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Effects of microbial fermented non-dairy creamer as an aquaculture feedstuff for Japanese red sea bream *Pagrus major*: A preliminary study

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Abstract
This study was designed to assess the use of microbial fermented, non-dairy creamer as a new aquaculture feed ingredient for juvenile red sea bream *Pagrus major* (Temminck & Schlegel, 1843) in a closed recirculating system. Fermented non-dairy creamer was supplemented 15, 10 and 5% in the diets (Diet 2, Diet 3, Diet 4). A diet with fish meal was used as a control (Diet 1). A steady growth was observed in all the treatments. The satisfactory weight gain was found in the fermented creamer diets. It was observed that the protein and lipid percentage was increased in the fermented product compared to the raw creamer. A significantly higher value of total final weight, final mean weight gain and daily weight gain was observed in the 15% microbial fermented, non-dairy creamer diet (Diet 2). Gross yield, specific growth rate (%) and growth rate (%) was found highest in the 15% fermented non-dairy creamer, compared to other treatments ($P<0.05$). The protein percentage in the body carcass was almost similar to all the treatments. The carcass lipid percentage increased significantly in the higher level of the fermented creamer diet compared to the control. This preliminary study has demonstrated that microbial fermented creamer could be an important aquaculture feed ingredient and however, more research needs to be done on different species of fishes.

Keywords: Non-dairy creamer, fermentation, red sea bream, *Pagrus major*, growth, aquaculture

Introduction

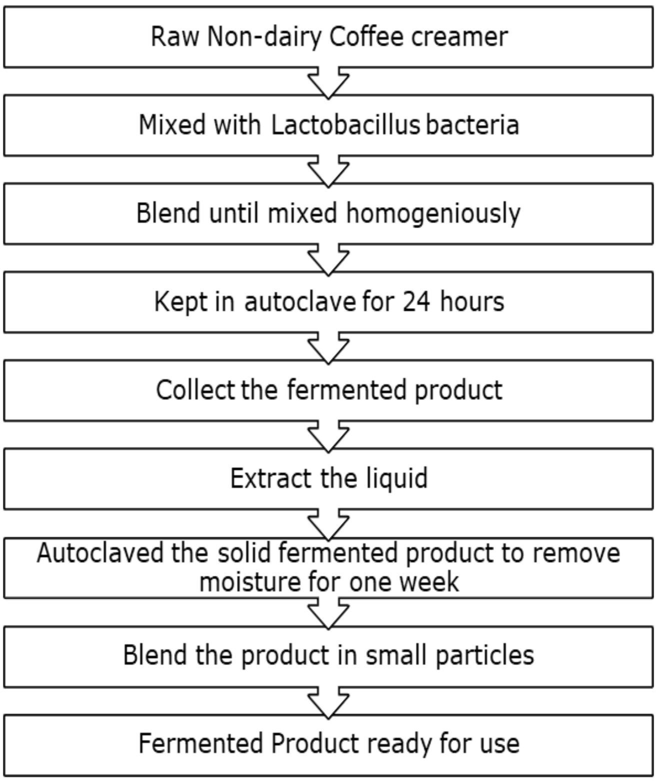
Aquaculture production of fish and shellfish is one of the fastest growing industries in the world [1]. This steady growth will in turn increase the demand of key raw materials for use in aquaculture feed. Feed is the most important input for commercial aquaculture. Fishmeal is the main component of aquaculture feed and with increasing the production, demand of fishmeal is also increasing. The main constraint of the industry is the availability of high quality and cost effective protein sources. This has stimulated the evaluation of a variety of alternative dietary protein sources for partial or complete replacing fish meal and fish oil. Continuous efforts have been made to find alternative sources [2, 3] and plant ingredients found to be the cheaper sources. Presence of ant nutritional factors is one of the constraints of using plant ingredients [4, 5]. In addition to this residual of antibiotics in aquaculture products are being seriously concerned with consumers. Several researches have been conducted as a feed supplement for a substitute of antibiotic, however, fermented product found to be a promising solution as an alternative to chemical use in aquaculture feed as it could enhance the non-specific immune system and disease resistance [6]. Fermentation is a distinctive process which will upgrade the nutrient value, acceptability, digestibility of a feed ingredient. Moreover, bacterial fermentation could enhance growth and immune system. Fermentation also increases the availability of certain vitamins and folic acid in certain feed ingredients [7]. Anti-nutritional factors from plant based raw materials can be reduced by fermentation. It was reported that 30-40% inclusion of fermented linseed meal in the diet of Rohu fish *Labeo rohita* (Hamilton, 1822) fish [8]. Fermented black gram can be used in the diet of carps *Catla catla* (Hamilton 1822), *L. rohita* and *Cirrhinus mrigala* (Hamilton 1822). Improvement of nutritive value of grass pea *Lathyrus sativus* (L.) seed meal in the formulated diet for rohu, *L. rohita* fingerlings after fermentation with a fish gut bacterium. Fermentation of grass pea with *Bacillus* sp. reduces the crude fiber [9].

