX 423, Warrnambool, Vic. 3280, Australia. E-mail: michael.salini@gmail.com

<sup>†</sup> Present address:Ridley Aquafeed, 12 Neon St, Narangba, Qld, Australia

was rapidly oxidized for energy production, whereas oleic acid (18:1n-9) was primarily deposited in the intramuscular fat in Atlantic salmon. In other species such as Atlantic cod (Gadus morhua), reduced lipid deposition in the liver was likely caused by selective  $\beta$ -oxidation of 16:0 and 18:0 saturated fatty acids (SFA) present in palm oil (PO) and rapeseed oil (Jobling et al. 2008). In agreement with this, polka dot grouper (Cromileptes altivelis) more readily oxidized lipid in diets containing SFA (coconut fat) for energy rather than MUFA (olive oil); however, this was at the expense of efficient growth (Smith et al. 2005). Further to this, the authors of a recent study on European sea bass (Dicentrarchus labrax) speculated that a single fatty acid (e.g. 18:1n-9) might be more efficient at sparing LC-PUFA from β-oxidation than oil blends (Eroldogan *et al.*) 2013).

Using an *in vivo* whole-body fatty acid balance approach for determining fatty acid metabolism in rainbow trout, Turchini & Francis (2009) found that the most heavily oxidized fatty acids were also the most dominant. However, in the fish oil-fed trout, the most predominant dietary fatty acid (16:0) was not readily oxidized for energy production, but rather was preferentially elongated to 18:0 or desaturated to 16:1n-7. The same research group also found that the rate of b-oxidation of LC-PUFA was reduced when Murray cod (Maccullochella peelii peelii) were fed diets containing abundant MUFA and SFA (Turchini et al. 2011). Moreover, a recent study on hybrid striped bass (White Bass Morone  $chrysops \times \text{Striped } Bass$  M. Saxatilis) concluded that diets containing SFA-rich lipid and to a lesser extent MUFA-rich lipid were able to conserve tissue fatty acid profiles possibly due to preferential  $\beta$ -oxidation (Trushenski et al. 2015).

Consistent responses of a range of fish species indicate that SFA and MUFA provided in surplus are heavily oxidized as substrates for energy, effectively sparing LC-PUFA for biological needs and deposition. Likewise, it is unfavourable to provide excess n-3 LC-PUFA, typical of FO, as these FA can also be readily  $\beta$ -oxidized (Torstensen et al. 2000; Turchini et al. 2013). Recent studies have shown that barramundi are capable of utilizing poultry oil, characterized by high MUFA content; however, to what extent SFA are preferentially utilized or able to 'spare' LC-PUFA remains unclear (Salini et al. 2015a). The proposed experiment examined the effect of diets containing a 2 : 1 or 1 : 2 ratio of SFA to MUFA in the lipid as it was hypothesized that these lipid classes would be metabolized differently. The digestibility of the diets was also measured,

and mass balance computations were used to estimate the fatty acid metabolism in vivo.

# **Materials and methods**

#### Ingredient and diet preparation

A single basal diet was formulated and prepared without the addition of dietary lipids. The dry ingredients were passed separately through a hammer mill (Mikro Pulverizer, type 1 SH, New Jersey, USA) such that the maximum particle size was less than  $750 \mu m$ . All dry ingredients were then thoroughly mixed using an upright commercial mixer (Bakermix, Model 60 A-G, NSW, Australia). Fish meal was defatted prior to use by manually mixing hexane and fish meal (2 : 1) in a large drum. The mix was soaked for 3 h before draining the excess hexane and repeating the process a second time. The fish meal was oven-dried overnight at 60 °C to a constant dry matter. The chemical composition of the main dietary ingredients is presented in Table 1. The lipid blends were first heated in an oven to 70 °C and then mixed gently. The basal diet was then separated into smaller batches, and aliquots of lipid (8.2% of the diet) were added to form the three treatment diets. Fresh water was added at approximately 30% of dry mash weight and mixed to form consistent dough and then the dough was subsequently screw-pressed through a 4-mm die. The pellets were dried overnight at 60 °C to a constant dry matter. The dietary treatments provided protein at 600 g kg<sup>-1</sup> and lipid at 130 g kg<sup>-1</sup> with an energetic value of 22 MJ  $kg^{-1}$ . The three dietary treatments consisted of a control diet with added FO (designated as CTRL-D), a diet containing a blend of FO and olive oil (designated as MUFA-D) and a diet containing a blend of FO and refined palm products (designated as SFA-D). This study used a blend of two common palm fractions: refined, bleached and deodorized palm oil (RBDPO) and palm flake, a highly refined stearin fraction. The RDBPO fraction used in this study contained approximately 35% MUFA, whereas the palm flake product was mostly SFA with a fatty acid profile dominated by roughly equal percentages of 16:0 and 18:0 FA. This blend of oils was used to achieve the desired SFA to MUFA ratio of the diets. The diets were stored at  $-20$  °C until required. The formulation and chemical composition of the three diets are presented in Table 2.

