（学位第３号様式）

学 位 論 文 要 旨

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題　目	Nutritional studies on the utilization of probiotics and fermented soybean meal for cultured fish species （海産養殖魚におけるプロバイオティクスと発酵大豆ミールの利用性に関する栄養学的研究）

With a growing global population, to maintain the current level of per capita consumption, global fish production needs to increase and most come from aquaculture. As the scale of intensive aquaculture increases, the frequency of diseases is increasing. The need to improve the disease resistance, feed efficiency, and growth performance of farmed organisms is very important. In addition, due to the shrinking fishmeal resources, feed protein sources are desired to replace fishmeal, but it is difficult to replace fishmeal alone with plant-based materials because of the antinutritional factors and low crude protein content. Therefore, the present study firstly investigated the effect of *Bacillus subtilis natto* (BSN) on the growth performance and health level of red sea bream and Japanese flounder. For this purpose, a study on the fermentation of soybean meal (SBM) by BSN was conducted. After evaluating the nutritional value of fermented soybean meal (FSM), a study was conducted on the feeding performance of FSM for red sea bream.

In the first part, BSN was evaluated on the growth, digestive enzyme activity, blood chemistry, oxidative status, immune response, and the growth-related genes' expression in the skeletal muscle of red sea bream. Five diets were prepared to contain BSN at 0, 1 x 10^7, 1 x 10^8, 1 x 10^9, and 1 x 10^{10} CFU/kg diet. After 56 days, the fish fed diets containing BSN resulted in an increase in growth performance, immune response, and intestinal microbial abundance in a dose-dependent manner when compared to the control diet. When compared to the control, the number of *Bacillus, Lactobacillus,* and total bacteria in the intestinal were significantly increased, and *Escherichia coli* was decreased in all BSN groups. Considering these promising results, we suggest that the supplementation of BSN improves the growth and health condition of red sea bream. Then the beneficial effects of BSN and *Lactobacillus plantarum* (LP) were tested in Japanese flounder feeds. A control and three other diets were prepared by supplementation with BSN or LP separately and mixed. After 60 days, both probiotic supplemented groups exhibited significant enhancement in growth performance, digestive enzyme gene expression, and there was a strong interaction in intestinal immunity and intestinal microbial abundance, in particular.

In the second part, the response surface methodology (RSM) was employed to explore the relationships of fermentation conditions such as temperature, time, water-substrate ratio, and layer thickness on the degree of protein hydrolysis (DH) and the crude protein content (CP) of SBM. The optimum conditions for achieving the higher DH (15.96%) and CP (55.76%) were 43.82 °C, 62.32 h, 1.08 of water-substrate ratio, and a layer thickness of 2.02 cm. Major antinutritional factors in SBM were significantly reduced during the process and almost all SBM protein macromolecules were decomposed. Together with the cost-effectiveness of solid-state fermentation (SSF), BSN-FSM products have great potential to improve the plant composition and replace high-cost ingredients in aquafeed. Using this fermented product (live BSN >10^8 CFU/g) as a base, five isonitrogenous diets where fishmeal was replaced with FSM at 0% (FM), 18% (FSM1), 36% (FSM2), 54% (FSM3), and 70% (FSM4), respectively were fed to red sea bream. After 56 days, the replacement level under 54% did not affect weight gain, feed conversion efficiency, fish somatic indices, and survival compared to control. Immune levels and intestinal histomorphology in FSM1 and FSM2 groups were superior to FM or FSM4 groups. The number of goblet cells in the proximal intestine exhibited significantly higher levels in FSM1 and FSM2 when compared to FM. These findings suggested that protein from FSM can substitute up to 54% of fishmeal without negative effects on growth, nutrient utilization, immune response, or intestinal morphology of red sea bream.