Non-dairy creamer whiteners are liquid or granular substances intended to substitute for milk or cream as an additive to coffee, tea, hot chocolate or other beverages. To replicate the mouthfeel of milk fats, nondairy creamers often contain hydrogenated vegetable oils, which are potential media for most probiotic bacteria in fermentation process. One of the perks of non-dairy creamers is that they keep longer than milk or cream which makes it a potential bulk material for aquaculture. However, production plants discharge a large proportion of coffee creamer during maintenance. Discarded creamer might pollute the surrounding environment. These creamers are rich is lipid (23%). However, no research has been found directly utilizing the non-dairy creamer for the aquatic animals but this process could be modified by fermentation. Therefore, effective uses of the waste creamer have been sought. The unused creamer could be used as an important aquaculture feed ingredient. *P. major* fingerlings in the late to early development stages have high energy requirement which could only be supplied by animal derived fats and protein. However non-dairy ferment is also rich in bacterially processed single cellular proteins and fatty acids. That is why the present research was designed to use the non-dairy creamer after bacterial fermentation for the Japanese red sea bream *P.* major which been an important aquaculture species in Japan as feed additive.

Materials and Methods

Preparation of the fermented creamer:

The following flow charts showed the steps of preparing the fermented non-dairy creamer



Preparation of feed

The feed was prepared using the formulation mentioned in Table 1. Fishmeal was used as a source of protein. Protein level was adjusted with the supplementation with soybean mean, rice bran and rice powder. The feed composition also includes 2% vitamin mixture and 3% mineral mixtures. Different level (5, 10, 15%) of fermented non-dairy creamer were used in the experimental diets. A diet without non-dairy creamer used as control. All the diets with average protein level more than 40% were maintained as for marine fish. Fishmeal was used as the primary protein source in all the diets and rice powder was used as the carbohydrate source. All the ingredients were ground and sieved to a particle size <100 µm and then mixed together completely. Carboxy-methyl-cellulose was uses as a binder. Feed ingredients were milled, mixed with other feed additives. The mixture was primed with distilled water to produce a suitable mash for extrusion and pelleted through 1.5 mm dye. Resulting extruded pellets were dried for 48 hours at 40 °C then stored in an airtight polythene bag and kept in frozen condition over the whole experimental period.

Table 1: Formulation of experimental diets for Japanese red sea bream *P. major* cultured in closed recirculating system

Items	Diet 1 (Control)	Diet 2 (15%)	Diet 3 (10%)	Diet 4 (5%)
Ingredients (%)				
White fish meal	60	60	60	60
Soybean meal	15	2	5	10
Rice bran	8	4	6	6
Rice powder	8	10	10	10
Fermented coffee creamer	0	15	10	5
Fish oil	2	2	2	2
Mineral premix	3	3	3	3
Vitamin premix	2	2	2	2
Carboxy-methyl-cellulose	2	2	2	2

Animal source and acclimatization

The experiment was conducted at the laboratory of shallow sea aquaculture of the graduate school of Bioresources, Mie University, Japan in a closed recirculating system. Healthy red sea bream was collected from the June 2016 of Mie prefecture and immediately transferred to the laboratory with maintaining the temperature. They were transferred to the fiberglass tanks in one close recirculating system. Prior to experiment, the animals were acclimatized to the laboratory conditions for two weeks. During the period fishes were fed commercial feed four times a day.