## Barramundi husbandry and growth

Juvenile barramundi (Lates calcarifer) were sourced from the Betta Barra fish hatchery (Atherton, Qld, Australia),

**Table 1** Chemical composition of ingredients used in experimental diets. All values are presented as g kg<sup>-1</sup> DM unless otherwise stated

	DF Fish meal	Poultry meal	Soy isolate	Wheat gluten	Wheat flour	Casein	Wheat starch	Fish oil	Olive oil	Palm Oil	Palm Flake
Composition											
Dry matter (g $kg^{-1}$ )	984	958	958	927	839	924	836	992	987	100	99
Protein	789	641	895	823	112	870	5	4	4	3	4
Ash	163	138	46	1	6	11	3	1	<b>ND</b>	<b>ND</b>	<b>ND</b>
Lipid	46	151	57	121	22	5	<b>ND</b>	956	973	963	986
CHO <sup>1</sup>	1	70	$\overline{2}$	55	860	113	992	39	23	34	10
Gross energy (MJ $kg^{-1}$ )	18.9	20.4	21.8	21.2	15.3	21.9	14.5	39.3	39.5	39.5	39.3
Fatty acids $(\%)^2$											
16:0	25.8	25.4	17.8	$\overline{\phantom{0}}$				22.9	9.9	51.9	46.2
18:0	8.7	8.6	4.5	$\overline{\phantom{0}}$			$\overline{\phantom{0}}$	5.1	3.0	4.6	51.3
18:1	15.5	43.8	25.1	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	18.6	73.8	34.6	0.4
$18:2n-6$	1.7	11.0	46.5	$\overline{\phantom{0}}$			$\overline{\phantom{0}}$	2.0	11.0	6.7	<b>ND</b>
$18:3n-3$	0.8	1.0	5.1	$\overline{\phantom{0}}$		$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	1.0	1.0	<b>ND</b>	<b>ND</b>
20:4n-6	2.5	0.6	<b>ND</b>	$\overline{\phantom{0}}$			$\overline{\phantom{0}}$	1.5	<b>ND</b>	<b>ND</b>	<b>ND</b>
$20:5n-3$	9.0	0.5	<b>ND</b>	$\overline{\phantom{0}}$		$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	11.3	<b>ND</b>	<b>ND</b>	<b>ND</b>
$22:5n-3$	2.2	<b>ND</b>	<b>ND</b>	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	2.1	<b>ND</b>	<b>ND</b>	<b>ND</b>
22:6n-3	19.9	<b>ND</b>	<b>ND</b>	$\overline{\phantom{0}}$			$\overline{\phantom{0}}$	14.2	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>SFA</b>	39.8	36.1	22.6	$\overline{\phantom{0}}$		$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	36.4	13.4	58.6	99.6
<b>MUFA</b>	22.2	50.8	25.8	$-$			$\overline{\phantom{0}}$	29.1	74.6	34.7	0.4
$C_{18}$ PUFA	3.4	12.0	51.6	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	4.9	12.0	6.7	<b>ND</b>
LC-PUFA	34.5	1.2	<b>ND</b>	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	$\overline{\phantom{0}}$	-	29.7	<b>ND</b>	<b>ND</b>	<b>ND</b>
$n-3$	32.7	1.6	5.1	$\overline{\phantom{0}}$			$\overline{\phantom{0}}$	30.5	1.0	<b>ND</b>	<b>ND</b>
n-6	5.2	11.6	46.5					4.1	11.0	6.7	<b>ND</b>

ND, not detected, N/A, not analysed, values <0.1 are reported as <0.1.

<sup>1</sup> CHO, carbohydrate calculated by difference (e.g. CHO = 1000 – (protein + lipid + ash).<br><sup>2</sup> All fatty acids are presented as percentage of the total fatty acids. Quantitative data can be obtained by multiplying the tot (mg  $q^{-1}$  lipid) by specific fatty acids (%). 18:1, sum of 18:1n-7, 18:1n-9 cis, 18:1n-9 trans; saturated fatty acids (SFA), sum of 12:0, 14:0, 16:0, 18:0, 20:, 22:0, 24:0; monounsaturated fatty acids (MUFA), sum of 14:1n-5, 16:1n-7, 18:1n-7, 18:1n-9 (cis and trans), 20:1n-7, 20:1n-9, 22:1n-9, 24:1n-9; polyunsaturated fatty acids (PUFA), sum of 18:2n-6 (cis and trans), 18:3n-6, 18:3n-3, 18:4n-3; long-chain polyunsaturated fatty acids (LC-PUFA), sum of 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 2-:3n-3, 20:5n-3, 22:5n-3, 22:6n-3; n-3 and n-6, sum of omega-3 and omega-6 PUFA and LC-PUFA.

on-grown in a 10 000-L tank and fed a commercial diet (Marine Float; Ridley Aquafeed, Narangba, Qld, Australia). Prior to commencement of the experiment, the fish were transferred to a series of experimental tanks (300 L) with flow-through sea water (salinity = 35 g kg<sup>-1</sup>; dissolved oxygen  $4.6 \pm 0.15$  mg L<sup>-1</sup>) maintained at  $30.0 \pm 0.01$  °C (mean  $\pm$  SD) with a supply rate of about 3 L min<sup>-1</sup> to each of the tanks. At the beginning of the experiment, the tanks held 26 fish of 47.0  $\pm$  0.3 g (mean  $\pm$  SD, n = 234 individually weighed fish). The three experimental diets were randomly distributed among the nine tanks with each treatment having three replicate tanks. The diets were fed once daily to apparent satiety as determined over three separate feeding events, for 8 weeks. Any uneaten feed was collected shortly after and a correction factor was applied (Helland et al. 1996). Briefly, the correction factor was calculated as the proportion of soluble losses after immersion in water for 1 h.

