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Marginal efficiencies of long chain-polyunsaturated fatty acid use by barramundi (*Lates calcarifer*) when fed diets with varying blends of fish oil and poultry fat.

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Abstract

An experiment was conducted with barramundi (*Lates calcarifer*) juveniles to examine the marginal efficiency of utilisation of long chain-polyunsaturated fatty acids (LC-PUFA). A series of five diets with blends of fish (anchovy) oil and poultry fat (F100:P0, F60:P40, F30:P70, F15:P85, F0:P100) were fed to 208 ± 4.1 g fish over a 12-week period. The replacement of fish oil with poultry fat had no impact on growth performance (average final weight of 548.3 ± 10.2 g) or feed conversion (mean = 1.14 ± 0.02). Analysis of the whole body composition showed that the fatty acid profile reflected that of the fed diet. However it was also shown that there was a disproportional retention of some fatty acids relative to others (notably LOA, 18:2n-6 and LNA, 18:3n-3). By examining the body mass independent retention of different fatty acids with differential levels of intake of each, the marginal efficiencies of the use these nutrients by this species were able to be determined. The differential retention of fatty acids in the meat was also examined allowing the determination of oil blending strategies to optimise meat n-3 LC-PUFA levels.

1. Introduction

The replacement of fish oil (FO) with alternative oil sources continues to be a high priority for aquafeed production worldwide. The barramundi (*Lates calcarifer*), also known as the Asian seabass, is an obligate carnivorous fish central to an expanding aquaculture industry in the Indo-Pacific region with a reported long chain-polyunsaturated fatty acid (LC-PUFA) requirement in juvenile fish of around 1.2% (Williams et al., 2006). However, variable responses of barramundi to FO replacement studies have led to questions being raised about the upper limits of FO substitution in this species (Glencross and Rutherford, 2011; Morton et al., 2014; Raso and Anderson, 2003; Tu et al., 2013). Poultry fat (PF) is produced as a by-product of the chicken processing industry and is characterised by its high 18:1n-9 and 18:2n-6 (oleic acid; OLA and linoleic acid; LOA, respectively) content (Turchini et al., 2009). Poultry fat is commonly used to replace FO in fish diets providing an excellent source of energy, however it is characterised by a lack of n-3 LC-PUFA (Turchini et al., 2009).

Depletion of LC-PUFA in the diet of barramundi can potentially lead to reduced productivity and the onset of essential fatty acid deficiency symptoms (Catacutan and Coloso, 1995; Glencross and Rutherford, 2011; Williams et al., 2006). Moreover, a lack of dietary LC-PUFA will also likely be reflected in the flesh, diminishing the human nutritional value of the product (Turchini et al., 2009). It is well established that the regular consumption of food rich in n-3 LC-PUFA is a fundamental part of a balanced diet and the Food and Agricultural Organisation (FAO) advocate the consumption of 250 to 2000 mg/d (EPA and DHA) for adults (FAO, 2010).

Highly variable or disproportionate retention of lipid or indeed specific fatty acids may indicate metabolic changes as a direct result of the fed diet. Thomassen et al. (2012) found that Atlantic salmon (*Salmo salar*) consuming a diet containing rapeseed oil with supplemental 20:5n-3 (eicosapentaenoic acid; EPA) oil had significantly higher 22:5n-3 (docosapentaenoic acid; DPA) retention via elongation of C20 to C22. Moreover, these fish selectively retained 22:6n-3 (docosahexaenoic acid; DHA) efficiently and in absolute terms the proportion of DHA was significantly improved when compared to those fish fed only rapeseed oil with no supplemental EPA. Similarly, a dramatic reduction of LC-PUFA retention was demonstrated in both barramundi and Atlantic salmon as dietary LC-PUFA increased (Glencross and Rutherford, 2011; Glencross et al., 2014). In contrast, Atlantic cod retained more LC-PUFA as intake increased (Hansen et al., 2008).

Efficient feed utilisation has a determinant effect on costs and outputs in aquaculture systems. In economics, a future return on an investment is estimated based on financial inputs and is termed the marginal efficiency of capital (Kalecki, 1937). Similarly, this concept can be applied in aquaculture nutrition in order to better understand the relationship between dietary inputs and fish outputs over time. It differs from deposition or retention in that it is not just a mass-balance model but rather is a bioenergetic approach based on the weight independent relationships between the intake and gain of a specific nutrient. The exact fate of ingested nutrients is difficult to measure, however calculation of the marginal (partial) efficiency can provide a clearer understanding of the discrete contributions of a dietary nutrient. The slope coefficient of the linear relationship is termed the efficiency of utilisation for production (k_{pf}), protein (k_p) and lipid (k_f) and this can be used to estimate the response over a range of nutrient intake levels independent of mass (NRC, 2011). Moreover, the slope of the regression can be further extrapolated until recovered energy for growth is equal to zero thus providing an estimate of nutrient maintenance requirements (NRC, 2011).

A number of studies have used this bioenergetic approach in determining the marginal efficiencies and estimating maintenance requirements of energy, lipid and protein in a variety of fish species. The marginal efficiencies of protein (k_p) and lipid (k_f) of a range of species including Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European seabass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), white grouper (*Epinephelus aeneus*) and yellow-tail kingfish (*Seriola lalandi*) generally range between k_p 0.53 - 0.64 and k_f 0.72 - 0.91 (Booth et al., 2010; Bureau et al., 2006; Helland et al., 2010; Lupatsch et al., 2003). In barramundi, Glencross and Bermudes (2010) showed that over a range of temperatures from 25 to 32˚C the partial efficiency of energy (k_{pf}) was relatively consistent at 0.56 and protein (k_f) was relatively consistent at 0.51.Despite the apparent importance of essential fatty acids (EFA) the energetic efficiencies and maintenance requirements of these nutrients do not appear to have been investigated in fish using a bioenergetics approach.

It was hypothesised that barramundi would reach a critical limit of FO substitution when absolute levels of dietary LC-PUFA dropped below estimated requirement of around 1.2% (Williams et al., 2006). Therefore a series of diets were developed to examine the effects of diluting fish oil with poultry fat on the growth and feed utilisation performance of juvenile

barramundi and to determine the consequences of this on the fillet fatty acid profiles. This study also aimed to develop a strategy for fish oil replacement with defined impacts on meat n-3 LC-PUFA levels.

