Summary

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Title	Nutritional evaluation of fermented rapeseed meal for marine cultured species

Key word（**Rapeseed meal**）（**Fermentation**）（***Pagrus major***）（***Paralichthys olivaceus***）

While aquaculture has continued to grow faster than any other food production industry, the main problem facing the sector today is the provision of suitable and sufficient fish feed. Indeed, the global production of commercial aquafeeds increased gradually and is expected to reach 71 million metric tons by 2020 with fishmeal's production, the all-time preferred ingredient in aquafeeds, expected to remain constant or decrease slightly in the future, evoking some concerns regarding the normal development of the aquaculture industry (FAO, 2018; Rana et al., 2009).

To develop economical aquaculture system, alternative sources of high-quality protein are then required to replace high-cost fishmeal and plant protein sources have been identified as a good candidate because of their relatively wide availability. As a co-product of oil extraction, rapeseed meal is well positioned to be a viable source of plant protein because of the volume of production and the nutritional and functional qualities of its protein (Poulsen and Blaabjerg, 2017). Rapeseed meal production was around 39.29 million metric tons in 2018 but its production is still lagging far behind the one of soybean meal because of the presence of antinutrients that limit its use in aquafeeds (USDA, 2018).

Several attempts have been made to improve rapeseed meal quality. Heat treatment reduced phytic acid and improves the meal's digestibility but heat-stable secondary compounds remain and amino destruction occurs (Drew et al., 2007). Alkaline or acidic treatment provides purified rapeseed meal but nitrogen losses, protein denaturation were observed and using purified ingredients in large amount can become costly. Solid state fermentation offers a simple and cheaper alternative as it serves the dual purpose of value addition and antinutrients reduction in meals (Katz, 2013; Famularo et al., 2005; Kiers et al., 2003). That is why the first objective of the present study was to investigate to effectiveness of microbial fermentation with fungi to improve rapeseed meal biological detoxification and nutritional characteristics. In addition, nutrients digestibility, digestive enzyme activity, and partial replacement of fishmeal by rapeseed meal or fermented rapeseed meal in practical diet for Red sea bream (*Pagrus major*) and Olive flounder (*Paralichthys olivaceus*) were evaluated.

Trials were carried out at the Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima

University, Japan. Fish were kept in 100-L polycarbonate tanks under a flow through sea water system where each tank was equipped with an inlet, outlet, continuous aeration, and maintained under natural light/dark regime. Fish were given test diets following specific feeding protocols, and samples were collected and analyzed.

In the first part of the study, solid state fermentation of rapeseed meal with *Saccharomyces cerevisiae* (RM-Yeast) and *Aspergillus oryzae* (RM-Koji) was used to change the characteristics of the meal. The fermentation process increased crude protein content by 17%, decreased lipid content by 25%, reduced antinutrients and selectively affected amino acids contents in fermented meals. In addition, small-size peptides (< 20kD) were increased in RM-Koji. Consequently, a second and third trials were conducted to evaluate the effects of fishmeal replacement by RM-Yeast and RM-Koji on red sea bream performances.

In the second trial, fish were fed five diets were RM-Yeast substituted fishmeal at 0, 18.75, 37.5, 56.25 and 75%. Only groups fed diet with 75% substitution recorded significantly lower ($P < 0.05$) growth, feed utilization and unfavorable blood parameters. Lysozyme and peroxidase activities in fish, and reaction to low salinity water were not significantly affected ($P > 0.05$) by RM-Yeast, but 18.75 and 37.5% substitution levels exerted better oxidative status. We concluded thus, that 56.25% of fishmeal protein could be substituted by RM-Yeast without negative effects on fish growth, feed utilization and general health condition.

In the third trial, a blend of RM-Koji replaced 0, 25, 50, 75 and 100% of fishmeal in diet for red sea bream. At the end, growth performances were significantly increased ($P < 0.05$) in 25% replacement diet, while growth, feed utilization, protease and protein digestibility were not affected ($P > 0.05$) by up to 50% replacement of fishmeal compared to control. In addition, lysozyme, bactericidal and peroxidase activities were increased in groups fed 25% - 50% replacement levels, together with improved oxidative condition.

A fourth trial was conducted to compare the effects of simple rapeseed meal and RM-Koji as fishmeal substitution in diet for red sea bream. 50% fishmeal was replaced by either rapeseed meal or RM-Koji. Groups fed fishmeal and RM-Koji based diets exerted significantly higher growth, hemoglobin and improved triglyceride levels than those fed simple rapeseed meal ($P < 0.05$). Also, bactericidal, lysozyme, respiratory burst, and peroxidase activities were increased in RM-Koji fed groups over simple rapeseed meal fed groups. Surprisingly, oxidative status of fish, measured through malondialdehyde concentration and reactive oxygen metabolites, were significantly improved in test groups over control.

In the last trial, nutrients digestibility (ADC) for fishmeal, rapeseed meal, RM-Yeast and RM-Koji were determined for Olive flounder using test diets composed of 70% reference diet and 30% of each of the

test ingredients following the indicator method with 0.5% chromic oxide (Cr_2O_3) as inert marker. ADC for crude protein of RM-Yeast and RM-Koji were significantly higher ($P < 0.05$) than that for rapeseed meal. In addition, protease, lipase and amylase activities in juveniles' Olive flounder fed rapeseed meal were lower, while RM-Koji recorded the highest activity, followed by RM-Yeast and fishmeal respectively.

Microbial fermentation with fungi enhances the nutritional quality of rapeseed meal, offering an effective approach to improving the quality of unconventional proteins sources. Considering the characteristics of the fermented meals, RM-Yeast and RM-Koji could be used as specialized feed ingredients endowed with functional properties. The results of the present study indicate that relatively high inclusion of fermented rapeseed meal in diet for marine fish is possible, opening the opportunity for the development of practical feeds with high levels of plant-protein sources. Our study demonstrated that RM-Yeast and RM-Koji could be included by up to 50% in diet for red sea bream without compromising growth and health condition of fish. At the same level, RM-Koji induces better growth and immune responses than simple rapeseed meal in red sea bream juveniles. In addition, the study revealed that, at 30% inclusion level, Olive flounder cannot effectively utilize rapeseed meal and derivate when compared to fishmeal. However, feedings Olive flounder with fermented rapeseed meal leads to better nutrients digestion together with intestinal enzymes improvements. Considering the lower price of rapeseed products compared to fishmeal, together with the high value addition aspects of the fermented meals, the obtained results revealed the biological and economic benefits of fungi mediated rapeseed meal in cultured fish.

However, it has been reported that high inclusion level of rapeseed meal in diet leads to negative health outcomes for juvenile grass carp (Yuan et al., 2015). Is then worth investigating the effect of high rapeseed products and/or long term feeding effects on cultured fish health. Also, since the success of the fermentation depends on the inoculation density of the fungi, it will be interesting to evaluate the effect of different level of fermentation on fish performance in further research. Finally, more research are needed to understand how the microbial fermentation of rapeseed meal modulated specific genes expression leading to growth, stress resistance and immune responses improvement in cultured fish.

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