



THE ROLE OF BRANCHED-CHAIN AMINO ACIDS IN THE AQUACULTURE OF RED DRUM, *Sciaenops ocellatus* L. – DEFINING DIETARY REQUIREMENTS AND ELUCIDATING ANTAGONISTIC EFFECTS

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First, the minimum dietary requirements of the branched-chain amino acids (BCAAs) (leucine [Leu], isoleucine [Ile] and valine [Val]) were determined for juvenile red drum in three independent 49-d feeding trials. Experimental diets were prepared by supplementing a basal diet with gradual incremental levels of Leu (9.0-29.0 g/kg), Ile (5.0-20.0 g/kg) or Val (6.8-14.0 g/kg). Triplicate groups of fish were fed each diet to apparent satiation twice daily, after which growth performance parameters were measured. Incremental levels of dietary Leu, Ile and Val significantly affected weight gain, feed efficiency and protein retention. A broken-line regression model estimated the minimum Leu, Ile and Val requirements of red drum to be 15.7 g/kg, 11.1 g/kg and 12.4 g/kg of diet, respectively.

Secondly, the effects of imbalanced dietary levels of BCAAs on growth performance and amino acid utilization of juvenile red drum were determined. A control diet was prepared by keeping the levels of Leu, Ile and Val at the established minimum requirements. Six experimental diets were prepared by supplementing the control diet with an excess of Leu (62.0 g/kg), an excess of Ile (44.0 g/kg), an excess of Val (50.0 g/kg), an excess of Leu and Ile (62.0 and 44.0 g/kg), an excess of Ile and Val (44.0 and 50.0 g/kg), and an excess of Leu and Val (62.0 and 50.0 g/kg). Diets were fed to fish in triplicate aquaria at a rate approaching apparent satiation, twice daily, for 49 d. Growth performance parameters were calculated and the postprandial concentration of BCAAs in plasma was analyzed. Weight gain was significantly affected by an excess of dietary Leu, confirming an antagonistic effect due to the imbalanced concentration of BCAAs in the diet. However, postprandial levels of BCAAs in plasma did not indicate that an excess of Leu blocked the intestinal absorption or promoted the catabolism of Ile and/or Val in red drum.

Ultimately, these results will facilitate the production of aquaculture feeds composed of alternative protein ingredients while maintaining an ideal amino acid profile for optimal performance of red drum.



Dietary leucine modulates growth performance, glucose metabolism, antioxidant and immunity related signaling molecules in juvenile blunt snout bream, *Megalobrama Amblycephala*

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Leucine plays an important role in growth, nutrient metabolism and immune response for fish species. An 8-week feeding trial was conducted to estimate the dietary leucine requirement and investigate the effects of dietary leucine levels on the growth performance, antioxidant, immunity, glucose metabolism signaling molecules in juvenile blunt snout bream. Fish were fed six practical diets with graded leucine levels ranging from 0.90% to 2.94%. Results showed that compared to fish fed diet with 0.90% leucine, significantly higher final weight (FW), weight gain rate (WG) and specific growth rate (SGR), and significantly lower feed conversion ratio (FCR) were observed in fish fed with 1.72% dietary leucine level. The highest whole body protein and lipid content were observed in fish fed with 2.14% leucine level. The optimal dietary leucine level for juvenile blunt snout bream was obtained at 1.40% and 1.56%, respectively, based on WG and SGR using second-order polynomial regression model at 95% of maximum response. 1.72% dietary leucine level significantly improved the antioxidant capacity of fish by regulating the plasma superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, total antioxidant capacity (T-AOC) activity, catalase (CAT) activity, aspartate aminotransferase (AST) activities and malondialdehyde (MDA) content, and also significantly influenced the antioxidant gene expression involved in nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway by regulating Nrf2, heme oxygenase-1 (HO-1), GPx, copper/zinc superoxide dismutase (Cu/Zn-SOD), manganese superoxide dismutase (Mn-SOD). 1.72% dietary leucine level significantly enhanced the mRNA levels of insulin signaling pathway related genes including target of rapamycin (TOR), insulin receptor substrate 1 (IRS-1), phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt), and subsequently affected expression of glucose transporter 2 (GLUT2), glucokinase (GK), phosphoenolpyruvate carboxykinase (PEPCK). However, excessive dietary leucine level (2.94%) resulted in high mRNA and protein levels of S6K1 and high level of plasma glucose. Results of our study demonstrate the benefits of dietary leucine on growth, glucose metabolism, antioxidant and immune status to maintain the health in juvenile blunt snout bream. Our results also indicate excessive dietary leucine level show a negative effect on growth and glucose metabolism in this fish species.



