

**Growth, Feed Utilization, Health Condition and Immune Response by Partially and Totally Replacing Fishmeal with Shochu Distillery By-Product in the Diet of Juvenile Japanese Flounder, *Paralichthys olivaceus***

<sup>1</sup>Mosa Sanzida Sultana, <sup>2</sup>Shunsuke Koshio, <sup>3</sup>Jobaer Md. Alam, <sup>2</sup>Manabu Ishikawa, <sup>2</sup>Saichiro Yokoyama and <sup>4</sup>Ikemura Tomoyuki

<sup>1</sup>Fisheries Science on Resources and Environments, The united Graduate School of Agricultural Sciences, Kagoshima University, Korimoto 1-21-24, Kagoshima, 890-0065, Japan

<sup>2</sup>Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Shimoarata 4-50-20, Kagoshima, 890-0056, Japan

<sup>3</sup>Department of Oceanography, University of Dhaka, Dhaka-1000, Bangladesh

<sup>4</sup>Graduate School of Fisheries Science, Kagoshima University, Shimoarata 4-50-20, Kagoshima, 890-0056, Japan

**Abstract:** A feeding trial was conducted to evaluate the potentiality of higher level of SDBP to partially and completely replacement the fishmeal in practical diets for Japanese flounder, *Paralichthys olivaceus*. This study was conducted by using five different levels of SDBP such as 0, 20, 25 and 30% by replacing fishmeal partially respectively and again SDBP 30% by replacing fishmeal completely (Non-FM). The control diet was formulated on the based of fishmeal (FM100). Fifteen fish (initial mean weight 2.65g) were placed in 100L polycarbonate circulate tank with triplicates (total 45 fish per treatment). The test diets were hand-delivered twice a day up to satiation level and fish were cultured under the flow-through system for 56 days. The average water temperature during the whole period of the feeding trial ranged from 14-19. Final weight and weight gain % was increased when we replaced the fish meal partially by SDBP and significantly higher value was found in SDBP 20% group than fish meal based control and completely replacing fish meal group. SGR also followed the similar trend like weight gain. Feed intake (FI) was significantly higher in SDBP 20% group but FER was significantly higher in SDBP 25% group. PER was significantly affected D3 group than D5 group. Survival and HSI did not differ significantly among the groups. Plasma GOT and T-pro was significantly affected by SDBP 20%. Oxidative stress parameters were not significantly affected by any group. Incase of immune response higher level of SDBP group showed the significantly higher activity than fishmeal based control group. This study demonstrated that dietary SDBP supplementation with low fish meal diet would be effective for the performances and quality of Japanese flounder.

**Key words:** Oxidative condition • Immune response • *Paralichthys olivaceus*

## INTRODUCTION

Japanese flounder, *Paralichthys olivaceus*, is one of the most important culture species in Japan due to its good growth characteristics and high market value [1]. Besides this Japanese flounder is highly popular and commercially important aquaculture species in Japan. To

culture this fish most farmers are using commercially manufactured feeds, which contents high amount of fishmeal (FM). As aquaculture production has still been increasing in the world, thereby increasing the demand for fish feeds still depends heavily on the availability of fishmeal [2]. So reducing the fishmeal in the diet formulations, without reducing fish performance is one of

**Corresponding Author:** Mosa Sanzida, Fisheries Science on Resources and Environments, The united Graduate School of Agricultural Sciences, Kagoshima University, Korimoto 1-21-24, Kagoshima, 890-0065, Japan.

the most important views for fish nutritionist. Though the fish nutritionist are trying to replace fishmeal partially or completely from the diets of many fish species but information of Japanese flounder is really limited. For that in this study we tried to replace fishmeal partially and completely by using shochu distillery by-product (SDBP) with some crude ingredients. Here we used squid and krill meal to improve the utilization of SDBP and soybean meal. Because Md. Kader *et al.* [3] reported that squid meal can improve the utilization of soybean protein and allow the substitution of higher level of fishmeal in red sea bream diet. Besides this Tomomi Kamizono *et al.* [4] reported that SDBP content growth promoting factor which is active in broiler chicken.

