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The effect of marine and non-marine phospholipid rich oils when fed to juvenile barramundi (*Lates calcarifer*)

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ABSTRACT

An experiment was conducted to assess the response of juvenile barramundi (*Lates calcarifer*) to four diets containing either marine- or non-marine derived neutral lipid (NL) or polar lipid (PL) sources for eight weeks in a 2 × 2 factorial design. The four diets contained 8.2% added lipid composed of a 1% fish oil base with 7.2% test lipid (n−3 NL: Fish oil, n−3 PL: Krill oil, n−6 NL: Soybean oil, n−6 PL: Soybean lecithin). The results demonstrated that the different lipid sources (either n−3 or n−6 omega series from either NL or PL class) had significant effects on growth performance and feed utilisation with some interaction terms noted. Growth was negatively affected in the n−6 NL fish and the feed conversion (FCR) was highest in the n−6 PL fish. Digestibility of total lipid and some specific fatty acids (notably 18:2n−6 and 18:3n−3) were also negatively affected in the n−6 PL fish. Analysis of the whole body neutral lipid fatty acid composition showed that these mirrored those of the diets and significant interaction terms were noted. However, the whole body polar lipid fatty acids appeared to be more tightly regulated in comparison. The blood plasma biochemistry and hepatic transcription of several fatty acid metabolism genes in the n−6 PL fed and to a lesser extent in the n−6 NL fed fish demonstrated a pattern consistent with modified metabolic function. These results support that there are potential advantages in using phospholipid-rich oils however there are clear differences in terms of their origin.

Statement of relevance: Juvenile barramundi may benefit from dietary phospholipid.

1. Introduction

The phospholipids form the structural bilayer of cell membranes providing integrity and fluidity (Hazel and Williams, 1990; Tocher et al., 2008). Central to their biological importance is their structure with lipoproteins that assist in the extracellular transport of lipids thus improving parameters such as growth, survival and health throughout the organism (Tocher et al., 2008). However, the total lipid content in fish is mostly composed of neutral lipid in the form of triacylglycerol (TAG) which is a more readily available energy source (Glencross, 2009).

There is evidence to suggest that most larval and early juvenile fish have a dietary requirement for intact phospholipids as endogenous biosynthesis is not sufficient (Coutteau et al., 1997). Coutteau et al. (1997) reported that the phospholipid requirement of fish and crustaceans varied depending on the life stage and history. Freshwater fish generally have lower dietary requirements, of around 2% whereas marine fish generally had higher requirement ranging up to 7% however that gradually reduced as fish grew. Early studies found that in both rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) the phospholipid requirement of first swim-up sized fish (<0.2 g) was 4% supplied in the form of soybean lecithin (Poston, 1990a, 1990b). However, larger salmon (~7.5 g initial) showed no improvement in terms of growth suggesting that endogenous synthesis of phospholipid is sufficient to support the requirement of the fish and that high dietary levels had a negative effect on survival (Poston, 1990a). It should also be noted that the latter study, and possibly others, refer to a requirement of phospholipid containing ingredients rather than the precise phospholipid content which is often unclear.

With very few exceptions, provision of marine derived phospholipid to cultured fish is limited. Moreover, there are few studies on the effect of dietary phospholipids in juvenile fish greater than 5 g as it is generally accepted that they don't have a requirement based on the historical evidence presented for Atlantic salmon (Poston, 1990a). Recently, the influence of dietary phospholipid from either krill oil or soybean lecithin was investigated in Atlantic salmon from first feeding up to smolt (0 to 70 g range) (Taylor et al., 2015). These authors demonstrated a range of improvements among the parameters tested and concluded that

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Atlantic salmon have a dietary requirement for intact phospholipid particularly in early development. Therefore, with the continual reduction of fish meal (FM) and fish oil (FO) in commercial feeds and the complex biochemistry of the phospholipids particularly in juvenile fish, further investigation is warranted. Moreover, the preferential incorporation and retention of phospholipid fatty acids are important in maintaining phospholipid quality and also to fulfil other downstream roles of the phospholipid classes (Linares and Henderson, 1991).

Recent *in-* and *ex-vivo* methods have so far demonstrated that barramundi or Asian seabass (*Lates calcarifer*) are not capable of any measureable long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis (Alhazzaa et al., 2011a; Mohd-Yusof et al., 2010; Tu et al., 2012). However, some notable effects on the phospholipid composition of tissues were identified, which may suggest that juvenile barramundi have a requirement for intact phospholipids in order to prevent the onset of deficiency (Alhazzaa et al., 2011b; Tu et al., 2013). It appears that when dietary PL are not sufficient then very selective retention of tissue phospholipids occurs in barramundi and other species, until depletion, this being a mechanism to prevent the onset of PL deficiency and secondary pathologies as a result (Skalli and Robin, 2004; Tocher et al., 2008; Tu et al., 2013).

Most phospholipid requirement studies to date have used soybean lecithin containing high levels of n−6 PUFA, while others have used egg lecithin or various other marine sources such as fish roe lecithin (Cahu et al., 2009). Recent studies have clearly demonstrated the potential of marine derived phospholipid sources to improve larval and juvenile fish performance (Betancor et al., 2012; Taylor et al., 2015). To date, information is scarce on the effect phospholipid in juvenile barramundi diets. Therefore, an experiment was designed to compare the metabolic effect of marine and non-marine neutral lipid (NL) and polar phospholipid (PL) sources using a two-by-two factorial approach in juvenile barramundi. The biochemical and molecular mechanisms underpinning the role of phospholipids were also investigated.

