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

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Abstract

Barramundi (*Lates calcarifer*), a catadromous teleost of commercial interest, perform well when fed a wide range of different dietary oils in varying combinations. However, as critical limits of fish oil (FO) are approached, a range of alternative oils are being explored that are typically richer in shorter chain and more saturated fatty acids. In this study, the response of juvenile barramundi (47.0 g/fish initial weight) fed isolipidic and isoenergetic diets with 8.2% added oil was tested. The experimental test diets were either 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 a period of eight weeks. The replacement of FO with diets containing mostly olive oil (diet MUFA-D) and mostly refined palm oil (diet SFA-D) did not impact the growth performance or feed utilisation parameters of fish in this study. However, the composition of the tissues generally reflected the diets and mass-dependent retention of energy was observed in the SFA-D and MUFA-D fed fish. The *in vivo* beta-oxidation activity was consistent with the diet fatty acid composition with the most dominant FA being heavily oxidised. *In vivo* elongation and desaturation activity indicated both a demand for initial synthesis of fatty acids and energy production. However, despite the similarities, some vagaries in the results were observed and discussed. 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.

Introduction

In the context of feeding fish alternative oils, many studies have focused on the essential fatty acids and their bioactive long-chain derivatives, eicosapentaenoic and docosahexaenoic acid (20:5n-3 and 22:6n-3). However, it is suggested that saturated and monounsaturated fatty acids (SFA and MUFA) are preferred substrates for β-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 seabass, must rely heavily on 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, Echium, usually included as blends (Williams et al. 2006, Alhazzaa et al. 2011, Raso & Anderson 2003). Moreover, it is also clear that higher dietary levels of long-chain polyunsaturated (LC-PUFA), originating from FO, are oxidised 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 β-oxidation (Menoyo et al. 2003). In the same species, β-oxidation was found to be dependent on energy demand and that when fatty acids were provided in excess to synthetic demands they were increasingly β-oxidised for energy production (Stubhaug et al. 2007, Torstensen et al. 2004). Denstadli et al. (2011) found that the medium chain length decanoic acid (10:0) was rapidly oxidised 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 β-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, polka dot grouper (*Cromileptes altivelis*) more readily oxidised 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 (eg. 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 oxidised fatty acids were also the most dominant. However, in the fish oil fed fish the most predominant dietary fatty acid (16:0) was not readily oxidised 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 β-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* × 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 oxidation (Trushenski et al. 2015).

Consistent responses of a range of fish species indicate that SFA and MUFA provided in surplus are heavily oxidised 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 β-oxidised (Turchini et al. 2013, Torstensen et al. 2000). Recent studies have shown that barramundi are capable of utilising

poultry oil, characterised by high MUFA content, however to what extent SFA are preferentially utilised or able to 'spare' LC-PUFA remains unclear. 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 hypothesised that these lipid classes would be metabolised differently. The digestibility of the diets was also measured and mass-balance computations 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 hammermill (Mikro Pulverizer, type 1 SH, New Jersey, USA) such that the maximum particle size was less than 750 µm. 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 left to soak for 3h 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 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 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 60%, lipid at 13% with an energetic value of 22 MJ/kg. 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). The present study used a blend of two common palm fractions, refined, bleached and deodorised palm oil (RBDPO) and palm flake, a highly refined stearin fraction. The RDBPO fraction used in the present 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), on-grown in a 10,000L 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 (300L) with flow-through seawater (salinity =35 g/kg; dissolved oxygen 4.6 ± 0.15 mg /L) maintained at 30.0 ± 0.01 ºC (mean ± SD) with a supply rate of about 3 L/min to each of the tanks. At the beginning of the experiment, the tanks held 26 fish of 47.0 ± 0.3 g (mean ± SD, n =234 individually weighed fish). The three experimental diets were randomly distributed amongst 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 eight 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 ºC 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 then attached to the evacuation line of a swirl separator and left overnight. The following morning, the collection chamber was removed and the chilled faeces were captured 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, 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, Germany). Dry matter was calculated gravimetrically following oven drying at 105ºC 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, 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, USA), based on N x