Rearing condition

Fishes were housed in each of the twelve 10 L rectangular fiberglass tanks within a single water recirculation system. During the experiments, continuous aeration was provided and water flow was maintained 1 L min^{-1} $tank^{-1}$. Water temperature was maintained at 28±0.5 °C and fishes were subjected to a photoperiod 12:12 h (D:L) using indoor fluorescent lights. Outflow water was passed through the protein skimmer to remove low molecular weight particles and then filtered by several layers of mechanical and biological filtration systems. The temperature was maintained by using digital thermal control system. UV was used to disinfect the inflow water. Artificial sea water was used during the experiment and salinity was maintained between 31 to 33 g L^{-1}, dissolved oxygen was maintained above 5.0 mg L^{-1}. During the experiment, 5% water of the recirculating system was exchanged each day and salt water ice was used to reduce the water temperature before adding to the system.

Water quality analysis

Weekly water samples were collected for the analysis of total phosphorus (TP), ammonia nitrogen (NH3-N) using HACH portable data logging colorimeter DR-850. pH and dissolved oxygen were measured using HACH portable meter.

Chemical Analysis of diets and fishes

Proximate composition of the experimental diets and animals were conducted by the official methods of analysis [10]. The dry matter remaining after drying samples at 105 ºC was combusted to ash at the 550b ºC for 4 h. Total nitrogen content was estimated by the Kjeldahl method. Crude protein was determined indirectly (N × 6.25). The crude fat was determined by soxhlet extraction method. The ash content was measured using muffle furnace combustion at 550 ºC.

Data calculation

Daily weight gain (WG), mean weight gain (MW), specific growth rate (SGR) were calculated as follows:

WG (g day-1) = Wt-W0/T

MW (g) = Wt-W0

SGRw (% d-1) = (lnWt-lnW0)/T×100

Where Wt and W0 are final and initial wet body weight (g) of sea cucumber respectively, T is the time of the experiment (days).

Statistical analysis

Means were calculated for each treatment (i.e. n=3). Mean values were analysed using SPSS 16.0 for Windows statistical package (SPSS, Chicago, USA). Values of growth performance, proximate analysis, water quality parameters were subjected to one-way analysis of variance (ANOVA) and significance differences (*P*<0.05) among treatments means were tested using least significant difference (LSD). The data are presented as mean ± SE of three replicate groups.

Results

Chemical composition of experimental diets

The proximate analysis of the ingredients showed that raw non-dairy creamer contained a very low percentage of protein (1%), however the protein percentage increased very much in the fermented creamer (12%). Non-dairy creamer milk richer in lipid and the lipid percentage increased significantly in the fermented product, from 23% in the raw material to 65% in the final product. Results showed that total lipids content of the initial liquid waste non-dairy samples is 23%. After fermentation, results showing that it had a better nutritional composition of 65% lipid content in the solid product as compared with non-fermented samples. Solidification during fermentation and cold drying allows significant moisture lost during the process, since non-dairy creamer has been supplied as non-Newtonian liquid form. Fermentation process also helps the removal of excessive water content of the creamer and forms a solid and much denser product with a higher lipid content.

White fish meal consisting of 66% protein ratio used as the main animal protein source for both feed formulations. Fermented cream is also rich in carbohydrate (17%). The moisture percentage decreased significantly in the final fermented product. Table 2 shows the proximate analysis of ingredients and Table 3 shows the proximate analysis results of the experimental diets.

Table 2: Chemical composition of ingredients used for the preparation of experimental diets for culturing Japanese red sea bream *P. major* in closed recirculating system (% dry matter basis)

Items Proximate composition	Non-dairy creamer	Fermented creamer	Soybean meal	Rice bran	Rice powder	White fish meal
Protein	1.00	12.20	47.2	24.4	10.4	65.8
Lipid	23.60	65.10	2.9	3.3	2.1	6.2
Carbohydrate	4.70	17.30	37.7	63.8	80.6	8.4
Ash	0.70	1.70	6.9	4.8	1	17.3
Moisture	70.00	3.70	5.3	3.7	5.7	2.3

The assessment of the accuracy of the test diet formulation is summarized by the results of the proximate analysis of the experimental diets is given in Table 3. The results reveal that there was practically no difference in the protein content of the experimental diets. The moisture content was also close to each other. Carbohydrate content was varied from 12.9% to 13.6% without any significant difference between treatments (P>0.05).

