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A preliminary study toward the improvement of low fishmeal diet utilization in a Yamanashi strain of rainbow trout *Oncorhynchus mykiss*

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Abstract: For future sustainable aquaculture practices, utilization of a low fish meal diet in a Yamanashi strain of rainbow trout was compared with another strain. In addition, efficacy of selective breeding for the low fishmeal diet was preliminarily examined in the Yamanashi strain. A 50% fishmeal diet (FMD) and a soybean and corn gluten meal-based 5% fishmeal diet (LFMD) were fed to apparent satiation to the juveniles of Yamanashi control strain (YC), Shiga Samegai strain (SS) and Yamanashi strain selectively-bred for one generation with LFMD (YS). Growth and feed utilization for the respective diets were similar between YC and SS strains. Among fish fed LFMD, apparent dietary crude protein and fat digestibility coefficients were higher in YC than those in SS. Selectively-bred YS showed superior growth and feed efficiency to YC for the respective diets due largely to increased feed intake. However, limited improvements in the physiological conditions were observed in YS fed LFMD. An additional trial under restricted feeding of LFMD revealed that growth and feed efficiency ratio of YS were superior to YC. These results suggest that selective breeding of Yamanashi strain rainbow trout with a low fishmeal diet is effective for improvement of this diet utilization.

Key words: Rainbow trout; Yamanashi strain; Low fishmeal diet; Selective breeding

The steady development of finfish and crustacean aquaculture worldwide has already made the supply of fishmeal, traditionally a main ingredient in aquaculture feeds, tight and aquaculture sections are being forced to develop aquaculture practices with less use of fishmeal (FAO 2016). In contrast to the global trend of reducing fishmeal levels in aquaculture feeds (Tacon and Metian 2008; Shepherd and Jackson 2013), e.g., 19.5% of fishmeal in Norwegian salmon feed in 2012 (Ytrestøyl et al. 2015), in Japan levels of more than 40% of fishmeal on average were included in aquaculture feeds in 2017 (http://www.maff.go.jp/j/chikusan/sinko/lin/l_siryo/cyosa/attach/pdf/kako-30, accessed on Aug. 21, 2018). Thus, more effort must be devoted to reducing the fishmeal levels in aquaculture feeds in Japan.

In salmonid fish, especially rainbow trout *Oncorhynchus mykiss* and Atlantic salmon *Salmo salar*, there is a large body of knowledge concerning the utilization of alternative protein sources for fishmeal (Gaylord et al. 2010; Espe et al. 2012). With the use of plant protein ingredients, adverse effects of anti-nutritional factors (ANF) have been shown to occur (Francis et al. 2001; Krogdahl et al. 2010). In a rainbow trout strain at Samegai, Japan, the effects of soybean

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ANF on the nutritional physiology have been intensively investigated, and practical strategies to alleviate the adverse effects of soybean ANF have been proposed (Yamamoto et al. 2007, 2010). On the other hand, differences in the sensitivity to soybean ANF between rainbow trout strains have also been reported (Venold et al. 2012; Barnes et al. 2015). In developing a suitable low fishmeal feed for a given rainbow trout strain, the specific physiological responses to plant ANF should be elucidated.

As an alternative way for aquaculture fish to be able to effectively utilize low fishmeal feeds in the future, efficacy of selective breeding using low or no fishmeal diets in rainbow trout (Overturf et al. 2013; Callet et al. 2017) and amago salmon *O. masou ishikawae* (Yamamoto et al. 2016) have recently been reported. In their breeding programs, Yamamoto et al. (2016) consistently fed a low fishmeal diet (fishmeal, 5%) from juveniles to broodstock in amago salmon, while Callet et al. (2017) used a fishmeal free diet until 14 months, and thereafter fed a commercial feed containing fishmeal to assure reproductive performance. On the other hand, treatment of gilthead seabream *Sparus aurata* broodstock with a reduced fish oil diet improved the ability of the progeny to utilize a low fishmeal-fish oil diet (Izquierdo et al. 2015), known as a “nutritional programming” (Lucas 1998). If such a nutritional effect through broodfish can be expected in addition to the effect by growth selection on a low fishmeal diet, a more efficient line could be obtained by continuous feeding of the low fishmeal diet for both growth selection and broodstock management.

We have successfully obtained a F1 generation from parents of Yamanashi strain rainbow trout which had been fed a soybean-corn gluten meal-based 5% fishmeal diet from juveniles for a period of 2 years and 4 months, during which they were selected for growth and then grew for reproduction (Miura et al. 2019). To understand the potential performance and suitability of the Yamanashi strain for a low fish meal diet and selective breeding, respectively, juveniles from the Samegai strain were also tested as well as juveniles of the selectively bred F1 and the original Yamanashi strain. A fishmeal-based diet and a 5% fishmeal diet were fed to these juveniles and the growth performance and physiological conditions were evaluated.

Materials and Methods

Experimental diets

A fishmeal-based diet (FMD, 50% fishmeal) and a low fishmeal diet (LFMD) composed of 5% fishmeal, 50% defatted soybean meal and 20% corn gluten meal (Table 1) that had compositions similar to those used in amago salmon studies, and made into dry pellets (Yamamoto et al. 2015, 2016). Crude protein and fat contents, and digestible methionine and lysine contents between diets were adjusted. The diets were stored at −20°C until use.

