Effect of Feed Ingredients on Digestive Enzyme Secretion in Fish

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Effect of Feed Ingredients on Digestive Enzyme Secretion in Fish

Koji MURASHITA*1, Haruhisa FUKADA*2, Noriyuki TAKAHASHI*2, Noriko HOSOMI*2, Hiroyuki MATSUNARI*1, Hirofumi FURUITA*1, Hiromi OKU*1, and Takeshi YAMAMOTO*1

Abstract: In response to the limitation in the global supply of fish meal, the traditional protein source used in aquaculture feed, efforts have increasingly been focused on the use of alternative protein sources of plant origin. However, plant ingredients may cause growth retardation in aquaculture fish species. Feed nutrients must be digested for their utilization, and so pancreatic digestive enzymes have essential roles for the digestion. Also, cholecystokinin (Cck) is known to be a hormone that stimulates the secretion of digestive enzymes in vertebrates. To improve the utilization of plant based diets in fish, we investigated the effects of various feed ingredients on secretion of digestive enzymes in red seabream *Pagrus major* and yellowtail *Seriola quinqueradiata*, which are commercially important aquaculture species in Japan.

We first investigated the effect of soybean meal on digestive enzyme secretion in red seabream. Activities of pancreatic digestive enzymes (trypsin, chymotrypsin, lipase, amylase) in the intestinal content of a soybean meal based diet (SBM) fed fish were lower than those of a fish meal based diet (FM) fed fish. Also, lower gene expression levels of the digestive enzymes in the hepatopancreas were observed in the SBM fed fish compared with the FM fed fish. These data indicate the FM diet stimulated the secretion/synthesis of pancreatic digestive enzymes to a greater degree than the SBM diet. Then, we tried to identify the stimulation factor in fish meal. Administration of a FM water-soluble fraction increased the gene expression of *trypsin, lipase, cck and cck receptor* (*cck-1r*) in yellowtail, suggesting the enzyme stimulation factor may exist in the water-soluble fraction of FM. Supplementation of the enzyme stimulation factor, although not identified yet, may improve the utilization of plant based diets in aquaculture fish species.

Key words: red seabream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*), pancreatic digestive enzymes, fish meal, soybean meal

Fish meal (FM), which is primarily produced from small pelagic fish such as jack mackerel and anchovy, has long been used as the major protein ingredient for fish feeds in aquaculture. However, as the wild stocks of these pelagic fish species become increasingly limited, development of alternative feeds is considered essential to the expansion of sustainable aquaculture worldwide. In response to such a limitation in the global supply of FM, efforts have increasingly been focused on the use of alternative protein sources of plant origin. Especially, soybean meal (SBM) is known to be the most common fish meal-replacement due to its price, high availability in the market and relatively well-balanced amino acid profile. However, fish fed with diets containing high concentrations of plant protein often show low growth performance and digestibility of feed (Gaylord *et al*., 2008; Glencross *et al*., 2007).

Feed nutrients must be digested for their utilization, and pancreatic digestive enzymes have essential roles for the digestion; trypsin and chymotrypsin are the main pancreatic proteases, lipase is the major pancreatic lipolytic enzyme, and amylase is known as the major pancreatic digestive

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*1 National Research Institute of Aquaculture, Fisheries Research Agency, Tamaki, Mie 519-0423, Japan
E-mail: kojim at affrc.go.jp
*2 Faculty of Agriculture, Kochi University, 200 Monobe, Nankoku, Kochi 783-8502, Japan

enzyme for carbohydrates. The exocrine pancreatic enzymes secretion is controlled by both neuronal and hormonal factors (Konturek *et al.*, 2003). In most vertebrates, a peptide hormone cholecystokinin (Cck) is known to be one of the key physiological regulators of pancreatic secretion (Einarsson *et al.*, 1997; Kofuji *et al.*, 2007). Recently, the *cck* gene has been identified in several fish species such as Atlantic herring *Clupea harengus* (Kamisaka *et al.*, 2005), Atlantic salmon *Salmo salar* (Murashita *et al.*, 2009), yellowtail *Seriola quinqueradiata* (Murashita *et al.*, 2006) and red drum *Sciaenops ocellatus* (Webb *et al.*, 2010), and is mainly expressed in the anterior part of the intestine (including the pyloric caeca) (Murashita *et al.*, 2006). Further, the *cck receptor* (*cck-1r*) gene has also been identified in yellowtail and is principally expressed in the pyloric caeca (Furutani *et al.*, 2013).

To improve the utilization of plant based diets in fish, we investigated the effects of various feed ingredients on secretion of digestive enzymes in red seabream *Pagrus major* and yellowtail, which are commercially important aquaculture species in Japan.