Effects of Non-dairy creamer on growth performance of red sea bream

The growth performance of red sea bream fed diet supplementation with fermented, non-dairy creamer is shown in Table 4. The mean initial weight of red sea bream was 1.3±0.01 g and was not significantly different among the treatments. A steady growth was observed in all the treatments during the experiment. The satisfactory weight gain was found with the diets containing fermented non-dairy creamer (Figure 1). Survival rate was not different among the treatments. Final mean weight gain and total weight gain of fishes was significantly different between treatments (Table 4, Figure 2, 3).

Table 3: Chemical composition of experimental diets prepared for Japanese red sea bream *P. major* supplemented with fermented non-dairy coffee creamer (% dry matter basis)

Items Proximate composition	Diet 1	Diet 2	Diet 3	Diet 4
Protein	48.7	42.4	45.2	47.2
Lipid	16.3	27.4	22.7	21
Carbohydrate	13.6	12.9	12.9	12.3
Ash	16.3	13.5	14.4	14.9
Moisture	5.1	3.8	4.8	4.5

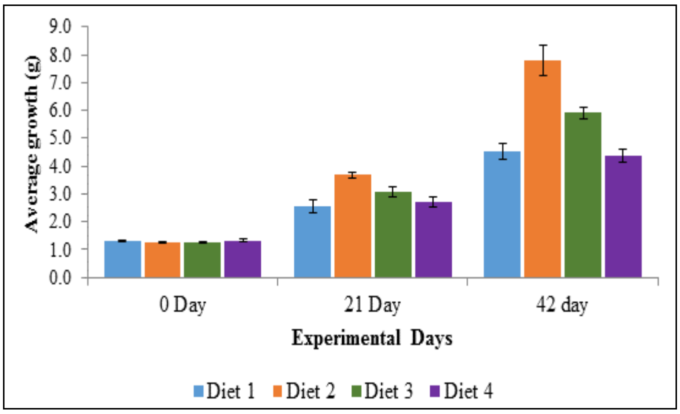


Fig 1: Growth performance of red sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

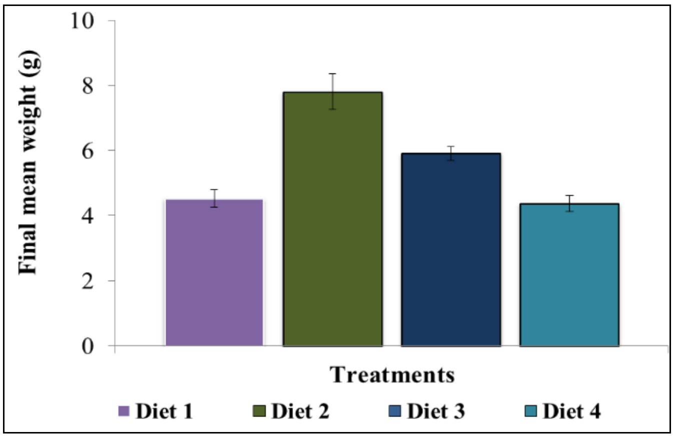


Fig 2: Final mean weight (g) of red sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

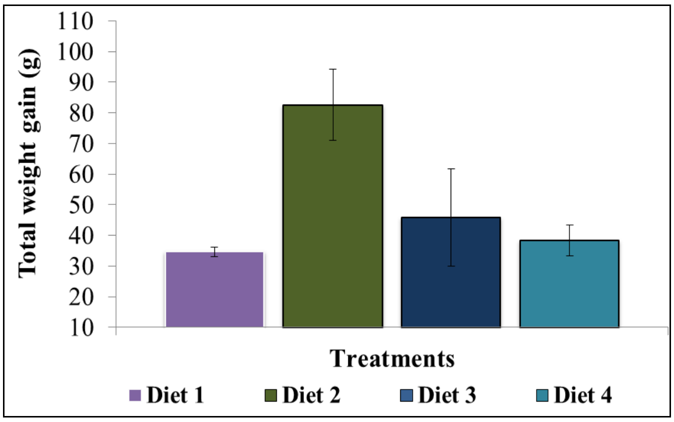


Fig 3: Total weight (g) of red sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