Experimental fish and feeding

Eyed eggs from the original Yamanashi strain rainbow trout served as a control (YC) and growth-selected Yamanashi strain rainbow trout reared for one generation on a 5% fishmeal

Table 1. Formulation and proximate composition of the test diets

Diet	FMD	LFMD
Ingredients (% wet weight)		
Jack mackerel meal (CP = 70%)	50.00	5.00
Defatted soybean meal (CP = 46%)	5.60	50.00
Corn gluten meal (CP = 62%)	3.80	20.90
Wheat flour (CP = 16%)	24.00	8.16
Gelatinized potato starch	2.60	2.22
Pollock visceral oil	3.94	7.00
Vitamin mix[a]	0.50	0.50
Choline chloride	0.25	0.25
Mineral mix (P free)[b]	1.20	1.20
Calcium dihydrogen phosphate	0.64	1.60
Potassium dihydrogen phosphate	0.64	1.60
Yttrium oxide (15%)	0.50	0.50
L-Lysine HCl	-	1.62
L-Methionine	-	0.46
Betaine	-	0.50
Cellulose	5.81	0.21
Nutrients (% dry matter basis)		
Crude protein	48.4	48.1
Crude fat	9.6	9.8
Crude starch	20.0	19.1
Ash	10.4	7.6
Phosphorus	1.9	1.5

CP, crude protein; *FMD*, fishmeal-based diet; *LFMD*, low fishmeal diet

[a] Yamamoto et al. (2007).

[b] Inorganic phosphorus sources are excluded from Yamamoto et al. (2007).

diet (YS) (Miura et al. 2019), both obtained from Oshino Branch, Yamanashi Prefectural Fisheries Technology Center (Oshino, Yamanashi), were transferred to Tamaki Station, National Research Institute of Aquaculture (Tamaki, Mie). YC and YS were obtained by artificial insemination from 8 males and 10 females, and 7 males and 10 females, respectively on Nov. 27, 2015. The Yamanashi strain is partly descended from the Donaldson line (Donaldson and Olson 1957). Eyed eggs from Shiga Samegai strain (SS) that had not been selected on low fishmeal diets were obtained from Shiga Prefectural Samegai Trout Farm (Maibara, Shiga) and transferred to the Tamaki Station. The Samegai strain is a classical one introduced approximately 140 years ago. These eyed eggs were maintained in a wet laboratory, hatched, and reared on commercial fishmeal-based trout feeds (Feed-One, Yokohama, Japan) under conditions identical to the previous studies in amago salmon (Yamamoto et al. 2015, 2016).

When fish reached approximately 5 g, they were acclimatized to LFMD for 6 days in 150 *l* tanks. Then, they were transferred to 60 *l* tanks (80 fish/tank) and further acclimatized to the rearing conditions using LFMD for 7 days. Well water was supplied to each tank at a flow rate of 4 *l*/min, and aeration was provided using an air stone. The water temperature was almost constant during the experiment at 16.3 ± 0.2℃. After acclimation, fish were sorted to a uniform size (7.9 ± 0.6 g) and the fish number per tank was adjusted to 65. Each diet was assigned to triplicate tanks (3 fish groups × 2 diets × 3 tanks) and the fish were fed to apparent satiation twice daily, 6 days a week for 58 days. Total number of fish and bulk weight were measured on days 24 and 42. Fish were deprived of food for 48 h before body weight measurement and anesthetized in 0.01% tricaine.

To exclude the effect of increased feed consumption under satiation feeding as a result of selective breeding on feed efficiency ratio, growth performances between YC and YS were compared under a restricted feeding condition. This experiment was conducted at the Oshino Branch. Eyed eggs of the same YC and YS used in the satiation feeding experiment were managed at the Oshino Branch, and fish were reared until the start of feeding trials according to the method used in the satiation feeding experiment. The effects of feeding LFMD and a commercial fishmeal-based diet (CFMD, Marubeni Nisshin Feed, Tokyo) were evaluated in two independent trials. The commercial diet contained 57% fishmeal, and 51% crude protein and 10% crude fat on dry matter basis. In the first trial, fish with an average body weight of 17.9 ± 0.4 g were allocated to 217 *l* tanks (30 fish/tank) in duplicate after they were acclimatized to the rearing conditions using LFMD for 7 days. Well water (12.5 ± 0.1℃) was supplied at a rate of 6 *l*/min. Then, LFMD was fed to fish according to the standard feeding table (Leitritz and Lewis, 1976) three times daily, five days a week for 16 weeks. This feeding table had been developed based on various feeding trials in rainbow trout and is still used by public and private hatcheries in Japan as a reliable one. In the second trial, fish with an average body weight of 30.9 ± 0.6 g were allocated to the 217 *l* tanks (30 fish/tank) in duplicate, and fed CFMD in the same manner as in the first trial. In both trials, fish were anesthetized in 0.02% FA100 (DS Pharma Animal Health, Osaka), weighed and the daily ration sizes were adjusted biweekly.

Sampling procedure

At the start of the satiation feeding experiment, a total of 20 fish from each fish group was taken and stored at −20℃ for proximate composition analysis. After 58 days of feeding, all fish were weighed individually. Blood was withdrawn from 3 fish per tank with a heparinized syringe, and immediately subjected to hemoglobin concentration analysis using Hemoglobin-B-test (Wako Pure Chemicals, Osaka, Japan). Plasma was separated from the rest of the blood samples by centrifugation (3,000 rpm for 10 min at 4℃) for nutrient concentration analysis. Then, from the same fish, the stomach, pyloric caeca, gallbladder and liver were dissected out and weighed for calculation of their somatic indices. The samples of stomach were emptied by visual observation. Segments of the liver and distal intestine were also taken and fixed in 10% phosphate-buffered formalin (Mildform®10MN, Wako) for histological examination. The rest of the liver was used for taurine concentration analysis. The plasma, gallbladder and liver samples were stored at −80℃ until analysis. Another 5 fish from each tank were taken and stored at −20℃ for proximate composition analysis. The remaining fish in the tanks were fed their respective diets for another 4 days. At 16 h after the last meal, 3 fish from each tank were taken, anesthetized and the intestine (from mid intestine to rectum) was dissected out. The intestinal sample was gently squeezed, and the digesta was taken into a 1.5 m*l* tube and freeze dried for determination of bile acid concentration.