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, PA, 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, California, USA) fitted with a DB-23 (60m x 0.25mm x 0.15 μm, cat 122-2361 Agilent Technologies, California) capillary column and flame ionisation detection was used. The temperature program was 50–175 ºC at 25 ºC /min then 175–230 ºC at 2.5 ºC /min. The injector and detector temperatures were set at 250 ºC and 320 ºC, 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, PA, USA). 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 formula: (Maynard & Loosli 1979):

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

Where Y_{diet} and Y_{faeces} represent the yttrium content in the diet and faeces respectively and the $Parameter_{diet}$ and $Parameter_{faeces}$ 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 (Maynard & Loosli 1979):

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

Where $Nutrient_f$ is the nutrient/energy content of the fish from each replicate upon termination of the experiment and the $Nutrient_i$ is the mean nutrient/energy content of the initial fish (n=6 fish). $Nutrient_c$ is the amount of nutrient/energy consumed during the experiment. The computation of apparent *in vivo* fatty acid elongation, desaturation and β-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 fish/d). 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 ± 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 utilisation

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 four weeks of feeding and then again after eight weeks and although there was a tendency towards lower growth in the SFA-D and MUFA-D 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 macro nutrients 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 composition on a wet weight basis was not significantly affected by the diets; however, the GE was significantly lower in the SFA-D fed fish (Table 5). Generally, the fatty acid composition of the diets was reflected in the whole fish and also the liver tissue. 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 was significantly higher in the CTRL-D fish compared to the MUFA-D 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 (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 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 LC-PUFA and n-3 composition were significantly higher in the CTRL-D 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 fish (Table 3). There was significantly higher β-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 β-oxidation for any of the dominant saturates (including 12:0, 14:0, 16:0 and 18:0) however there was a significant increase in β-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 β-oxidation of 18:1 fatty acids in the MUFA-D diet compared to the other treatment diets (Table 6).

There was significantly higher fatty acid *de novo* 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-D and 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 Δ-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 Δ-5 or Δ-6 desaturation activity detected in any of the diet treatments (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 β-oxidation in the SFA-D and MUFA-D 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 of dietary LC-PUFA (~11% LC-PUFA in SFA- and MUFA-D vs 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 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 (Ng et al. 2004, Olsen & Ringø 1998). 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), post-smolt 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, the results of most studies and those of the present study conclude that MUFA rich lipid is an ideal energy source capable of 'sparing' the more valuable LC-PUFA from β-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 (Shapawi et al. 2008, Ng et al. 2003) and digestibility of a range of species was 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, Ng et al. 2004, Turchini et al. 2013, Caballero et al. 2002). Moreover, in the present study the digestibility of SFA's decreased with increasing chain length, consistent with other studies

(Caballero et al. 2002, Ng et al. 2004, Johnsen et al. 2000). 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 six months. Japanese seabass (*Lateolabrax japonicas*) growth performance 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 in order to understand the apparent fate of specific fatty acids (Turchini et al. 2007). Theoretically, β-oxidation of specific fatty acids should be recorded if they are provided in excess (Turchini et al. 2013, Eroldogan et al. 2013, Stubhaug et al. 2007). However, in the present study there was no recorded β-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 β-oxidation results, it appears that MUFA is marginally better at sparing LC-PUFA from 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 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 MUFA rich (canola oil) fed trout (Turchini et al. 2013). This is broadly 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 up-regulation of genes related to lipogenic activity in response to vegetable oil observed in other species (Tocher et al. 2002, Bell et al. 2001, Bell 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 utilised more efficiently for growth and other cellular processes.

There was also elongation and desaturation activity occurring in the CTRL-D fed fish. This may indicate that the total lipid content of the diet was limiting as intended based on the recommended 14 to 16 % specification for growing barramundi (Glencross 2006). However, there was clearly adequate n-3 LC-PUFA in the diets based on the known requirement data for barramundi of around 1.2% (Glencross & Rutherford 2011, Williams et al. 2006).