Gross yield, specific growth rate (%) and weight gain (%) among the treatments were significantly different ($P<0.05$) (Figure 4, 5, 6). Highest total weight gain, gross yield, specific growth rate and percent weight gain were observed in the 15% (Diet 2) fermented non-dairy creamer diet compared to the control (Table 4).

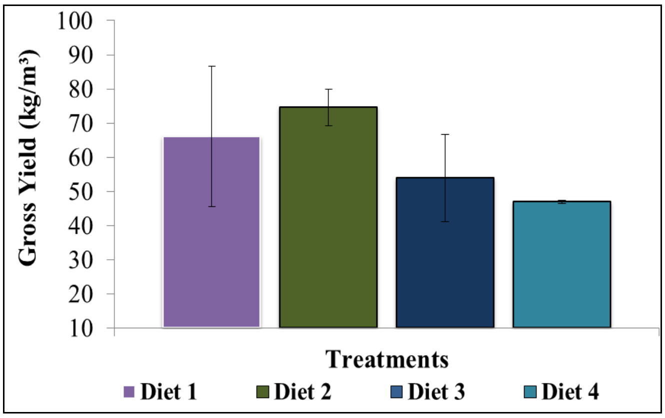


Fig 4: Gross yield (kg/m^3/year) of red sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

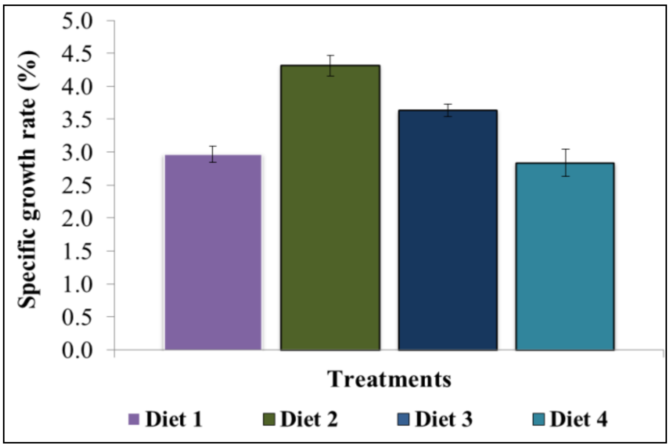


Fig 5: Specific growth rate (%) of red sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

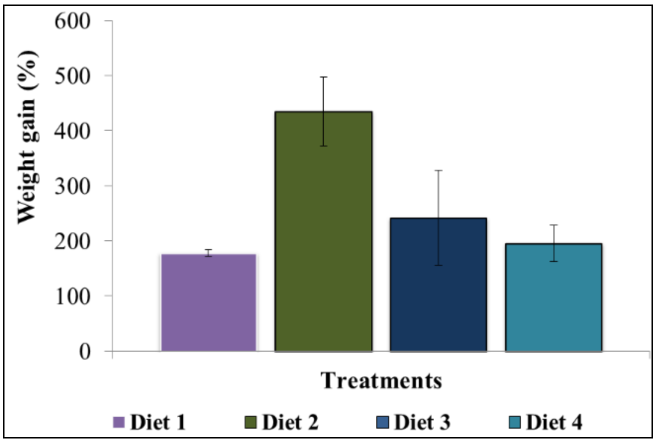


Fig 6: Weight gain (%) of red sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

Average length (mm) of fishes increased gradually with the time of the experiment (Figure 7). The length-weight relationship of fishes showed that fishes increased their body weight with proportionate to the size (Figure 8) and the R^2 value was close to one (0.99).