Fecal collection

After the intestinal digesta sampling, fish from 3 tanks in each treatment were pooled and reared in 2 conical tanks for fecal collection (Yamamoto et al. 1998). The fish were acclimatized to the new conditions for 4 days and fed the respective diets. The test diets were fed to apparent satiation twice daily and fecal materials were collected just before each meal. Fecal samples for the 4 days were pooled and freeze-dried for determination of concentrations of the nutrients and a marker (yttrium).

Chemical and biochemical analyses

Diets and whole fish body samples were subjected to moisture (drying at 110℃ for 10 h), crude protein [semi-micro Kjeldahl method ($N \times 6.25$)], crude fat (di-ethylether extraction) and ash (5 h combustion at 600℃) analyses. Amino acid contents of the protein ingredients were determined using an amino acid analyzer L-8800 (Hitachi, Tokyo, Japan) according to the methods of Yamamoto et al. (2002). Hepatic taurine concentration was also determined using L-8800 after the liver samples being extracted in 0.6 N perchloric acid. An inductively coupled plasma emission spectroscopy ICPE-9000 (Shimadzu, Kyoto, Japan) was used for determination of dietary and fecal yttrium and phosphorus contents after nitric acid hydrolysis using a microwave oven ETOS-D (Milestone General, Kawasaki, Japan).

Plasma total protein, total cholesterol, triacylglycerol and glucose concentrations were determined using a commercial analyzer SPOTCHEM SP-4410 (Arkrey, Kyoto, Japan). Biliary bile acid concentration was determined using a clinical investigation kit (Total bile acid-test, Wako), and conjugated bile salt composition was determined using high performance liquid chromatography as described previously (Yamamoto et al. 2016).

Histological examination

Liver and distal intestinal samples fixed in formalin were dehydrated in ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). The sections were examined microscopically. The morphological conditions were evaluated according to Yamamoto et al. (2015, 2016) and categorized by numerically scoring morphological abnormalities in specific tissue sites as follows.

Hepatocytes

Nuclei: 0, round and clearly stained; 1, atrophied and darkly stained; 3, darkly stained and necrosed

Intercellular space: 0, not expanded; 1, expanded

Capillary: 0, not expanded; 1, expanded

Cell cords: 0, orderly arranged; 1, irregularly structured

Cytoplasm: 0, clearly stained; 1, intermediate between 0 and 2; 2, darkly stained and cell boundaries were unclear

Mucosal folds and submucosa of the distal intestine

Microvilli: 0, long enough; 1, shortened; 2, degenerated

Pinocytotic and absorptive vacuoles: 0, well developed; 1, not developed; 2, disappeared

Degenerated large vacuoles: 0, absent; 1, present

Submucosa: 0, not inflamed; 1, inflamed

Stratum compactum: 0, layers were well-formed; 1, layers were irregular

Statistics

Data are presented as means ± SD in the text and figures, or means and pooled standard error of the mean (SEM, $n = 6$) in the tables. The scored morphological conditions of the liver and distal intestine are shown as means ($n = 3$).

In the satiation feeding experiment, the growth performance parameters and the results of biological and biochemical analyses were subjected to a two-way analysis of variance (ANOVA) to evaluate the effects of fish (YC, SS or YC, YS) and diet (FMD, LFMD). Statistical examinations for the pair of SS and YS were not conducted. The data were arcsine transformed prior to ANOVA, if necessary. When a significant interaction effect between fish and diet was found, one-way ANOVA followed by the Tukey-Kramer test was performed to evaluate the differences between all 4 treatments (2 fish groups × 2 diets). A probability level of less than 0.05 was considered as significant. All statistical analyses were performed using StatView (SAS Institute, Cary, NC, USA).

In the restricted feeding experiment, the growth performance parameters were tested by Welch's *t*-test using Statcel 3 (OMS Publishing, Saitama, Japan).

Results

Growth performances of rainbow trout under satiation feeding are shown in Table 2. Irrespective of fish strains (YC, SS), final body weight, weight gain, specific growth rate (SGR) and feed efficiency ratio (FER) of fish fed LFMD were inferior (ANOVA, $P < 0.001$) to fish fed FMD. These parameters were similar between YC and SS fed the respective diets. However, significant interactions between the effects of fish and diet were observed on the growth parameters due to the relative feed consumption (% BW) of LFMD, which was lower in YC than in SS (Tukey-Kramer test, $P < 0.05$).

Except for relative feed consumption, the effects of fish and diet on the growth parameters and FER were significant in YC and YS without significant interactions. Irrespective of diets, growth parameters and FER were higher (ANOVA, $P < 0.001$) in YS than those in YC, and the growth of YS fed LFMD was similar to YC fed FMD. A significant interaction was observed in relative feed consumption due to a significantly higher value of YS fed LFMD than YC fed the same diet (Tukey-Kramer test, $P < 0.05$).