2. Materials and Methods

2.1. Ingredient and diet preparation

A single basal diet was formulated to provide protein at 53 %, lipid at 16 % with an energetic value of 22 MJ/kg. 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 ingredients were then thoroughly mixed in using an upright commercial mixer (Bakermix, Model 60 A-G, NSW, Australia). The chemical composition of the main dietary ingredients is presented in Table 1. The single batch of basal diet was produced using a laboratory-scale twin-screw extruder with intermeshing, co-rotating screws (MPF24, Baker Perkins, Peterborough, United Kingdom). The pellets were extruded through a 4 mm tapered die and obtained a 1.5-fold increase in diameter by expansion Pellets were cut off at lengths of 5-6 mm using a variable speed 4-blade cutter and dried overnight at 60 ˚C to a constant dry matter. The dietary treatments were generated by vacuum-infusion of the different oils and blends of FO and PF (8.5% diet) to batches of dried pellets. The five dietary treatments contained 100% FO, 60% FO, 30% FO, 15% FO and 0%FO with a concomitant increase in PF in each (F100:P0, F60:P40, F30:P70, F15:P85 and F0:P100). The diets were then stored at -20˚C until required. The formulation and chemical composition of the five diets are presented in Table 2.

2.2. 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 (600L) with flow-through seawater (salinity =35 PSU; dissolved oxygen 6.3 ± 0.17 mg /L) of 29.7 ± 0.09 ºC (mean ± SD) at a flow rate of about 3 L/min being supplied to each of the tanks. At the beginning of the experiment, the tanks held 20 fish of 209±26 g (mean ± SD, n =300 individually weighed fish). The five experimental diets were randomly distributed amongst the fifteen tanks with each treatment having three replicate tanks.

2.3. Sample collection, preparation and digestibility analysis

Five 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 analysis. Upon termination of the experiment after 82 days, an additional three fish from each tank were euthanized and pooled then stored at -20 ºC until analysis. A thin strip of flesh was dissected and the skin removed from the left side of each sampled fish. This sample, analogous to the Norwegian quality cut (NQC) was taken posterior to the dorsal fin to include both dorsal and ventral muscle (NS.9410, 1994).

Upon termination of the growth assay, the fish were fed their respective dietary treatments before faeces were collected using established protocols (Glencross et al., 2005). Briefly, the fish were netted from their tanks and anesthetised then gentle abdominal pressure was applied to the distal intestine to extract the faeces. Care was taken by the operator to avoid contamination of the sample with foreign material and hands were rinsed after each stripping. The faecal sample was placed into a small plastic vial on ice before being stored in a freezer -20˚C until analysis.

2.4. Chemical analysis

Prior to analysis the diets were each ground to a fine powder using a bench grinder (KnifeTec™ 1095, FOSS, Denmark). The initial and final fish were all processed using the same method. 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 by gravimetric analysis following oven drying at 105ºC for 24 h. Total yttrium concentrations were determined 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). 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 gravimetrically 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). 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 coefficients (ADC) using the formula:

$$ADC_{diet} = 1 - \left(\frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}}\right)$$

Where Y_{diet} and Y_{faeces} represent the yttrium content in both the diet and faeces, respectively and $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter (dry matter, protein, lipid and energy) in the diet and faeces, respectively (Maynard and Loosli, 1979).

Fatty acid composition was determined following the method of Coutteau and Sorgeloos (1995). 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 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 carrier gas was hydrogen at a flow rate of 40 mL/min. 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).

2.5. Nutrient deposition and marginal efficiency assessment

Nutrient deposition 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:

$$Deposition\ (\%) = \left(\frac{Nf - Ni}{Nc}\right) \times 100$$

Where *Nf* and *Ni* are the final and initial nutrient composition (g/fish) of the fish on a wet basis, respectively, and *Nc* is the amount of the nutrient consumed (g/fish) during the study period (Maynard and Loosli, 1979).

The marginal efficiency of the utilisation of specific fatty acids was determined by regressing the mass-independent nutrient gain relative to the mass-independent nutrient intake. The slope of the regression is considered to be the marginal efficiency constant ($k_{fatty\ acid}$) (NRC, 2011). The mass-independent gain was calculated using the formula:

$$Marginal\ gain = Gain_{FA} / GMW^{0.9} / t$$

Where Gain $_{FA}$ is the specific fatty acid gained (g/fish) on a weight specific (geometric mean live-weight g/fish) basis, transformed to an exponent of 0.9 which has been shown to define the relationship between lipid utilisation and fish size and t is the duration of the study period (Glencross and Bermudes, 2011). The mass-independent intake was calculated in the same manner as marginal gain above, however the Gain $_{FA}$ is substituted with the Intake $_{FA}$ of the specific fatty acid using the formula:

$$Marginal\ intake = Intake_{FA} / GMW^{0.9} / t$$

2.6. Statistical analysis

All data are expressed as mean ± SEM unless otherwise specified. All data were checked for normal distribution and homogeneity of variance by qualitative assessment of residual and normal Q-Q plots using the RStudio package v.0.98.501 (R Core Team, 2012). All dietary effects were analysed by linear regression using the RStudio package v.0.98.501 (R Core Team, 2012) with significance among treatments defined as $P < 0.05$.

3. Results

3.1. Growth performance and feed utilisation

During the 82 d growth period, the fish responded to the experimental diets, growing consistent with the predicted model growth (Glencross and Bermudes, 2012). Survival was 100% in all treatments. No significant differences were observed among the treatment diets in terms of growth performance (Table 3). During the growing period, there was greater than 2.5-fold increase in weight among the groups of fish with final fish weights ranging between 545 to 553 g. Similarly, there were no significant differences in feeding parameters with FCR values ranging from 1.12 to 1.15 (Table 3). The diet dry matter, protein, lipid and energy digestibility were also unaffected by the modified lipid profile of the treatment diets (Table 3). The digestibility of individual fatty acids was not significantly affected by treatment (Table 3).

When compared to the initial samples the dry matter, lipid and gross energy content of the carcass of all treatments were numerically higher upon termination of the experiment (Table 4). However, there were no significant differences in the final whole body composition of the fish fed the treatment diets. In terms of the fatty acid composition, some significant effects among the dietary treatments were observed (Table 4). The fatty acid composition of the

whole body was in most cases a reflection of the dietary treatments and changed accordingly. DPA was the only exception to this as it was not detected in the diets containing less than 30% FO, while whole body DPA levels were maintained at levels similar to that of the initial fish ($R^2 = 0.76$, $P < 0.001$). There was a significant difference in whole body ARA ($R^2 = 0.47$, $P < 0.05$) and this fatty acid appears to be well conserved compared to the initial fish. Both EPA and DHA whole body proportions were significantly reduced by the dietary treatments ($R^2 = 0.90$, $P < 0.001$ and $R^2 = 0.76$, $P < 0.001$, respectively).There was no significant difference in SFA composition despite the altered dietary profiles.

