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The effect of poultry protein concentrate and phosphorus supplementation on growth, digestibility and nutrient retention efficiency in barramundi *Lates calcarifer*

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ABSTRACT

Two experiments were conducted on juvenile barramundi testing the replacement of regular poultry by-product meal (PBM) with a premium grade poultry protein concentrate (PPC). In the first experiment, a series of five iso-nitrogenous and iso-calorific dietary treatments composed of incrementally increasing levels of PPC from 0% to 20%, substituting PBM were formulated (PPC0, PPC5, PPC10, PPC15, PPC20). The diets were restrictively pair-fed to 63g barramundi for 56 days. In the second experiment, a series of four iso-nitrogenous, iso-calorific and iso-phosphoric dietary treatments composed of incrementally increasing levels of PPC from 0% to 20% substituting PBM were formulated (PPC0 + P, PPC6.7 + P, PPC13.3 + P, PPC20 + P). An additional dietary treatment was formulated without any phosphorous supplementation (PPC20-P). The diets were restrictively pair-fed to 69g barramundi and the PPC20 + P and PPC20-P were also tested when fed to satiation for 42 days. Experiment 1 demonstrated a clear linear trend towards poorer growth performance and feed utilisation when increasing the level of PPC in the diet. Significant differences were noted for final weight, weight gain, SGR and FCR ($P < 0.05$) in the fish fed diets PPC15 and PPC20 compared to the PPC0. Protein retention efficiency and the apparent biological value of protein and energy was lower ($P < 0.05$) in the PPC20 compared to the PPC0 fed fish. The apparent digestibility of crude protein, gross energy, phosphorous and several amino acids all increased with increasing level of PPC in the diet. In experiment 2, using iso-phosphoric diets, the reverse trend was demonstrated whereby increasing PPC improved the performance of barramundi. Significant differences were noted for the final weight, weight gain, SGR and FCR ($P < 0.05$). When comparing the PPC20 + P and the PPC20-P fed fish, there was a clear negative impact of not supplementing phosphorus, which was further compounded in fish fed to satiation (interaction, $P < 0.05$). There were no significant differences in nutrient retention with the exception of calcium which increased with increasing PPC, and energy retention being higher in fish fed PCC20 + P than PCC20-P treatments. The results of these two experiments demonstrate that the effective replacement of regular PBM with high quality PPC is achievable and when the diets are supplemented with phosphorus to an inclusion > 1.5%, promotes faster growth, for fish fed restrictedly and to satiation. The results indicate a pressing need to redefine the phosphorus requirement of barramundi juveniles, notably to ensure the scope for growth is not affected when testing the nutritional value of novel protein sources.

1. Introduction

Reliable raw materials supply to fuel the expansion of the aquafeed industry is a major challenge to ensure the provision of sustainable seafood to a growing population with insatiable demand for quality protein. The feasibility for alternative protein sources to replace fishmeal in freshwater and marine fish and prawns has been a major research topic for several decades (Gatlin et al., 2007; Hardy, 2010; Tacon and Metian, 2008). Currently, three main categories of fishmeal and fish oil replacements are available and used at commercial scales: terrestrial plant meals, rendered animal by-products, and seafood processing wastes (Klinger and Naylor, 2012). Other fishmeal and oil replacers such as insect meal (Henry et al., 2015), algae (Maisashvili et al., 2015) and microorganisms (yeast, bacteria, and microalgae) are

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showing good prospects but need to be more cost-effectively produced at scale to be incorporated into commercial fish and prawn feeds (Gamboa-Delgado and Márquez-Reyes, 2016). Partial replacement of fishmeal (> 50%) is now regularly achieved in most omnivorous and carnivorous species at the commercial scale (Klinger and Naylor, 2012), and more recent innovations have allowed successful validation of zero fishmeal diets for carnivorous fish such as barramundi (Glencross et al., 2016). Rendered animal by-products such as poultry meal, blood meal, feather meal, meat and bone meal, are increasingly being relied upon as high quality alternative sources of protein for fish feeds to replace fishmeal. Their advantages compared to plant protein tend to be more complete amino acid profile and for some of them high levels of available lysine, methionine and phosphorus. Reports are mixed to the extent to which these by-product meals are used in aquafeeds today (Naylor et al., 2009). In Australia and New Zealand, there has been significant research and development and commercial application of poultry by-product meal and oil (Salini et al., 2015; Glencross et al., 2011; Booth et al., 2017). On a world scale, their low use compared to plant protein tends to be attributed in large part by regulations, such as the previous ban in the European Union (i.e., it recently lifted its ban on non-ruminant processed animal protein for fish feed), and consumer acceptance due to social concerns associated with disease transmission (Klinger and Naylor, 2012). Another major reason for the historically limited use of rendered products has been their variable nutritive value due to poor digestibility and quality variability (Bureau et al., 1999). Reports have demonstrated that rendered products can be variable and have poor nutritive value in some species however this is not always the case (Bureau et al., 1999; Glencross, 2006). Williams et al. (2003) found that certain rendered animal derived meals did not impact negatively on laboratory scale or semi-commercial trials with barramundi.