Japanese are well known for their high consumption of shochu, which results in large amounts of by-products. Shochu is a distilled Japanese alcoholic beverage that is produced from rice, sweet potato, barley, buckwheat or sugarcane mainly in Kyushu, Okinawa and the Southern Islands of Japan. The production of shochu reached about 958,000 kl in 2004 [5] and demand for this beverage is still increasing. Recent increases in shochu production have resulted in an enormous output of distillery by-product and thus the waste has been dumped into the ocean, causing environmental pollution. However, it is prohibited to discard it into the ocean by London treaty. So using SDBP as a feed stuff for farm animals and fishes may be the best way to solve this pollution issues. Tomomi Kamizono *et al.* [4] and Mosa. Sanzida Sultana *et al.* [6] reported that SDBP contains growth promoting factor and this growth promoting factor can be extracted by ether and fractionated by a Sephadex LH-20 column [7, 8]. Besides this it also contains various functional ingredients such as citric acid, polyphenols and vitamins.

Therefore, the present study was conducted to investigate the viability of use higher level of SDBP with some crude ingredients by evaluating several parameters such as growth, feed utilization, health status and immune response of Japanese flounder fed partially and completely replacing fishmeal diet.

## MATERIALS AND METHODS

**Experimental System:** Juveniles Japanese flounder were collected from a local hatchery, in Miyazaki prefecture, Japan and transported to the Kamoike Marine Production Laboratory, faculty of Fisheries, Kagoshima University, Japan. The feeding trial using juveniles average initial body weight of 2.65g was carried out in 100 L polycarbonated tanks (filled with 80 of water) at 15

fishes/tank where each tank was equipped with an inlet, outlet and continuous aeration. Each treatment having triplicates. All fish were fed twice daily up to apparent satiation. Uneaten diets were collected, oven dried and weighed for getting real feed intake. Periodic sampling was conducted every 2 weeks to monitor instantaneous growth and mortality in tanks. The tanks were maintained under a natural light and dark regime. The seawater was pumped from the deep basin of Kagoshima Bay, Japan; gravel filtered and supplied to the system. A flow rate of 1.5 l/min was maintained throughout the experimental period (56 days).

Initial sample from stock tank (20 fishes) were taken for body chemical composition. In order to minimize error in body weight data, fish were starved 24 h before final (56 days) sampling. Total number of fish and individual body weight of fish from each tank was recorded. Two fish from each tank were randomly taken and kept at -20 for body chemical composition. Using heparinized syringes, blood was collected from the caudal vein of five in each replicate tank and pooled. Plasma samples were obtained by centrifugation at  $3000 \times g$  for 15 minutes using a high speed refrigerated microcentrifuge (MX-160; Tomy Tech USA Inc., Tokyo, Japan) kept at -80. Fish were dissected for liver and muscle and weighed and store in -80 for measuring the hepatosomatic index and other analysis.

**Test Diets:** Table 1 summarized the composition of experimental diets. All the dietary components were obtained commercially except SDBP. SDBP obtained from Biochemistry and feed chemistry lab. So in this study we used condensed liquid SDBP. The liquid part of SDBP was first separated by a decanter and the liquid part was condensed [9].

Five isonitrogenous (more than 44% crude protein), isolipidic (10-11% total lipid) diets were formulated; where diet 1 was a 100% fish meal (FM) based control diet (D1). Diets 2 to 4 were prepared as follows by replacing FM protein with SDBP 20% or D2; SDBP 25% or D3; SDBP 30% or D4; and SDBP 20% or non-fishmeal or D5 (100% fishmeal replacement group). Here fish meal and soybean meal is the source of protein, wheat meal is the source of carbohydrate, pollack liver oil is the source of lipid and SDBP itself is functional ingredients. Krill and squid meal is the source of protein. The diets were prepared by mixing all ingredients in food processor for 30 min. Pellet size was 1.5 mm and pellets were oven (DK 400 Yamato Scientific, Tokyo, Japan) dried for 2 h at 60. The diets were stored in a cold room.