# Sample collection, preparation and digestibility analysis

Ethical clearance was approved for the experimental procedures by the CSIRO Animal Ethics Committee A12/2013. Six fish of similar size from the original stock were euthanized by an overdose of AQUI-S™ (Lower Hutt, New Zealand) at the beginning of the experiment and stored at  $-20$  °C until biochemical analysis. A further six fish were dissected, and a sample of liver tissue was then removed and placed into 1.5-mL screw-top vial and kept on dry ice before being transferred to a  $-80$  °C freezer until biochemical analysis.

Prior to the termination of the growth assay, faeces were collected using established settlement protocols for the digestibility assessment (Blyth et al. 2014). Briefly, a collection chamber was filled with water and frozen and then attached to the evacuation line of a swirl separator and left





ND, not detected, N/A, not analysed, values <0.1 are reported as  $< 0.1$ .

<sup>1</sup> Ridley aquafeeds, Narangba, Qld, Australia.

<sup>2</sup> Manildra Group, Rocklea, Qld, Australia.

<sup>3</sup> Bulk Powders, [www.bulkpowders.com.au.](http://www.bulkpowders.com.au)

<sup>4</sup> Vitamin and mineral premix (IU kg-1 or g kg<sup>-1</sup> of premix): vitamin A, 2.5MIU; vitamin D3, 0.25 MIU; vitamin E, 16.7 g; vitamin K3, 1.7 g; vitamin B1, 2.5 g; vitamin B2, 4.2 g; vitamin B3, 25 g; vitamin B5, 8.3; vitamin B6, 2.0 g; vitamin B9, 0.8; vitamin B12, 0.005 g; biotin, 0.17 g; vitamin C, 75 g; choline, 166.7 g; inositol, 58.3 g; ethoxyquin, 20.8 g; copper, 2.5 g; ferrous iron, 10.0 g; magnesium, 16.6 g; manganese, 15.0 g; zinc, 25.0 g;

<sup>5</sup> Yttrium oxide; Stanford Materials, Aliso Viejo, California, United States.

Sydney Essential Oil Co. (Sydney, NSW, Australia).

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 $7$  CHO, carbohydrate calculated by difference (e.g. CHO = 1000 -(protein  $+$  lipid  $+$  ash).

<sup>8</sup> Refer to Table 1 for details.

overnight. The following morning, the collection chamber was removed and the chilled faeces were collected in a plastic sample container and stored at  $-20$  °C until chemical analysis.

### Chemical analyses

Prior to analysis, the diets were each ground to a fine powder using a bench grinder (KnifeTec™ 1095, FOSS Analytical, Hillerød, Denmark). The whole fish were passed through a commercial meat mincer (MGT – 012, Taiwan) twice to obtain a homogeneous mixture. A sample was taken for dry matter analysis and another sample was freeze-dried along with the faecal samples until no further loss of moisture was observed (Alpha 1-4, Martin Christ, Osterode am Harz, Germany). Dry matter was calculated gravimetrically following oven drying at 105 °C for 24 h. Total yttrium concentrations were determined in the diet and faecal samples after nitric acid digestion in a laboratory microwave digester (Ethos One, Milestone, Italy) using inductively coupled plasma-mass spectrophotometry (ICP-MS) (ELAN DRC II; Perkin Elmer, Waltham, MA, USA) following McQuaker et al. (1979). Crude protein was calculated after the determination of total nitrogen by organic elemental analysis (CHNS-O, Flash 2000; Thermo Scientific, Waltham, MA, USA), based on  $N \times 6.25$ . Total lipid content was determined following extraction of the lipids using chloroform : methanol  $(2:1)$  following Folch et al. (1957). Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550 °C for 12 h. Gross energy was determined by adiabatic bomb calorimetry (Parr 6200 Calorimeter, USA).

Fatty acid composition was determined following the methods of Christie (2003). Lipids were esterified by an acid-catalysed methylation, and 0.3 mg of an internal standard was added to each sample (21 : 0; Supelco, St. Louis, MO, USA). The fatty acids were identified relative to the internal standard following separation by gas chromatography (GC). An Agilent Technologies 6890N GC system (Agilent Technologies, Santa Clara, CA, USA) fitted with a DB-23 (60 m  $\times$  0.25 mm  $\times$  0.15 µm, cat 122-2361 Agilent Technologies) capillary column and flame ionization detection was used. The temperature program was 50– 175 °C at 25 °C min<sup>-1</sup> and then 175–230 °C at 2.5  $^{\circ}$ C min<sup>-1</sup>. The injector and detector temperatures were set at 250 and 320 °C, respectively. The column head pressure was set to constant pressure mode at 170 kPa using hydrogen as the carrier gas. The peaks were identified by comparing retention times to the internal standard and further referenced against known standards (37 Comp. FAME mix; Supelco). The resulting peaks were then corrected by the theoretical relative FID response factors (Ackman 2002) and quantified relative to the internal standard.