2. Materials and methods

2.1. Ingredient and diet preparation

The diets were formulated to provide digestible protein at ~55%, lipid at ~12% with a digestible energy value of ~19 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. A single batch of basal diet was prepared then divided up and warmed aliquots of the oil mixtures were thoroughly mixed in. Water was added at approximately 30% of the mash weight and then mixed until consistent dough was formed. The pellets were extruded through a 4 mm die attached to a screw-press pasta machine and cut off at lengths of 5 to 6 mm. The pellets were dried overnight at 60 °C to a constant dry matter and stored in a freezer until required. The formulation and chemical composition of the three diets are presented in Table 2.

2.2. Barramundi husbandry and growth

Juvenile barramundi (*L. calcarifer*) were sourced from the Betta Barra fish hatchery (Atherton, QLD, Australia), on-grown in a 10,000 L tank and fed a commercial diet (Marine Float; Ridley Aquafeed, Narangba, QLD, Australia). Prior to commencement of the experiment the fish were transferred to a series of experimental tanks (300 L) with flow-through seawater (salinity = 35 PSU; dissolved oxygen 4.6 ± 0.15 mg/L) maintained at 30.0 ± 0.01 °C (mean ± SD) with a supply rate of about 3 L/min to each of the tanks. The tanks were maintained in an environment-controlled laboratory with the photoperiod set to a constant 12:12 h cycle. At the beginning of the experiment, the tanks held 26 fish of 47.0 ± 0.3 g (mean ± SD, n = 312 individually weighed fish). The four experimental diets were randomly distributed among the twelve tanks with each treatment having three replicate tanks. The fish

Table 1
Chemical composition of ingredients used in experimental diets, all values are g/kg DM unless otherwise stated.

	Fish meal[a]	Poultry meal	Soy isolate	Wheat gluten	Wheat flour	Casein	Wheat starch	Fish oil	Krill oil	Soybean oil	Soybean lecithin
Composition											
Dry matter (g/kg)	98.4	95.8	95.8	92.7	83.9	92.4	83.6	99.2	99.9	100.0	98.0
Protein	78.9	64.1	89.5	82.3	11.2	87.0	0.5	0.4	4.5	1.0	7.5
Ash	16.3	13.8	4.6	0.1	0.6	1.1	0.3	0.1	2.9	ND	9.8
Lipid	4.6	15.1	5.7	12.1	2.2	0.5	ND	95.6	92.6	94.6	75.7
Carbohydrate	0.1	7.0	0.2	5.5	86.0	11.3	99.2	3.9	ND	4.5	7.1
Gross energy (mJ/kg)	18.9	20.4	21.8	21.2	15.3	21.9	14.5	39.3	36.3	39.5	29.7
Fatty acids (mg/g lipid)											
16:0	149.0	161.7	NA	NA	NA	NA	NA	128.4	107.3	93.7	111.5
18:0	50.9	55.4	NA	NA	NA	NA	NA	29.0	6.3	35.4	23.7
18:1	89.8	277.0	NA	NA	NA	NA	NA	104.0	90.0	220.7	53.3
18:2n−6	10.4	71.4	NA	NA	NA	NA	NA	11.7	12.2	430.6	326.1
18:3n−3	4.7	7.1	NA	NA	NA	NA	NA	5.8	7.1	49.7	41.8
20:4n−6	16.4	4.5	NA	NA	NA	NA	NA	9.1	3.2	ND	ND
20:5n−3	57.6	3.7	NA	NA	NA	NA	NA	70.3	144.5	ND	ND
22:5n−3	13.0	ND	NA	NA	NA	NA	NA	12.4	0.0	ND	ND
22:6n−3	152.2	ND	NA	NA	NA	NA	NA	105.3	94.8	ND	ND
SFA	231.5	230.1	NA	NA	NA	NA	NA	205.5	175.3	137.1	135.8
MUFA	129.1	322.0	NA	NA	NA	NA	NA	163.2	135.5	221.7	53.3
C_{18}PUFA	20.0	78.5	NA	NA	NA	NA	NA	27.5	39.6	483.6	367.9
LC-PUFA	244.4	8.2	NA	NA	NA	NA	NA	200.5	242.6	ND	ND
Total n−3	222.7	3.7	NA	NA	NA	NA	NA	198.0	259.7	ND	ND
Total n−6	36.7	83.0	NA	NA	NA	NA	NA	29.9	22.5	483.6	367.9

^ 18:1, sum of 18:1n−7, 18:1n−9 cis, 18:1n−9 trans; saturated fatty acids (SFA), sum of 12:0, 14:0, 16:0, 18:0, 20:, 22:0, 24:0; monounsaturated fatty acids (MUFA), sum of 14:1n−5, 16:1n−7, 18:1n−7, 18:1n−9 (cis and trans), 20:1n−7, 20:1n−9, 22:1n−9, 24:1n−9; polyunsaturated fatty acids, with 18 carbon atoms (C_{18}PUFA), sum 18:2n−6 (cis and trans), 18:3n−6, 18:3n−3, 18:4n−3; long chain polyunsaturated fatty acids, with 20 or more carbon atoms (LC-PUFA), sum 20:2n−6, 20:3n−6, 20:4n−6, 22:4n−6, 20:3n−3, 20:5n−3, 22:5n−3, 22:6n−3; n−3, sum of omega 3 C_{18}PUFA and LC-PUFA; n−6, sum of omega 6 C_{18}PUFA and LC-PUFA.

[a] Fish meal was defatted using hexane. Please see Materials and methods for details. NA, not analysed; ND, not detected.