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Comparative study on the effects of L-methionine or 2-hydroxy-4-(methylthio) butanoic acid as dietary methionine source on growth performance and anti-oxidative responses of turbot (*Psetta maxima*)

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ABSTRACT

A growth trial was conducted to evaluate the effects of L-methionine (L-Met) or 2-hydroxy-4-(methylthio) butanoic acid (HMTBa) as dietary methionine source on growth, whole-body composition, and ascorbic acid concentrations in serum and liver of turbot (*Psetta maxima*). Five levels (0.3, 0.6, 0.9, 1.2 and 1.5% dry matter) of L-Met or HMTBa were added to a practical basal diet, which was limiting in methionine (0.59%) and cystine (0.42%). The basal control diet and 10 experimental diets were fed to groups ($n = 5$) of juvenile turbot (initial weight: 5.6 g), which were reared in a flow-through seawater system. Fish were fed twice daily to satiation for 75 days. Fish fed the basal diet displayed significantly ($P < 0.05$) lower specific growth rate (SGR), feed efficiency (FE), feed intake (FI), protein efficiency ratio (PER), productive protein value (PPV), whole-body protein and lipid contents, but higher whole-body moisture and ash contents compared to those fed with L-Met or HMTBa supplemented diets. Ascorbic acid concentrations in serum increased significantly with dietary L-Met or HMTBa levels ($P < 0.05$), but not in liver ($P > 0.05$). On the basis of SGR or FI, the dietary total methionine requirement of juvenile turbot was estimated to be 1.58 and 1.59% (3.31 and 3.27% of dietary protein) based on L-Met or 1.56% and 1.49% (3.25% and 3.19% of dietary protein) based on HMTBa, respectively, using second-order polynomial regression analysis. Furthermore, fish fed HMTBa, in addition, showed higher PER and ascorbic acid concentrations in serum than those fed L-Met. In conclusion, turbot can use HMTBa as effectively as or better than L-Met to achieve a higher maximum performance.

1. Introduction

Turbot (*Psetta maxima*) is a flatfish species in Europe, which is increasingly being cultured in China since its introduction in 1992 (FAO, 2010). It is a fast-growing fish that is much sought after by consumers (Regost et al., 2001). There are several studies on protein and lipid requirement of this species (Cho et al., 2005; Lee et al., 2003; Li et al., 2011; Regost et al., 2001). However, there is little published data on the quantitative requirement of essential amino acids for turbot (Fournier et al., 2002; Kaushik, 1998; Peres and Oliva-Teles, 2008).

The turbot is a highly carnivorous species with a protein requirement of 49.4% (Lee et al., 2003), and feed formulations for this species have typically incorporated high levels of fish meal. Due to increasing costs, there has been a trend towards decreasing fish meal levels in dietary formulations and replacing it with plant ingredients. Methionine is an essential amino acid required by terrestrial vertebrates as well as various fish species for normal growth and metabolic functions (Luo et al., 2005). In many fish diet, methionine is usually the first limiting amino acid, especially those containing high levels of plant protein feedstuffs such as soybean meal, peanut meal and copra meal (Mai et al., 2006). It is suggested from the previous studies that requirements of dietary methionine for fish range from 1.8 to 4.0% of dietary protein (Wilson, 2002). Methionine is produced commercially by chemical processes and most commonly available in the DL-form. L-methionine is the natural isomer. And D-methionine form is converted to L-methionine in animals. Previous studies showed that fish can use DL-methionine as effectively as L-methionine (Goff and Gatlin, 2004; Robinson et al., 1978). The 2-hydroxy-4-(methylthio) butanoic acid (HMTBa) is an organic acid, which bears a hydroxyl group on the α-carbon instead of the amino group found in methionine. Because HMTBa can be converted to L-methionine within the body of the animal through broadly distributed enzymatic systems (Dibner, 2003), it has been widely used in diets of poultry, swine and ruminant. The use of HMTBa in aquatic animals has been reported in several fish and shrimp species (Cheng et al., 2003; Huai et al., 2010; Zhao et al., 2010), but not in turbot.

