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Effects of dietary soybean meal on the bile physiology in rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

To clarify the effect of dietary soybean meal (SBM) on bile synthesis and enterohepatic circulation in rainbow trout, three isonitrogenous and isolipidic diets; a fishmeal based diet (diet FM), a SBM based non-fishmeal diet (diet SBM) and a 1% bile acid (taurocholate) supplemented SBM diet (diet C-tau) were prepared and acute/chronic effects of the diets on the bile physiology was examined. In the acute experiment (< 24 h), fish fed the SBM based diets (diet SBM and C-tau) had higher expression levels of bile acid synthesis genes, *cyp7a1-2*, *cyp8b1-1* and *cyp8b1-2* in the liver compared to fish fed the FM diet. In the chronic experiment (10 weeks), fish fed the SBM and C-tau diets had significantly lower expression levels of *cyp7a1* (*-1* and *-2*), *cyp8b1* (*-1* and *-2*) genes compared with fish fed the FM diet. On the other hand, expression of bile acid synthesis inhibitor gene *shp-2* was higher in fish fed the SBM diet compared with fish fed the FM and C-tau diets. Furthermore, fish fed the SBM based diet had reduced bile acid absorption rate. These data suggest that SBM up-regulates bile acid synthesis on a short-term basis, while long-term SBM administration down-regulates the bile acid synthesis and the recovery of bile through the enterohepatic circulation. The gallbladder weights of fish fed the C-tau diet were similar to those of fish fed the FM diet whereas the actual bile acid absorption ability was still suppressed in the C-tau group. This study contributes to the general knowledge of bile physiology in teleost fish under different nutritional conditions and to develop tailored and sustainable aquaculture feeds based on SBM.

1. Introduction

Fishmeal (FM), which is primarily produced from small pelagic fish, has long been used as the major protein ingredient for fish feeds in aquaculture industry. In response to limitations in the global supply of FM, efforts are increasingly focused on identifying alternative plant protein sources for replacing FM. Defatted and heat-treated soybean meal (SBM) is currently considered to be one of the most valuable alternative protein source for FM in fish feeds due to its price, availability and relatively well balanced amino acid profile (Gatlin et al., 2007). However, besides growth retardation and morphological changes in the intestine/liver in salmonid species (Iwashita et al., 2008a; Urán et al., 2008; van den Ingh et al., 1991), SBM based diets are known to cause abnormalities in the bile physiology, such as a reduction in amounts of bile in the body and change in conjugated bile salt composition in some teleost species (Nguyen et al., 2011; Romarheim et al., 2006; Yamamoto et al., 2007). In addition, Yamamoto et al. (2007) reported that supplementation of bile acid to a SBM based diet improve growth performance and various physiological abnormalities in rainbow trout *Oncorhynchus mykiss*, indicating a detailed understanding of fish bile physiology is important to improve utilization of plant protein ingredients.

Bile acids are important physiological detergents that promote biliary secretion of lipids and toxic metabolites, and enhance intestinal absorption of fat and related nutrients in animals (Hofmann, 2009). The bile acids are synthesized from cholesterol in the liver and cholic acid (CA) and chenodeoxycholic acid (CDCA) are the major primary bile acids synthesized in humans (Chiang, 2009). Cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1) are known to be crucial for the bile acids synthesis in the liver; CYP7A1 is the first rate-limiting enzyme of bile acid synthesis, while CYP8B1 determines the ratio of CA to CDCA (Chiang, 2011). On the other hand, the small heterodimer partner (SHP) inhibits bile acids synthesis via suppression of *CYP7A1* and *CYP8B1* expression (Chiang, 2002). After being

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synthesized in the liver, bile acids are excreted into the gallbladder via bile salt export pump (BSEP; also referred to as ATP-binding cassette sub-family B member 11, ABCB11) until they are secreted into the intestine during digestion. After secretion to the intestine, most of the bile acids are reabsorbed and shuttled into the portal circulation by apical sodium-dependent bile salt transporter (ASBT) and heteromeric organic solute transporter (OSTa and OSTb), and the bile acids returned to hepatic sinusoid are taken up mainly by Na^+-taurocholate cotransport peptide (NTCP) (Chiang, 2011).

Short amino acid residues of soybean protein has a high bile acid-binding ability (Choi et al., 2002), and soybean protein can prevent reabsorption of bile acids in the ileum of mammals (Nagaoka et al., 1999; Sugano et al., 1990). Such a mechanism can partially explain the reason why the amount of bile in the gallbladder is reduced in teleosts fed SBM. However, it is still controversial whether SBM affects the bile acid production and reabsorption/enterohepatic circulation system in fish (Gu et al., 2014; Kortner et al., 2013), and also if there are species differences depending on their natural feeding habits. Moreover, although Kortner et al. (2013) showed that short-term SBM administration may up-regulate bile synthesis in Atlantic salmon, it is still unclear if SBM has any other temporary effects on the bile physiology since most of the other reported trials have only been based on long-term experiments (Gu et al., 2014; Murashita et al., 2013a; Yamamoto et al., 2010). Moreover, while the mechanisms associated with bile synthesis/enterohepatic circulation system in mammals as mentioned above have been examined extensively, relatively little information is currently available on these mechanisms in fish. Recently, we have cloned and analyzed the tissue expression profiles for a series of bile physiology related genes in rainbow trout (*Oncorhynchus mykiss*) including *cyp7a1-1/-2*, *cyp8b1-1/-2*, *shp-1/-2*, *osta-1/-2*, *ntcp* and *asbt*, and our data indicates that the liver and distal intestine are the important tissues for bile synthesis and enterohepatic circulation in trout (Murashita et al., 2014, 2013b). Furthermore, the expression levels of the genes were drastically affected by a meal and/or diurnal rhythm in rainbow trout (Murashita et al., 2013b). In the present study, therefore, to clarify the acute/chronic effect of SBM on bile synthesis and enterohepatic circulation in rainbow trout, we examined postprandial responses of SBM based diet on the expression of bile related genes in short-term and long-term trials. Also, we examined the bile acid absorption rate using an isolated intestine collected from fish fed SBM diet to reveal whether the actual bile acid absorption is affected by SBM.

