Supplemental Effects of Shochu Distillery By-product on Performance of Japanese Flounder, *Paralichthys olivaceus*, with Low Fishmeal Diet

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Abstract: Since the development of functional aquafeeds is one of the priority areas for sustaining aquaculture, the study was conducted to determine the efficacy of shochu distillery by-product (SDBP) when dietary fishmeal was lowered. This study targeted Japanese flounder, Paralichthys olivaceus, as an experimental model fish by using four different dietary levels of SDBP such as 0, 4, 8, and 14%, respectively. Twelve fish (initial mean weight=0.5g) were placed in 100L polycarbonate circular tank with four replicates (total 48 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 11 to 15°C. The results showed that body weight gain (%) and specific growth rates (% day¹) increased with increased level of dietary SDBP although statistical significance was not detected. Significantly improvement of feed efficiency ratio and protein efficiency ratio were found in higher level of SDBP supplemented groups than control and lower supplemented ones. Survival rates were not significantly affected by supplementation of SDBP. In determining the oxidative condition of the fish the thiobarbituric acid reactive substances (TBARS) were measured, showing that muscle TBARS of fish showed the decreasing tendency with increased dietary SDBP levels. Furthermore, it was found that linoleic acids of neutral lipid fraction in fish liver significantly increased with increased dietary SDBP supplementation. This study demonstrated that dietary SDBP supplementation would be effective for the performances and quality of Japanese flounders.

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1. Introduction

The expansion of aquaculture production has been accompanied by rapid growth of aquafeed productions. On the other hand, the availability of fishmeal, which is a major protein source in aquafeeds, has decreased and this situation is not expected to improve in the near future (Forster and Ogata 1998). Thus, the challenge facing the aquaculture industry is identify economically viable to and environmental-friendly alternatives to fishmeal. Although plant proteins are potential candidates in this category, most of the plant feed stuff contains anti-nutritional factors, and indicates imbalance amino acid profile, leading decline of feed palatability or acceptability (Chatzifotis et al. 2008, Kader et al. 2010, Kissil et al. 2000, Kikuchi et al. 1999, Kubitza et al. 1997). Therefore, the development of feeds containing plant proteins with stimulant factors is an effective approach to maintain feed attractiveness and induce adequate feed consumption rate for fish (Kissil et al. 2000, Papatryphon et al. 2000).

Shochu is popular Japanese traditional liquor made from a variety of materials including rice, sweet potato, barley, corn, brown sugar, etc. Increases in shochu production have resulted in an enormous output of distillery by-product. Recently, dumping a by-product of the liquor into the ocean is prohibited because of London treaty (Kamizono et al. 2010, Sanzida et al. 2011, Yoshimoto et al. 2004). Thus, using shochu distillery by-product (SDBP), which is the waste of shochu industry, as a feed additive would be one of the ways to solve the disposal issues. It was already investigated that sweet potato SDBP contains a growth promoting factor butoxy butyl alcohol (BBA) which is active in broiler chicken (Kamizono et al. 2010), and feeding of SDBP increased muscle α-tocopherol and glycogen contents (Ohtsuka et al. 1998) and decreased the muscle thiobarbituric acid reactive substances (TBARS) in broiler chicken (Sanzida et al. 2011). On the other hand SDBP also contains large amounts of functional ingredients such as polyphenols, vitamin C and E (Kamizono et al.

2010). It is also rich in amino acids such as alanine, glycine, arginine, glutamine, lysine, asparagines, threonine, and phenylalanine etc. (Sudoh 1975). Several studies have reported about the effect of SDBP on growth, feed intake, feed efficiency ratio, blood parameter, oxidative condition and protein degradation of land animals. The previous reports have clarified SDBP is a potential feed additive for land animals (Hayashi et al. 2009, Ohtsuka et al. 1998).

However, very limited study on SDBP in aquatic animal nutrition as a feed additive has yet been conducted. This is first experiment to use SDBP as an additive in feeds for marine fish such as Japanese flounder so far. Japanese flounder, *Paralichthys olivaceus*, is one of the most important cultured species due to its good meat quality characteristics and high market value (Kikuchi et al. 1999, Moe Thu 2009).

