

論文の内容の要旨

水圏生物科学専攻

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論文題目 Genetic basis and genetic improvement of heterobothriosis resistance and growth performance in the tiger pufferfish, *Takifugu rubripes*, fed with low fishmeal diet

(低魚粉飼料給餌下におけるトラフグのヘテロボツリウム症耐性と成長能力に関する遺伝基盤と選抜育種の可能性に関する研究)

Fishmeal produced from wild-captured fish is an important component of diets used in the aquaculture industry. However, the rising price of fishmeal and the necessity for sustainable fishing have encouraged the aquaculture industry to explore alternative protein resources. To reduce the amount of fishmeal, a wide range of potential replacements has been tested mainly using plant proteins and recently the potential of single cell proteins from microorganisms such as bacteria, yeasts, and microalgae have been suggested. However, these novel diets are still under development and remain untested for the majority of aquaculture species.

The availability of low fishmeal (LFM) diet would be largely depending on species specific characteristics of digestive system, and LFM diets may diminish growth performances of farmed fish. Even though, it is expected that growth performances of fish fed with LFM diets can be improved by harnessing selective breeding technologies. Selective breeding is the process to selectively improve economically important traits in farmed populations by producing genetically superior progenies through recurrent mating among high-potential individuals and has dramatically improved farming efficiency of major crops and live stocks. In aquaculture, its potential has been widely recognized and routinely practiced in several species, mainly salmonids and tilapias. Recently, genomic selection (GS) is attracting the most attention as a rapid selective breeding method, which leverage the genomic information to predict the genetic ability for artificial selection. Thanks to the developments in the next-generation sequencing technologies, the genome-wide single nucleotide polymorphisms (SNPs) is available for aquaculture species at low costs, and the feasibility of GS has been proven in a wide range of aquaculture species.

In this study, I have investigated the availability of GS for parasitic resistance against heterobothriosis, caused by a monogenean parasite *Heterobothrium okamotoi*, and growth performances in the tiger pufferfish, *Takifugu rubripes*, reared under a LFM diet. The species is a major aquaculture species in Japan that commands a high market price. Currently, the standard feeds for the tiger pufferfish are composed of extruded dry pellets

(EP) that contain a high level of animal protein (70–80%) mainly from fishmeal; thus, it is expected that the combination of the use of low fishmeal diets and GS can significantly reduce feed costs while facilitating sustainable aquaculture.

Chapter 1. Availability of genomic selection for heterobothriosis resistance and body size under a standard feed

I have first investigated the genetic basis of heterobothriosis resistance and growth trait, i.e., standard length (SL), and feasibility of GS for these traits using a small number of samples. The test population was produced by artificial mating among 11 wild males and 10 wild females. At four months old, 240 individuals were subjected to an artificial infection experiment. At 32 days post infection, SL were measured (mean \pm S.D. = 9.83 ± 0.78 , cm) and the number of parasites on branchial cavity walls (*H. okamotoi* counts, HC: 15.85 ± 9.15) was counted for each specimen. Subsequently, genome wide 6,707 SNPs were genotyped for all individuals. Genome-wide association study (GWAS) showed no SNPs significantly associated with each trait, implying these traits are polygenic. Genomic heritability (h^2 , mean \pm S.E.) estimated under genomic best linear unbiased predictor (GBLUP) model was moderate for each of the traits (HC: 0.308 ± 0.123 ; SL: 0.405 ± 0.131), suggesting the high potential for genetic improvement by GS.