Materials and Methods

Effect of soybean meal on digestive enzymes secretion in red seabream: Juvenile red seabream were used for this experiment. Isonitrogenous and isolipidic FM based and SBM based diets were prepared (diet FM and diet SBM). The fish were fed the experimental diets for 6 weeks, and the hepatopancreas and intestinal content of the fish were sampled (n = 6). Activities of pancreatic digestive enzymes (trypsin, chymotrypsin, lipase and amylase) in the intestinal content (n = 7) were analyzed according to Murashita *et al.* (2007). Gene expression levels of the enzymes in the hepatopancreas were evaluated by real-time quantitative PCR (qPCR) using the specific primers for red seabream digestive enzymes.

Effect of fish meal on gene expression levels of *trypsin*, *lipase*, *cck* and *cck-1r* in yellowtail: Water-soluble and water-insoluble fractions of FM were prepared. The same amount of the two FM fractions on crude protein basis were orally administrated to juvenile yellowtail according to Murashita *et al.* (2008). The pyloric caeca was sampled from the fish,

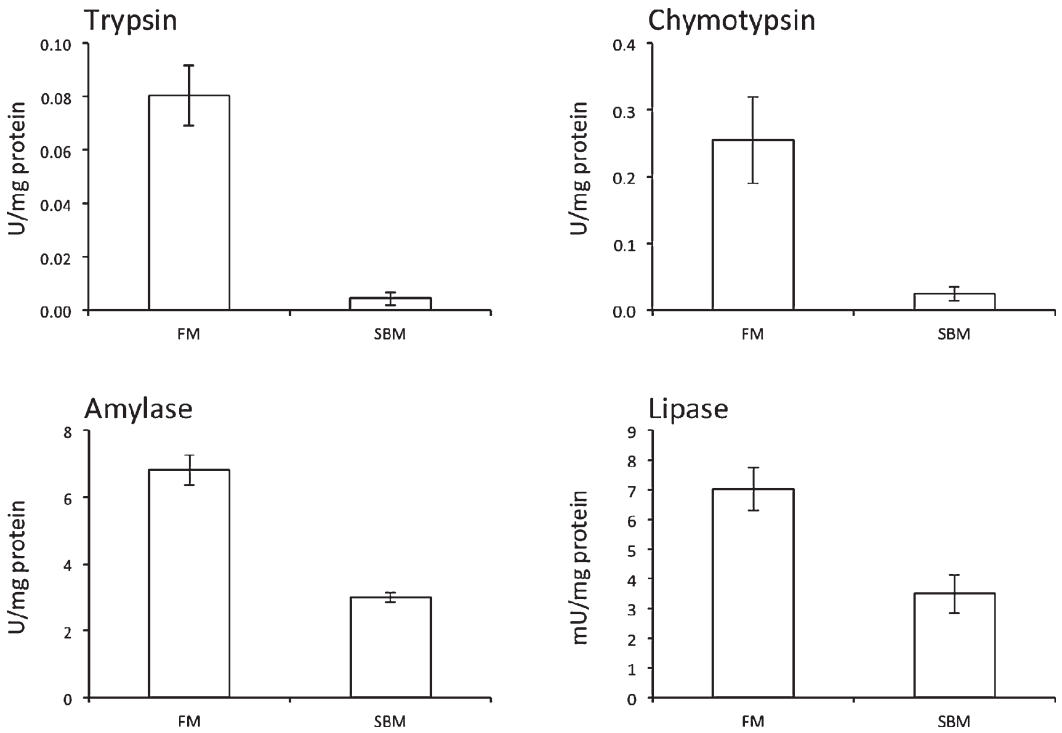

Fig. 1. Effects of fish meal (FM) and soybean meal (SBM) based diets on activities of the pancreatic digestive enzymes in intestinal content of red seabream. Values are mean ± SE (n = 7 fish).

and the gene expression levels of *trypsin, lipase, cck* and *cck-1r* were analyzed by qPCR according to Furutani *et al.* (2013).

Results and Discussion

Effect of soybean meal on digestive enzymes secretion in red seabream: Activities of all four pancreatic digestive enzymes examined in the intestinal content of SBM fed fish were lower than those of FM fed fish (Fig. 1), indicating the amounts of enzymes secreted into the intestine in the SBM fed fish were lower than those of the FM fed fish. Also, lower gene expression levels of the digestive enzymes in the hepatopancreas were observed in the SBM fed fish compared with the FM fed fish (Fig. 2), which is in line with the report in yellowtail; orally administrated FM increased the *trypsin* and *lipase* gene expressions in the pyloric caeca, but not in fish administrated SBM (Furutani *et al.*, 2012). These data indicate that SBM does not fully stimulate the secretion/synthesis of the pancreatic digestive enzymes, or, FM strongly stimulates the digestive enzymes secretion/synthesis.