In this study, effects and efficacy of dietary SDBP on growth, feed efficiency, whole body composition, and oxidative condition of juvenile Japanese flounder, *Paralichthys olivaceus*, were investigated to clarify the function of SDBP as an additive when fed low dietary fishmeal.

2. Materials and Methods

2.1. Experimental system

Japanese flounder Juveniles were purchased from a local hatchery in Miyazaki prefecture, Japan, and transported to the Kamoike Marine Production Laboratory, faculty of Fisheries, Kagoshima University, Japan. A feeding trial using juveniles (average initial body weight of 0.5g) was carried out in 100 L polycarbonate circular tanks (filled with 80 of water) 12 fishes/tank where each tank was equipped with an inlet, outlet and continuous aeration. Each treatment has four replicates. All fish were fed twice daily up to apparent satiation. Uneaten diets were collected, oven dried and weighed for the calculation of actual feed intake. Periodical sampling was conducted every 2 weeks to monitor growth and mortality of fish 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 1.5L/min was maintained throughout the experimental period. The average water temperature during the whole period of the feeding trial ranged from 11 to 15°C. Since the culture system was flow-through, water quality was kept clean during the trial. Initial sampling using 20 fishes were taken for body chemical composition and amino acid analysis of the fish. In order to minimize variations on body weight data, fish were starved for 24h before the final sampling. Three fish from each tank were randomly taken and keep at -20°C for body chemical composition and amino acid analysis. Fish were dissected for liver weighed and store in -80°C for fatty acid analysis. A feeding trial was conducted for 56 days.

2.2. Test diets preparation

Table 1 summarizes the composition of experimental diets. All dietary components were obtained commercially except the shochu distillery by-product (SDBP). The liquid part of SDBP was first separated by a decanter and the liquid fraction was condensed followed by the method of Hayashi et al. 2009. The process of making SDBP used for the feeding trial was conducted at Biochemistry and Feed Chemistry laboratory, Faculty of Agriculture, Kagoshima University, Japan.

Four test diets were formulated, in which Diet1 was a control diet containing 60% of fishmeal (FM), and Diets2 to 4 were prepared by supplementing 4%, 8%, and 14% of SDBP, respectively, while FM was reduced down to 45%. To make isocaloric, isolipidic, and iso-energetic diets, wheat flour was adjusted. Major sources of protein, lipid, and carbohydrate are FM and soybean meal, pollack liver oil, and wheat flour, respectively. The diets were prepared by mixing all ingredients in food processor for 30 min. Pellet size was 1.2 mm diameter and pellets were oven-dried (DK 400 Yamato Scientific, Tokyo, Japan) for 2h at 60°C. The diets were stored in a cold room during the trial. 2.3. Amino acid analysis

Amino acid analysis of diet samples were conducted using high performance liquid (HPLC, chromatography Shimadzu. Japan) according to Teshima et al. 1986. For the analysis of total amino acid, about 2 mg of dry sample was spiked with internal standard norleucine and hydrolyzed with 4 N-methanesulfonic acid at 110°C for 22h. The pH of hydrolysate was adjusted to 2.15 to 2.5 and diluted to 5ml sodium citrate, filtered (0.45µm) and stored at 4°C. To quantify free amino acid, 40 mg of sample was mixed with 100µl norleucine as internal standard (0.6mg), 900ul cold distilled water and 2.5 ml cold 10% trichloroacetic acid (TCA) and was homogenized by using polytron homogenizer (Kinematica, Gmbh LITTAU, Lucerne, Switzerland). Samples were then centrifuged at $3000 \times g$ for 15 minutes at 4°C washed with diethyl ether to remove TCA from the homogenate. The pH of homogenate was then adjusted to 2.2 and diluted to 5 ml sodium citrate, filtered (0.45μ) and stored at 4°C for HPLC injection.

2.4. Analysis of chemical composition, and fatty acid composition of whole body and liver

Proximate compositions of whole body in each treatment were analyzed for moisture, crude protein, total lipid and ash, in triplicate, using standard AOAC methods (AOAC 1990).