2. Materials and methods

2.1. Diets

The formulation and proximate composition of the experimental diets are presented in Table 1. Three isonitrogenous and isolipidic diets were prepared. Jack mackerel meal (steam dried) was used as the primary protein source in the control diet (diet FM). In a soybean meal based diet, the fishmeal was totally replaced by common defatted and heat-treated SBM (hulled) for animal feed (Nisshin OilliO, Tokyo, Japan) and a small amount of soy protein isolate (Fuji oil, Osaka, Japan) (diet SBM). Since taurocholate (C-tau) is known to be a main component of trout bile (Yamamoto et al., 2007), to evaluate the effect of bile acid supplementation on bile physiology, C-tau supplemented soybean meal based diet was also prepared (diet C-tau). The soybean meal based diets were supplemented with amino acids to simulate the digestible amino acid contents of diet FM (Yamamoto et al., 2002).

2.2. Animals and samples

2.2.1. Fish

Rainbow trout, *Oncorhynchus mykiss*, purchased from the Shiga Prefectural Samegai Trout Farm (Maibara, Shiga, Japan) were used in this study. Fish were handled and treated following the "Guidelines for Animal Experimentation" at National Research Institute of Aquaculture, FRA, Japan. Fish were reared at the Tamki Laboratory of the National Research Institute of Aquaculture (Tamaki, Mie, Japan) where they were maintained in indoor 200 L tanks supplied with a continuous flow of well water at 15 °C. The fish were fed a commercial pellet feed (Nippon Formula Feed Mfg Co., Ltd., Yokohama, Japan) by hand twice a day (08:00 and 16:00) for a period of three months, until experiments were carried out.

Table 1
Ingredients and proximate composition of the experimental diets.

	FM	C-tau	SBM
Ingredients (% wet weight)			
Jack mackarel meal	52.00	–	–
Defatted soybean meal	–	53.00	53.00
Soy protein isolated	–	10.00	10.00
C-tau	–	1.00	–
Pollok oil	9.47	12.38	12.37
Wheat flour	18.40	14.00	14.00
α-Starch	5.80	–	–
Vitamins[a]	0.50	0.50	0.50
Choline chloride	0.25	0.25	0.25
Minerals[b]	3.00	5.00	5.00
Cellulose	10.58	0.21	1.22
Betain	–	0.50	0.50
L-Histidine	–	0.76	0.76
L-Isoleucine	–	0.04	0.04
L-Leucine	–	0.14	0.14
L-Lysine	–	0.97	0.97
DL-Methionine	–	0.67	0.67
L-Tyrosine	–	0.04	0.04
L-Threonine	–	0.31	0.31
L-Valine	–	0.24	0.24
Proximate composition (% dry matter basis)			
Crude protein	42.4	41.6	41.5
Crude fat	15.5	14.9	15.2
Ash	9.5	7.4	7.1

[a] Yamamoto et al., 2002.
[b] Yamamoto et al., 2007.

2.2.2. Acute effect trial

Prior to the experiment, 140 fish (35.3 ± 0.8 g) were transferred from the 200 L tanks to seven 60 L tanks (20 fish/tank) and maintained for one week for acclimation to the experimental conditions using the commercial pellet feed. After 48 h fasting, eight fish were sampled from one of the tanks as 0-time fish, and then to the remaining tanks, each test diet was fed (duplicated randomized tanks) by hand to apparent satiation at 08:00. Eight fish were sampled from each group (four fish/tank) at 6 and 24 h after feeding. At the time of sampling, fish were sacrificed by an overdose of MS-222, and the liver and hind-gut were collected and stored in RNAlater (Thermo Fisher Scientific, MA, USA) at −80 °C until RNA isolations were performed. The gallbladder was also dissected out, with care not to lose any contents, and weighed.

2.2.3. Chronic effect trial

Prior to the experiment, 240 fish were transferred from the 200 L tanks to six 60 L tanks (40 fish/tank) and reared for one week with the SBM diet to enable the fish to acclimate to the experimental treatments since SBM based diet tend to have lower palatability and result in lower ingestions rates. After 48 h fasting, the number of fish was adjusted to 30–34 (mean BW, 19.8 ± 0.2 g) to give a fish density of approximately 650 g $tank^{-1}$. Then, each test diet was fed to duplicate tanks by hand to apparent satiation twice daily (08:00 and 17:00), 6 days a week for 10 weeks. Upon measuring the terminal BW, eight fish were sampled from each group (four fish/tank) at 0 (48 h fasted, before feeding), 6 and 24 h after feeding. At the time of sampling, fish were sacrificed by an overdose of MS-222, and the liver and hind-gut were collected and stored in RNAlater (Thermo Fisher Scientific) at −80 °C until RNA