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 synthesise 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 the present study demonstrate that the inclusion of 2:1 or 1:2 ratios of SFA and 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 metabolised 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 utilisation 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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Table 1 Chemical composition of ingredients used in experimental diets, all values are presented as g/kg 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)	984	958	958	927	839	924	836	992	987	1003	994
Protein	789	641	895	823	112	870	5	4	4	3	4
Ash	163	138	46	1	6	11	3	1	ND	ND	ND
Lipid	46	151	57	121	22	5	0	956	973	963	986
Carbohydrate [#]	1	70	2	55	860	113	992	39	23	34	10
Gross energy (MJ/kg)	18.9	20.4	21.8	21.2	15.3	21.9	14.5	39.3	39.5	39.5	39.3
Fatty acids (%) ^											
16:0	25.8	25.4	17.8	-	-	-	-	22.9	9.9	51.9	46.2
18:0	8.7	8.6	4.5	-	-	-	-	5.1	3.0	4.6	51.3
18:1	15.5	43.8	25.1	-	-	-	-	18.6	73.8	34.6	0.4
18:2n-6	1.7	11.0	46.5	-	-	-	-	2.0	11.0	6.7	ND
18:3n-3	0.8	1.0	5.1	-	-	-	-	1.0	1.0	ND	ND
20:4n-6	2.5	0.6	ND	-	-	-	-	1.5	ND	ND	ND
20:5n-3	9.0	0.5	ND	-	-	-	-	11.3	ND	ND	ND
22:5n-3	2.2	ND	ND	-	-	-	-	2.1	ND	ND	ND
22:6n-3	19.9	ND	ND	-	-	-	-	14.2	ND	ND	ND
SFA	39.8	36.1	22.6	-	-	-	-	36.4	13.4	58.6	99.6
MUFA	22.2	50.8	25.8	-	-	-	-	29.1	74.6	34.7	0.4
PUFA	3.4	12.0	51.6	-	-	-	-	4.9	12.0	6.7	ND
LC-PUFA	34.5	1.2	ND	-	-	-	-	29.7	ND	ND	ND
n-3	32.7	1.6	5.1	-	-	-	-	30.5	1.0	ND	ND
n-6	5.2	11.6	46.5	-	-	-	-	4.1	11.0	6.7	ND

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

[#] Carbohydrate calculated by difference (eg. carbohydrate = 1000 – (protein + lipid + ash)

^ All fatty acids are presented as a percentage of the total fatty acids. Quantitative data can be obtained by multiplying the total FA (mg/g 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 of14:1n-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 18:2n-6 (cis and trans), 18:3n-6, 18:3n-3, 18:4n-3; long chain polyunsaturated fatty acids (LC-PUFA), sum 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 6 PUFA and LC-PUFA.

Table 2 Formulation and composition of experimental diets. All values are g/kg DM unless otherwise stated.

	CTRL-D (Fish oil)	SFA-D (Palm oil)	MUFA-D (Olive oil)
Formulation			
DF Fish meal [a]	150	150	150
Poultry meal [a]	150	150	150
Soy protein isolate [b]	150	150	150
Wheat gluten [b]	150	150	150
Wheat flour [b]	109	109	109
Casein [c]	100	100	100
Pregelled wheat starch [b]	80	80	80
DL-Methionine [c]	10	10	10
Di-calcium phosphate	10	10	10
Pre-mix vitamins and minerals [d]	8	8	8
Yttrium oxide [e]	1	1	1
Fish oil [a]	82	41	41
Olive oil [f]	0	0	41
Palm Oil [f]	0	15	0
Palm Flake [f]	0	26	0
Composition as analysed			
Dry matter (g/kg)	940	952	979
Protein	598	601	585
Ash	64	63	60
Lipid	126	137	135
Carbohydrate [#]	204	192	217
Gross energy (MJ/kg DM)	21.5	21.6	22.2
Fatty acids (%) ^			
Total FA (mg/g lipid)	801.7	734.7	717.4
16:0	22.9	32.0	18.4
18:0	5.5	16.1	4.8
18:1	22.7	20.6	42.6
18:2n-6	10.0	10.5	13.3
18:3n-3	1.3	1.0	1.3
20:4n-6	1.2	0.7	0.7
20:5n-3	8.1	4.1	4.0
22:5n-3	1.6	0.9	0.9
22:6n-3	10.6	5.4	5.4
SFA	34.5	51.8	26.4
MUFA	31.3	25.0	47.3
PUFA	12.7	12.1	15.3
LC-PUFA	21.5	11.0	10.9
n-3	22.9	12.0	12.2
n-6	11.3	11.1	14.0