Fatty acid composition of liver was analyzed according to Querijero et al. 1997. Total lipid was extracted by homogenizing 0.2g sample according to Bligh and Dver 1959. Fatty acid esters (FAMEs) were then produced from total lipids aliquots and methylated with boron triflouride (BF3) in methanol. Methyl tricosanoate (Nu-Check Prep. Inc.Elysian, MN, U.S.A.) was used as an internal standard at 1000 mg/ml hexane. FAMEs were analyzed using gas chromatography (Shimadzu GC 17A, Tokyo, Japan). The temperature of injector and detector (FID) were both set at 250°C. High-purity helium was used as the carrier gas at a flow rate of 1ml/min. The samples (1.0µl) were manually injected into an injection port and identified fatty acids were presented as area percentage of total fatty acid.

Table 1. Composition of basal diet (% of dm basis)

| | _ | Dietary Groups | | |
|-----------------------|--------|----------------|--------|--------|
| Ingredients | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| BFM^1 | 60.0 | 45.0 | 45.0 | 45.0 |
| SBM^2 | 0.0 | 22.0 | 22.0 | 22.0 |
| SDBP ³ | 0.0 | 4.0 | 8.0 | 14.0 |
| Fish oil ⁴ | 5.0 | 6.0 | 6.0 | 6.0 |
| Wheat flour | 16.0 | 4.5 | 3.0 | 1.0 |
| Vit mix ⁵ | 3.0 | 3.0 | 3.0 | 3.0 |
| Min mix ⁶ | 3.0 | 3.0 | 3.0 | 3.0 |
| L-lysine | 0.5 | 0.5 | 0.5 | 0.5 |
| DL-Met | 0.5 | 0.5 | 0.5 | 0.5 |
| α-cellulose | 12.0 | 11.5 | 9.0 | 5.0 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 |

¹Brown fish meal, Nippon Suisan, Tokyo, Japan

²Soybean meal, J-Oil Mills, Kanagawa, Japan

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

⁴Riken Vitamin, Tokyo, Japan

⁵Vitamin mixture according to Yokoyama et al. (2006) with slight modification

⁶Mineral mixture according to Kader et al. (2010)

2.5. Determination of lipid oxidation

The measurements of thiobarbituric acid reactive substances (TBARS) in liver and muscle were carried out using a method adapted from Yagi 1987. 0.2g sample mixed with 1.5ml of 20% (w/v) trichloroacetic acid (TCA, pH 3.5), 1.5ml of 0.8% BHT in acetic acid, 0.2ml of 8.1% SDS, 0.05ml of 0.8% BHT and 1ml of distilled water added to a 15ml test tube and then the test tubes were keep at 5°C refrigerator for 1h. The tubes were then heated on water bath for 1h at 100°C. 1ml of distilled water and 5ml of n-butanol:pyridine (15:1) solution were added after samples were cooled down and centrifuged at $3000 \times g$ for 10min at 4°C. The supernatant was analyzed by a spectrophotometer at 532nm.

2.6. Equation of growth performance parameter

The following equations were applied for the calculations:

Weight gain or WG (%)=(final weight (g)-initial

weight (g)) x100/initial weight (g) Specific growth rate (SGR %, day-1)=100x {Ln (final weight)-Ln (initial weight)}/duration) Survival (%)=100x(final no. of fish/initial no. of fish) Feed intake (FI (g)/fish/56 days)=(dry diet given-dry uneaten diet)/no. of fish Feed efficiency ratio (FER) =live weight gain (g)/dry feed intake (g) Protein efficiency ratio (PER) = live weight gain (g)/dry protein intake (g) 2.7. Statistical analysis

Statistical analysis was conducted by super ANOVA (Tukey Kramer test) to identify the significant differences among treatments (p<0.05).

3. Results

3.1. Diet analysis

The composition of experimental diets was shown in Table 1. Diets contained 40-44% of crude protein, 10-11% of total lipid, and 10-12% of ash. Total (Table 2) and amino acids had a tendency that the values were relatively higher in all SDBP groups compared with Diet1, particularly, for indispensable amino acids. Free amino acids data was not shown here individually but the total free amino acids followed the similar trend like total amino acids (Diet 1: 2.94; Diet 2: 3.80; Diet 3: 4.58; Diet 4: 5.14, respectively).