Table 1: Composition of basal diets (% of dry matter basis)

Ingredients	Con	S20%	S25%	S30%	Non-FM
Fish meal (BF) <sup>1</sup>	57	28	28	28	0
Soybean meal <sup>2</sup>	0	25	25	23.5	2.3
SDBP <sup>3</sup>	0	20	25	30	30
Krill meal (com) <sup>4</sup>	0	6.5	6.5	6.5	30
Squid meal (com) <sup>5</sup>	0	6.5	6.5	6.5	30
Wheat flour <sup>6</sup>	23	5	0	0	0
CMC <sup>7</sup>	0.5	0.5	0.5	0.5	0.5
Vitamin mix <sup>8</sup>	1	1	1	1	1
Mineral mix <sup>9</sup>	1	1	1	1	1
L-lysine	0	0.5	0.5	0.5	0.5
DL-methionine	0	0.5	0.5	0.5	0.5
Fish oil <sup>10</sup>	1.5	1.5	1.5	1.5	0.5
α-cellulose	16	4	4	0.5	3.7
Total	100	100	100	100	100

<sup>1</sup>Nippon Suisan, Tokyo, Japan

<sup>2</sup>J-Oil Mills, Kanagawa, Japan

<sup>3</sup>Shochu Distillery By-product (SDBP) obtained from Faculty of Agriculture, Kagoshima University

<sup>4</sup>Nippon Suisan Co. Ltd., Tokyo, Japan

<sup>5</sup>Nippon Suisan Co. Ltd., Tokyo, Japan

<sup>6</sup>Riken A-three, Tokyo, Japan

<sup>7</sup>Carboxymethyl cellulose

<sup>8</sup>According to Yokoyama *et al.*, (2006) with slight modification

<sup>9</sup>According to kader *et al.*, (2010)

<sup>10</sup>Riken Vitamin, Tokyo, Japan

Con: Control diet

S20%: SDBP 20%

S25%: SDBP 25%

S30%: SDBP 30%

Non-FM: Non fish meal

**Proximate Analysis of Whole Body:** Whole body in each treatment was analyzed for moisture, crude protein, total lipid and ash, in triplicate, using standard AOAC methods [10].

**Amino Acid Analysis:** Amino acid analysis of diets samples was analyzed using high performance liquid chromatography (HPLC, Shimadzu Corp.) according to Teshima *et al.* [11]. To quantify free amino acid, 40 mg of sample was mixed with 100μl norleucine as internal standard (0.6mg), 900μl cold distilled water and 2.5 ml of cold 10% trichloroacetic acid (TCA) and was homogenized by using polytron homogenizer (Kinematica, GmbH LITTAU, Lucerne, Switzerland). Samples were then centrifuged at 3000×g for 15 minutes at 4 and washed with diethyl ether to remove TCA from the homogenate. The PH of the homogenate was then adjusted to 2.2 and diluted to 5 ml sodium citrate, filtered (0.45μ) and stored at 4 for HPLC injection.

**Health Condition or Blood Parameter, Oxidative Stress and Immune Responses:** To measure the health condition we used plasma. So plasma chemical parameters were measured spectrophotometrically with an automated

analyzer (SPOTCHEM™ EZ model SP-4430, Arkray, Inc. Kyoto, Japan). Biological antioxidant potential (BAP) and reactive oxygen metabolites (d-ROMs) were also measured spectrophotometrically from blood plasma with an automated analyzer FRAS4, Diacron International s.r.l., Grosseto, Italy by following Morganti *et al.* [12]. *Escherichia coli* strain IAM11239 was used for the assay of plasma bactericidal activity which was performed according to Yamamoto and Iida [13]. Plasma lysozyme activity was measured with turbidimetric assays [14].

**Equation of Growth Performance Parameter:** The following equations were followed.

$$\text{Weight gain (\%)} = \frac{(\text{final weight} - \text{initial weight}) \times 100}{\text{initial weight}}$$

$$\text{Specific growth rate (SGR \% day}^{-1}\text{)} = \frac{\text{LN (final weight)} - \text{LN (initial weight)}}{\text{duration}} \times 100$$

$$\text{Survival (\%)} = 100 \times \frac{\text{final no. of fish}}{\text{initial no. of fish}}$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{live weight gain (g)}}{\text{dry feed intake (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{live weight gain (g)}}{\text{dry protein intake (g)}}$$

**Statistic Analysis:** All data were tested using one-way analysis of variance (Package Super-ANOVA, version 1.11; Abacus Concepts, Berkery, CA, USA). The level of significance between individual treatments ( $p < 0.05$ ) was evaluated by Tukey Kramer test. Results were presented as means  $\pm$  standard deviations.

## RESULTS

**Test Diet Analysis:** All diets were isonitrogenous and isolipidic. Crude ash content of the diet was 7-10%. The dietary content of free amino acids was higher in SDBP group than fishmeal based control group (data was not shown here).