#### Calculations and statistical analysis

Differences in the ratio of dry matter, protein, lipid and energy to yttrium in the diet and faeces were calculated to determine the apparent digestibility using the following formula: (Maynard & Loosli 1979):

$$
AD_{\text{dict}} = \left(1 - \left(\frac{Y_{\text{dict}} \times \text{Parameter}_{\text{faces}}}{Y_{\text{faces}} \times \text{Parameter}_{\text{dict}}}\right)\right) \times 100
$$

where  $Y_{\text{die}t}$  and  $Y_{\text{faces}}$  represent the yttrium content in the diet and faeces, respectively, and the Parameter<sub>diet</sub> and Parameter<sub>faeces</sub> represent the nutritional parameter (dry matter, protein, lipid or energy) and specific fatty acids in the diet and faeces, respectively. Nutrient retention efficiencies were calculated as the ratio of the nutrient or specific fatty acid gained relative to their respective consumption during the study period using the formula given below (Maynard & Loosli 1979):

$$
Retention % = \left(\frac{Nutrient_f - Nutrient_i}{Nutrient_c}\right) \times 100
$$

where Nutrient<sub>f</sub> is the nutrient/energy content of the fish from each replicate upon termination of the experiment and the Nutrient, is the mean nutrient/energy content of the initial fish ( $n = 6$  fish). Nutrient<sub>c</sub> is the amount of nutrient/energy consumed during the experiment. The computation of apparent in vivo fatty acid elongation, desaturation and  $\beta$ -oxidation was performed using the whole-body fatty acid balance method (WBFABM) following Turchini et al. (2007). Briefly, this involved determination of the appearance/disappearance of specific fatty acids by mass balance. The resulting values of net appearance/disappearance were then transformed to a molecular weight basis per gram of body weight per day (nmol  $g^{-1}$  fish d<sup>-1</sup>). Subsequent back calculations along the known fatty acid bioconversion pathways were used to determine the fate of specific fatty acids.

All data are expressed as mean  $\pm$  SEM (*n* = 3) unless otherwise specified. Data with values <0.05 are reported as 0.1. All data were checked for normal distribution

and homogeneity of variance by qualitative assessment of residual and normal Q-Q plots. All data were analysed by one-way ANOVA or t-test using the RStudio package v.0.98.501 (R Core Team 2012). Any percentage data were arcsine-transformed prior to analysis. Levels of significance were compared using Tukey's HSD a posteriori test with significance among treatments defined as  $P < 0.05$ .

### **Results**

#### Growth and feed utilization

During the 56-d growth assay, the fish in all treatments responded readily to the experimental diets and growth in the CTRL-D group was consistent with the predicted model growth, achieving 106% of the modelled potential (Glencross & Bermudes 2012). Live-weight measurements were conducted after 4 weeks of feeding and then again after 8 weeks, and although there was a tendency towards lower growth in the SFA-D-fed and MUFA-D-fed fish at both time points, there were no significant differences (Table 3). The same trend was observed in terms of daily feed intake and growth rate with no significant differences, and there was no difference in FCR values (Table 3). There were no differences in terms of survival with only one fish that died and was removed from the system over the term of the experiment (Table 3).

The apparent digestibility of macronutrients and specific fatty acids was affected by the diets (Table 4). Both dry matter (DM) and lipid were significantly less digestible in the SFA-D-fed group of fish. There were no significant differences in either protein or gross energy (GE) digestibility among the treatments (Table 4). There were no significant differences in the digestibility of individual and total LC-PUFA among the diets, each showing almost complete digestion in most cases. Similarly, there were no significant differences in the digestibility of individual and total PUFA and 18:1. However, the saturated fatty acids were significantly affected, with 18:0 and 16:0 being less digestible in the SFA-D-fed fish compared to the other treatments. The total SFA digestibility was also significantly reduced in the SFA-D-fed fish (Table 4).

#### **Biochemical analysis**

The whole-body DM, protein and lipid compositions on a wet weight basis were not significantly affected by the diets;

Table 3 Growth performance and feed utilization of juvenile barramundi fed experimental diets for 8 weeks



ND, not detected, N/A, not analysed, values <0.1 are reported as <0.1.

 $1 P < 0.05$ \*; One-way ANOVA, DF 2,6, post hoc Tukey's HSD. Superscript letters indicate different levels of significance between treatment diets; percentage data were arcsine-transformed prior to analysis.



ND, not detected, N/A, not analysed, values <0.1 are reported as <0.1.

 $1 P < 0.05$ \*,  $P < 0.01$ \*\*; One-way ANOVA, DF 2,6, post hoc Tukey's HSD; t-test, DF 4, was used to test two variables; superscript letters indicate different levels of significance between treatment diets; percentage data were arcsine-transformed prior to analysis.

<sup>2</sup> Refer to Table 1 for details.

however, the GE was significantly lower in the SFA-D-fed fish (Table 5). Whole-body total fatty acids were not significantly different; however, there were some significant differences between the specific fatty acids. Among the LC-PUFA, the whole-body 22:6n-3 and 22:5n-3 were significantly higher in the CTRL-D-fed fish compared to the MUFA-D-fed fish. The whole-body 18:2n-6 was significantly highest in the MUFA-D and lowest in the CTRL-D (Table 5). The whole-body 18:1 was significantly highest in the MUFA-D-fed fish, whereas 18:0 and 16:0 were lowest in the MUFA-D-fed fish (Table 5). The total LC-PUFA and the total n-3 fatty acids were significantly lowest in the MUFA-D-fed fish (Table 5).