Effect of fish meal on gene expression levels of *trypsin*, *lipase*, *cck* and *cck-1r* in yellowtail: Administration of a FM water-soluble fraction increased the gene expressions of *trypsin, lipase, cck* and *cck-1r* in yellowtail, whereas those of fish administrated a FM water-insoluble fraction did not (Fig. 3, brief results), suggesting the enzyme stimulation factor may exist in the water-soluble fraction of FM. We are trying to identify the enzyme stimulation factor from the FM water-soluble fraction.

The main component of the FM water-soluble fraction may be very small peptides and/or free amino acids (data not shown). It is known that some amino acids strongly stimulate CCK release in rodents (Daly *et al.*, 2013); such a mechanism might also exist in fish. Supplementation of the enzyme stimulation factor may improve the utilization of plant based diets in aquaculture fish species.

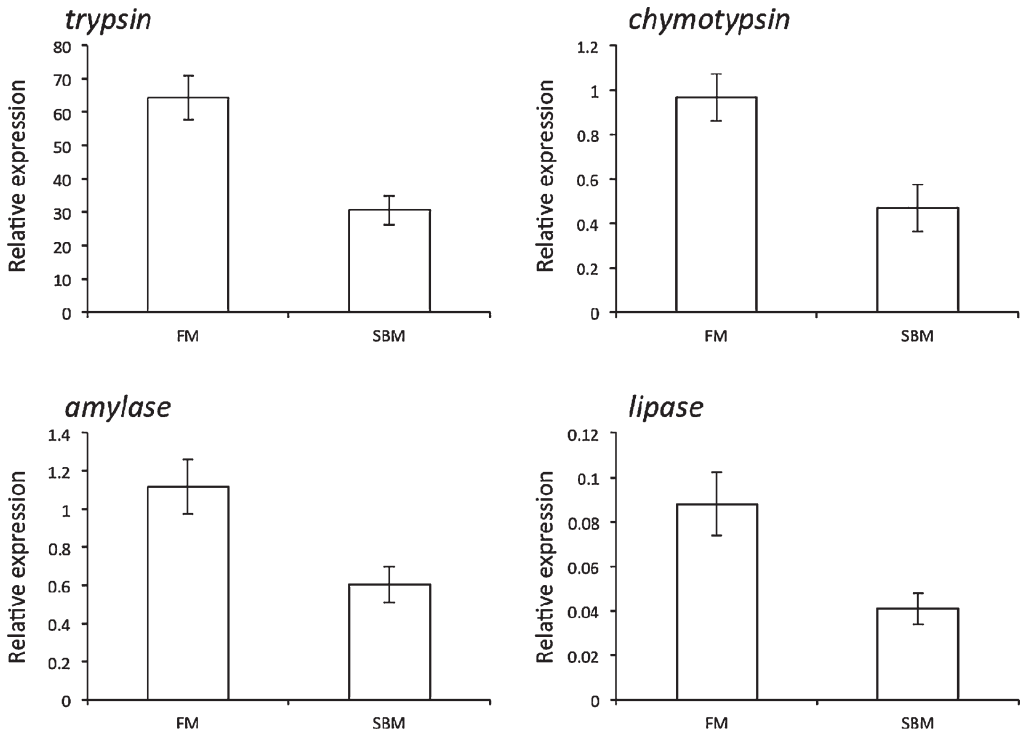

Fig. 2. Effects of fish meal (FM) and soybean meal (SBM) based diets on gene expression of pancreatic digestive enzymes in the hepatopancreas of red seabream. Data for each gene is represented as mean calculated copy number normalized against red seabream *ef1a* copy numbers. Values are mean ± SE (n = 6 fish).

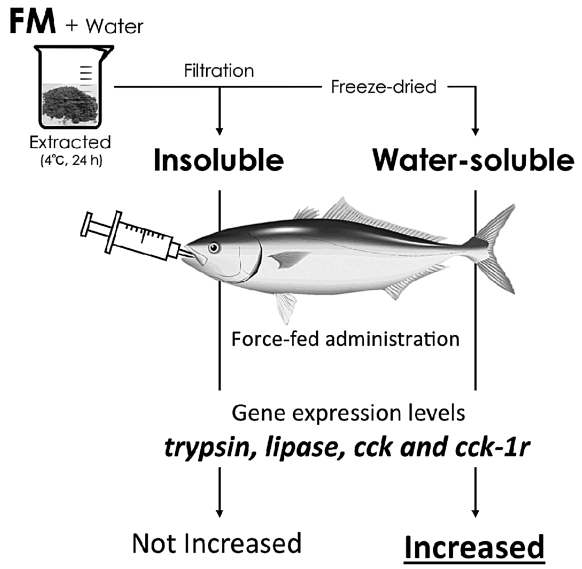

Fig. 3. Effect of fish meal on gene expression levels of *trypsin*, *lipase*, *cck* and *cck-1r* in yellowtail. The schematic diagram of the experiment is presented with brief results.