Feasibility of GS can be evaluated from predictive ability of the GS model. I have calculated prediction ability under 12 models, including GBLUP, Bayesian regressions (Bayes A, Bayes B, Bayes C, Bayesian ridge regression, Bayesian LASSO, and Bayesian RKHS), and machine learning procedures (feedforward neural networks and multi-task feedforward deep neural network). Moderate predictive ability was observed in each model for both HC (0.248–0.344) and SL (0.340–0.481), confirming the feasibility of GS for both traits. Notably, undesirable genetic correlation was observed between HC and SL ($r_g = 0.228$) under a multivariate GBLUP model. To examine the possibility of simultaneous improvements in both traits, in silico selection was performed for ten generations under six scenarios: random mating, selection on HC, selection on SL, simultaneous selection based on genomic Smith-Hazel index, and that based on the desired gains index. The simulation results suggested that selection on one trait may have negative response on the other and the desired gains index can help achieving simultaneous genetic improvements in the two traits.

Chapter 2. Genomic prediction for heterobothriosis resistance and body size traits in a middle-scale population

As described above, the feasibility of GS on heterobothriosis resistance and body size was confirmed using a small population produced from wild parents at a small-scale experiment. In this chapter, I have further tested the availability of GS using a middle-scale population produced using commercially raised individuals (ten males and four females) derived from families cultured for several generation. I have also tested the effects of the number of SNPs on the predictive ability. A total of 1,100 fish at four months old were subjected to an artificial infection trial to collect phenotypes of HC (3.31 ± 3.26) and SL (12.97 ± 0.95 , cm). Genotypes of 12,548 SNPs were obtained for each fish after imputation. As in the case of the small-scale experiment, GWAS study revealed polygenetic nature of both traits. HC and SL showed low and moderate heritability, respectively (HC: $h^2 = 0.162 \pm 0.041$; SL: $h^2 = 0.537 \pm 0.045$). The predictive abilities estimated under three models, GBLUP, Bayes C and Bayesian RKHS, were also low for HC (0.253–0.258) and moderate for SL (0.520–0.524). The

low heritability and predictive ability for HC seen in the middle-scale experiment could be largely due to the phenotypic distribution leans to zero; nevertheless, the values still support the possibility of GS for heterobothriosis resistance. To examine the needed SNP density for GP, a panel of SNP density (12,548, 10,000, 7500, 5,000, 2,500, 1,000, 500, 100, 50 SNPs) were randomly sampled for 50 times to calculate predictive abilities. The results showed that average predictive abilities (\pm S.E.M.) of GP for HC using 5,000 SNPs (0.256 ± 0.001) are comparable to the one using 12,548 SNPs (0.258 ± 0.000) but decreased as the number of SNPs was less than 5,000.

Chapter 3. Availability of low fishmeal diets for the tiger pufferfish

Exploitation of alternative protein sources for LFM diets has been highly demanded for the tiger pufferfish. In this study, I have investigated the availability of single cell proteins such as yeast and bacteria as replacement of fishmeal as no systematic study on the practicality of using single cell proteins in this species even though the high potential of these proteins has been realized in several fish species.

Growth performance, blood chemistry, resistance to heterobothriosis, and transcriptomic responses in the liver were compared between groups fed either a standard diet (FM, of which fishmeal ingredients occupied 80% of the total mass) or one of the four LFM diets; in three diets, 37.5% of the fishmeal (30% of the total mass) was replaced by plant (PP), bacterial (BAC), or yeast (LY)-derived ingredients; in the fourth, 62.5% (50% of the total mass) was replaced by yeast (HY). After a three-month feeding trial, the HY-fed fish was significantly larger in SL ($p = 0.031$) and body weight ($p = 0.008$) compared to control fish but lower in hematocrit value (Ht, $p = 0.009$). Mean total cholesterol levels in BAC-, LY- and HY-fed fish were lower than in control fish ($p = 0.015, 0.007, \text{ and } 0.047$, respectively) while no significant difference was found for triglyceride, total protein, and glucose levels in plasma among the groups. Heterobothriosis resistance was also not different among diet groups, and the difference in Ht between HY and the other groups was diminished at the end of the 36-day infection trial. These analyses indicate that a LFM diet in which 67.5% of fishmeal is replaced by yeast protein (i.e., containing 50% of yeast protein in total mass) may be a suitable replacement for the commercial FM diet as it promotes growth but does not induce severe deleterious effects. The gene expression analyses in the liver showed up-regulation in genes on the terpenoid backbone biosynthesis and steroid synthesis pathways in LFM diet groups suggesting the importance of these pathway to be adjusted themselves to LFM diets for the species.