The liver fatty acid composition was affected in a similar fashion to that of the whole-body composition where 22:6n-3, 22:5n-3, 20:5n-3 and 20:4n-6 had significantly higher composition in the CTRL-D-fed fish. However, there were no significant differences among the MUFA-D or SFA-d diets (Table 5). There was no significant difference in PUFA composition of the liver. The total

Table 4 Apparent digestibility coefficients of macronutrients and specific fatty acids of experimental diets fed to juvenile barramundi. All data  $(n = 3)$ per treatment) are reported as apparent digestibility percentage (%)

**Table 5** Whole-body composition data  $(n = 3$  per treatment, g kg<sup>-1</sup> live basis). Whole-body and liver fatty acid data  $(n = 3$  per treatment,  $%$  total)

	Initial	CTRL-D	SFA-D	MUFA-D	TEST <sup>1</sup>
Whole-body composition					
Dry matter	$265 \pm 0.4$	$329 \pm 0.5$	$318 \pm 0.3$	$324 \pm 0.3$	$F = 2.1, P = 0.21$
Protein	$188 \pm 0.5$	$206 \pm 0.7$	$191 \pm 0.8$	$200 \pm 0.6$	$F = 1.2, P = 0.36$
Lipid	$51\,\pm\,0.4$	$95 \pm 0.6$	$91 \pm 0.2$	$94 \pm 0.2$	$F = 0.3, P = 0.73$
Gross energy (MJ $kg^{-1}$ )	$61 \pm 0.2$	$84 \pm 0.2^{\circ}$	$77 \pm 0.1^b$	79 $\pm$ <0.1 <sup>ab</sup>	$F = 8.0*$
Whole-body fatty acids $(\%)^2$					
Total FA (mg $g^{-1}$ lipid)	681.0 $\pm$ 35.1	616.6 $\pm$ 19.2	$665.7 \pm 68.1$	647.7 $\pm$ 44.7	$F = 0.4, P = 0.69$
16:0	$26.1 \pm 0.2$	$26.9 \pm 0.5^{\circ}$	$28.0 \pm 0.2^a$	$22.4 \pm 0.1^b$	$F = 74.3***$
18:0	$7.8 \pm 0.2$	7.4 $\pm$ 0.1 <sup>b</sup>	$9.9 \pm 0.2^{\circ}$	$6.4 \pm 0.1a$	$F = 148.8***$
18:1	$37.9 \pm 0.8$	$30.1 \pm 0.5^{\circ}$	$30.9 \pm 0.2^a$	41.7 $\pm$ 0.1 <sup>b</sup>	$F = 371.0***$
$18:2n-6$	$9.7 \pm 0.3$	$8.8 \pm 0.2^{\circ}$	$10.2 \pm 0.2^b$	$11.0 \pm 0.1^c$	$F = 37.4***$
$18:3n-3$	$1.0 \pm 0.1$	$1.0 \pm \le 0.1$	$1.0 \pm < 0.1$	$1.1 \pm 0.1$	$F = 3.3, P = 0.11$
20:4n-6	$0.4 \pm 0.0$	$0.7 \pm 0.1^a$	$0.5 \pm \le 0.1^{\rm b}$	$0.5 \pm \le 0.1^b$	$F = 14.7$ **
$20:5n-3$	$1.4 \pm 0.1$	$3.7 \pm 0.3^a$	$2.6 \pm 0.1^b$	$2.2 \pm 0.1^b$	$F = 13.1**$
$22:5n-3$	$0.8 \pm 0.0$	$1.6 \pm 0.1^a$	$1.3 \pm 0.1^{ab}$	$1.2 \pm \le 0.1^{\rm b}$	$F = 5.3*$
$22:6n-3$	$2.1 \pm 0.2$	$5.5 \pm 0.6^{\circ}$	$4.3 \pm 0.2^{ab}$	$3.8 \pm 0.1^b$	$F = 6.1*$
<b>SFA</b>	$38.5 \pm 0.2$	$39.3 \pm 0.7^{\circ}$	$41.4 \pm 0.4^{\circ}$	$31.9 \pm 0.1^b$	$F = 102.4***$
<b>MUFA</b>	$45.7 \pm 0.5$	$37.7 \pm 0.7^{\rm a}$	$36.6 \pm 0.1^a$	$46.6 \pm 0.1^b$	$F = 198.1***$
$C_{18}$ PUFA	$11.0 \pm 0.1$	$11.7 \pm 0.3^{\circ}$	$13.2 \pm 0.3^b$	$13.9 \pm 0.1^{\rm b}$	$F = 26.6$ **
LC-PUFA	$4.7 \pm 0.2$	$11.4 \pm 1.1^a$	$8.8 \pm 0.4^{\circ}$	$7.6\,\pm\,0.1^{\rm b}$	$F = 7.7*$
$n-3$	$4.3 \pm 0.0$	$11.9 \pm 1.2^{\circ}$	$9.3 \pm 0.4^{\circ}$	$8.2 \pm 0.1^{\rm b}$	$F = 7.0*$
$n-6$	$11.4\,\pm\,0.1$	$11.2\,\pm\,0.2^{\mathrm{a}}$	$12.7 \pm 0.2^b$	$13.4 \pm 0.1^{\rm b}$	$F = 38.1***$
Liver fatty acids $(\%)^2$					
16:0	$27.8 \pm 0.4$	$30.8 \pm 0.4^{ab}$	33.7 $\pm$ 0.7 <sup>b</sup>	$27.6 \pm 1.3^a$	$F = 12.9**$
18:0	$11.5 \pm 0.2$	$10.6 \pm 0.2^{\circ}$	$13.9 \pm 0.4^b$	$9.8 \pm 0.8^a$	$F = 17.5***$
18:1	34.8 $\pm$ 0.3	$31.1 \pm 0.1^a$	$33.3 \pm 0.8^{\rm a}$	$39.8 \pm 0.6^b$	$F = 59.3***$
$18:2n-6$	$6.4 \pm 0.2$	$4.8 \pm 0.2$	$4.3 \pm 0.6$	$6.9 \pm 1.2$	$F = 3.0, P = 0.12$
$18:3n-3$	$0.6 \pm 0.0$	$0.5 \pm < 0.1$	$0.4 \pm 0.1$	$0.6 \pm 0.2$	$F = 1.5, P = 0.29$
20:4n-6	$0.7 \pm 0.0$	$0.8 \pm 0.1^a$	$0.5 \pm \le 0.1^{\rm b}$	$0.5 \pm 0.1^b$	$F = 37.4***$
$20:5n-3$	$1.7 \pm 0.1$	$2.7\,\pm\,0.1^{\rm a}$	$1.3 \pm 0.1^{\rm b}$	$1.5 \pm 0.3^b$	$F = 18.0**$
$22:5n-3$	$1.1 \pm 0.1$	$1.4 \pm 0.1^a$	$0.7 \pm 0.1^{\rm b}$	$0.9 \pm 0.1^{\rm b}$	$F = 14.4***$
22:6n-3	$4.4 \pm 0.1$	$4.8 \pm 0.1^a$	$2.7 \pm 0.2^b$	$2.8 \pm 0.3^{b}$	$F = 15.0**$
<b>SFA</b>	42.9 $\pm$ 0.3	$45.5 \pm 0.5^{ab}$	50.5 $\pm$ 0.9 <sup>b</sup>	$40.5 \pm 1.8^a$	$F = 17.4**$
<b>MUFA</b>	$40.0 \pm 0.3$	$37.9 \pm 0.3^{\circ}$	$38.2 \pm 0.7^{\rm a}$	$44.8\,\pm\,0.6^{\text{b}}$	$F = 48.9***$
$C_{18}$ PUFA	$8.5 \pm 0.1$	$6.2 \pm 0.2$	$5.5 \pm 0.7$	$8.4 \pm 1.2$	$F = 3.4, P = 0.10$
LC-PUFA	$8.6 \pm 0.1$	$10.4 \pm 0.1^{\circ}$	5.7 $\pm$ 0.4 <sup>b</sup>	$6.3 \pm 0.7^b$	$F = 29.6***$
$n-3$	$8.6 \pm 0.1$	$9.7 \pm 0.1^a$	5.6 $\pm$ 0.5 <sup>b</sup>	$6.1 \pm 0.9^b$	$F = 17.6$ **
$n-6$	$8.5 \pm 0.2$	$6.8 \pm 0.2$	$5.7 \pm 0.7$	$8.6 \pm 1.0$	$F = 3.9, P = 0.08$