Effects of dietary leucine levels on growth performance, feed utilization, neuro-endocrine growth axis and TOR- related signaling molecule expression in juvenile hybrid grouper (*Epinephelus coguttatus* ♀ × *Epinephelus lanceolatus* ♂)

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A 9-week growth trial was conducted to evaluate the effects of dietary leucine levels on growth performance, feed utilization, neuro-endocrine growth axis and TOR- related signaling molecule expression in juvenile hybrid grouper (*Epinephelus coguttatus* ♀ × *Epinephelus lanceolatus* ♂). Seven isoenergetic, isoproteic (53.5% of dry matter) and isolipidic (7% of dry matter) were formulated to contain graded dietary leucine levels (1.7%, 2.25%, 2.8%, 3.35%, 3.9%, 4.45% and 5.0%). Dietary amino acid nitrogen contents were adjusted to be constant by replacing leucine with aspartic acid and glycine (1:1) mixture. Each experimental diet was fed to triplicate groups of 12 hybrid grouper juveniles (average initial body weight: 6.92 ± 0.03 g/fish) which were stocked into small floating cages (L 120 cm × W 70 cm × H 50 cm). Fish were fed twice daily (08:00 and 16:00) to apparent satiation. Results indicated that weight gain% (WG%) were significantly influenced by different dietary leucine levels. WG% was increased as dietary leucine level increased, reaching a peak value at the 3.35% dietary leucine level, and then thereafter, WG% was declined as dietary leucine level continued to be raised. The quadratic regression analysis of weight gain against dietary lysine levels indicated that the optimal dietary leucine requirement for hybrid grouper was estimated to be 3.31%. Gene expressions of growth hormone (GH) in pituitary and insulin like growth factor I (IGF-I), growth hormone receptor 1(GHR1) in liver were significantly affected by different dietary leucine levels. Fish fed 3.35% and 3.9% had higher GH expressions than fish fed other levels of leucine. For hepatic IGF-I and GHR1 genes, fish in the 3.35% leucine treatment had higher expressions than fish in other treatments. The TOR-related signaling molecule expression, such as target of rapamycin (TOR) and EIF4E binding protein1 (4EBP1), were also significantly influenced by different dietary leucine levels, and fish fed 3.35% dietary leucine had higher expressions than fish fed other levels of dietary leucine. Generally, the optimal dietary leucine requirement for best growth of hybrid grouper was estimated to be 3.35% of dry matter.



Dietary methionine spares taurine in sub-adult yellowtail kingfish (*Seriola lalandi*)

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Taurine (Tau) is a β -sulfonic amino acid, deriving from the transsulfuration pathway of methionine (Met) and is an accepted supplement to promote growth and health in commercial finfish aquaculture. Previous studies on omnivorous and carnivorous fish species have altogether shown suboptimal, but species-specific, de novo syntheses of Tau with deficiencies associated to inferior vital indices. Recommendations on Tau supplementation in *Seriola* spp. feeds range from 2.6–6.4g kg⁻¹ to ≥ 10 g kg⁻¹ diet. Met, a Tau precursor, potentially spares and therefore alters Tau biosynthesis in yellowtail kingfish (YTK). Met recommendations for the closely related *Seriola quinqueradiata* indicate that 11g kg⁻¹ diet is adequate. However, recommendations for YTK are absent. Findings on the Met-Tau interactions could potentially contribute towards a more economically and environmentally sustainable feed.

A dose-response approach was applied to quantify the dietary Tau requirement in YTK. Further, sparing effects of dietary Met on Tau was tested utilising an orthogonal design to identify dietary interactions. Fourteen isonitrogenous and isoenergetic diets were prepared using practical raw ingredients with either of two levels of Met (10.9 \pm 0.6 [LowMet]) or 17.2 \pm 1.6g kg⁻¹ [HighMet]) and either of seven levels of taurine ranging from 1.6 \pm 0 to 20.2 \pm 0.3g kg⁻¹. Triplicate groups of fourteen fish (53.3 \pm 0.4g fish⁻¹) were fed one of the fourteen diets over seven weeks.

Based on growth data, YTK do not require additional Tau supplementation when fed the HighMet diet, demonstrating that adequate dietary Met can completely spare Tau supplementation. Growth performance of YTK fed at the HighMet diet exceeded that of the LowMet diet even at the highest Tau level. Minimum dietary Tau for YTK when fed the LowMet diet is 7.7g kg⁻¹ diet, whereas minimum dietary Tau level for optimal feed utilization was 6.2g kg⁻¹ diet. FCR of YTK fed the HighMet diet were independent of dietary Tau content. Digestibility of diets and nutrients, amino acid deposition, enzyme activity and pathophysiology of liver were also analysed (results pending).