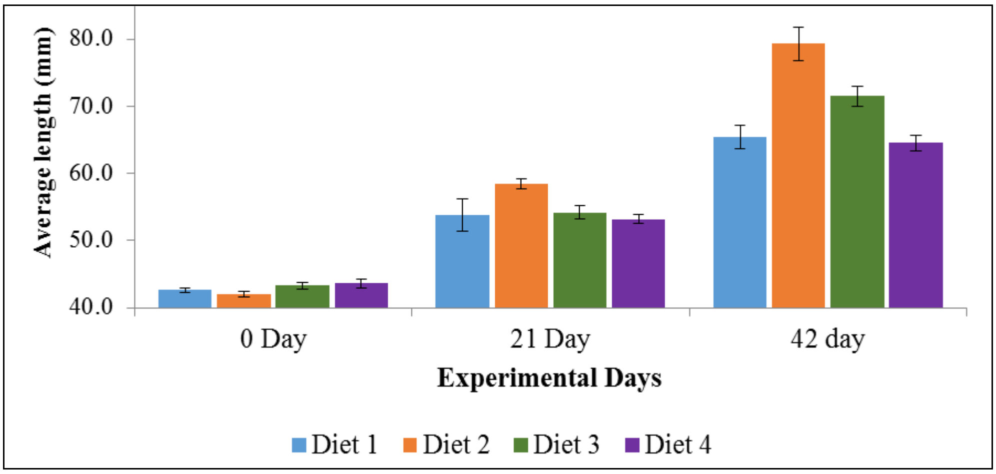


Fig 7: Average length of red sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

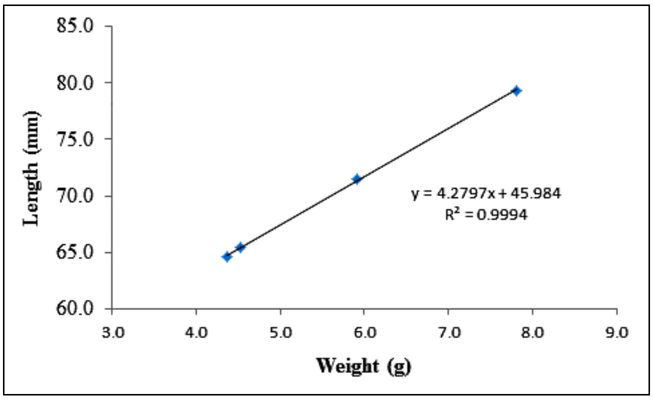


Fig 8: Length-weight relationship of sea bream *P. major* fed diets with fermented non-dairy creamer during the experiment.

Effects of fermented on nutrient composition of non-dairy creamer

The proximate composition of ingredients is presented in Table 5. It was observed that fresh non-dairy creamer contained 1% protein and 23% lipid, however, after fermentation the nutrient level increased very much and found that fermented non-dairy creamer contained 12% protein and 65% lipid, that indicating a good sources of protein and lipid for the aquaculture diet. Table 6 is showing the proximate composition of experimental diets for red sea bream in the recirculating system. It was observed that there were no big differences in the crude protein, carbohydrate and ash content among the treatments. The crude lipid level increased with the use of fermented creamer. All the diet contained more than 40% protein.

Table 4: The growth performance Japanese red seabream *P. major* fed diets with different levels of fermented non-dairy coffee creamer reared in closed recirculating system