ND, not detected, N/A, not analysed, values <0.1 are reported as <0.1.

 $1 P < 0.05$ \*,  $P < 0.01$ \*\*,  $P < 0.001$ \*\*\*; One-way ANOVA, DF 2,6, post hoc Tukey's HSD; superscript letters indicate different levels of significance between treatment diets; percentage data were arcsine-transformed prior to analysis.

<sup>2</sup> Refer to Table 1 for details.

LC-PUFA and n-3 compositions were significantly higher in the CTRL-D-fed fish, and there was no significant difference in n-6 composition of the liver (Table 5).

#### Mass balance computations

There were no significant differences in protein or lipid retention; however, there was significantly higher energy retention in the CTRL-D-fed fish (Table 3). There was significantly higher b-oxidation of specific n-3 series fatty acids (22:6n-3, 20:5n-3 and 18:4n-3) and n-6 series fatty acids (18:3n-6 and 20:4n-6) in the CTRL-D diet compared to the other treatment diets (Table 6). There was no recorded  $\beta$ -oxidation for any of the dominant saturates (including 12:0, 14:0, 16:0 and 18:0); however, there was a significant increase in  $\beta$ -oxidation of n-7 series FA (16:1n-7 and 18:1n-7) in the CTRL-D diet compared to the other treatment diets. There was a significant increase in the  $\beta$ oxidation of 18:1 fatty acids in the MUFA-D diet compared to the other treatment diets (Table 6).

There was significantly higher *de novo* fatty acid production of 12:0 (neogenesis) in the MUFA-D-fed fish compared to the other treatment diets (Table 6). The recorded elongation activity of 14:0 to 14:1n-5 was significantly higher in the CTRL-D- and MUFA-D-fed fish, while the elongation of 16:0 to 16:1n-7 was highest in the CTRL-Dand SFA-D-fed fish. Elongation of 20:5n-3 to 22:5n-3 was only recorded in the fish fed the SFA-D and MUFA-D diets, and there was no significant difference (Table 6). The  $\Delta$ -9 desaturation activity was significantly highest in the SFA-D-fed fish followed by the control-fed fish, and there was none recorded in the MUFA-D-fed fish (Table 6). There was no  $\Delta$ -5 or  $\Delta$ -6 desaturation activity detected in any of the treatment diets (data not presented).