Chapter 4. Genomic prediction for heterobothriosis resistance and body size under a short-term treatment of low fish meal diet

As the availability of the LFM diet including high proportion of yeast protein (HY) was demonstrated selected as the best diet after the evaluation, I have examined heritability and the possibility of genomic selection in two different populations: Pop-C, consisting of 993 individuals (three-month old) produced from the commercial broodfish, and Pop-CW, 1,017 individuals (three-month old) produced from commercial broodfish and wild individuals). After 99 days (Pop-C) and 128 days (Pop-CW) of dietary treatments with HY diet, fish were subjected to an artificial infection trial. These specimens were subsequently genotyped by means of GRAS-Di, resulting 19,227 and 21,272 imputed SNPs for Pop-C and Pop-CW, respectively. The predictive

abilities estimated under three models, i.e., GBLUP, Bayes C and Bayesian RKHS, were high for both HC (Pop-C: 0.520–0.522; Pop-CW: 0.564–0.567) and SL (Pop-C: 0.601–0.604; Pop-CW: 0.736–0.740), indicating the feasibility of GS for parasite resistance and growth performance under short-term HY diet feeding. Meanwhile, a strong genetic correlation between HC and SL were observed in both populations ($r_g = 0.661$ and 0.585 , respectively), and therefore breeders need to pay close attention to this undesired correlation for selection.

Chapter 5. Genomic prediction for body size under a long-term treatment of low fish meal diets

I have demonstrated the feasibility of genomic prediction for heterobothriosis resistance and body size of the tiger pufferfish fed with HY-diet for a short period of several months. However, a long-term treatment of HY-diet may result in different outcomes. In this chapter, the ability of genetic prediction was investigated using a total of 936 three-month old fish, sharing the same parental individuals with Pop-C in Chapter 4. These specimens were fed with HY-diet for 19 months from juvenile (three months old) to the harvest size, during which standard length was recorded four times: SL_1 (198th day of the long-term feeding treatment), SL_2 (319th day), SL_3 (422nd day), and SL_4 (569th day). A total of 16,471 imputed SNPs were obtained. None of these SNPs showed significant association with SL at each sampling point. High genetic correlation between each pair of SL_i ($r_g = 0.917$ – 0.973) suggested high proportion of shared genetic architecture. Predictive abilities accessed by Bayesian RKHS were moderate and similar between SL at each sampling point (0.589–0.632), and GEBVs showed high correlation between each pair of SL_i (Pearson's $r = 0.915$ – 0.962) suggested harvest size can be predicted at early stage under HY-diet feeding condition. As the importance of genes related to terpenoid backbone biosynthesis and steroid biosynthesis for utilization of LFM diet was suggested in the gene expression analysis, it is expected that this transcriptomic information can be utilized to improve predictive ability. The predictive ability for SL_4 was estimated under the GBLUP model using weighted genomic relationship matrix (i.e., weighted SNPs near and within the genes in terpenoid backbone biosynthesis and steroid biosynthesis); however, predictive ability was lower when weighted genomic relationship matrix was used (0.623–0.627), suggesting minimal genetic differences in genes relating these pathways among individuals.

In conclusion, I found that both heterobothriosis resistance and SL are polygenic traits in the tiger pufferfish populations fed with the standard diet and the HY based LFM diet and demonstrated the feasibility of GS for these traits in the populations fed with either of the diets. It is expected that these findings and investigation strategies can be applied for the establishment of breeding populations with a high tolerance of low fishmeal diets, and thus contribute facilitating sustainable aquaculture of the tiger pufferfish and the other aquaculture fishes.