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

[#] Carbohydrate calculated by difference (eg. carbohydrate = 1000 – (protein + lipid + ash)

a Ridley aquafeeds, Narangba, QLD, Australia

b Manildra Group, Rocklea, QLD, Australia

c Bulk Powders, www.bulkpowders.com.au

d Vitamin and mineral premix (IU kg-1 or g/kg 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
e Yttrium oxide; Stanford Materials, Aliso Viejo, California, United States
f Sydney Essential Oil Co. (Sydney, NSW, Australia)
^ Refer to Table 1 for details.

Table 3 Growth performance and feed utilisation of juvenile barramundi fed experimental diets for eight weeks.

	CTRL-D	SFA-D	MUFA-D	TEST ^
Week 0 weight (g)	46.9 ± 0.1	47.3 ± 0.2	47.1 ± 0.1	F=2.2, P=0.20
Week 4 weight (g)	139.2 ± 1.5	135.3 ± 7.8	136.0 ± 3.0	F=0.9, P=0.45
Week 8 weight (g)	238.3 ± 1.2	231.2 ± 9.0	230.9 ± 6.2	F=0.4, P=0.66
Feed intake (g/fish)	209.6 ± 2.7	202.4 ± 5.0	200.7 ± 4.9	F=0.6, P=0.58
Growth rate (g/fish/d)	3.4 ± 0.1	3.3 ± 0.2	3.3 ± 0.1	F=0.5, P=0.65
FCR	1.10 ± 0.1	1.12 ± 0.1	1.09 ± 0.1	F=2.0, P=0.21
Survival (%)	98.0 ± 0.1	100.0 ± 0.0	100.0 ± 0.0	F=1.0, P=0.42
Protein retention (%)	34.1 ± 1.1	29.8 ± 1.9	32.5 ± 0.9	F=2.5, P=0.16
Lipid retention (%)	48.6 ± 4.5	40.2 ± 1.7	45.9 ± 0.7	F=2.4, P=0.17
Energy retention (%)	37.8 ± 0.6[a]	33.5 ± 1.0[b]	34.4 ± 0.3[b]	F=10.1*

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

^ 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.