There were no significant differences in protein or energy retention efficiency in the whole body among the treatment groups (Table 3). However, there was a slight increase in lipid retention efficiency values with increasing PF. Whole body retention efficiency of linolenic acid (LOA) was not significantly affected by increasing intake and the values ranged between 55% to 86% ($y = 0.03x – 0.49$, $R^2 = 0.13$, $P = 0.55$, figure 1a). Similarly, the retention efficiency of LNA was not significantly affected by increasing intake with the retention efficiency values ranging between 45% and 69% ($y = 0.65x – 0.10$, $R^2 = 0.65$, $P = 0.13$, figure 1b). There was disproportionate retention of LC-PUFA's including arachidonic acid (ARA), EPA and DHA which are presented in Figures 1c-e**.** The ARA retention efficiency in the whole body decreased sharply from 74% to 47% with increasing intake however the dietary intake values of ARA were very low ($y = -1.08x + 1.03$, $R^2 = 0.81$, $P < 0.05$). Similarly, the retention efficiency of EPA decreased from 49% at the lowest dietary inclusion to 32% at the highest inclusion ($y = -0.04x + 0.52$, $R^2 = 0.90$, $P < 0.05$). The whole body retention efficiency of DHA was 73% at the lowest dietary inclusion levels then showed a curvilinear decline to around 37% ($y = 0.72x^{-0.44}$, $R^2 = 0.97$, $P < 0.05$).

3.2. Nutrient marginal efficiencies

The marginal efficiencies of nutrient utilisation were calculated using a bioenergetic approach to determine the discrete effects on nutrient utilisation and maintenance requirements for juvenile barramundi (Figures 2 a-e). The marginal efficiency of LOA (k_{LOA}) was determined as 82% ($y = 0.82x – 0.03$, $R^2 = 0.75$) and the maintenance requirement estimated at 0.068 $g/kg^{0.9}/d$. Similarly, the marginal efficiency of LNA (k_{LNA}) was determined as 104% ($y = 1.04x – 0.01$, $R^2 = 0.88$) and the maintenance requirement was estimated at 0.012 $g/kg^{0.9}/d$. The marginal efficiencies of the LC-PUFA's were contrasting to those of the PUFA's. The marginal efficiency of ARA (k_{ARA}) was low at 19% ($y = 0.19x + 0.005$, $R^2 =$

0.43) and the regression suggests there was no maintenance requirement for this fatty acid. Similarly, the marginal efficiencies of EPA and DHA ($k_{EPA\ and\ DHA}$) were also low at 30% (y = 0.30x + 0.0, R^2 = 0.95) and 27% (y = 0.27x + 0.01, R^2 = 0.95), respectively and the regression model suggested that there was also no maintenance requirement for these LC-PUFA's. A summary of the marginal efficiencies and the intake to gain ratio of key fatty acids are presented in table 5.

3.3. Fillet quality assessment

The consequence of changing dietary lipids in juvenile barramundi was evaluated in terms of fillet quality using a standard sample of flesh (NQC). There were no significant differences in macro nutrient composition of the NQC samples (Table 6). Overall, the dietary fatty acid profiles were closely reflected in the flesh with the exception of DPA which was barely present in the diets F100:P0 and F60:P40 and not detected in the other diets. Consequently, some significant differences in NQC fatty acid composition were observed (Table 8). Myristic acid (14:0) was the only saturate affected showing a reduced concentration with increasing FO substitution (R^2 = 0.80, P < 0.001). Oleic acid (OLA; 18:1n-9) was significantly increased with FO substitution (R^2 = 0.50, P < 0.01). Both LOA and LNA composition increased with increasing FO substitution (R^2 = 0.65, P < 0.001 and R^2 = 0.58, P < 0.01, respectively). ARA composition in the fillet did not change however n-3 LC-PUFA (EPA and DHA combined) deposition in the flesh showed an increasing curvilinear response relative with increasing intake (Figure 3).

4. Discussion

The benefits of regular consumption of seafood rich in n-3 LC-PUFA are well known. These fatty acids are implicated in a range of physiological and metabolic processes and many studies have demonstrated their positive benefit in the prevention and management of cardio-vascular disease and inflammation (Calder, 2012). The FAO's guideline to consume a dose of 250 mg/d EPA and DHA is an achievable yet rarely met target due to the production and subsequent consumption of the predominant vegetable oils including soybean, palm and canola (FAO, 2010). This study investigated the potential effects of fish oil substitution with poultry fat in diets fed to juvenile barramundi and provides empirical support for the development of a strategy to manage the LC-PUFA content in the fillet. However, in addition to this objective the study also provides some unique insights into fatty acid metabolism in

this species based on understanding the marginal utilisation of these nutrients and comparing that with what we know about similar parameters for utilisation of other nutrients.

4.1. Growth performance and feed utilisation

This study demonstrated that the growth and feed utilisation of barramundi was not significantly affected by the complete replacement of fish oil with poultry fat. Until recently, variable responses to fish oil replacement and fatty acid requirement studies have led to further questions being raised about lipid metabolism in this species (Catacutan and Coloso, 1995; Glencross and Rutherford, 2011; Morton et al., 2014; Raso and Anderson, 2003; Williams et al., 2006). The fish in this study achieved a minimum 2.5-fold increase in weight over the twelve week study period. Experimental duration is of particular interest in the realm of fatty acid nutrition as the increasing use of alternative oils has an definitive influence on flesh quality (Robin et al., 2003). The NRC (2011) suggest that 300% or a 3-fold increase in weight should be respected and a range of other studies suggest different methods of assessment (Glencross et al., 2003; Jobling, 2004; Morton et al., 2014; Robin et al., 2003). Clearly the fish in this study achieved sufficient biological turnover as evidenced by the whole fish and flesh fatty acid profiles mirroring the diets similar to that of other studies (Glencross et al., 2014; Tu et al., 2013; Turchini et al., 2011). Moreover, past studies have demonstrated that fatty acid profiles of fish can be manipulated in as little as two weeks (Castell et al., 1994; Skonberg et al., 1994).

Poultry fat (PF) is a commonly utilised alternative lipid source for aquafeeds in Australia, having been widely used for over a decade, despite containing little or no n-3 LC-PUFA. In this study, FO substitution with PF covered a range of LC-PUFA inclusion from 22.0 to 4.8 g/kg, theoretically surpassing the reported minimum requirement of 12 g/kg LC-PUFA (Williams et al., 2006). It is noteworthy that this species is a catadromous, obligate carnivore and the reduced levels of LC-PUFA fed in this study should probably have induced a range or EFA deficiency symptoms similar to those previously reported (Catacutan and Coloso, 1995; Glencross and Rutherford, 2011). Indeed, when other marine carnivorous fish were fed diets with high levels of FO substitution, this led to growth retardation (Glencross et al., 2003; Izquierdo et al., 2005; Montero et al., 2005). We suggest that the absence of problems seen in the present study may be reflective of the use of larger fish which had previously been raised on high LC-PUFA diets and therefore their underpinning n-3 LC-PUFA requirements in the demanding early juvenile growth phase had already been met and that there was limited

subsequent turnover. Alternatively, it may also be that the actual requirement for EFA by this species is much lower than previously thought. In this study, the digestibility of lipid and individual fatty acids were not significantly affected. Further suggesting that despite the contrasting differences in fatty acid profiles of PF and FO, the diets were digested by barramundi equally efficiently.