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Studies with terrestrial animals (e.g., broiler chicks, laying hens and pigs) showed variable results with regard to the relative biological efficiency (RBE) of HMTBa to L-methionine (L-Met) or DL-methionine (DL-Met) (Harms and Russell, 1994; Kim et al., 2006; Lemme et al., 2002; Liu et al., 2004; Yi et al., 2006). Varied responses have also been observed in aquatic animals, such as channel catfish (Robinson et al., 1978), hybrid striped bass (Keembiyehetty and Gatlin, 1995; Kelly et al., 2006; Li et al., 2009), red drum (Goff and Gatlin, 2004) and Pacific white shrimp (Forster and Dominy, 2006). Some studies have considered HMTBa as a dilution of methionine with the same form of dose–response and same plateau, and have used slope-ratio model or nonlinear common plateau asymptotic regression models to compare the different sources (Littell et al., 1997). However, other research has suggested that relative efficacies vary with dose (Kratzer and Littell, 2006), and that different dose responses and plateau responses occur for HMTBa and DL-Met as seen in broilers (Vázquez-Añón et al., 2006a) and turkeys (González-Esquerra et al., 2007). However, there is little published information on the response to doses of different methionine sources in aquatic animals.

Levine et al. (1999) showed that methionine residues in a wide variety of proteins play important roles in anti-oxidative defense activity from *Escherichia coli* as an in vitro model system. HMTBa increased plasma ascorbic acid concentrations and decreased thiobarbituric acid reactive substance (TBARs) concentrations in liver of hybrid striped bass (Li et al., 2009). Meanwhile, it improved anti-oxidative status and depressed lipid and protein oxidation in intestine, hepatopancreas, serum and muscle of juvenile Jian carp (Feng et al., 2011; Xiao et al., 2012).

Therefore, this study was conducted to comparatively evaluate the effects of L-methionine or HMTBa as dietary methionine sources on growth, feed utilization and anti-oxidative response in turbot (*P. maxima*).

2. Materials and methods

2.1. Experimental diets

A basal practical diet (Table 1) was formulated to contain 48% (dry matter) crude protein and 12% crude lipid. A crystalline L-amino acid premix was supplemented to diet according to the whole body amino acid pattern of turbot (Kaushik, 1998) except for methionine and cystine (Table 2). The basal diet contained 0.59% methionine and 0.42% cystine as determined by amino acid analyzer (S7130, Sykam, Munich, Germany). Graded levels (0.3, 0.6, 0.9, 1.2 and 1.5%) of either L-Met or HMTBa on an equivalent basis were added to the basal diet, respectively. Crystalline L-Met was used as the source of dietary L-Met. HMTBa was supplemented in the form of Mera™ Met (an 84% Ca salt of HMTBa, Novus International Inc., St. Charles, MO, USA). Final L-methionine concentrations in the five L-Met supplemented diets (Diet 2–6) were 0.91, 1.15, 1.49, 1.70 and 2.02%, respectively. And final HMTBa contents in the five Mera™ Met supplemented diets (Diet 7–11) were 0.31, 0.61, 0.97, 1.23 and 1.39%, respectively as determined by the method of Ontiveros et al. (1987). The reverse-phase high-pressure liquid chromatography (HPLC; HP 1100, HP, Palo Alto, USA) with a Zobar C18 column (4.6 mm × 250 mm) was used. Mobile

Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

Ingredients	Diet number (% of methionine source added)										
	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6	Diet7	Diet8	Diet9	Diet10	Diet11
	(0)	L-Met (0.3)	L-Met (0.6)	L-Met (0.9)	L-Met (1.2)	L-Met (1.5)	HMTBa (0.3)	HMTBa (0.6)	HMTBa (0.9)	HMTBa (1.2)	HMTBa (1.5)
Fish meal[a]	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6
Soybean meal[a]	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6
Beer yeast[a]	5	5	5	5	5	5	5	5	5	5	5
Amino acid mixture[b]	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Wheat meal[a]	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31
Fish oil	7	7	7	7	7	7	7	7	7	7	7
Lecithin	1	1	1	1	1	1	1	1	1	1	1
Mineral premix[c]	2	2	2	2	2	2	2	2	2	2	2
Vitamin premix[d]	2	2	2	2	2	2	2	2	2	2	2
Choline chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Ca $(H_2PO_4)_2$•H_2O	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Attractant	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mold inhibitor	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-methionine[e]	0	0.3	0.6	0.9	1.2	1.5	0	0	0	0	0
Mera™ Met[f]	0	0	0	0	0	0	0.36	0.71	1.07	1.43	1.79
Glutamic acid[e]	1.79	1.49	1.19	0.89	0.59	0.29	1.43	1.08	0.72	0.36	0
Proximate composition (n = 3)											
L-methionine (%)	0.59	0.91	1.15	1.49	1.70	2.02	0.60	0.59	0.58	0.61	0.58
HMTBa (%)	0	0	0	0	0	0	0.31	0.61	0.97	1.23	1.39
Crude protein (%)	48.3	48.2	48.0	48.2	48.1	48.4	47.8	47.7	47.6	47.4	47.2
Crude lipid (%)	12.4	12.3	12.5	13.1	12.7	12.9	12.9	12.9	13.0	13.0	13.1
Moisture (%)	5.7	5.9	5.9	8.6	5.3	4.9	8.3	6.1	5.9	6.6	7.4
Ash (%)	6.8	6.7	6.7	6.7	6.7	6.7	6.8	6.9	7.0	7.0	7.2

[a] Fish meal, obtained from Liu He Group (Shandong, China), crude protein 68.3%, crude lipid 14.2%; soybean meal, obtained from Great Seven Bio-tech (Shandong, China), crude protein 48.4%, crude lipid 2.0%; beer yeast, obtained from Great Seven Bio-tech (Shandong, China), crude protein 53.4%, crude lipid 1.4%; wheat meal, obtained from Great Seven Bio-tech (Shandong, China), crude protein 17.0%, crude lipid 0.6%.

[b] Amino acid premix (g/100 g diet): arginine 1.52, histidine 0.57, isoleucine 0.60, leucine 0.90, lysine 1.20, phenylalanine 0.74, threonine 0.76, valine 0.54, alanine 2.03, aspartic acid 1.39, glycine 2.52, serine 0.96, tyrsine 0.62, proline 0.14.

[c] Mineral premix (g/kg diet): $MgSO_4$•H_2O, 1.200; $CuSO_4$•$5H_2O$, 0.010; $FeSO_4$•H_2O, 0.080; $ZnSO_4$•H_2O, 0.050; $MnSO_4$•H_2O, 0.045; $CoCl_2$•$6H_2O$, 0.050; $Ca(IO_3)_2$, 0.060; Na_2SeO_3, 0.020; zeolite, 18.485.

[d] Vitamin premix (g/kg diet): thiamin, 0.025; riboflavin, 0.045; pyridoxine HCl, 0.020; vitamin B12, 0.010; vitamin K3, 0.010; inositol,0.800; pantothenic acid, 0.060; niacin acid, 0.200; folic acid, 0.020; biotin, 0.060; retinal acetate, 0.032; cholecalciferol, 0.005; α-tocopherol,0.240; ascorbic acid, 2.000; ethoxyquin 0.003; microcrystalline cellulose, 16.470.

[e] 99.56%, Jizhoucity Huayang Chemical Co., LTD., China

[f] 84% (Calcium salt of HMTBa; Novus International Inc., St. Charles, MO, USA).