Quantitative and qualitative aspects of the protein nutrition of barramundi (*Lates calcarifer*) larvae fed formulated foods.

Thesis submitted by
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Leo Nankervis

Abstract

Formulated ‘artificial’ diets have the potential to overcome inherent nutritional and financial drawbacks associated with live foods used for larvae fish culture. Artificial formulations also provide a vehicle for accurate manipulation of nutritional constituents, enabling investigations into the nutrient requirements of fish larvae. In order to develop species-specific larval food formulations, optimal macronutrient requirements and appropriate nutrient sources must be established. The nutritional value of a food ingredient is determined by its nutritional profile and nutrient availability, and therefore nutrient digestibility is an essential part of the evaluation of novel food ingredients. Since the proteolytic capacity of barramundi larvae is limited, optimal sources of dietary protein must be established to optimise both their amino acid profile, and protein digestibility. An integrated approach was therefore adopted in this study to evaluate protein sources for barramundi larvae in terms of their amino acid profile and digestibility and in their capacity to support optimal growth and survival when incorporated into food formulations. Furthermore, the endocrinal mediation of the nutritional control of growth is investigated through thyroid hormone analysis, and nutritional effects on digestive physiology are examined through pepsin development.

The thyrotropic hormone system is a major regulatory mechanism for the control of growth in teleosts. Thyroid hormones (triiodothyronine, T3 and L-thyroxine, T4) mediate extrinsic processes, such as nutrition, to regulate growth in juvenile and adult fish, and are regulated by nutritional quality and quantity. While thyroid hormones regulate growth, survival, development and metamorphosis in fish larvae, data are

lacking on an endocrine-nutrition link at the larval stage. By applying an endocrinal approach to the nutritional control of growth, we may achieve a better understanding of the underlying processes governing the physiological status of fish.

The research described in this thesis was therefore designed to clarify the quantitative and qualitative protein requirement of barramundi, *Lates calcarifer*, larvae, and to investigate possible nutritional links to thyroid hormone concentration.

Dietary protein and energy contents were initially manipulated in larval food formulations to determine baseline macronutrient inclusion levels. Barramundi larvae (14 days after hatch, DAH) were fed microbound diets (MBD), varying in gross dietary protein (45, 50 and 55%) and energy (18 and 21 $MJ.kg^{-1}$) for a period of 14 days. All fish were then sacrificed, measured for total length and a sub-sample taken for dry weight analysis. Carcass T3 and T4 were measured by radioimmunoassay, following chloroform/NH_3OH extraction.

In following experiments, marine animal meals (fish meal, squid powder, *Artemia* meal, mussel meal, prawn meal and krill meal) were evaluated for their suitability for inclusion into MBD for barramundi larvae. Each of these meals was included in dietary formulations to a total of 50% gross protein. These formulations were evaluated in terms of growth and survival of barramundi larvae, amino acid composition of protein sources, protein digestibility and resulting carcass thyroid hormone levels. To improve the limited digestibility of fish meal, subsequent experiments incorporated fish meal hydrolysates and acid-denatured fish meal into MBD for evaluation in growth trials with barramundi larvae.

An optimal diet was found to contain at least 21 $MJ.kg^{-1}$ dietary energy and derived its protein from a combination of fish meal and squid powder (9:1 ratio). The limited digestibility of fish meal was improved approximately two-fold by acid denaturation, and the moderate inclusion of denatured fish meal into food formulations improved larval growth significantly, while the entire replacement of untreated fish meal with denatured fish meal did not improve growth above that of diets containing intact fish meal. The reasons for this are unclear, though carcass pepsin level was depressed for larvae fed the formulation containing no intact fish meal, indicating that larvae may adapt to less digestible protein sources. The high leaching rates typically attributed to MBD are assumed to be responsible for the poor growth and/or survival of larvae fed diets containing autolysates and hydrolysates in this study.

Thyroid hormone levels had no direct correlation to dietary energy level, protein source or protein inclusion level, though T4 correlated with growth independently of these nutritional manipulations. This finding indicates that T4 is important in the growth process of barramundi larvae, but is not directly mediated by specific nutritional inputs.

This study developed a microbound diet which supported up to 58% survival and significant growth in barramundi larvae from 14-28 DAH. The diet that supported the best rates of growth and survival contained 21 $MJ.kg^{-1}$ utilisable dietary energy and at least 50% dietary protein, comprised of a 9:1 ratio of fish meal to squid powder.

This study utilised integrated methodology to improve diet composition to increase growth and survival in barramundi larvae fed MBD, and to investigate the underlying mechanisms behind growth promotion. Growth trials remain the most conclusive way to determine optimal nutrient requirements for formulated foods, however, biochemical composition and physical properties can be used to narrow-down the wide range of nutrient sources available. Digestibility is of critical importance to the study of protein sources in food formulations for fish larvae, and a major area for the improvement of native animal meal protein sources.

The diet developed in this study is a critical step in the development of species-specific weaning and larval diets for barramundi. Amino acid profiles for optimal growth have been refined, and the digestibility of fish meal has been increased to improve larval growth, thus potentiating early weaning protocols and diminishing *Artemia* requirements. The development of co-feeding and weaning protocols with this diet is expected to increase growth through optimised amino acid profile and increased energy and fatty acid availability, while reducing costs in barramundi hatcheries through reduced *Artemia* requirements.

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Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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