The LC-PUFA content of the F0:P100 diet was only 3.5% of the total lipid (equivalent to 4.8 g/kg diet) with this residual LC-PUFA coming from the fish meal appearing sufficient to maintain biological functions in the fish. The inclusion of 15% fish meal in the present study is based on the findings of Glencross et al. (2011) as the use of a fully purified diet containing no fish meal was shown to be a risky approach and lacks practical application (Tu et al., 2013). In light of this, the results of this study demonstrate that in terms of growth performance, 100% of the FO can be effectively replaced by poultry fat in growing barramundi however discrete effects on deposition and marginal efficiencies of specific fatty acids were apparent.

4.2. Retention and marginal efficiencies

Bioenergetic models have come a long way to understanding how different nutrients are deposited in the body of an animal and that macro nutrients such as protein and lipid have different energetic efficiencies (Bureau et al., 2006). The individual fatty acids have vastly different metabolic fates and hence are likely to have different energetic efficiencies. For example, the n-3 and n-6 series eicosapolyenoic fatty acids are implicated in a competing nature for the synthesis of the autocrine hormones (Tocher, 2003). Arguably they may also have different allometric relationships, as do protein and lipid, and this remains to be explored (Glencross and Bermudes, 2011). In the present study we have relied on the determined exponent of total lipid utilisation of $LW^{0.90}$ to define the energetic relationships of the different fatty acids, though we acknowledge that this is still an assumption.

The present study demonstrated that the marginal efficiencies of LC-PUFA (k_{DHA}, k_{EPA} and k_{ARA}) were 0.27, 0.30 and 0.19, respectively and the PUFA (k_{LNA} *and* k_{LOA}) were significantly higher at 1.04 and 0.82, respectively. At first glance, the examination of the retention and marginal efficiencies of LC-PUFA in this study gives further evidence to the hypothesis that barramundi are unable to elongate and desaturate precursor FA to form essential LC-PUFA (Mohd-Yusof et al., 2010). As evidenced by the direct accumulation of those fatty acids

(LOA and LNA) in a manner directly reflective of their intake levels, indicating no loss of these nutrients from what was consumed.

Clearly in the case of DHA, EPA and ARA, retention efficiency in the whole body decreased as dietary intake of those nutrients increased (Figure 1c-e). In agreement, the marginal efficiencies of these nutrients were low compared to other the shorter-chain and more saturated FA. These responses are likely due to the physiological requirements of these nutrients in growing barramundi and suggest that they were catabolised into other forms or used for energy. The strong conservation of DHA in the whole body at low dietary intakes may be indicative of a priority for retaining this nutrient (essentiality) as the organism attempts to retain what it has to sustain necessary functions, but from our data it is implied that these necessary functions require a transformation of the nutrient into another metabolite or energy (Tocher, 2003). However, by extrapolating the marginal efficiency of the DHA regression it appears as if there is no maintenance requirement for LC-PUFA in the barramundi. This is contrasting to that identified for many other nutrients (and energy), each of which have clear maintenance requirement levels.

This observation may be partly explained by the extrapolation method in itself which has been found to slightly underestimate maintenance requirements compared to a factorial approach (Bureau et al., 2006). However, it is also likely that the dietary formulations in this study all supplied LC-PUFA in excess or at least beyond the point of sensitivity for this species. Given that the barramundi is clearly very effective at selective LC-PUFA retention it probably has a very low requirement for these specific fatty acids. As discussed earlier, we are also assuming that the relationships of the different fatty acids to animal live-weight is consistent with that of total lipid ($LW^{0.90}$), though this needs to be further validated and this, and other further evidence may still be required to draw conclusions on the use of this method to estimate baseline metabolic requirements of individual LC-PUFA.

Recent fatty acid studies have demonstrated similar metabolic responses for a range of species. In Atlantic salmon (Glencross et al., 2014; Torstensen et al., 2004), barramundi (Glencross and Rutherford, 2011) and gilthead seabream (Montero et al., 2001) there was a curvilinear decline in the retention efficiency of DHA in response to increasing DHA level in the diet. In contrast, DHA and EPA retention efficiency in the Atlantic cod (*Gadus morhua*) increased in response to increasing dietary supply (Hansen et al., 2008) . In the latter

example, the authors argued that DHA was probably used as a substrate for energy production at lower dietary intake. Many studies have demonstrated that despite up regulation of genes involved in FA synthesis pathways, most fish species are unable to completely compensate for a lack of dietary LC-PUFA (Alhazzaa et al., 2011; Betancor et al., 2014; Francis et al., 2007; Geay et al., 2010; Tu et al., 2013). Interestingly in this study, the reduced retention of EPA may be attributable in part, to the apparent concomitant appearance of DPA via elongation of C20 to C22. This is a likely scenario as the barramundi attempts to synthesise DHA from dietary precursor fatty acids in response to the treatment diets. However to achieve this goal it requires a Δ5 desaturation enzyme that probably does not exist in the species hence the building up of DPA (Mohd-Yusof et al., 2010). Another possible explanation for the accumulation of DPA may be that the animal is β-oxidising available DHA to EPA for eicosanoid production and DPA is an intermediate in this process.

Moreover, the high retention and low marginal efficiency of ARA and EPA could be related to the requirement for eicosanoid production under inadequate or stressful dietary conditions. The eicosanoids particularly the n-6 series are implicated in a range of physiological roles such as ion transfer and osmoregulation (Castell et al., 1994) and the n-3 series eicosanoids have an anti-inflammatory role (Wall et al., 2010). In addition, the majority of eicosanoid products are derived from ARA in fish adding to the idea that ARA may be implicated in a range of biological processes in barramundi (Henderson, 1996; Tocher, 2003). Few studies have investigated ARA metabolism in barramundi, however current evidence suggests that ARA may be implicated in reduced growth and increased sub-clinical signs of essential fatty acid deficiency (Glencross and Rutherford, 2011). In agreement, the results of the present study emphasized the relative importance of ARA and future work in this area is warranted.

4.3. Fillet fatty acid composition

Much awareness is now placed on the consumption of seafood, rich in LC-PUFA, in an attempt to ameliorate a range of largely preventable diseases (Calder, 2012; Wall et al., 2010). Many nutritional feeding studies have focused on the replacement of FO with alternative oils and also how this translates to the flesh (Sales and Glencross, 2011; Turchini et al., 2009). Past studies have demonstrated that fish fillet adiposity can be altered by the use of common vegetable oils (Bell et al., 2002) however the same response in this study was not found with poultry fat. Fillet lipid levels remained constant among the treatment groups and the fillet fatty acid profiles were largely reflective of the diets. The fatty acids deposited in

the flesh of barramundi were clearly proportional to their respective diet with few exceptions which is typical of most fish (Turchini et al., 2009).