Growth and feed conversion data indicate that current recommended concentrations of dietary Met may be insufficient for sub-adult YTK. At LowMet levels a minimum intake of 0.03g Tau fish⁻¹ day⁻¹ will provide adequate growth in YTK.



Evaluation of synchronicity of methionine in the hemolymph of Pacific white shrimp *Litopenaeus vannamei* fed diets containing different methionine sources

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A series of feeding trials were conducted to investigate the absorption of amino acids and compare the methionine (Met) levels in the hemolymph of Pacific white shrimp, *Litopenaeus vannamei* fed diets containing different supplemental Met sources. For each trial, the shrimp were preconditioned to the diets for four days. Prior to feeding, a group of shrimp were bled to determine the fasted levels of free amino acids (AA), and then, offered feed for a defined period and hemolymph were sampled and analyzed for postprandial amino acid profile. In trial 1, feed was offered to shrimp for 30 minutes then hemolymph samples were taken every 30 minutes (mins) over 5 hours (hrs). Results indicated that all AA were near their peak after feeding and the level declined within 30 mins of fasting to near fasting levels. The second trial and third trial focused on evaluating synchronicity of Met in relation to other AA in the hemolymph of shrimp fed two different Met sources (DL-Met or DL-Met-Met). Fasted shrimp were offered diets for 15 mins and hemolymph samples were taken every 15 mins within 1 h after feeding. The results generally confirmed the fast absorption and clearance of AA in shrimp and indicated that the diets containing different Met sources resulted in higher Met level in the hemolymph compared to the basal diet. Shrimp fed with the diet containing Met-Met exhibited significantly higher Met level (expressed in % total AA or Lys) in the hemolymph compared with the basal diet or the diet supplemented with DL-Met which showed intermediate Met level. In order to understand how quickly Met and other AA are absorbed, fasted shrimp were offered diets for a total of 60 mins during which samples were taken while feed was present. The pattern for AA uptake is similar with a clear upswing in AA levels in 10-15 mins after the initiation of feeding. The current study demonstrates that the uptake of amino acids in shrimp corresponds to the digestive physiology of the animal, there is no apparent asynchronous uptake of amino acids and there was a difference across sources.



Research progress of color formation and its nutritional regulation of Chinese mitten crab (*Eriocheir sinensis*)

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The color of *E. sinensis* is closely related to the content, composition and the existing forms of various carotenoids in the tissues. In recent years, a series of studies have been conducted to investigate the color formation and its nutritional regulation on the coloration of *E. sinensis*. The main results are as follows: (1) the main carotenoids in the ovaries are astaxanthin, β -carotene, zeaxanthin and lutein, and their contents and composition in the tissue are closely related to their dietary carotenoids composition. The main carotenoids in the hepatopancreas are β -carotene and astaxanthin, and the content of β -carotene accounts for more than 80% of total carotenoids. The major carotenoid in the shell is staxanthin, while the contents of the other carotenoids are very low. (2) Astaxanthin and β -carotene are two dominated carotenoids that affect the color of the ovaries. There are three forms of astaxanthin in the crab tissues, including free astaxanthin, astaxanthin diesters (AD) and astaxanthin monoesters (AM). Generally, AD and AM are major form of astaxanthin in ovaries, while the free astaxanthin content accounts for 90% in the crab shell. (3) Based on various evaluation indicators (i.e. gonadal development, coloration, antioxidant capacity, immune performance and biochemical composition), our studies have shown that the appropriate dietary natural astaxanthin in the fattening diets are 60 mg/kg and 40 mg/kg, respectively for females and males. Dietary natural astaxanthin can significantly improve the color, immune response and antioxidant capacity of the crabs. (4) As for different astaxanthin sources, dietary natural astaxanthin has proved to be more effective than synthetic astaxanthin, as its biological value was 2-3 times to that of synthetic astaxanthin. (5) The utilization efficiency of dietary astaxanthin is closely related to the dietary lipid content, and the the higher dietary lipid content could facilitate the astaxanthin deposition for *E. sinensis*. (6) Crustacyanin (CRCN) plays an important role in the coloration and transportation of astaxanthin, which was highly expressed in the hepatopancreas, ovaries and epidermis of the crabs. These results provide the valuable information for the understanding of color formulation of *E. sinensis*.