**Survival and Growth Performance:** Table 2 represents the growth performance and feed utilization data Survival (%) of fish did not differ significantly ( $p > 0.05$ ) among treatments. Survival was almost 100% in each group. Final weight, weight gain% and SGR was significantly higher in SDBP 20% group than fishmeal based control and Non-Fm group. Rest two groups were not significantly differ from others group but they also showed the increasing tendency than fishmeal based control and Non-FM group. Feed intake was significantly higher in SDBP 20% group. But FER and PER was not followed the similar trend. FER was significantly higher in

SDBP 25% group and PER was significantly higher in D3 group. HSI did not differ significantly among the groups but they maintained some trend.

**Whole Body Composition:** Table 3 represents the whole body proximate composition of fish. In comparison with the fish meal based control diet, other dietary treatments had no significant influences on the whole body moisture, crude protein, total lipid and crude ash contents at the end of feeding trial.

**Blood Parameter, Oxidative Condition and Immune Response:** Table 4 summarizes the blood parameters, oxidative condition and immune response of juvenile Japanese flounder after 56 days feeding trial. Most of the parameters of blood were not differ significantly among the dietary treatments. But Total cholesterol was tending to be decreased by SDBP groups than fishmeal based control group. GOT was significantly lower in SDBP 20% group than fishmeal based control group and T-Pro also significantly higher in SDBP 20% group. BAP and ROMs did not differ significantly among the treatments but they followed the positive trend. Bactericidal activity was significantly higher in higher level of SDBP and Non-FM group than fishmeal based control group. Lysozyme activity did not differ significantly among the treatments.

Table 2: Growth performance and feed utilization of juvenile japanese flounder fed test diets for 56 days

Parameters	Test diet				
	Con	S20%	S25%	S30%	Non-FM
Initial weight (g)	2.65 $\pm$ 0.03	2.65 $\pm$ 0.02	2.65 $\pm$ 0.02	2.65 $\pm$ 0.02	2.66 $\pm$ 0.05
Final weight (g)	24.04 $\pm$ 0.43a	27.36 $\pm$ 2.13b	25.45 $\pm$ 0.59ab	25.03 $\pm$ 0.77ab	22.77 $\pm$ 0.66a
Weight gain %	808 $\pm$ 8.5a	931 $\pm$ 77.1b	860 $\pm$ 18.2ab	845 $\pm$ 30.0ab	756 $\pm$ 14.1a
SGR (% day) <sup>1</sup>	3.94 $\pm$ 0.02ab	4.16 $\pm$ 0.13c	4.04 $\pm$ 0.03bc	4.01 $\pm$ 0.06abc	3.83 $\pm$ 0.03a
FI (g fish <sup>-1</sup> 56days <sup>-1</sup> ) <sup>2</sup>	19.82 $\pm$ 0.52a	22.24 $\pm$ 1.12b	19.98 $\pm$ 0.21a	20.87 $\pm$ 0.68ab	19.30 $\pm$ 0.44a
FER <sup>3</sup>	1.08 $\pm$ 0.03ab	1.11 $\pm$ 0.04ab	1.14 $\pm$ 0.02b	1.07 $\pm$ 0.00ab	1.04 $\pm$ 0.02a
PER <sup>4</sup>	2.49 $\pm$ 0.08ab	2.55 $\pm$ 0.09ab	2.64 $\pm$ 0.05b	2.49 $\pm$ 0.01ab	2.40 $\pm$ 0.04a
Survival (%)	100 $\pm$ 0.00	96 $\pm$ 3.85	98 $\pm$ 3.85	100 $\pm$ 0.00	100 $\pm$ 0.00
HSI <sup>5</sup>	1.95 $\pm$ 0.14	1.95 $\pm$ 0.01	1.94 $\pm$ 0.31	2.17 $\pm$ 0.10	2.24 $\pm$ 0.32

Values are means $\pm$ SD of triplicate groups. Absence of letters indicates no significant difference among the treatments.