Given the limited ability of barramundi to desaturate and elongate precursor FA to EPA and then DHA it was not surprising that these FA were strongly related to the dietary composition. However, the relationship was best described as curvilinear and the fillet concentration of LC-PUFA plateaued as the dietary concentration exceeded 6 % (Figure 3). There was a linear decrease in the fillet EPA concentration with increasing replacement of the fish oil that appeared to be related to a slight increase in DPA relative to the diet. The accumulation of DPA in the fillet is likely to be a result of chain length modification in the liver followed by transport to the adipose tissue for storage or eventual secondary processing, however the effect was minimal. Similarly, the fillet DHA concentration also decreased linearly with increasing fish oil replacement, however it was selectively retained in the meat at levels higher than the dietary supply. Similarly, studies have shown that fillet DHA levels in Atlantic salmon (Bell et al., 2001; Bell et al., 2002), European seabass (Mourente and Bell, 2006), gilthead sea bream (Izquierdo et al., 2005) and Murray cod (Turchini et al., 2011) can be elevated relative to the diet. Possible mechanisms underpinning this selective retention among species may be attributable to the high specificity of fatty acyl transferases for DHA (Bell et al., 2001) and reduced catabolism due to the complex peroxisomal β-oxidation required for the DHA molecule (Tocher, 2003). Furthermore, this study showed that OLA and palmitic acid (16:0) dominated within the flesh and these FA are known to be heavily oxidised as energy substrates, potentially promoting more efficient accumulation of other FA (Codabaccus et al., 2012; Turchini et al., 2011).

By extrapolation, the results of this study allow the estimation of fillet FA composition of barramundi (Figure 3). These observations are of practical use for predicting the outcome of feeding diets with reduced levels of FO to barramundi and promoting the most efficient use of the FO resource. It is estimated that feeding barramundi with approximately 11% LC-PUFA would result in a fillet concentration of 500 mg/100 g. The FAO recommend a minimum daily consumption of 250 mg of n-3 LC-PUFA (FAO, 2010). In recognition of the FAO guideline, a 350 g fillet portion would theoretically meet the weekly needs of adult consumers, aiming to maintain a healthy and balanced diet.

5. Conclusion

In conclusion, this study has demonstrated that poultry fat can completely substitute fish oil in growing barramundi. There were no aberrations to fish growth performance or feed utilisation parameters however there were clear differences in the retention and marginal efficiencies of LC-PUFA utilisation. The fish responded to increasing PF by improving the retention of LC-PUFA however the marginal efficiency of LC-PUFA was relatively low, and reasons for this need to be further explored. Moreover the increasing use of PF had a clear impact on the fillet LC-PUFA content, which needs to be carefully considered in order to manage the defined impacts on meat LC-PUFA.

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Figure 1 Whole-body retention of polyunsaturated fatty acids a) LOA ($y = 0.03x + 0.49$, $R2 = 0.13$, $P = 0.55$) and b) LNA ($y = 0.65x + 0.10$, $R2 = 0.60$, $P = 0.13$) and whole-body retention of long-chain polyunsaturated fatty acids c) ARA ($y = -1.08x + 1.03$, $R2 = 0.81$, $P < 0.05$) , d) EPA ($-0.04x + 0.52$, $R2 = 0.90$, $P < 0.05$) and e) DHA ($y = 0.72x-0.44$, $R2 = 0.97$, $P < 0.05$).

Figure 2 The linear relationship between the mass-independent intake relative to mass-independent gain (marginal efficiency) of specific fatty acids in juvenile barramundi. For comparitive purposes the dotted line indicates the slope of 1.0 ($y = x$). a) LOA ($y = 0.82x - 0.03$, $R2 = 0.75$) b) LNA ($y = 1.04x - 0.01$, $R2 = 0.88$) c) ARA ($y = 0.19x + 0.005$, $R2 = 0.43$) d) EPA ($y = 0.30x + 0.007$, $R2 = 0.95$) e) DHA ($y = 0.27x + 0.01$, $R2 = 0.95$).

Figure 3 Fillet (NQC) long-chain polyunsaturated fatty acid (LC-PUFA) deposition in juvenile barramundi expressed relative to intake ($y = -1.54x2 + 56.33x + 75.23$, $R2 = 0.99$) as mg/100g meat. By extrapolation the dotted line suggests a formulation of 11% total FA as LC-PUFA to achieve a fillet composition of 500 mg/100g.

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Table 1 Chemical composition of key ingredients. All data are g/kg DM unless otherwise stated. Fatty acid data are expressed as a percentage of total fatty acids (%).

	FM	WF	LM	WG	PM	FO	PF
Dry matter	933	868	903	921	953	998	997
Crude protein	706	112	432	816	671	9	7
Lipid	101	18	76	97	164	949	996
Ash	139	6	28	4	137	1	0
Gross energy (MJ/kg)	20.0	16.5	18.9	21.3	21.0	39.2	38.7
14:0	5.2	0.2	0.2	0.1	1.5	8.3	1.1
16:0	24.1	20.3	12.1	20.2	27.3	19.0	23.0
18:0	7.2	1.5	5.5	1.2	8.6	3.7	6.1
16:1n-7	5.3	0.4	0.1	0.1	6.4	10.3	5.6
18:1n-9	13.2	14.4	0.0	12.4	41.3	10.1	42.8
18:2n-6	1.5	56.5	41.3	59.4	8.9	1.6	16.2
18:3n-3	0.8	3.5	4.9	2.8	0.7	0.7	2.0
20:4n-6	0.1	0.0	0.0	0.0	0.0	1.1	0.0
20:5n-3	9.0	0.4	1.5	0.6	0.0	17.3	0.6
22:5n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:6n-3	17.3	0.0	0.0	0.0	0.0	13.6	0.0
SFA	39.1	22.4	18.5	22.1	37.8	33.3	30.1
MUFA	24.2	16.6	33.5	14.7	51.3	24.9	51.0
PUFA	3.5	60.0	46.3	62.2	9.5	5.2	18.2
LC-PUFA	30.3	0.4	1.5	0.6	0.2	32.0	0.6
n-3	28.7	4.0	6.4	3.4	0.7	35.3	2.6
n-6	5.2	56.5	41.3	59.4	9.1	2.7	16.2

FM fish meal, WF wheat flour, LM lupin meal, WG wheat gluten, PM poultry meal, FO fish oil, PF poultry fat

Table 2 Experimental diet formulation and composition. All data are g/kg DM unless otherwise stated. Fatty acid data are expressed as a percentage of total fatty acids (%).