Growth performances of rainbow trout under restricted feeding are given in Figs. 1 and 2. Final body weights of fish fed LFMD were 47.5 ± 0.7 g in YC and 53.5 ± 0.4 g in YS (*t*-test, $P < 0.01$), and those of fish fed CFMD were 102.3 ± 0.8 g in YC and 102.0 ± 0.2 g in YS (*t*-test, $P > 0.05$). When fish were fed LFMD, both SGR and FER were significantly higher (*t*-test, $P <$

Table 2. Growth performance of rainbow trout juveniles fed the test diets under satiation feeding

Fish	Diet	BW (g)		Gain		Feed intake	
		Initial	Final	% initial	SGR	% BW	FER
Individual treatment means							
YC	FMD	7.9	42.0^{A}	431^{A}	2.88^{A}	$2.66^{AB, b}$	1.07
	LFMD	7.9	29.3^{B}	271^{B}	2.26^{B}	$2.59^{B, c}$	0.93
SS	FMD	7.9	39.1^{A}	396^{A}	2.76^{A}	2.60^{B}	1.07
	LFMD	7.9	31.4^{B}	298^{B}	2.38^{B}	2.74^{A}	0.90
YS	FMD	7.9	53.5	579	3.30	2.66^{b}	1.16
	LFMD	7.9	40.0	406	2.80	2.74^{a}	1.02
Pooled SEM		0.034	0.293	3.509	0.030	0.037	0.024
Analysis of variance: P values (YC vs. SS)							
Fish			0.621	0.569	0.922	0.121	0.252
Diet			< 0.001	< 0.001	< 0.001	0.257	< 0.001
Fish × Diet			0.011	0.008	0.008	0.003	0.382
Analysis of variance: P values (YC vs. YS)							
Fish			< 0.001	< 0.001	< 0.001	0.001	< 0.001
Diet			< 0.001	< 0.001	< 0.001	0.912	< 0.001
Fish × Diet			0.534	0.445	0.086	< 0.001	0.867

FMD, fishmeal-based diet; *LFMD*, low fishmeal diet; *YC*, Yamanashi control group; *SS*, Shiga Samegai group; *YS*, Yamanashi selectively-bred group

Values are means ($n = 3$). Values with the same superscript letter within the same column are not significantly different ($P > 0.05$).

Gain (% initial) = 100 × final BW/(final BW-initial BW)

SGR (specific growth rate) (% BW/day) = 100 × [Ln (final BW) − Ln (initial BW)]/days

Feed intake (% BW/day) = 100 × dry feed intake/[(initial BW + final BW) × 0.5 × days]

FER (feed efficiency ratio) = wet weight gain/dry feed intake

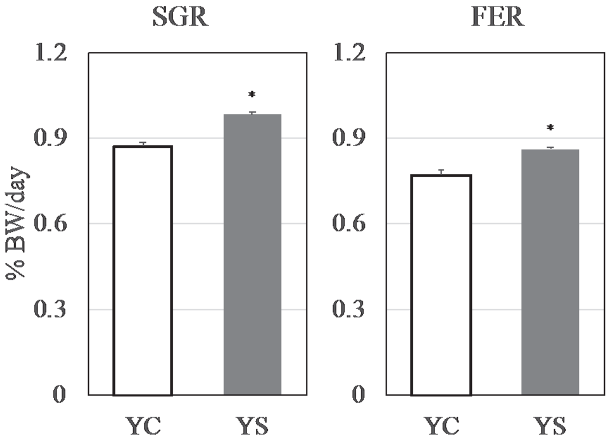

Fig. 1. Growth performance of rainbow trout juveniles fed a low fishmeal diet (LFMD) under restricted feeding
Values are means ± SD ($n = 2$).
Values with asterisks indicate significantly different compared to YC ($^{*}P < 0.05$).

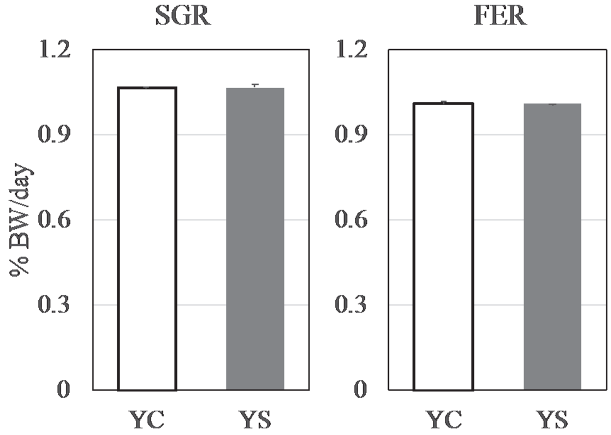

Fig. 2. Growth performance of rainbow trout juveniles fed a commercial fishmeal-based diet (CFMD) under restricted feeding
Values are means ± SD ($n = 2$). No significant differences are found between YC and YS ($P > 0.05$).

0.05) in YS than those in YC, whereas no significant differences were observed between YC and YS both fed CFMD.

Whole body proximate compositions of rainbow trout under satiation feeding are presented in Table 3. In YC and SS, significant diet effects (ANOVA, $P < 0.05$) were observed in moisture (FMD < LFMD) and crude fat contents (FMD > LFMD). In YC and YS, a significant fish effect was observed in ash contents (YC > YS) and significant diet effects were found in the contents of crude fat (FMD > LFMD) and ash (FMD < LFMD). A significant interaction between fish and diet effects was observed in crude protein contents; the crude protein content of YS fed LFMD was significantly higher than that of YC fed the same diet (Tukey-Kramer test, $P < 0.05$).

