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Dietary L-methionine requirement of juvenile grouper *Epinephelus coioides* at a constant dietary cystine level

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Abstract

The present experiment was conducted to determine the methionine requirement and the effect of dietary methionine levels on growth, feed utilization, body composition, haematological and morphometrical parameters in juvenile grouper. Diet 1 with fish meal as the sole protein source was used as a reference. Diets 2–7 were formulated to contain 48% crude protein and supplemented with graded concentrations of L-methionine, resulting in six levels of dietary methionine (0.55–1.81% of dry matter) at a constant dietary cystine level of 0.26%. Fish (initial weight of 13.25 ± 0.19 g, mean ± S.D.) were reared in twenty-one 500-l circular fibreglass tanks provided with flow-through seawater at 28–30.5 °C and salinity of 24–28 mg/l for 8 weeks. Fish fed the reference diet had significantly higher weight gain (WG), specific growth rate (SGR), feed utilization efficiency, protein and lipid contents of whole body, plasma protein and cholesterol concentrations ($P<0.05$). Among other treatments fed the diets containing CAAs, increasing dietary methionine up to 1.34% increased growth performance and feed utilization, whole-body protein and lipid contents, condition factor (CF), viscerosomatic index (VSI) and intraperitoneal fat ratio (IPR), beyond which they remained nearly unchanged. Plasma protein concentration showed no significant differences for fish fed the diet containing more than 1.11% methionine. Triacylglycerol increased with increasing dietary methionine levels, but showed a relatively lower value for fish fed the 1.59% methionine diet. Plasma glucose could not be related to dietary treatments. Cholesterol was the highest for fish fed the 1.34% methionine diet but showed no significant difference among other treatments. Fish fed higher-methionine diets exhibited relatively higher essential amino acid (EAA) contents in muscle than fish fed the lower methionine diet ($P<0.05$). Broken-line regression analysis of WG against dietary methionine level indicated that optimal dietary methionine requirement for juvenile grouper was 1.31% of the diet (corresponding to 2.73% of dietary protein on a dry weight basis).

Keywords: *Epinephelus coioides* juveniles; Methionine; Optimal requirement; Growth performance

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1. Introduction

Grouper is a popular food fish cultured in southeast Asia and a potentially important aquaculture species because of fast growth, efficient feed conversion and high market value (Boonyaratpalin, 1997; Millamena, 2002). They are also economically important and regarded as a good candidate for culture in southern China (Luo et al., 2004a). The traditional culture of grouper in southern China relies heavily on the use of trash fish, which is difficult to store, has variable nutritional quality, low feed conversion and may not be available throughout the year (Luo et al., 2004a). Thus, practical feeds formulated on the basis of their nutritional requirements are needed as an alternative to the use of trash fish. The protein requirement for this fish species has been reported to be from 40% to 60% of the diet (Teng et al., 1978; Chen and Tsai, 1994; Shiau and Lan, 1996; Boonyaratpalin, 1997; Luo et al., 2004a).

Protein quantity and dietary amino acid composition are two major factors that influence growth of fish. Determining the essential amino acid (EAA) requirements of cultured fish is of importance due to the effects of these nutrients on growth, feed costs and nitrogen pollution (Small and Soares, 1999). Methionine is an essential amino acid required by terrestrial vertebrates as well as various fish species for normal growth and metabolic functions. It is the first limiting amino acid in some plant protein sources used for fish diet formulation (Poston, 1986; Goff and Gatlin, 2004). In a previous study, we demonstrated that fermented soybean meal could be used to provide a significant proportion of the dietary protein for grouper (Luo et al., 2004b). However, one of the factors limiting its use at higher inclusion levels appeared to be a deficiency of methionine. Methionine deficiency results in slow growth, reduced feed efficiency and the development of lanticular cataracts in certain fish species (Walton et al., 1982; Keembiyehetty and Gatlin, 1993). The presence of the nonessential amino acid cystine in the diet represents a sparing effect in that it reduces the need for methionine. In this case, cystine can only be synthesized from methionine so if cystine is present in the diet, methionine may be spared from cystine synthesis. Thus, the requirement for total sulfur amino acids can be met by either methionine alone or the proper mixture of methionine and cystine (Moon and Gatlin, 1991). Therefore, it is important to consider the dietary cystine content to quantify the methionine requirement of the cultured species for maximum growth and efficient feed utilization. Dietary methionine requirements have been estimated for several species of fish (Wilson, 2002), but has not been reported for grouper. The present experiment was conducted to determine the methionine requirement at a constant dietary cystine level of 0.26% for juvenile grouper.

2. Materials and methods

2.1. Experimental diets

Ingredients and proximate composition of the experimental diets are presented in Table 1 and amino acid composition (g per 100 g dry diet) of dietary ingredients are presented in Table 2. The diet 1 containing 69% fish meal without CAA supplementation was used as a reference. Other six diets (diets 2–7) were formulated to contain 48% protein, about 25% of which was supplied by fish meal and soybean protein concentrate (SPC), and the remaining by a mixture of CAAs without methionine and cystine. The CAA (L-form, Shanghai Kangda Amino Acid Company, Shanghai, China) premix was supplemented to simulate an amino acid profile found in 48% whole chicken egg protein (Kim et al., 1992a) except for methionine and cystine. The dietary protein level was slightly below the requirement for optimal growth of juvenile grouper, 50.2%, with fish meal as a sole protein source (Shiau and Lan, 1996), to ensure the maximum utilization of methionine for growth and limited catabolism for energy. Estimated digestible energy (DE) was 357 kcal per 100 g dry diet for the reference diet and 342 kcal per 100 g dry diet for the diets 2–7 assuming that energy is 4, 9 and 4 kcal per g for protein, lipid and carbohydrate, respectively (Garling and Wilson, 1976). Incremental levels (from 0 to 1.25% methionine) of crystalline L-methionine were added, resulting in six dietary treatments with methionine levels from 0.55 to 1.81 g per 100 g diet in about 0.25 g increments. The basal diet used to quantify the methionine requirement of juvenile grouper contained 0.55% methionine and 0.26% cystine from fish meal and SPC. The diets were maintained isoni-