	F100:P0	F60:P40	F30:P70	F15:P85	F0:P100
Formulation					
Fish meal [a]	150	150	150	150	150
Fish oil [b]	85	51	25	12	0
Wheat flour [c]	119	119	119	119	119
Wheat gluten [d]	85	85	85	85	85
Lupin meal [e]	100	100	100	100	100
Poultry meal [f]	455	455	455	455	455
Poultry fat [g]	0	34	59	72	85
Premix [h]	5	5	5	5	5
Yttrium oxide [i]	1	1	1	1	1
Composition					
Dry matter	908.2	921.7	977.6	977.6	981.3
Crude protein	518.8	536.9	543.8	531.3	539.5
Crude lipid	149.7	151.2	157.0	159.4	161.9
Ash	92.9	90.3	91.3	94.9	93.8
Gross energy (MJ/kg)	21.9	22.4	22.6	22.7	22.6
14:0	4.7	3.4	2.4	1.9	1.4
16:0	21.1	22.0	22.7	22.8	22.9
18:0	5.6	6.1	6.5	6.6	6.7
16:1n-7	7.7	6.8	6.0	5.7	5.4
18:1n-9	24.0	30.0	34.8	36.7	38.9
18:2n-6	8.9	11.6	13.9	14.8	15.6
18:3n-3	1.1	1.4	1.5	1.6	1.7
20:4n-6	0.9	0.8	0.7	0.6	0.5
20:5n-3	8.8	5.9	3.3	2.3	1.2
22:5n-3	1.2	0.9	0.0	0.0	0.0
22:6n-3	7.6	5.4	3.5	2.7	1.8
SFA	33.7	27.1	25.6	25.4	24.9
MUFA	36.9	46.8	50.8	52.3	54.3
PUFA	11.7	14.0	16.0	16.8	17.4
LC-PUFA	17.3	12.1	7.5	5.5	3.5
n-3	19.4	13.7	9.0	7.0	4.7
n-6	10.0	12.4	14.5	15.3	16.2

a Fish meal; Ridley aquafeeds, Narangba, QLD, Australia
b Fish (anchovy) oil; Ridley aquafeeds, Narangba, QLD, Australia
c Plain wheat flour; Manildra Group, Rocklea, QLD, Australia
d Wheat gluten; Manildra Group, Rocklea, QLD, Australia
e Lupinus angustifolius cv. Coromup; Coorow Seeds, WA, Australia.
f Poultry meal; Ridley aquafeeds, Narangba, QLD, Australia
g Poultry fat; Ridley aquafeeds, Narangba, QLD, Australia
h Vitamin and mineral premix includes (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
i Yttrium oxide; Stanford Materials, Aliso Viejo, California, United States

Table 3 Growth, feed utilisation and nutrient deposition parameters.

	F100:P0	F60:P40	F30:P70	F15:P85	F0:P100	Pooled SEM	Regression R^2, P#
Fish performance							
Initial (g/fish)	208.8	211.2	208.6	207.1	207.5	1.13	0.04, 0.48
Week-12 (g/fish)	545.0	546.4	553.6	546.6	549.7	4.20	0.01, 0.70
Gain (g/fish)	336.2	335.1	345.0	339.5	342.2	4.08	0.03, 0.57
Growth rate (g/d)	4.1	4.0	4.2	4.1	4.1	0.05	0.03, 0.55
Feed Intake (g/fish)	384.4	386.0	394.6	380.6	382.6	4.54	0.00, 0.93
FCR (feed/gain)	1.14	1.15	1.14	1.12	1.12	0.01	0.11, 0.22
Survival (%)	100.0	100.0	100.0	100.0	100.0	0.00	0.50, 0.10
Diet digestibility (%)							
Dry matter	57.5	55.3	53.9	56.7	59.1	4.00	0.00, 0.94
Protein	76.2	74.2	74.5	76.2	77.6	2.20	0.00, 0.84
Lipid	93.7	91.7	93.5	92.9	92.4	0.65	0.02, 0.67
Energy	71.7	69.4	68.4	70.6	70.4	2.41	0.00, 0.85
16:0	88.6	86.8	91.1	91.6	89.7	1.17	0.06, 0.45
18:0	81.7	80.4	86.6	88.1	84.5	1.74	0.11, 0.30
18:1n-9	95.0	94.4	96.3	96.4	96.7	0.48	0.22, 0.12
18:2n-6	93.6	93.4	95.5	95.9	97.2	0.78	0.29, 0.07
18:3n-3	96.5	95.3	96.4	97.4	97.8	0.66	0.07, 0.42
20:4n-6	95.0	100.0	97.4	100.0	93.2	1.32	0.00, 0.91
20:5n-3	99.2	98.5	99.4	95.3	97.0	0.82	0.13, 0.25
22:5n-3	100.0	100.0	100.0	100.0	100.0	0.00	0.45, 0.14
22:6n-3	98.0	96.1	96.0	94.9	94.8	0.99	0.14, 0.23
SFA	90.9	89.7	93.0	92.0	90.9	0.86	0.01, 0.72
MUFA	91.5	90.7	93.9	93.1	93.2	0.69	0.14, 0.23
PUFA	94.8	94.1	95.7	96.2	97.2	0.67	0.18, 0.17
LC-PUFA	98.6	97.7	97.6	95.6	95.3	0.89	0.18, 0.17
n-3	98.6	97.3	97.6	95.9	96.4	0.74	0.13, 0.25
n-6	93.9	93.9	95.5	96.1	97.0	0.75	0.25, 0.10
Nutrient deposition (%)							
Protein	33.5	31.0	33.7	34.2	35.2	0.01	0.04, 0.47
Lipid	64.2	82.1	73.9	87.2	81.5	0.03	0.31, *
Energy	36.7	42.1	40.6	42.3	40.1	0.01	0.12, 0.22

Linear regression of all replicates with 1,13 *df*, $P < 0.05$ *

Table 4 Whole body chemical composition of the experimental fish on a live-weight basis. All data are g/kg unless otherwise stated. Fatty acid data are expressed as a percentage of total fatty acids (%).