Items	Diet 1	Diet 2	Diet 3	Diet 4
Initial weight (g)	19.45±0.26	19.04±0.19	19.23±0.37	19.86±0.70
Final total weight (g)	54.07±1.81^{b}	101.62±11.52^{a}	65.10±15.57^{b}	58.27±4.30^{b}
Initial mean weight (g)	1.30±0.02	1.27±0.01	1.28±0.03	1.32±0.05
Final mean weight (g)	4.53±0.27^{b}	7.81±0.55^{a}	5.91±0.21^{c}	4.37±0.25^{b}
Survival rate (%)	80.00±3.87	86.63±6.67	73.33±17.64	88.87±4.43
Daily mean weight gain (g/day)	0.07±0.01^{b}	0.16±0.01^{a}	0.11±0.01^{c}	0.07±0.01^{b}
SGR (%)	2.97±0.12^{b}	4.13±0.16^{a}	3.64±0.10^{c}	2.84±0.21^{b}
Gross yield (g/L)	5.11±0.18^{b}	10.16±1.15^{a}	6.51±1.56^{b}	5.83±0.43^{b}
Gross yield (kg/m^3/year)	47.00±1.58^{b}	88.31±10.01^{a}	56.58±13.53^{b}	50.64±3.73^{b}
Total weight gain (g/L)	3.46±0.16^{b}	8.26±1.16^{a}	4.57±0.1.60^{c}	3.84±0.86^{b}
Percent weight gain (%)	177.99±5.93^{b}	434.40±62.40^{a}	241.79±86.19^{b}	195.73±33.10^{b}

Table 5: Chemical composition of ingredients used for the preparation of experimental diets for culturing Japanese red sea bream *P. major* reared in closed recirculating system (% dry matter basis)

Items Proximate composition	Coffee cream	Fermented coffee cream	Soybean meal	Rice bran	Rice powder	White fish meal
Protein	1.00	12.20	47.2	24.4	10.4	65.8
Lipid	23.60	65.10	2.9	3.3	2.1	6.2
Carbohydrate	4.70	17.30	37.7	63.8	80.6	8.4
Ash	0.70	1.70	6.9	4.8	1	17.3
Moisture	70.00	3.70	5.3	3.7	5.7	2.3

Table 6: Chemical composition of experimental diets prepared for Japanese red sea bream *P. major* supplemented with fermented non-dairy coffee creamer reared in closed recirculating system (% dry matter basis)

Items Proximate composition	Diet 1	Diet 2	Diet 3	Diet 4
Protein	48.7	42.4	45.2	47.2
Lipid	16.3	27.4	22.7	21
Carbohydrate	13.6	12.9	12.9	12.3
Ash	16.3	13.5	14.4	14.9
Moisture	5.1	3.8	4.8	4.5

Effects of fermented non-dairy creamer on biochemical composition of fishes

Table 7 shows the proximate analysis of body muscle of red sea bream. It was observed that crude protein content in the body muscle was almost similar in all the experimental treatments. Highest crude lipid was observed in the fish fed diet with 15% fermented creamer and significantly higher percentage of carbohydrate in the same treatment ($P<0.05$).

Table 7: The biochemical compositions (%) of the whole body of Japanese red sea bream *P. major* fed diets with fermented coffee cream in closed recirculating system

Items	Diet 1	Diet 2	Diet 3	Diet 4
Protein	18.2	16.0	17.2	16.2
Lipid	2.7	4.3	3.4	4.0
Carbohydrate	0.3	1.3	0.1	0.8
Ash	6.5	6.6	6.7	6.8
Moisture	72.3	71.8	72.6	72.2

Effects of fermented non-dairy creamer on water quality parameters

Water quality parameter are shown in Table 8. All the water quality parameters were within the acceptable range in the recirculating system. Ammonia-nitrogen and phosphorus increased with the time of the experiment as feed particle dissolved in the water. However, none of the water quality parameters were out of the acceptable range for red sea bream.

Table 8: Water quality parameters in the closed recirculating system during the experimental period

Parameters	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	Average
DO (ppm)	8.20	7.70	7.00	6.00	6.20	6.5	6.93±0.39
pH	8.40	8.60	8.10	8.30	8.20	8.1	8.28±0.09
NH_3-N (mg/L)	0.07	0.14	0.58	0.45	0.27	0.2	0.28±0.09
Phosphorus (mg/L)	0.08	0.20	0.34	0.46	1.05	1.7	0.64±0.28

Discussion

Management of feed is the most important part for aquaculture operation. Profitability of aquaculture depends on the feed cost. Generally fishmeal is the main ingredients for fish feed, being expensive day by day [11]. Fermented products has been used as feed for animals and fishes. It was reported that the nutritive values of the feed improved due to the fermentation [12, 13, 14]. Various types of enzymes such as amylases, polysaccharides, cellulases, proteases, lipases and lignocellulases can be produced by microorganisms [15].