<sup>1</sup>SGR: specific growth rate=100x(ln final weight–ln initial weight)/days

<sup>2</sup> FI: feed intake (g dry diet fish<sup>-1</sup> 56 days)

<sup>3</sup>Feed efficiency ratio=total live weight gain (g)/total dry feed intake (g)

<sup>4</sup>Protein efficiency ratio=live weight gain (g)/dry protein intake (g)

<sup>5</sup>HSI: hepatosomatic index (%) =wt of liver/ wt of fish $\times$ 100

Con: Control diet

S20%: SDBP 20%

S25%: SDBP 25%

S130%: SDBP 30%

Non-FM: Non fish meal

Table 3: Whole body proximate analysis (%) of juvenile Japanese flounder fed test diets for 56 days

	Initial	Con	S20%	S25%	S30%	Non-FM
Moisture	80.29	75.89±0.81	76.29±0.57	76.21±0.41	76.42±0.58	77.43±0.36
Crude protein	14.03	16.87±0.15	16.69±0.08	16.87±0.04	16.85±0.35	16.20±0.3
Total lipid	2.81	3.06±0.24	2.48±0.11	2.29±0.07	2.79±0.25	2.78±0.3
Crude ash	3.24	2.93±0.00	3.08±0.15	2.99±0.07	3.07±0.1	3.25±0.02

Values are means±SD of triplicate groups. Absence of letters indicates no significant differences among the treatments.

Values are expressed as wet weight basis.

Con: Control diet

S20%: SDBP 20%

S25%: SDBP 25%

S30%: SDBP 30%

Non-FM: Non fish meal

Table 4: Blood parameters, oxidative condition and immune response of juvenile Japanese flounder fed test diets for 56 days

Parameters	Test diet					
	Con	S20%	S25%	S30%	Non-FM	
Glu(mg/dl) <sup>1</sup>	22±2	22±2.65	21.33±0.58	21.67±0.58	21.67±2.08	
T-cho (mg/dl) <sup>2</sup>	320±33	282±21	268±13	277±9	275±15	
BUN (mg/dl) <sup>3</sup>	5±0	5±0	5±0	5±0	5±0	
T-Bil (mg/dl) <sup>4</sup>	0.2±0	0.2±0	0.2±0	0.2±0	0.2±0	
GOT (IU/L) <sup>5</sup>	78±15.6b	40.5±3.5a	62.5±0.7ab	46.5±2.1ab	66.5±7.8ab	
GPT (IU/L) <sup>6</sup>	10±0	10±0	10±0	10±0	10±0	
T-Pro (g/dl) <sup>7</sup>	3.33±0.06bc	3.4±0c	3.17±0.12ab	3.37±0.06bc	3.1±0.1a	
TG (mg/dl) <sup>8</sup>	281±43	271±25	328±18	306±16	296±13	
<i>Oxidative stress parameters</i>						
BAP (µMol/l) <sup>9</sup>	3393±429	4275±812	4329±926	4473±1073	4298±996	
d-ROMs (U.Carr) <sup>10</sup>	146±29	101±4	109±25	102±1	108±4	
<i>Immune response parameters</i>						
BC (×10 <sup>7</sup> CFU) <sup>11</sup>	44±2c	38±3abc	43±0bc	30±6ab	25±1a	
Lysozyme activity (unit)	0.92±0.19	1.06±0.38	1.29±0.59	0.79±0.40	1.04±0.38	

Values are means ±STD of triplicate groups. Same letters are not significantly different (p>0.05). Absence of letters indicates no significant difference among the treatments.

<sup>1</sup>GLU: glucose, <sup>2</sup>T-cho: total cholesterol, <sup>3</sup>BUN: blood urea nitrogen, <sup>4</sup>T-bil: total bilirubin, <sup>5</sup>GOT: glutamyl oxaloacetic transaminase, <sup>6</sup>GPT: glutamic - pyruvate transaminase, <sup>7</sup>T-pro: total protein, <sup>8</sup>TG: triglyceride, <sup>9</sup>biological antioxidant potential, <sup>10</sup>reactive oxygen metabolites, <sup>11</sup>BC: bactericidal activity.

Con: Control diet

S20%: SDBP 20%

S25%: SDBP 25%

S30%: SDBP 30%

Non-FM: Non fish meal

## DISCUSSION

In this study, the effects of FM replacement partially and completely by higher level of SDBP were studied not only on growth performance but also on the oxidative condition and immune response status of juvenile Japanese flounder. This study clearly demonstrated that supplementation of SDBP could improve the low and non-FM diet in growth performances, oxidative condition and immune responses of Japanese flounder.

