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Imbalanced dietary levels of branched-chain amino acids affect growth performance and amino acid utilization of juvenile red drum *Sciaenops ocellatus*

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ABSTRACT

Imbalanced dietary levels of branched-chain amino acids (BCAAs) are known to produce antagonistic effects in pigs, rats and humans, affecting the concentration of BCAAs in plasma and ultimately depressing growth. In fish, antagonism involving BCAAs has not been fully characterized or understood. The objective of this study was to determine the effects of imbalanced dietary levels of BCAAs on growth performance and amino acid utilization of juvenile red drum. A control diet was prepared by combining lyophilized red drum muscle and crystalline amino acids, while keeping leucine (Leu), isoleucine (Ile) and valine (Val) at the previously quantified minimum dietary requirement levels for red drum. Six experimental diets were prepared by supplementing the control diet with (1) an excess of Leu, (2) an excess of Ile, (3) an excess of Val, (4) an excess of Leu and Ile, (5) an excess of Ile and Val, and (6) an excess of Leu and Val. Red drum juveniles were stocked in 38-L glass aquaria, and diets were fed to fish in triplicate aquaria, twice daily, for 45 d. At the end of the feeding trial, growth performance was evaluated and postprandial levels of BCAAs in plasma were analyzed. Growth performance of red drum was significantly depressed by an excess of dietary Leu, but not by an excess of Ile nor Val. The postprandial concentration of plasma Leu, Ile or Val was significantly higher in fish fed an excess of Leu, Ile or Val, respectively. 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, as has been reported in other species. However, excess Leu did significantly reduce the postprandial concentration of α-ketoglutarate in plasma, possibly indicating a higher ratio of transamination due to the imbalanced postprandial concentration of BCAAs in plasma. In conclusion, an antagonistic effect due to excess dietary Leu was confirmed in juvenile red drum. This study represents a step forward in understanding the nature of the antagonistic effects among BCAAs in fish.

List of abbreviations

α-KG	α-Ketoglutarate
AA	Amino acid
BCAA	Branched-chain amino acid
BCKA	Branched-chain α-keto acid
BCKDH	Branched-chain α-keto acid dehydrogenase kinase
CP	Crude protein
HSI	Hepatosomatic index
IAA	Indispensable amino acid
Ile	Isoleucine
IPF	Intraperitoneal fat
Leu	Leucine
RDM	Red drum muscle
UPLC	Ultra-performance liquid chromatography
Val	Valine

1. Introduction

The branched-chain amino acids (BCAAs) are a group of indispensable amino acids (IAAs) composed of leucine (Leu), isoleucine (Ile) and valine (Val) that are largely found in the hydrophobic interior core of globular proteins, playing important structural roles and being

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primarily deposited in body protein, notably in skeletal muscle (National Research Council and Committee on the Nutrient Requirements of Fish and Shrimp, 2011). As has been reported in other organisms (Zhang et al., 2017), studies with different fish species (Kawanago et al., 2014, 2015; Lansard et al., 2011) have increasingly recognized the BCAAs as anabolic nutrient signals, communicating the presence of an ingested protein-containing meal to peripheral tissues, and promoting protein synthesis in muscle. In addition, in other monogastric species (i.e., pigs and rats), BCAAs are known to be regulators of lipolysis, thereby enhancing glucose consumption and utilization, promoting improved meat quality, benefitting embryo growth, enhancing intestinal development and intestinal amino acid (AA) transport, as well as up-regulating innate and adaptive immune responses (Zhang et al., 2017).

All three BCAAs share the same common transporter for intestinal absorption, and their oxidation is catalyzed by two common BCAA transaminase enzymes, therefore, most organisms metabolize these three AAs using the same enzymatic system. Because of this, imbalanced dietary levels of BCAAs are known to produce antagonistic effects in pigs, rats and humans, disturbing the postprandial concentration of BCAAs in plasma and ultimately affecting growth. This has been mainly attributed to competitive inhibition during intestinal absorption and increased oxidation through the activation of the branched-chain α–keto acid dehydrogenase kinase (BCKDH) (Block and Harper, 1984; Matsumoto et al., 2014; May et al., 1991; Swendseid et al., 1965).

In fish, antagonism involving BCAAs has not been fully understood. Chance et al. (1964) observed that excess Leu and Ile in diets depressed growth of Chinook salmon (*Oncorhynchus tshawytscha*). In channel catfish (*Ictalurus punctatus*), an excess of Leu and Ile depressed growth of fish fed diets deficient in BCAAs, but not of fish fed diets where BCAAs met the minimum dietary requirements (Robinson et al., 1984). An antagonistic effect of excess Leu on the concentration of BCAAs in plasma and tissue was found in rainbow trout (*O. mykiss*) (Yamamoto et al., 2004), in which lower concentrations of Ile and Val were found in plasma of fish fed diets formulated with high Leu concentrations. In contrast, Choo et al. (1991) did not observe any effect of excess dietary Leu on the concentration of plasma Val and Ile in rainbow trout.