Relative to the total intake of specific fatty acids, there was a significantly greater proportion of total LC-PUFA deposited in the SFA-D- and MUFA-D-fed fish; however, there was no difference between these two groups (Table 7). Likewise, there was proportionally less  $\beta$ -oxidation in the SFA-D-fed and MUFA-D-fed fish; however, there was no difference between these two groups (Table 7). There was only a minor proportion of the consumed LC-PUFA converted or excreted in the groups of fish.

# **Discussion**

The present study demonstrated that the inclusion of a 2 : 1 or 1 : 2 ratio of either SFA or MUFA did not affect the growth performance of juvenile barramundi. The simultaneous reduction in dietary LC-PUFA (~11% LC-PUFA in SFA- and MUFA-D versus 21.5% LC-PUFA in CTRL-D), provided that they were still above reported requirements of 1.2% LC-PUFA, also had no effect on performance (Williams et al. 2006). Moreover, the feed intake and FCR values were unaffected. This is consistent with a range of species showing that substitution of FO with lipid rich in either SFA or MUFA does not affect fish growth performance (Turchini et al. 2009). The fish in the present study more than tripled in size suggesting that trial duration or nutrient turnover was not a confounding issue. The fish in the SFA-D and MUFA-D treatments did show a numerical reduction in growth; however, this was not confirmed statistically. It is uncertain whether longer trial

Table 6 Whole-body fatty acid mass balance computations of  $\beta$ -oxidation, elongation and desaturation activity of juvenile barramundi fed experimental diets for 8 weeks. All data ( $n = 3$  per treatment) are reported as nmol  $g^{-1}$ fish  $d^{-1}$  basis



ND, not detected, N/A, not analysed, values <0.1 are reported as <0.1.

 $1$  P < 0.05\*, P < 0.01\*\*, P < 0.001\*\*\*; One-way ANOVA, DF 2,6, post hoc Tukey's HSD; t-test, DF 4, was used to test two variables. Superscript letters indicate different levels of significance between treatment diets; percentage data were arcsine-transformed prior to analysis.  $2$  Computations following Turchini et al. (2007). Refer to Table 1 for details.



Table 7 Calculated summary of LC-PUFA flux in juvenile barramundi. All data are  $(n = 3)$  reported as a percentage based on the total intake of each fatty acid (nmol  $g^{-1}$  fish  $d^{-1}$ )

ND, not detected, N/A, not analysed, values <0.1 are reported as <0.1.

 $1 P < 0.05* P < 0.01**$ ; One-way ANOVA, DF 2,6, post hoc Tukey's HSD; t-test, DF 4, was used to test two variables; superscript letters indicate different levels of significance between treatment diets; percentage data were arcsine-transformed prior to analysis.

 $2$  Assumed that no further conversion occurs (Turchini et al. 2007).

duration would have resulted in significant differences as recent studies with barramundi have demonstrated that changes to the lipid profile of the diets can have rapid metabolic effects (Salini et al. 2015b). Lipid and in particular the saturated fatty acids are generally less digestible at lower environmental temperature and as a result less energy availability for growth (Olsen & Ringø 1998; Ng et al. 2004). The barramundi is a tropical species adapted to high water temperature and potentially better able to cope with dietary SFA- and MUFA-rich lipid.

The replacement of FO with MUFA-rich lipid such as that from poultry oil had no effect on growth or FCR in a range of species including rainbow trout (Oncorhynchus mykiss) juveniles (Fonseca-Madrigal et al. 2005), postsmolt Atlantic salmon (Salmo salar) (Bell et al. 2002) or large 1.5-kg Atlantic salmon (Torstensen et al. 2000), juvenile red hybrid tilapia (Oreochromis sp.) (Bahurmiz & Ng 2007), humpback grouper (Cromileptes altivelis) (Shapawi et al. 2008) and African catfish (Clarias gariepinus) (Ng et al. 2003). However, consistent among these studies when FO was completely replaced by MUFA-rich lipid was the modified tissue FA profile resulting in reduced concentration of the beneficial n-3 LC-PUFA. The same effect was clearly noted in the present study. However, proportional to the LC-PUFA intake, from the results of most studies

and those of the present study we can conclude that MUFA-rich lipid is an ideal energy source capable of 'sparing' the more valuable LC-PUFA from b-oxidation.

An important consideration when formulating with SFA-rich palm oil (PO) is the fraction used (Ng & Gibon 2011). Past studies demonstrated that growth performance (Ng et al. 2003; Shapawi et al. 2008) and digestibility of a range of species were not affected by different PO fractions (Bahurmiz & Ng 2007), while the digestibility of palm products was significantly reduced compared to FO. Recent studies have also concluded that other sources of lipid rich in SFA such as beef tallow did not affect the growth of Atlantic salmon (Emery et al. 2014) or rainbow trout (Trushenski et al. 2011). Consistent with these reports, the present study demonstrated that the blend of palm products used did not compromise growth performance in barramundi, while there were notable reductions to the lipid and specific fatty acid digestibility.

In the present study, the digestibility of total lipid was significantly reduced in the SFA-fed fish; however, this did not lead to a reduction in energy availability or any changes in whole-body lipid composition or retention. The reduction in lipid digestibility was evidently a result of the greatly reduced digestibility of the saturates, including both 16:0 and 18:0 FA. This is in agreement with other studies

showing that the digestibility of the saturated fatty acids was reduced when a range of species were fed SFA-rich oil (Torstensen et al. 2000; Caballero et al. 2002; Ng et al. 2004; Turchini et al. 2013). Moreover, in the present study, the digestibility of SFAs decreased with increasing chain length, consistent with other studies (Johnsen et al. 2000; Caballero et al. 2002; Ng et al. 2004). The reduced rate of lipolysis and absorption of longer chain SFA, such as 18:0, is caused by the lower ability of these FA to form lipid emulsions prior to digestion (Olsen et al. 1998).