Table 3. Whole body proximate composition (%) of rainbow trout juveniles fed the test diets under satiation feeding

Fish	Diet	Moisture	Crude protein	Crude fat	Ash
Individual treatment means					
YC	FMD	72.2	16.7^{ab}	8.4	2.4
	LFMD	73.1	16.5^{b}	7.5	2.6
SS	FMD	72.2	16.8	8.3	2.4
	LFMD	72.7	16.8	7.9	2.3
YS	FMD	72.5	16.6^{ab}	8.3	2.3
	LFMD	73.0	16.9^{a}	7.5	2.4
Pooled SEM		0.146	0.068	0.145	0.024
Analysis of variance: P values (YC vs. SS)					
Fish		0.403	0.063	0.413	0.113
Diet		0.026	0.388	0.039	0.142
Fish × Diet		0.513	0.163	0.374	0.089
Analysis of variance: P values (YC vs. YS)					
Fish		0.749	0.258	0.907	0.035
Diet		0.084	0.687	0.024	0.037
Fish × Diet		0.623	0.036	0.859	0.208

FMD, fishmeal-based diet; *LFMD*, low fishmeal diet; *YC*, Yamanashi control group; *SS*, Shiga Samegai group; *YS*, Yamanashi selectively-bred group
Values are means ($n = 3$). Values with the same superscript letter within the same column are not significantly different ($P > 0.05$). The moisture, crude protein, crude fat and ash contents of the initial fish were 76.3, 15.6, 5.2 and 2.4% for the YC group, 75.2, 15.9, 5.8 and 2.5% for the SS group, and 76.2, 15.8, 4.9 and 2.5% for the YS group, respectively.

Hematological parameters of rainbow trout under satiation feeding are given in Table 4. The effects of fish and diet were similar in both pairs of YC-SS and YC-YS. In fish fed LFMD, blood hemoglobin concentrations and plasma total protein and total cholesterol concentrations significantly decreased (ANOVA, $P < 0.05$) compared to fish fed FMD. Plasma glucose concentrations were higher in fish fed FMD than in fish fed LFMD, and those of YC were higher than SS and YS.

Organ somatic indices of rainbow trout under satiation feeding are shown in Table 5. In YC and SS, hepatosomatic indices (HSI) were lower (ANOVA, $P < 0.05$) in YC than SS irrespective of diet, and those of fish fed FMD were higher (ANOVA, $P < 0.001$) than fish fed LFMD. The somatic indices of stomach and pyloric caeca were not significantly affected by fish and diet, however, both indices of YC were numerically lower than those of SS. The hepatic taurine concentrations in YC fed LFMD was lower than YC fed FMD, while the taurine concentrations were similar in SS fed both diets (interaction, $P < 0.05$).

In YC and YS, a significant interaction

Table 4. Blood hemoglobin and plasma nutrient concentrations of rainbow trout juveniles fed the test diets under satiation feeding

Fish	Diet	Hemoglobin (g/d*l*)	Total protein (g/d*l*)	Total cholesterol (mg/d*l*)	Triacyl-glycerol (mg/d*l*)	Glucose (mg/d*l*)
Individual treatment means						
YC	FMD	9.4^{a}	3.7	453	242	86
	LFMD	6.9^{c}	3.1	229	260	73
SS	FMD	9.4	3.9	415	267	75
	LFMD	7.7	3.3	241	245	66
YS	FMD	8.2^{b}	3.7	420	278	77
	LFMD	7.5^{bc}	3.2	238	272	65
Pooled SEM		0.149	0.082	11.652	4.891	1.063
Analysis of variance: P values (YC vs. SS)						
Fish		0.297	0.209	0.639	0.722	0.025
Diet		< 0.001	0.003	< 0.001	0.859	0.008
Fish × Diet		0.262	0.825	0.358	0.153	0.519
Analysis of variance: P values (YC vs. YS)						
Fish		0.229	0.857	0.732	0.096	0.013
Diet		< 0.001	0.018	< 0.001	0.645	0.002
Fish × Diet		0.004	0.857	0.546	0.380	0.813

FMD, fishmeal-based diet; *LFMD*, low fishmeal diet; *YC*, Yamanashi control group; *SS*, Shiga Samegai group; *YS*, Yamanashi selectively-bred group
Values are means (n = 3). Values with the same superscript letter within the same column are not significantly different ($P > 0.05$).

Table 5. Liver, stomach and pyloric caeca weights and hepatic taurine concentration of rainbow trout juveniles fed the test diets under satiation feeding

Fish	Diet	Liver		Stomach (% BW)[a]	Pyloric caeca (% BW)[a]
		HSI (% BW)[a]	Taurine (mg/g)		
Individual treatment means					
YC	FMD	1.08^{a}	3.75^{A}	2.45	1.86
	LFMD	0.90^{b}	3.26^{B}	2.55	1.91
SS	FMD	1.13	3.40^{AB}	2.59	2.08
	LFMD	0.96	3.45^{AB}	2.86	2.02
YS	FMD	1.10^{a}	3.75	2.91	2.00
	LFMD	0.80^{c}	2.90	3.03	2.14
Pooled SEM		0.025	0.067	0.066	0.050
Analysis of variance: P values (YC vs. SS)					
Fish		0.039	0.416	0.081	0.065
Diet		< 0.001	0.055	0.140	0.967
Fish × Diet		0.894	0.023	0.478	0.513
Analysis of variance: P values (YC vs. YS)					
Fish		0.093	0.234	< 0.001	0.039
Diet		< 0.001	0.002	0.273	0.239
Fish × Diet		0.019	0.234	0.930	0.556

FMD, fishmeal-based diet; *LFMD*, low fishmeal diet; *YC*, Yamanashi control group; *SS*, Shiga Samegai group; *YS*, Yamanashi selectively-bred group; *HSI*, hepatosomatic index
Values are means (n = 3). Values with the same superscript letter within the same column are not significantly different ($P > 0.05$).
[a] Organ (liver, stomach, pyloric caeca)-somatic index (%BW) = 100 × organ weight/body weight

(ANOVA, $P < 0.05$) between the effects of fish and diet was observed on HSI. Although HSI of both fish groups fed FMD were similar, that of YS fed LFMD was significantly lower than YC fed the same diet (Tukey-Kramer test, $P < 0.05$). A similar trend was observed in the hepatic taurine concentrations. The somatic indices of stomach and pyloric caeca in YS were higher than those in YC regardless of diet.