	Initial	F100:P0	F60:P40	F30:P70	F15:P85	F0:P100	Pooled SEM	Regression R^2, P[#]
Dry matter	28.9	33.2	35.7	35.5	35.4	35.2	0.01	0.24, 0.06
Crude protein	20.9	20.3	22.1	21.0	20.6	21.1	0.01	0.58, 0.10
Crude lipid	5.3	9.1	10.6	10.1	11.5	11	0.01	0.58, 0.10
Gross energy (MJ/kg)	6.3	8.1	8.9	8.9	9.1	8.7	0.18	0.15, 0.16
14:0	2.7	4.3	3.6	2.4	2.0	1.6	0.29	0.94, ***
16:0	22.9	23.8	26.1	23.6	23.2	23.6	0.32	0.02, 0.65
18:0	7.3	7.0	7.9	7.3	7.3	7.4	0.10	0.08, 0.36
16:1n-7	5.7	7.5	6.0	4.2	5.7	3.0	0.76	0.01, 0.71
18:1n-9	37.6	32.6	39.6	39.3	40.2	42.5	1.05	0.77, ***
18:2n-6	10.9	8.2	10.1	11.7	12.1	13.2	0.52	0.96, ***
18:3n-3	1.1	0.8	0.9	1.2	1.2	1.4	0.06	0.71, ***
20:4n-6	0.6	0.7	0.5	0.6	0.5	0.5	0.03	0.47, *
20:5n-3	1.7	3.9	2.3	1.9	1.3	0.8	0.36	0.90, ***
22:5n-3	1.0	1.3	0.9	0.9	0.7	0.6	0.09	0.76, ***
22:6n-3	3.8	4.2	2.7	2.7	2.2	1.9	0.33	0.76, ***
SFA	34.1	36.6	38.9	34.3	33.3	33.3	0.59	0.30, 0.06
MUFA	44.8	41.8	41.9	44.9	47.2	46.6	0.74	0.76, ***
PUFA	13.0	10.2	11.7	13.7	13.9	15.2	0.53	0.91, ***
LC-PUFA	7.8	10.7	7.0	6.8	5.4	4.6	0.80	0.80, ***
n-3	8.2	11.3	7.7	7.5	6.0	5.2	0.81	0.82, ***
n-6	12.5	9.6	11.0	13.1	13.2	14.7	0.53	0.91, ***

Linear regression of all replicates with 1,13 *df*, $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***

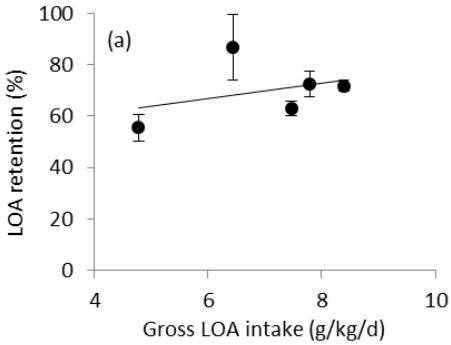
Table 5 Summary of marginal efficiencies of specific fatty acids by juvenile barramundi when fed diets with varying blends of fish oil and poultry fat.

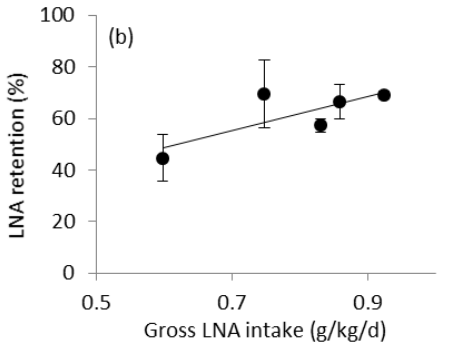
Fatty acid	Efficiency constant *k*	R^2	Intake:gain ratio
18:2n-6; LOA	0.82	0.75	1.2:1
18:3n-3; LNA	1.04	0.88	1.0:1
20:4n-6; ARA	0.19	0.43	5.3:1
20:5n-3; EPA	0.30	0.95	3.3:1
22:6n-3; DHA	0.27	0.95	3.7:1

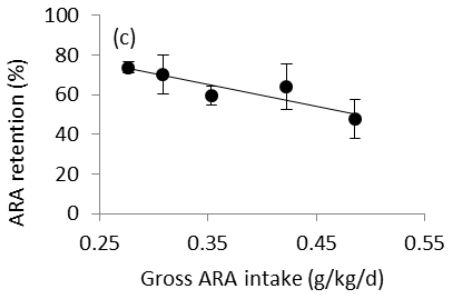
Table 6 Fillet (NQC) composition on a live-weight basis. All data are g/kg unless otherwise stated. Fatty acid profiles of NQC samples from each treatment are expressed as mg/100g meat.

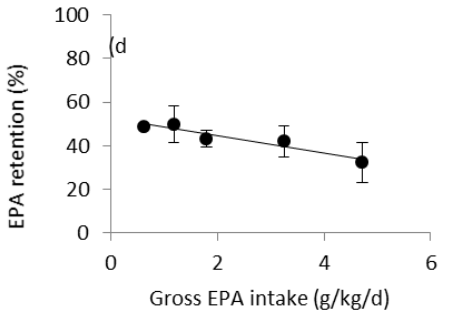
	F100:P0	F60:P40	F30:P70	F15:P85	F0:P100	Pooled SEM	Regression R^2, P[#]
Dry matter	33.2	35.7	35.5	35.4	35.2	0.01	0.20, 0.10
Crude protein	26.5	28.5	28.3	28.3	27.4	0.01	0.08, 0.30
Crude lipid	4.9	5.2	5.6	6.3	5.4	0.01	0.15, 0.16
Gross energy (MJ/kg)	8.2	8.8	8.9	9.1	8.8	0.13	0.24, 0.06
14:0	4.0	2.9	2.1	1.8	1.5	0.24	0.80, ***
16:0	22.9	23.0	22.4	22.8	23.1	0.13	0.11, 0.22
18:0	7.1	7.4	7.2	7.3	7.4	0.09	0.14, 0.17
16:1n-7	7.1	6.2	5.6	5.4	5.1	0.18	0.15, 0.15
18:1n-9	30.9	34.4	37.8	39.3	40.8	0.96	0.50, **
18:2n-6	8.4	10.3	11.9	12.6	13.2	0.46	0.65, ***
18:3n-3	0.9	1.1	1.2	1.3	1.2	0.04	0.58, **
20:4n-6	1.0	0.9	0.8	0.7	0.7	0.03	0.15, 0.15
20:5n-3	5.2	3.7	2.4	1.7	1.0	0.41	0.84, ***
22:5n-3	1.7	1.4	1.1	0.9	0.7	0.10	0.62, ***
22:6n-3	6.2	5.2	4.0	3.4	2.8	0.36	0.61, ***
SFA	35.0	34.1	32.4	32.5	32.7	0.35	0.02, 0.57
MUFA	39.5	41.9	44.8	45.9	47.1	0.75	0.38, *
PUFA	10.6	12.5	14.1	14.7	15.1	0.45	0.56, **
LC-PUFA	14.2	11.2	8.5	6.7	5.2	0.90	0.71, ***
n-3	15.1	12.2	9.4	7.6	5.8	0.93	0.67, ***
n-6	9.6	11.5	13.3	13.9	14.5	0.48	0.64, ***

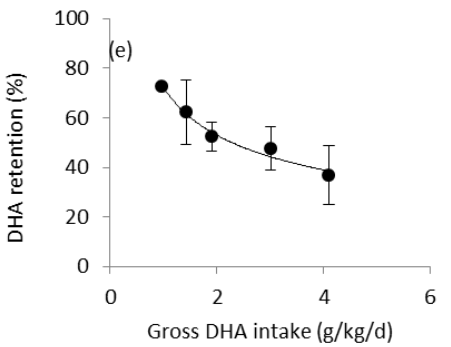
Linear regression of all replicates with 1,13 *df*, $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***