In contrast to the present study, negative effects of the high-level inclusion of SFA (e.g. as occurs with high-level use of PO) have been reported and that these effects are arguably more pronounced in carnivorous and/or marine fish with higher n-3 LC-PUFA requirements. Fountoulaki et al. (2009) found that gilthead sea bream growth was negatively impacted by reduced digestibility of SFA-rich lipid over an extended growth trial of 6 months. Growth performance of Japanese sea bass (Lateolabrax japonicas) was significantly reduced with a high inclusion of SFA-rich lipid after only 50 days of growth and similarly reduced digestibility was inferred to be the cause (Gao *et al.* 2012). In agreement, Turchini et al. (2013) found that over a long duration (27 weeks), rainbow trout fed SFA-rich lipid (at 75% replacement level) showed depressed performance compared to that of a control diet.

An in vivo whole-body fatty acid balance method (WBFABM) was used in the present study to understand the apparent fate of specific fatty acids (Turchini et al. 2007). Theoretically, b-oxidation of specific fatty acids should be recorded if they are provided in excess (Stubhaug et al. 2007; Eroldogan et al. 2013; Turchini et al. 2013). However, in the present study, there was no recorded  $\beta$ oxidation of any SFA despite the relatively high proportion of SFA in all three diets. This effect is likely to be caused by the low lipid levels of all three diets, a strategy that was intended to highlight the potential effects of the lipid classes. Based on the  $\beta$ -oxidation results, it appears that MUFA is marginally better at sparing LC-PUFA from  $\beta$ oxidation (Table 6), which is in agreement with past studies (Turchini et al. 2011; Codabaccus et al. 2012). However, in the present study, the final composition of the fish suggests that the SFA-D-fed fish were significantly more efficient at depositing or 'sparing' LC-PUFA in the whole body (Table 5). To resolve this discrepancy, it is necessary to look at the net intake and total intake budgets to clarify the situation (Table 7). When expressed proportional to FA intake, the SFA-D- and MUFA-D-fed fish consequently deposited almost exactly the same n-3 LC-PUFA

and the differences between the two diets were insignificant.

Previous studies have demonstrated that limited de novo FA production (neogenesis) occurs when diets with adequate SFA were fed to Atlantic salmon (Emery et al. 2014) or rainbow trout (Turchini et al. 2013). However, neogenesis was evident in trout fed MUFA-rich (canola oil) diet (Turchini et al. 2013). This is mostly similar to the results obtained in the present study in that neogenesis in barramundi was significantly higher in the MUFA-fed fish. This may be partly explained by the upregulation of genes related to lipogenic activity in response to vegetable oil that is observed in other species (Bell et al. 2001, 2002; Tocher et al. 2002). In contrast, dietary PUFA clearly demonstrated a suppression of the lipogenic enzyme, fatty acid synthase (FAS), in the rat (Blake & Clarke 1990). Moreover, in rainbow trout hepatocytes, FAS expression was strongly inhibited by PUFA (18:3n-3 and 20:5n-3). These results and those of the present study confirm that the energetically expensive process of initial synthesis of palmitic acid from acetyl- and malonyl-CoA is avoided by the presence of dietary SFA, thus allowing energy to be utilized more efficiently for growth and other cellular processes.

Elongation and desaturation activities also occurred in the CTRL-D-fed fish, suggesting that the total lipid content of the diet was limiting as intended based on the optimal specification of at least 18% for growing barramundi (Glencross et al. 2013). However, there was clearly adequate n-3 LC-PUFA in the diets based on the known requirement data for barramundi of around 1.2% (Williams et al. 2006; Glencross & Rutherford 2011). Consistent with Glencross & Rutherford (2011), there was no elongation of 20:5n-3 to 22:5n-3 in the control-fed fish. However, in the SFA-D- and MUFA-D-fed fish, there was a slight increase in the elongation of available 20:5n-3 to 22:5n-3 possibly in an attempt to achieve 22:6n-3 synthesis. However, barramundi, like most marine fish, are not equipped with the complete set of enzymes required to endogenously synthesize sufficient LC-PUFA from precursor FA (Mohd-Yusof et al. 2010). Moreover, a recent study also demonstrated a similar increase in 22:5n-3 from 20:5n-3, lending further support to the elongation results obtained in the present study (Salini et al. 2015a).

# **Conclusions**

The results of this study demonstrate that the inclusion of a 2 : 1 or 1 : 2 ratio of SFA or MUFA did not lead to a

reduction in growth performance of juvenile barramundi. However, a range of other metabolic modifications were observed, notable was the LC-PUFA sparing effect of both MUFA and SFA. Additionally, SFA and MUFA were preferentially metabolized and deposited in the whole body and liver tissue proportional to their respective intake. The low digestibility of specific fatty acids (18:0 and 16:0) is consistent with other studies and may have an impact in the long-term utilization of the SFA-rich diet. These results clearly indicate that consideration must be given to the proportion of either SFA or MUFA during diet formulation, as these two classes of fatty acids can influence the in vivo metabolism of fatty acids and the final fatty acid composition of the whole fish.

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