Inconsistency in product quality is known to affect parameters such as digestibility and this should be considered prior to any product development (Bureau et al., 1999; Glencross et al., 2007). The composition and freshness of rendered animal protein, as well as processing conditions can have significant effects on their nutritional quality. Heat treatment can result in changes to protein or damage to amino acids and other nutrients through oxidation of sulfhydryl bonds into disulphide bonds, non-peptide crosslinking, Maillard reactions, oxidative degradation, and even pyrolysis (Bureau et al., 1999). For instance, the apparent digestibility for protein of two poultry by-product meals was 87% and 91% and that for energy of 77% and 87% in rainbow trout (Bureau et al., 1999). *In vitro* digestibility, crude protein, fat and ash content for 94 commercial meat and bone meal produced in New Zealand was found to be highly variable (CV = 10-43%) (Hendriks et al., 2002). The digestibility of these products have now improved through improvements in selection of raw ingredients and processing techniques (Klinger and Naylor, 2012).

Improving the utilisation of protein and phosphorus in aquaculture is at the forefront of improving fish performance, through improving nutrient retention and growth rates, as well as improving water quality through minimising nitrogen and phosphorus discharge into the environment (Vielma and Lall, 1998; Lupatsch and Kissil, 1998; Storebakken et al., 1998; Åsgård and Shearer, 1997). In most fish, the main signs of phosphorus deficiency include problems associated with skeletal deformities and modulation of intermediate metabolism leading to poor growth and poor feed efficiency (NRC, 2011; Sugiura et al., 2004). Barramundi, or Asian sea bass, is a commercially relevant species produced in both in freshwater (11,130 tonnes) and seawater (65,713 tonnes) with a global value of USD320 millions. Major producing countries include Malaysia (29,133t), Thailand (16,500t), Taiwan (14,015), Indonesia (6,558t), Saudi Arabia (3,888t) and Australia (3,770t) (FAO statistics, 2018). Barramundi's nutritional requirements have been studied since the 1980s (Glencross, 2006). Juveniles have a protein requirement between 450 to 550 g kg^{-1}, protein to energy ratio of 25 to 30 mg KJ^{-1} and a protein (nitrogen) utilisation efficiency estimated at 46%. Barramundi have a limited capacity to utilise dietary carbohydrates, and require a typically carnivorous fish diet (Glencross et al., 2017). While most nutrient requirements are known in barramundi, little is known regarding even the most basic mineral requirements with the exception of phosphorus and selenium (Ilham, 2016; Le et al., 2013; Glencross, 2006). The phosphorus requirement was reported to be between 5.5 and 6.5 g kg^{-1} (Boonyaratpalin, 1997). However, this study is likely not reflective of current ingredient or processing technology nor was it a complete assessment across the different stages of growth. Additionally, no data on phosphorus retention efficiency is available. Phosphorus loss in the environment can lead to eutrophication, particularly in freshwater (Wang et al., 2008), and as a consequence can also hinder farm productivity through discharge limits. Improving phosphorus retention efficiency is therefore of particular relevance to barramundi culture, conducted at various salinities.

In this study, two experiments were conducted to better characterise the nutritional value of a high quality poultry meal (PPC) against standard poultry by-product meal (PBM) in barramundi and the potential for phosphorus supplementation to improve the retention of these meals. The objectives for each experiment were:

1) To test the effect of substituting PBM with PPC in diets pair-fed to juvenile barramundi on growth, digestibility, and nutrient retention efficiency.
2) To test the effect of substituting PBM with PPC in diets pair-fed and satiation fed with and without phosphorus supplementation on barramundi growth and nutrient retention efficiency.

2. Material and methods

2.1. Culture system and fish measurements

The fish were sourced as fingerlings from Robarra hatchery, West Beach, South Australia for both experiments. Experiment 1 and experiment 2 (2016 and 2017 respectively) were carried out in 15 and 21, respectively, replicated 500 L conical bottom tanks (n = 3 per treatment) at the Bribie Island Research Centre, Queensland, Australia. The experimental tanks were set up with ~ 5 L/min flow of continuously aerated seawater (35 PSU) with water temperature of 30°C for the duration of both experiments. Photoperiod was set at 12L:12D. Water temperature and dissolved oxygen were logged in six random tanks, and averaged 29.8°C and 7.50 mg/L respectively for experiment 1, and averaged 30.0°C and 5.70 mg/L respectively for experiment 2.

Prior to each experiment, forty fish were weighed from a pooled population to minimum of 0.1 g accuracy to obtain a population size estimate. Only fish within one standard distribution of the mean were selected to be stocked into the experiments. Twenty five fish of 63.0g ± 4.5 SD in experiment 1 and twenty five fish of 69.5g ± 5.7 SD in experiment 2 were then allocated to each tank. Fish were anaesthetized using AQUI-S prior to weighing and allowed to recover in their allocated tank following weighing. This procedure was carried out on each weight check day.

2.2. Dietary treatments

2.2.1. Feed manufacture and feeding

A total of 10 diets were prepared for this study. Each of the ingredients were milled to < 750um before batching, ingredient chemical and amino acid composition can be found in Table 1. A total of 10kg of each of the experimental diets was mixed thoroughly (without the oil component) using an upright planetary mixer (BakerMix, Artarmon, NSW, Australia). The diets were then extruded through a laboratory-scale 24mm twin-screw extruder (MPF24:25, Baker Perkins, Peterborough, UK), using a 3.0 mm Ø die (~4 mm Ø pellets) using Standard CSIRO Extrusion protocols. The pellets were dried at 60°C for 24h, after which each diet was vacuum infused with the specific allocation of oil.