Chemical composition of the diets

In the present experiment fishmeal was used as protein source and 60% fishmeal was used in each treatment. Soybean meal and rice bran were replaced with fermented non-dairy creamer 5, 10 and 15%. Control diet contained 0% non-dairy creamer. Proximate analysis of the ingredients showed that fermentation process increased the protein, lipid and carbohydrate level in the final product. It was reported that fermentation enhanced the protein level in the fish feed [12]. Using the potato starch as fermented product found the improved protein content in the final product [16].

Fermentation helps to degrade the large molecules to single molecules. Shahabuddin *et al*. (2017) [17] used commercial enzymes such as mannanase, agarose and xylanase for the breakdown of long chain polysaccharides of *Pyropia yezoensis* (Ueda) to prepare spheroplasts. However, it was also tested for the fermentation of sugar beet pulp for producing single cell protein [18]. During fermentation different types of enzymes are produced by microbes and these enzymes help to degrade the starch, non-starch polysaccharides and other polymeric forms of the molecules in the substrate to soluble monomers with a beneficial increase in total protein, lipid and carbohydrate [19, 20].

Growth performance

There was no significant difference observed in the survival rate of red sea bream between the control and the other treatments. The average survival rate was more than 80%. Mortality observed in the experimental tanks was due to the cannibalism of the red sea bream as the tank size was 10 liter that might be relatively lower for the species. Though no difference in the feed intake, however with increasing the percentage of non-dairy creamer diet, decreased the feed intake. Final total weight and mean weight gain of fishes showed significantly higher value for the microbial fermented non-dairy creamer diet compared to control. It was observed that 75% replacement of fishmeal by fermented animal by product did not show any significant difference in the growth performance of *L. Rohita* [21]. However several studies suggested for the certain percentage of use of fermented product that could yield better growth of fish [17, 22, 23]. Enzyme treated soy protein showed remarkable higher growth performance in the red sea bream *P. Major* [24]. The red sea bream *P. major* showed an exponential growth in all the experimental diets. Exponential regression curve showed r^2 values of 0.99, and no significant differences were observed between the treatments. Similar result was observed for the Nile tilapia in the close recirculating system using enzyme treated spheroplast [11]. The regression curve indicated that fermented non-dairy creamer diets did not show any negative results in the weight gain of red sea bream.

Biochemical composition

Results of the present study indicate that the body protein content of red sea bream *P. major* was not affected by the addition of fermented non-dairy creamer. However, crude lipid content in the body muscle increased with increasing the percentage of fermented creamer. Samaddar *et al*. [21] reported that body protein of *L. rohita* was affected by the increasing the percentage of fermented blend. A significant reduction of daily protein retention was observed with increasing the percentage of fermented products. However, the experiment was carried out to replace the fishmeal with fermented blend.

In the present experiment, the proportion of fishmeal was similar to all the experimental diets. It was observed in the experiment that the lipid level in the diet increased with the increasing level of fermented non-dairy creamer. The carcass composition also showed the similar trend of increasing percentage of lipid content in the muscle of *P. major*. No significant difference was observed in the crude lipid level in the body of *L. rohita* fed diets with fermented animal by-product blend meal [21]. Controversy result also reported that high deposition of lipid in the body of fish fed fish offal supplemented feed. In this experiment the crude lipid level was pretty high although different level of lipid contained feed were consumed.

Conclusion

Fermentation is an environmental friendly technology that requires less energy. It will help in protecting biodiversity by using the unused and unutilized raw materials. Fermentation process effective improved the protein, lipid and carbohydrate value of the non-dairy coffee creamer. It also might help in improving digestibility and immunity of fishes. Bacteria in the fermentation process could help in the effective utilization of nutrient in the fish gut. The fermentation of creamer improved the growth and body composition of red sea bream. Thus microbial fermentation of coffee creamer provides a promising future as feed ingredient to a certain levels and help in reducing the feed cost. This will be an environmental friendly technology for utilizing the waste coffee creamer and thereby would increase the profitability of aquaculture. However, more research should be done on this issue to make it an industrial product.

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