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

	CTRL-D	SFA-D	MUFA-D	TEST ^
Macro nutrient digestibility (%)				
Dry matter	64.3 ± 1.3[ab]	60.3 ± 1.5[b]	69.4 ± 0.2[a]	F=9.6*
Protein	91.1 ± 0.3	92.0 ± 1.2	92.1 ± 1.4	F=0.2, P=0.85
Lipid	91.0 ± 0.7[a]	73.7 ± 2.6[b]	92.9 ± 1.2[a]	F=21.8**
Gross energy	87.3 ± 0.3	82.4 ± 3.3	89.2 ± 1.6	F=1.6, P=0.31
Fatty acid digestibility (%)[#]				
16:0	82.1 ± 1.4[a]	59.2 ± 3.8[b]	86.6 ± 1.7[a]	F=17.7*
18:0	77.5 ± 3.3[a]	42.4 ± 5.4[b]	82.1 ± 2.6[a]	F=15.5*
18:1	92.4 ± 0.3	89.2 ± 1.5	94.3 ± 1.0	F=3.6, P=0.12
18:2n-6	96.0 ± 0.1	94.3 ± 0.8	96.3 ± 0.9	F=1.5, P=0.33
18:3n-3	98.6 ± 1.1	100.0 ± 0.0	97.7 ± 0.6	T=0.6, P=0.60
20:4n-6	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	N/A
20:5n-3	99.1 ± 0.1	100.0 ± 0.0	98.6 ± 0.4	T=0.9, P=0.45
22:5n-3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	N/A
22:6n-3	98.5 ± 0.2	100.0 ± 0.0	97.3 ± 0.8	T=1.1, P=0.35
SFA	82.9 ± 1.6[a]	55.6 ± 4.2[b]	86.4 ± 1.8[a]	F=18.3**
MUFA	92.9 ± 0.4	90.0 ± 1.3	94.3 ± 1.0	F=2.9, P=0.17
PUFA	96.7 ± 0.1	95.0 ± 0.7	96.6 ± 0.9	F=1.2, P=0.38
LC-PUFA	98.9 ± 0.1	100.0 ± 0.0	98.1 ± 0.6	T=1.1, P=0.40
n-3	98.9 ± 0.1	100.0 ± 0.0	98.1 ± 0.6	T=1.2, P=0.36
n-6	96.4 ± 0.1	94.6 ± 0.8	96.5 ± 0.9	F=1.5, P=0.32

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

^ 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.

[#] Refer to Table 1 for details.

Table 5 Whole body composition data (n=3 per treatment, g/kg live basis). Whole body and liver fatty acid data (n=3 per treatment, % total).

	Initial	CTRL-D	SFA-D	MUFA-D	TEST ^
Whole body composition					
Dry matter	265 ± 0.4	329 ± 0.5	318 ± 0.3	324 ± 0.3	F=2.1, P=0.21
Protein	188 ± 0.5	206 ± 0.7	191 ± 0.8	200 ± 0.6	F=1.2, P=0.36
Lipid	51 ± 0.4	95 ± 0.6	91 ± 0.2	94 ± 0.2	F=0.3, P=0.73
Gross energy (MJ/kg)	61 ± 0.2	84 ± 0.2^{a}	77 ± 0.1^{b}	79 ± 0.1^{ab}	F=8.0*
Whole body fatty acids (%)$^{\#}$					
Total FA (mg/g lipid)	681.0 ± 35.1	616.6 ± 19.2	665.7 ± 68.1	647.7 ± 44.7	F=0.4, P=0.69
16:0	26.1 ± 0.2	26.9 ± 0.5^{a}	28.0 ± 0.2^{a}	22.4 ± 0.1^{b}	F=74.3***
18:0	7.8 ± 0.2	7.4 ± 0.1^{b}	9.9 ± 0.2^{c}	6.4 ± 0.0^{a}	F=148.8***
18:1	37.9 ± 0.8	30.1 ± 0.5^{a}	30.9 ± 0.2^{a}	41.7 ± 0.1^{b}	F=371.0***
18:2n-6	9.7 ± 0.3	8.8 ± 0.2^{a}	10.2 ± 0.2^{b}	11.0 ± 0.1^{c}	F=37.4***
18:3n-3	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	F=3.3, P=0.11
20:4n-6	0.4 ± 0.0	0.7 ± 0.0^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	F=14.7**
20:5n-3	1.4 ± 0.1	3.7 ± 0.3^{a}	2.6 ± 0.1^{b}	2.2 ± 0.1^{b}	F=13.1**
22:5n-3	0.8 ± 0.0	1.6 ± 0.1^{a}	1.3 ± 0.1^{ab}	1.2 ± 0.0^{b}	F=5.3*
22:6n-3	2.1 ± 0.2	5.5 ± 0.6^{a}	4.3 ± 0.2^{ab}	3.8 ± 0.0^{b}	F=6.1*
SFA	38.5 ± 0.2	39.3 ± 0.7^{a}	41.4 ± 0.4^{a}	31.9 ± 0.0^{b}	F=102.4***
MUFA	45.7 ± 0.5	37.7 ± 0.7^{a}	36.6 ± 0.1^{a}	46.6 ± 0.1^{b}	F198.1***
PUFA	11.0 ± 0.1	11.7 ± 0.3^{a}	13.2 ± 0.3^{b}	13.9 ± 0.1^{b}	F=26.6**
LC-PUFA	4.7 ± 0.2	11.4 ± 1.1^{a}	8.8 ± 0.4^{a}	7.6 ± 0.1^{b}	F=7.7*
n-3	4.3 ± 0.0	11.9 ± 1.2^{a}	9.3 ± 0.4^{a}	8.2 ± 0.1^{b}	F=7.0*
n-6	11.4 ± 0.1	11.2 ± 0.2^{a}	12.7 ± 0.2^{b}	13.4 ± 0.1^{b}	F=38.1***
Liver fatty acids (%)$^{\#}$					
16:0	27.8 ± 0.4	30.8 ± 0.4^{ab}	33.7 ± 0.7^{b}	27.6 ± 1.3^{a}	F=12.9**
18:0	11.5 ± 0.2	10.6 ± 0.2^{a}	13.9 ± 0.4^{b}	9.8 ± 0.8^{a}	F=17.5**