Survival did not differ significantly among the groups. It was almost 100%. Growth performance in terms of final weight, weight gain % and SGR were significantly increased by SDBP 20% than fishmeal based control and

non-FM group. Other groups did not differ significantly but SDBP content groups showed increasing tendency. These results suggest that dietary SDBP supplementation could be a potential feed additive to enhance the growth performance of juvenile Japanese flounder. Even in this scenario, totally replacing the fishmeal group showed the similar trend like 100% fishmeal group. This is might be because of growth promoting effect of SDBP. Similar growth promoting effects of dietary SDBP were reported in broiler [6, 7, 8]. Tomomi Kamizono *et al.* [4] also reported that SDBP contains a growth promoting factor active in broiler chickens and it can be extracted with ether. So from this result we could say SDBP not only effective feed additive for broiler chicken but also for juvenile Japanese flounder.

Feed intake (FI) also followed the similar trend like weight gain. This is because SDBP contents appetite stimulating factors in addition to growth promoting factor [7]. FER also significantly improved by SDBP. Bortov [15] reported that growth promoters not only improve the growth but also feed efficiency of broiler chicks because of their energy sparing effect. This is might be the cause to improve the FI and FER in SDBP group. So this growth promoter not only active in broiler chicken [4] but also in fish.

Blood chemical parameters are tools to indicate physiological stress as well as overall health condition of any species. In this study the values were considered to be within normal range of juvenile Japanese flounder compared with previous findings [16, 17]. GOT and GPT are the parameters which are usually used to evaluate the liver and kidney function. The rise in plasma level of GOT and GPT is attributed to the damage structural integrity of the liver because this enzymes are normally located in the cytoplasm and release into the circulation after cellular damage [4]. In this present study GPT was not affected by dietary SDBP but GOT was significantly affected by dietary SDBP. Significantly lower value was found in SDBP 20% group than FM based control group. Though there was no significant difference among the other groups but SDBP content groups showing the lower level than FM based control. This result similar to the result of Ahamed *et al.* [18] incase of broiler. T-pro content was significantly higher in SDBP 20% group than non-FM group and SDBP 25% group. Mahfudz *et al.* [8] reported that SDBP increases the protein content of cultured cells because of growth promoting factor. TG was not affected by dietary SDBP here. Oxidative stress was measured using the free radical analytical system (FRAS 4) assessing the derivatives of d-ROMs and BAP. Recently, in our laboratory, d-ROM and BAP has been used for evaluation of the oxidative stress condition of fish [19]. These parameters for all flounder were within normal range when compared with previous studies for this species. These parameters did not differ significantly among the dietary groups but SDBP content groups showed the positive effect than fish meal based control group. Immune response parameters were significantly affected by SDBP than fishmeal based control group incase of bactericidal activity but lysozyme activity did not differ significantly among the groups. But they are showing some positive trend in SDBP group. This is might be the cause of vitamin E and C content of SDBP which help to reduce the production of free radicals and cortisol [20]. As SDBP is fermented alcoholic product this is might be the cause to increase the immune response.

As far I know for immune response of fish by using SDBP this is the first work. So, further analysis is needed to clear this mechanism.

## CONCLUSION

Results, shows that SDBP may be used in replacing fishmeal partially or completely in marine fish such as Japanese flounder without any negative effect on growth performance, feed utilization, health parameters and immune responses. From the above result it might be concluded that SDBP is new promising candidate to make the quality feed.

## ACKNOWLEDGMENT

The first author is grateful for the Ministry of Education, Culture, Sports, Science and Technology (MONBUKAGAKUSHO), Japan for the scholarship. The research was partially funded by the Management Expenses Grants of the United Graduate School of Agricultural Sciences, Kagoshima University provided to SK.

## REFERENCES

1. Moe Thu, 2009. Study on effect of dietary bamboo charcoal in marine fish. Dissertation for the degree of Doctor of Philosophy (PhD); Kagoshima University, Japan, pp: 99.
2. Watanabe, T., 2009. Strategies for further development of aquatic feeds. *Fish Sci.*, 68: 242-252.
3. Md. Abdul Kader, Shunsuke Koshio, Manabu Ishikawa, Saichiro Yokoyama and Mahbuba Bulbul, 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. *Aquaculture*; 308: 136-144.
4. Tomomi Kamizono, Kazuki Nakashima, Akira Ohtsuka and Kunioki Hayashi, 2010. Effects of feeding hexane-extracts of a shochu distillery by-product on skeletal muscle protein degradation in broiler chicken. *Bioscience, Biotechnology and Biochemistry*, 74: 92-95.
5. Takashi Yamasaki, Tsunehiro Aki, Masami Shinizaki, Mashiro Taguchi, Seiji Kawamoto and Kazuhisa Ono, 2006. Utilization of shochu distillery waste water for production of polyunsaturated fatty acids and xanthophylls using *Thraustochytrid*. *Journal of Bioscience and Bioengineering*, 102(4): 323-327.