Bile acid status of rainbow trout under satiation feeding is given in Table 6. In both pairs of YC-SS and YC-YS, only the effects of diet were significant (ANOVA, $P < 0.001$) and biliary bile acid contents, proportions of biliary taurocholate and bile acid concentrations in the intestinal digesta were all lower in fish fed LFMD than in fish fed FMD.

Apparent digestibility coefficients of dietary nutrients in rainbow trout under satiation feeding are shown in Table 7. Significant interactions (ANOVA, $P < 0.05$) between the effects of fish and diet on the digestibility coefficients of crude protein, crude fat and phosphorus (P) were observed in YC and SS. Digestibility coefficients of FMD crude protein were similar between YC and SS, whereas the coefficient of LFMD crude protein in YC was higher (Tukey-Kramer test, $P < 0.05$) than SS. Crude fat digestibility coefficients of LFMD were lower than those of FMD, but the coefficient of LFMD was significantly higher in YC than in SS (Tukey-Kramer test, $P < 0.05$). Absorption rates of P from LFMD were higher than those from FMD, but the P absorption coefficient from FMD in YC was significantly lower than that in SS (Tukey-Kramer test, $P < 0.05$). No significant effects of fish and diet were observed on crude starch digestibility coefficients in YC and SS.

In YC and YS, no significant effects of fish and the interaction were observed. Digestibility

Table 6. Bile acid status of rainbow trout juveniles fed the test diets under satiation feeding

Fish	Diet	Gallbladder				Intestinal digesta
		Bile acids				Bile acids (μmol/g DM)
		GBSI (%)^{a}	Conc. (mM)	Content (mmol/kg BW)	C-tau (%)	
Individual treatment means						
YC	FMD	0.32	295	0.95	90.1	84.9
	LFMD	0.18	319	0.60	53.8	27.2
SS	FMD	0.33	306	1.03	89.3	74.5
	LFMD	0.18	330	0.60	52.3	30.2
YS	FMD	0.32	287	0.92	86.1	79.7
	LFMD	0.19	304	0.58	45.8	29.9
Pooled SEM		0.024	2.786	0.052	1.177	1.041
Analysis of variance: P values (YC vs. SS)						
Fish		0.853	0.350	0.506	0.708	0.239
Diet		< 0.001	0.068	< 0.001	< 0.001	< 0.001
Fish × Diet		0.712	0.994	0.569	0.976	0.054
Analysis of variance: P values (YC vs. YS)						
Fish		0.809	0.149	0.605	0.067	0.732
Diet		< 0.001	0.025	< 0.001	< 0.001	< 0.001
Fish × Diet		0.809	0.651	0.913	0.933	0.314

FMD, fishmeal-based diet; *LFMD*, low fishmeal diet; *YC*, Yamanashi control group; *SS*, Shiga Samegai group; *YS*, Yamanashi selectively-bred group; *C-tau*, taurocholate
Values are means ($n = 3$). Values with the same superscript letter within the same column are not significantly different ($P > 0.05$).
^{a}Gallbladder-somatic index (GBSI) = 100 × gallbladder weight/body weight

Table 7. Apparent nutrient digestibility coefficient (%) of rainbow trout juveniles fed the test diets under satiation feeding

Fish	Diet	Crude protein	Crude fat	Crude starch	Phosphorus
Individual treatment means					
YC	FMD	91.1^{AB}	98.6^{A}	57.8	38.2^{C}
	LFMD	92.1^{A}	95.0^{B}	56.2	69.0^{A}
SS	FMD	91.4^{AB}	98.6^{A}	55.0	54.1^{B}
	LFMD	90.7^{B}	92.0^{C}	55.1	65.6^{A}
YS	FMD	91.0	98.5	57.1	47.3
	LFMD	91.9	94.3	56.1	68.1
Pooled SEM		0.163	0.166	0.651	1.165
Analysis of variance: P values (YC vs. SS)					
Fish		0.048	< 0.001	0.085	0.025
Diet		0.476	< 0.001	0.432	< 0.001
Fish × Diet		0.014	< 0.001	0.377	0.004
Analysis of variance: P values (YC vs. YS)					
Fish		0.632	0.331	0.787	0.133
Diet		0.058	< 0.001	0.387	< 0.001
Fish × Diet		0.826	0.525	0.832	0.074

FMD, fishmeal-based diet; *LFMD*, low fishmeal diet; *YC*, Yamanashi control group; *SS*, Shiga Samegai group; *YS*, Yamanashi selectively-bred group
Values are means ($n = 2$). Values with the same superscript letter within the same column are not significantly different ($P > 0.05$).