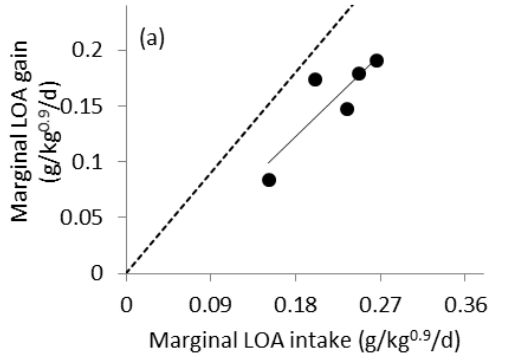

(a)
LOA retention (%)
100
80
60
40
20
0
4
6
8
10
Gross LOA intake (g/kg/d)

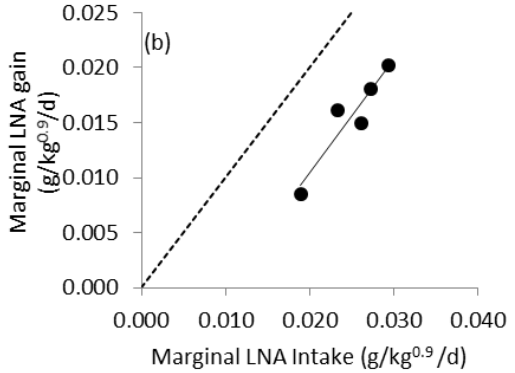

(b)
LNA retention (%)
100
80
60
40
20
0
0.5
0.7
0.9
Gross LNA intake (g/kg/d)

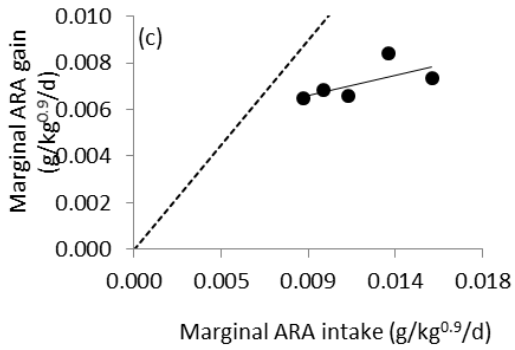

(c)
ARA retention (%)
100
80
60
40
20
0
0.25
0.35
0.45
0.55
Gross ARA intake (g/kg/d)

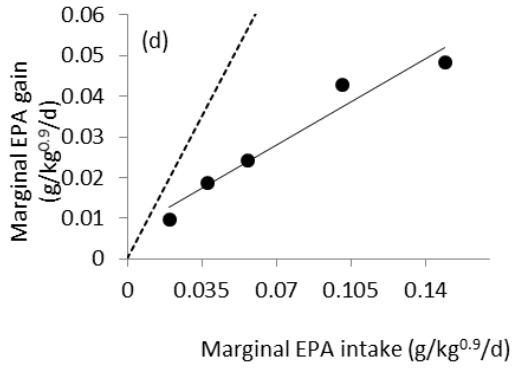

(d
EPA retention (%)
100
80
60
40
20
0
0
2
4
6
Gross EPA intake (g/kg/d)

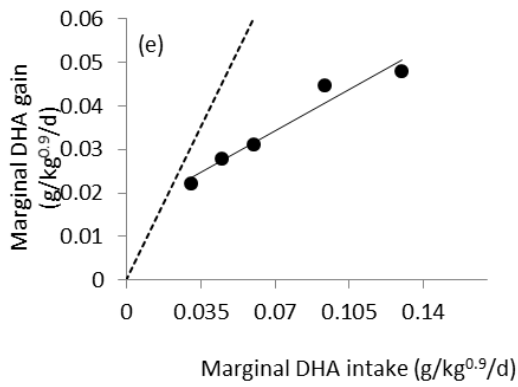

(e)
DHA retention (%)
100
80
60
40
20
0
0
2
4
6
Gross DHA intake (g/kg/d)

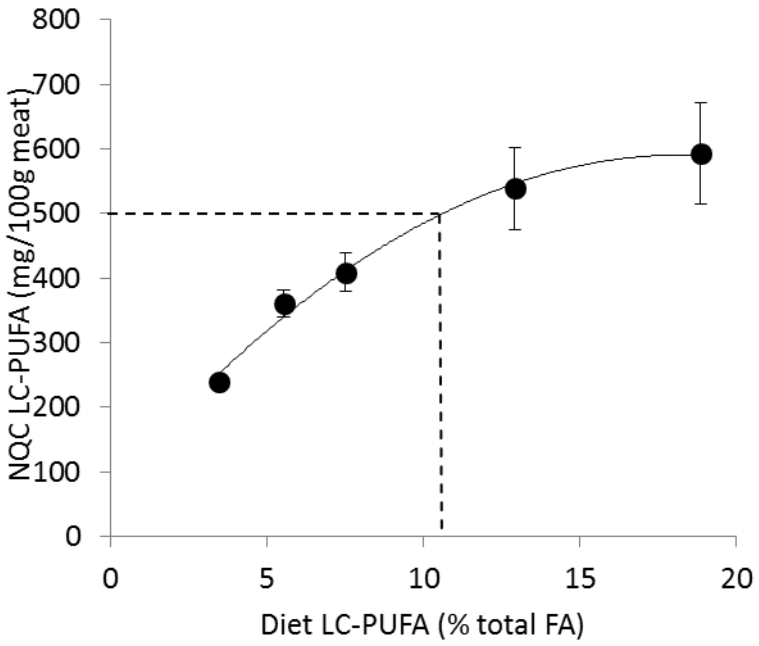

(a)
Marginal LOA gain (g/kg^0.9/d)
0.2
0.15
0.1
0.05
0
0
0.09
0.18
0.27
0.36
Marginal LOA intake (g/kg^0.9/d)


(b)
Marginal LNA gain (g/kg^0.9/d)
0.025
0.020
0.015
0.010
0.005
0.000
0.000
0.010
0.020
0.030
0.040
Marginal LNA Intake (g/kg^0.9 /d)


(c)
Marginal ARA gain (g/kg^0.9/d)
0.010
0.008
0.006
0.004
0.002
0.000
0.000
0.005
0.009
0.014
0.018
Marginal ARA intake (g/kg^0.9/d)


(d)
Marginal EPA gain (g/kg^0.9/d)
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.035
0.07
0.105
0.14
Marginal EPA intake (g/kg^0.9/d)


(e)
Marginal DHA gain (g/kg^0.9/d)
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.035
0.07
0.105
0.14
Marginal DHA intake (g/kg^0.9/d)


800
700
600
500
400
300
200
100
0
NQC LC-PUFA (mg/100g meat)
0
5
10
15
20
Diet LC-PUFA (% total FA)