6. Mosa Sanzida Sultana, Tomomi Kamizono, Kenta Furuso and Kunioki Hayashi, 2011. Shochu distillery by-product loses growth promoting activity during preservation. *Journal of Warm Regional Society of Animal Science*, 54(1): 99-105.
7. Mahfudz, L.D., K. Hayashi, M. Ikeda, K. Hamada, A. Ohtsuka and Y. Tomita, 1996. The effective use of shochu distillery by-product as a source of broiler feed. *Japanese Poultry Science*, 33: 1-7.
8. Mahfudz, L.D., K. Nakashima, A. Ohtsuka and K. Hayashi, 1997. Growth factors for a primary chick muscle cell culture from shochu distillery by-product. *Biosci. Biotech. Biochem.*, 61(11): 1844-1847.
9. Hayashi, K., M. Maeda, K. Kitahara, T. Tagoyama and A. Ohtsuka, 2009. Effect of shochu distillery by-product on productivity and meat quality in broiler. *Nihon Chikusan Gakkaiho*, 80: 35-39. (in Japanese with English abstract).
10. A.O.A.C., 1990. *Official Methods of Analysis of the Association of Official Analytical chemists*, 15<sup>th</sup> ed. Association of Official Analytical Chemists, Arlington, VA, USA.
11. Teshima, S., A. Kanazawa and Y. Kakuta, 1986b. Effects of dietary phospholipids on lipid transport in the juveniles prawn. *Bull. Jpn. Soc. Sci. Fish.*, 52: 159-163.
12. Morganti, P., C. Bruno, F. Guarneri, A. Cardillo, P. Del Ciotto and F. Valenzano, 2002. Role of topical and nutritional supplement to modify the oxidative stress. *Int. J. Cos. Sci.*, 24: 331-339.
13. Yamamoto, A. and T. Iida, 1995. Bactericidal activity of serum of all -female triploid rainbow trout. *Fish Pathol.*, 30: 123-124.
14. Takahashi, Y., T. Itami and K. Konegawa, 1986. Enzymatic properties of partially lysozyme from skin mucus of carp. *Bull. Jap. Soc. Sci. Fish.*, 52: 1209-1214.
15. Bartov, I., 1992. Effects of energy concentration and duration of feeding on the responses of broiler chicks to growth promoters. *British Poultry Science*, 33: 357-382.
16. Hernandez, L.H.H., S. Teshima, S. Koshio, M. Ishikawa, Y. Tanaka and M.S. Alam, 2007. Effects of vitamin A on growth, serum anti-bacterial activity and transaminase activities in the juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 262: 444-450.
17. Mamauag, R.E.P., S. Koshio, M. Ishikawa, S. Yokoyama, J. Gao, B.T. Nguyen and J.A. Ragaza, 2011. Soy peptide inclusion levels influence the growth performance, proteolytic enzyme activities, blood biochemical parameters and body composition of Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 321: 252-258.
18. Ahamed A. Saleh, Y.Z. Eid, T.A. Ebeid, K. Amber, N. Badawi and K. Hayashi, 2010. Effect of *Aspergillus niger* on broilers performance. *Egypt. Poultry Science*, 30: 1017-1029.
19. Kader, M.A., S. Koshio, M. Ishikawa, S. Yokoyama, M. Bulbul, Y. Honda, R.E. Mamauag and A. Laining, 2011. Growth, nutrient utilization, oxidative condition and element composition of juvenile red sea bream, *Pagrus major* fed with fermented soybean meal and scallop by-product blend as fish meal replacement. *Fisheries Science*, 77: 119-128.
20. Mosa Sanzida Sultana, 2010. Study on the nutritional value of shochu distillery by-product. Dissertation for the degree of Master of Science (MS); Kagoshima University, Japan, pp: 55.