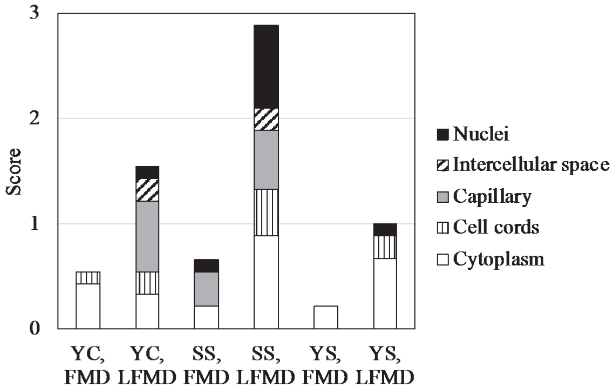

Fig. 3. Cumulative scores for morphological abnormalities in the liver of rainbow trout juveniles fed the test diets under satiation feeding
Values are means ($n = 3$).

coefficients of crude protein and crude starch were similar regardless of fish and diet. The digestibility coefficients of crude fat for LFMD were lower (ANOVA, $P < 0.001$) than those of FMD. P absorption coefficients were higher in fish fed LFMD than in fish fed FMD.

Morphological conditions (abnormalities) of the liver are summarized in Fig. 3. Irrespective of fish groups that were fed FMD, the conditions are almost normal except for some fish showing slightly inferior nutrient deposition in the cytoplasm that was stained mildly. Compared to YC fed LFMD, the nuclei in the hepatocytes of SS fed LFMD were atrophied and sometimes became necrotic, and the cytoplasm were more darkly stained. The capillaries in the liver of both fish groups fed LFMD were similarly expanded, which was not observed in YS fed LFMD.

Morphological conditions (abnormalities) of the distal intestine are summarized in Fig. 4. Compared to fish fed FMD, the microvilli of the mucosal folds were shortened in all three fish groups (YC, SS, YS) fed LFMD. Nevertheless, in these fish, especially YC and YS, pinocytotic and absorptive vacuoles were developed, meaning

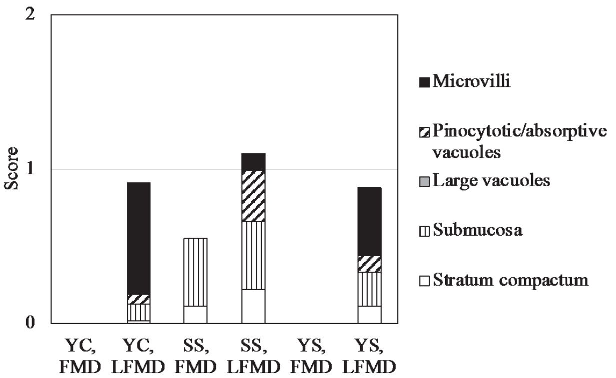

Fig. 4. Cumulative scores for morphological abnormalities in the distal intestine of rainbow trout juveniles fed the test diets under satiation feeding
Values are means ($n = 3$).

that the nutrient absorption function was not severely affected. In addition, degenerate large vacuoles were rarely observed in any fish fed LFMD. In SS, the number of fish showing an inflammatory response in the submucosa of the distal intestine was higher, irrespective of diets, than YC and YS. The morphological conditions of YC and YS fed FMD were considered as normal.

Discussion

Utilization of a low fishmeal diet between different strains

In salmonids (Smith et al. 1988; Wolters et al. 2009) and channel catfish (Li et al. 2006), growth responses between strains to low fishmeal diets have been known to differ. In this study, however, no notable differences were observed in growth and feed utilization between the Yamanashi (YC) and Samegai (SS) rainbow trout strains. Although statistically significant fish effects were not detected, interactions between the effects of fish and diet were found in the growth parameters and feed consumption rate; compared to SS, YC had a slightly higher growth rate on FMD, but a slightly lower growth rate on LFMD. These differences could be attributable to the feed consumption rates (FMD, YC > SS; LFMD, YC < SS). Since feed consumption rate, expressed as relative feed intake per unit body weight, decreases as fish grow (Lovell 1998), the higher feed consumption rate for LFMD relative to that for FMD in SS is a reasonable response. However, the lower feed consumption rate for LFMD relative to that for FMD in YC may mean that LFMD was less palatable in YC than in SS. The stomach-somatic indices of YC (2.55) and SS (2.86), both fed LFMD, may support the differences in their feed consumption rates.

It is well known that soybean meal causes morphological changes in the distal intestine, often called "enteritis", in salmonid fish (Krogdahl et al. 2010). Strain-specific differences in the physiological condition when fish are fed a plant-based diet have been observed in rainbow trout (Venold et al. 2012). In the present study, blood chemistry and bile acid status were not significantly different between the two strains. However, histological observations suggest that the liver and intestinal functions of YC fed LFMD, although they are possibly affected by soybean ANF, were better than SS fed the same diet. In addition, coefficients of apparent crude protein and fat digestibility in YC fed LFMD were significantly higher than those in SS. Since lipid digestibility is suggested to be affected by the intestinal morphological conditions in rainbow trout (Yamamoto et al. 2007; Romarheim et al. 2008), the differences in the digestibility of LFMD fat between the strains could results from their intestinal conditions. Thus, the relatively low FER of SS fed LFMD may have been due to these inferior intestinal conditions.