18:1	34.8 ± 0.3	31.1 ± 0.1[a]	33.3 ± 0.8[a]	39.8 ± 0.6[b]	F=59.3***
18:2n-6	6.4 ±0.2	4.8 ± 0.2	4.3 ± 0.6	6.9 ± 1.2	F=3.0, P=0.12
18:3n-3	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	F=1.5, P=0.29
20:4n-6	0.7 ± 0.0	0.8 ± 0.0[a]	0.5 ± 0.0[b]	0.5 ± 0.0[b]	F=37.4***
20:5n-3	1.7 ± 0.1	2.7 ± 0.1[a]	1.3 ± 0.1[b]	1.5 ± 0.3[b]	F=18.0**
22:5n-3	1.1 ± 0.1	1.4 ± 0.1[a]	0.7 ± 0.1[b]	0.9 ± 0.1[b]	F=14.4**
22:6n-3	4.4 ± 0.1	4.8 ± 0.1[a]	2.7 ± 0.2[b]	2.8 ± 0.3[b]	F=15.0**
SFA	42.9 ± 0.3	45.5 ± 0.5[ab]	50.5 ± 0.9[b]	40.5 ± 1.8[a]	F=17.4**
MUFA	40.0 ± 0.3	37.9 ± 0.3[a]	38.2 ± 0.7[a]	44.8 ± 0.6[b]	F=48.9***
PUFA	8.5 ± 0.1	6.2 ± 0.2	5.5 ± 0.7	8.4 ± 1.2	F=3.4, P=0.10
LC-PUFA	8.6 ± 0.1	10.4 ± 0.1[a]	5.7 ± 0.4[b]	6.3 ± 0.7[b]	F=29.6***
n-3	8.6 ± 0.1	9.7 ± 0.1[a]	5.6 ± 0.5[b]	6.1 ± 0.9[b]	F=17.6**
n-6	8.5 ± 0.2	6.8 ± 0.2	5.7 ± 0.7	8.6 ± 1.0	F=3.9, P=0.08

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

^ 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..

[#] Refer to Table 1 for details.

Table 6 The apparent *in vivo* fatty acid β-oxidation, de novo fatty acid synthesis and fatty acid bioconversion (elongation and desaturation) activity in juvenile barramundi fed experimental diets for eight weeks, deduced by the whole body fatty acid balance method. All data (n=3 per treatment) are reported as nmol/g fish/d basis.