Fish represent an important source of nutritious food and animal protein for most of the world's population. Therefore, research efforts looking to ensure aquaculture's production efficiency are of imminent importance (Food and Agriculture Organization, 2016). In this regard, red drum (*Sciaenops ocellatus*) is a marine carnivorous fish native to the Gulf of Mexico that has proven to be an excellent candidate for aquaculture due to its tolerance of a wide range of environmental conditions, growing from a weight of 1 g to 1 kg in 8–12 months. Because of this, red drum's production has been steadily increasing in the southeastern United States and several Asian countries (Food and Agriculture Organization, 2016; Gatlin, 2002).

The end goal of the present study was a refined understanding of potential antagonistic interactions among BCAAs in fish, which could possibly be encountered due to the imbalanced profile of BCAAs in some protein feedstuffs commonly used in aquaculture diets (e.g., corn gluten meal has a high concentration of Leu [94.0 g/kg] relative to the concentration of other IAA like lysine [11.0 g/kg] and arginine [19.0 g/kg] [National Research Council and Committee on the Nutrient Requirements of Fish and Shrimp, 2011]). With this in mind, the objective of this study was to elucidate the potential impacts of imbalanced dietary levels of BCAAs on red drum's growth performance and AA utilization.

2. Methods

2.1. Feeding trials

Fish husbandry and experimental protocols conducted in this study were approved by the Institutional Animal Care and Use Committee at Texas A&M University (AUP IACUC 2016-0075). Two independent feeding trials were conducted at the Aquacultural Research and Teaching Facility at Texas A&M University, College Station, TX, USA. First, a preliminary trial (Leu trial) was focused on characterizing the possible antagonistic effects of excess Leu in the diet of red drum. Secondly, a follow-up and more comprehensive feeding trial (BCAAs trial) was focused on elucidating possible antagonistic effects of excess Leu, Ile and/or Val. Juvenile red drum used in these feeding trials were obtained from the Sea Center Texas Marine Aquarium, Fish Hatchery and Nature Center operated by the Texas Parks and Wildlife Department in Lake Jackson, Texas, USA.

Both feeding trials were conducted in 38-L aquaria configured as a recirculating system (1 L/min), whereby waste water gravity-flowed to a settling chamber, then to a biological filter and was pumped through an ultraviolet light chamber and sand filter before being returned to the aquaria. Water quality was maintained within optimal levels for red drum. Salinity was maintained at 6–8 g/L by combining a synthetic seawater mixture (Fritz Industries, Mesquite, TX) with sodium chloride and well water, as recommended by the manufacturer. Low-pressure electrical blowers provided aeration via air stones to maintain dissolved oxygen levels near air saturation (6.4 ± 0.02 mg/L). Water temperature was maintained at 27 ± 0.5 °C by controlling ambient temperature with dual air-conditioning units. A 12 h light–12 h dark photoperiod was maintained with fluorescent lights controlled by automatic timers.

At the start of each feeding trial, juvenile red drum were stocked into aquaria to acclimate to experimental conditions for 7 d, during which time a control diet was fed to apparent satiation. After the conditioning period, triplicate aquaria were randomly assigned to each dietary treatment ($n = 3$). In the Leu trial, groups of 15 juvenile red drum were stocked into each aquarium at mean wet body weight (± standard deviation) of 3.51 ± 0.11 g/fish. Whereas, in the BCAAs trial, groups of 12 juvenile red drum were stocked into each aquarium at mean wet body weight (± standard deviation) of 4.56 ± 0.27 g/fish. The experimental fish were fed twice daily at a rate approaching apparent satiation with pre-weighed rations based on a percentage of total fish weight per aquarium (50–60 g/kg of total body weight) and visual feeding cues. Fish in each aquarium were group-weighed every 2 weeks and rations were adjusted accordingly. The preliminary Leu trial continued for a total of 30 d, while the BCAAs trial continued for a total of 45 d.

2.2. Experimental diets

For both feeding trials, a semi-purified control diet (Table 1) containing 430 g/kg of crude protein (CP) was prepared by combining lyophilized red drum muscle (RDM) meal as an intact protein source (105 g/kg of dietary CP) and supplementing with a premix of crystalline L-AAs simulating the AA pattern found in RDM, while keeping the levels of Leu, Ile and Val at the minimum dietary requirement levels for red drum's optimal performance (16.0, 11.0 and 12.0 g/kg of dry diet, respectively [Castillo and Gatlin, 2017]). Dietary lipid and dextrin were adjusted to provide 13.4 kJ of estimated digestible energy/g of diet (McGoogan and Gatlin, 1998). This diet composition has proven to make an excellent test diet for studies on AA nutrition of red drum because of its high palatability and nutritional quality, ensuring maximum growth potential of the experimental fish (Gatlin, 2002).

In addition to the control diet, for the preliminary Leu trial, two experimental diets were prepared by removing most of the supplemental Leu (Leu deficient diet – 9.00 g/kg of dry diet) and by supplementing an excess of Leu (62.0 g/kg of dry diet). Additionally, for the BCAAs trial, six experimental diets were prepared by supplementing the control diet with an excess of Leu (62.0 g/kg of dry diet), an excess of Ile (44.0 g/kg of dry diet), an excess of Val (50.0 g/kg of dry diet), an excess of Leu and Ile (62.0 and 44.0 g/kg of dry diet), an excess of Ile