Effect of selective breeding on the utilization of a low fishmeal diet in Yamanashi strain

The efficacy of selective breeding using low fishmeal diets has been reported in rainbow trout (Overturf et al. 2013; Callet et al. 2017) and amago salmon (Yamamoto et al. 2016). In the selectively bred trout compared to the control ones both fed no fishmeal diets, Overturf et al. (2013) observed improvements in both growth and feed conversion ratio (FCR), while Callet et al. (2017) found an improvement in growth but a decrease of FER although they did not consider the viewpoint that FER decreases with fish growth (Lovell 1998). In the present study, both growth and FER in the selectively bred trout fed a low fishmeal diet (LFMD) improved like the case in amago salmon (Yamamoto et al. 2016). It is generally accepted that FER increases to a certain level as the feed consumption rate increases (Lovell 1998). Indeed, the feed consumption rate for LFMD in YC was lower, despite the smaller body size, whereas that in YS was higher, than in their counterparts fed FMD. Thus the improvement in growth performance by the selective breeding on LFMD could be primarily due to the improvement in feed consumption as we had found in amago salmon (Yamamoto et al. 2016). In this study, an appreciable effect was obtained by the selection from one strain, which may be criticized in terms of genetic diversity (Gjedrem et al. 2012), however, genetic variations for fish responses to low fishmeal diets are reported to exist within a strain and a population (Pierce et al. 2008; Le Boucher et al. 2011).

Callet et al. (2017) observed improvements in lipid and starch digestibility of a fishmeal-free diet in rainbow trout selectively bred for 3 generations on no fishmeal diets. In the present study, no notable improvements by selective breeding were observed in nutrient digestibility. Yamamoto et al. (2015) also did not find particular improvements in nutrient digestibility in amago salmon selectively bred for 2 generations on a low fishmeal diet. On the other hand, increasing the frequency of satiation feeding in rainbow trout decreases crude starch digestibility (Yamamoto et al. 2007). This finding suggests that a larger amount of food decreases starch digestibility in rainbow trout. In the present study, YS fed on more LFMD than YC (% feed intake, YC < YS). Nevertheless, crude starch digestibility determined under satiation feeding was similar between YC and YS (Table 7). Whole body crude protein content of fish fed LFMD increased by the selective breeding, while the whole body crude fat contents were similar between YC and YS (Table 3). These findings suggest that impairment of carbohydrate digestion/absorption do not occur in YS fed a larger amount of food compared to YC, and absorbed lipid and carbohydrate are effectively used as energy sources in YS. In addition, FER of YS fed LFMD under restricted feeding (identical % feed intake) improved relative to YC fed LFMD (Fig. 1), suggesting that ingested nutrients were more effectively utilized in YS under the restricted feeding. Taken together, the improvements of growth and FER in YS fed LFMD under the satiation feeding might have also resulted from the improvement in nutrient utilization. In salmonids, there are strains that utilize carbohydrates more efficiently (Mazur et al. 1992; Blanc 2002).

Liver tissues of rainbow trout fed low fishmeal diets are often atrophied (Yamamoto et al. 2007, 2015, 2016). In this study, fish fed diet LFMD had smaller liver than fish fed FMD. Moreover, the liver of YS fed LFMD was smallest, and the hepatic taurine concentration was numerically lower than YC fed LFMD. In the Samegai strain rainbow trout, supplementation of taurine (0.14 g/100 g diet) to a soybean-corn gluten meal-based fishmeal free diet (methionine was supplemented) had no effect on the improvement of growth and feed utilization (Yamamoto et al. 2003). This is probably because hepatic and muscle taurine concentrations of fish fed a taurine un-supplemented diet were identical to fish fed a fishmeal-based diet (Yamamoto et al. 2003), which was reconfirmed in the Samegai strain of the current study. By contrast, Gaylord et al (2006, 2007) found supplemental effects of taurine to fishmeal free diets in rainbow trout. Salze and Davis (2015) suggest in their review that taurine may be conditionally essential in rainbow trout, depending on the fish growth rate. Thus, rainbow trout showing faster growth on low fishmeal diets by selective breeding may require taurine supplementation.

A strategy toward the improvement of low fishmeal diet utilization in Yamanashi strain

In conclusion, the Yamanashi strain rainbow trout has superior characteristics in terms of tolerance to soybean meal-induced tissue morphological changes but inferior in feed intake of the low fishmeal diet, compared to the Samegai strain. The latter defect of the Yamanashi strain was proven to be overcome by selective breeding on the low fishmeal diet. By contrast, improvements in the morphological conditions of liver and, especially distal intestine by selective breeding were limited, partly due to the

Yamanashi strain being potentially resistant to soybean ANF compared to the Samegai strain. In this report, the results of only one generation of selective breeding relative to 3 (Yamamoto et al. 2016; Callet et al. 2017) and 4 (Overturf et al. 2013) generations of selection are detailed. In the future continuous selective breeding, improvements in the physiological conditions as well as growth performance should be evaluated.

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山梨系ニジマスにおける低魚粉飼料の利用性改善の方向性の検討

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将来的な低魚粉時代に対応するため，山梨県が継代飼育しているニジマスにおける低魚粉飼料の利用性を知見が豊富な醒井系と比較するとともに，低魚粉飼料により成長選抜して交配する有効性について検討した。魚粉50%および植物性原料主体の魚粉 5%飼料を飽食量与えた山梨系稚魚の飼育成績は醒井系と同等であったが，魚粉 5%飼料における摂餌性や比肝重値がやや劣り，消化吸収率や肝臓と腸管の組織状態がやや優れた。一方，魚粉 5%飼料で選抜・交配して得られた山梨系 F1 稚魚では両飼料とも摂餌性が向上して通常の山梨系に比べて成長や飼料効率が改善したが，胆汁生理等に顕著な改善は見られなかった。さらに魚粉 5%飼料を制限給餌したところ，選抜 F1 稚魚では成長とともに飼料効率が改善した。以上のことから低魚粉飼料の利用性は家系により特徴があり，山梨系ニジマスでは低魚粉飼料により選抜交配を継続することが有効であることが示唆された。