	CTRL-D	SFA-D	MUFA-D	TEST ^
β-oxidation [#]				
16:0	ND	ND	ND	N/A
18:0	ND	ND	ND	N/A
18:1	54.0 ± 2.8[a]	7.7 ± 3.9[a]	580.4 ± 48.6[b]	F=127.6***
18:2n-6	298.3 ± 16.8	312.5 ± 27.4	240.5 ± 14.5	F=3.5, P=0.09
18:3n-3	ND	ND	ND	N/A
20:4n-6	64.3 ± 3.3[a]	25.8 ± 1.3 [b]	27.6 ± 0.7[b]	F=108.0***
20:5n-3	482.9 ± 23.9[a]	183.7 ± 12.8[b]	167.7 ± 4.8[b]	F=125.0***
22:5n-3	25.7 ± 9.9	ND	ND	N/A
22:6n-3	631.3 ± 50.9[a]	220.5 ± 17.3[b]	187.9 ± 3.7[b]	F=63.0***
SFA	ND	ND	ND	N/A
MUFA	348.4 ± 12.9[b]	38.5 ±13.8[c]	639.9 ± 60.9[a]	F=69.8***
PUFA	558.1 ± 32.0[a]	382.9 ± 44.0[b]	492.7 ± 19.8[a]	F=7.3*
LC-PUFA	1623.6 ± 115.5[a]	574.1 ± 37.9[b]	508.6 ± 15.4[b]	F=78.4***
n-3	1616.3 ± 116.4[a]	560.1 ± 38.3[b]	494.7 ± 15.1[b]	F=78.2***
n-6	565.4 ± 31.1[a]	396.9 ± 43.6[b]	506.6 ± 20.1[a]	F=7.1*
Neogenesis [#]				
12:0	188.3 ± 31.6[a]	166.0 ± 29.8[a]	312.3 ± 22.6[b]	F=7.7*
Elongation [#]				
12:0	202.6 ± 28.8	184.9 ± 29.9	285.5 ± 22.4	F=3.1, P=0.12
14:0	350.8 ± 30.0[b]	241.4 ± 30.2[a]	363.7 ± 20.0[b]	F=6.1*
16:0	216.8 ± 17.7[b]	235.9 ± 15.6[b]	107.2 ± 3.6[a]	F=25.4**
20:5n-3	ND	13.9 ± 4.8	12.9 ± 1.1	T=0.5, P=0.55
Δ-9 desaturation [#]				
18:0	98.0 ± 22.7[b]	288.3 ± 28.6[a]	ND	T=5.2**

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

[#] Computations following Turchini et al (2007). Refer to Table 1 for details.

^ 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.

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.

		CTRL-D	SFA-D	MUFA-D	TEST ^
22:6n-3[#]	% Converted	ND	ND	ND	N/A
	% Oxidised	58.1 ± 5.1	38.6 ± 3.5	36.7 ± 0.4	F=10.9*
	% Excreted	1.5 ± 0.1	ND	2.7 ± 0.6	T=2.0, P=0.11
	% Deposited	40.4 ± 5.0	61.4 ± 3.5	60.6 ± 0.3	F=11.4**
22:5n-3	% Converted	ND	ND	ND	N/A
	% Oxidised	20.5 ± 8.0	ND	ND	N/A
	% Excreted	ND	ND	ND	N/A
	% Deposited	79.5 ± 8.0	100 ± 0.0	100 ± 0.0	N/A
20:5n-3	% Converted	ND	3.5 ± 1.2	3.6 ± 0.3	T=0.1, P=0.96
	% Oxidised	63.4 ± 3.7	47.3 ± 3.9	47.3 ± 1.1	F=8.7*
	% Excreted	1.0 ± 0.1	ND	1.4 ±	T=1.5, P=0.21
	% Deposited	35.6 ± 3.6	49.2 ± 2.7	47.6 ± 0.7	F=7.8*
n-3 LC-PUFA	% Converted	ND	1.2 ± 0.4	1.2 ± 0.1	T=0.1, P=0.96
	% Oxidised	47.3 ± 5.6	28.6 ± 2.5	28.0 ± 0.5	F=9.7*
	% Excreted	0.8 ± 0.1	ND	1.4 ± 0.3	T=1.8, P=0.14
	% Deposited	51.8 ± 5.5	70.2 ± 2.1	69.4 ± 0.2	F=9.2*

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

^ P<0.001*** P<0.01** P<0.05*; 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.

[#]Assumed that no further conversion occurs (Turchini et al 2007).