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Murashita K., Fukada H., Rønnestad I., Kurokawa T., and Masumoto T., 2008: Nutrient control of release of pancreatic enzymes in yellowtail (*Seriola quinqueradiata*): Involvement of CCK and PY in the regulatory loop. *Comp. Biochem. Physiol. A*, **150**, 438–443.

Cholecystokinin (Cck) and neuropeptide Y (Npy)-related peptides are the key regulators of pancreatic enzyme secretion in vertebrates. Cck stimulates enzyme secretion whereas peptide Y (Py or Pyyb), a Npy-related peptide, plays an antagonistic role to that of Cck. In fish, very little is known about the effects of different nutrients on the synthesis of Cck and Py in the digestive tract, and the mechanism by which Cck and Py actually regulate digestive enzyme secretion is not well understood. In order to determine stimulating effects of different nutrients on the synthesis of Cck and Py in yellowtail (*Seriola quinqueradiata*), *cck* and *py* mRNA levels in the digestive tract were measured after oral administration of a single bolus of either phosphate-buffered saline (PBS: control), starch (carbohydrate), casein (protein), oleic acid (fatty acid) or tri-olein (triglyceride). In addition, in order to confirm the synthesis and secretion of digestive enzymes, the mRNA levels and enzymatic activities of three digestive enzymes (*lipase, trypsin* and *amylase*) were also analyzed. Casein, oleic acid and tri-olein increased the synthesis of *lipase*, *trypsin* and *amylase*, while starch and PBS did not affect the activity of any of these enzymes. *cck* mRNA levels rose, while *py* mRNA levels were reduced in fish administered casein, oleic acid and tri-olein. These results suggest that in yellowtail, Cck and Py maintain antagonistic control of pancreatic enzyme secretion after intake of protein and/or fat.

Furutani T., Masumoto T., and Fukada H., 2012: Response of cholecystokinin and digestive enzyme mRNA levels to various feed ingredients in yellowtail *Seriola quinqueradiata*. *Fish. Sci.* **78**, 1075–1082.

Cholecystokinin (Cck) is the key regulator hormone that stimulates the secretion of digestive pancreatic enzymes in vertebrates. In fish, little is known about the mechanism of induction of Cck in the digestive tract by different feed ingredients. To investigate the response of *cck* and digestive enzymes to fish feed ingredients in yellowtail *Seriola quinqueradiata*, we performed a series of experiments in which we measured the mRNA levels of *cck*, *trypsin*, and *lipase* after oral administration of a single bolus of various ingredients. We administered fish meal and fish oil in experiment 1; high and low concentrations of fish meal in experiment 2; and five different dietary protein sources (fish meal, soybean meal, soy protein concentrate, corn gluten meal, and glutamic acid fermentation by-products) in experiment 3. In experiments 1 and 3, only fish meal significantly increased the mRNA level of *cck* and digestive enzyme. In experiment 2, a high concentration of fish meal [20 % (w/v)] significantly increased the *cck* and *trypsin* mRNA levels, but a low concentration of fish meal [1 % (w/v)] did not. These results suggest that high concentrations of fish meal (the protein source in fish feed) has the most potent effect on stimulation of *cck* synthesis and secretion of digestive enzymes in yellowtail.

Furutani T., Masumoto T., and Fukada H., 2013: Molecular cloning and tissue distribution of cholecystokinin-1 receptor (CCK-1R) in yellowtail *Seriola quinqueradiata* and its response to feeding and *in vitro* CCK treatment. *Gen. Comp. Endocrinol.* **186**, 1–8.

In vertebrates, the peptide cholecystokinin (Cck) is

one of the most important neuroregulatory digestive hormones. Cck acts via Cck receptors that are classified into two subtypes, Cck-1 receptor (Cck-1r; formally Cck-a) and Cck-2 receptor (formally Cck-b). In particular, the Cck-1r is involved in digestion and is regulated by Cck. However, very little information is known about Cck-1r in fish. Therefore, we performed molecular cloning of *cck-1r* cDNA from the digestive tract of yellowtail *Seriola quinqueradiata*. Phylogenetic tree analysis showed a high sequence identity between the cloned yellowtail *cck receptor* cDNA and *cck-1r*, which belongs to the *cck-1r* cluster. Furthermore, the expression of yellowtail *cck receptor* mRNA was observed in gallbladder, pyloric caeca, and intestines, similarly to *cck-1r* mRNA expression in mammals, suggesting that the cloned cDNA is the yellowtail *cck-1r*. In *in vivo* experiments, the *cck-1r* mRNA levels increased in the gallbladder and pyloric caeca after feeding, whereas *in vitro*, mRNA levels of *cck-1r* and digestive enzymes in cultured pyloric caeca increased by the addition of Cck. These results suggest that Cck-1r plays an important role in digestion stimulated by Cck in yellowtail.