Table 2. Total amino acid contents of experimental diets (g/100g dry sample)¹

| | Dietary Groups | | | |
|--------------------------------------|----------------|--------|--------|--------|
| AA^2 | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| Indispensa | ıble | | | |
| Arg | 1.78 | 1.81 | 2.08 | 2.13 |
| His | 1.54 | 1.44 | 1.51 | 1.57 |
| Isol | 1.35 | 1.33 | 1.39 | 1.39 |
| Leu | 3.95 | 3.90 | 3.97 | 4.08 |
| Lys | 0.47 | 0.43 | 0.48 | 0.51 |
| Met | 0.58 | 0.96 | 0.40 | 0.99 |
| Phe | 1.88 | 1.90 | 2.04 | 2.12 |
| Thr | 1.19 | 1.18 | 1.20 | 1.25 |
| Try | 0.00 | 0.00 | 0.00 | 0.00 |
| Val | 1.55 | 1.46 | 1.48 | 1.59 |
| Dispensab | le | | | |
| Tau | 0.18 | 0.14 | 0.14 | 0.14 |
| Asp | 5.47 | 5.90 | 6.08 | 6.36 |
| Glu | 7.14 | 7.26 | 7.38 | 7.54 |
| Ser | 1.51 | 1.61 | 1.65 | 1.73 |
| Pro | 0.00 | 0.00 | 0.00 | 0.00 |
| Gly | 1.03 | 0.96 | 0.99 | 1.02 |
| Ala | 2.05 | 1.92 | 1.93 | 2.07 |
| Tyr | 1.62 | 1.64 | 1.73 | 1.78 |
| ΣΤΑΑ | 33.3 | 33.8 | 34.5 | 36.3 |
| Values are mean of triplicate groups | | | | |

¹Values are mean of triplicate groups.

²AA:Amino acids; Arg: Arginine; His: Histidine; Iso: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Phe: Phenylalanine; Thr: Threonine; Try: Tryptophan; Val: Valine; Tau: Taurine; Asp: Aspartic acid; Glu: Glutamic acid; Ser: Serine; Pro: Proline; Gly: Glycine; Ala: Alanine; Tyr: Tyrosine; Σ TAA; Total amino acid residues

3.2. Survival and growth performance

The results of survival rates and growth performances were shown in Table 3. After 56 days, the dietary treatment did not affect the survival rates of fish, and the survival rates were very high in all groups (more then 90%). Final weight gain, weight gain (%), and specific growth rate (SGR) of fish showed the increasing tendency with increased dietary SDBP levels. The highest value was obtained from the fish fed SDBP14% and the poorest was found in fish fed only fishmeal based control diet although there was no significant difference on those parameters among all groups. Similar feed intake was observed among all groups. On the other hand, the feed efficiency ratio (FER) significantly increased with increased dietary SDBP supplementation, and the highest values were obtained in fish fed a diet with SDBP14%. This value was significantly higher than those of control and SDBP4%, but not significantly different from SDBP8%. The protein efficiency ration (PER) (Table 3) was significantly higher in SDBP14% than SDBP4%. The values of fish fed the control, SDBP8%, and SDBP16% were not significantly different each other.

3.3. Whole body composition

Table 4 represents the whole body proximate analysis of fish. In comparison with the control and other dietary groups, there were no significant differences on the whole body moisture, crude protein, total lipid and ash contents at the end of the feeding trial.

3.4. Oxidative condition

Results of TBARS analysis from fish muscle and liver were shown in Table 5. Muscle and liver TBARS were not significantly affected by dietary SDBP supplementation although the SDBP groups showed a decreasing trend in muscles, but increasing one in livers.

3.5. Fatty acids of neutral lipid fraction in fish liver

The data of major fatty acids of neutral lipid fraction in fish liver were shown in Table 6. Linolenic acid contents significantly increased with dietary SDBP supplementations, and DHA data showed the increasing trend with increased dietary SDBP supplementations although there was no significant different. In SDBP supplemented groups, liver EPA increased with increased dietary SDBP supplementations although a statistical significance was not detected among groups.

| Table 3. Growth performance and feed utilization in | |
|--|--|
| juvenile Japanese flounder fed test diets for 56 days ¹ | |

| Dietary groups | | | |
|-----------------|---|---|---|
| Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| | | | |
| 973 | 1046 | 1183 | 1261 |
| ±167 | ±212 | ±392 | ±135 |
| | | | |
| 4.22 | 4.33 | 4.50 | 4.67 |
| ±0.29 | ±0.33 | ±0.51 | ±0.17 |
| 4.81 | 5.03 | 4.99 | 4.98 |
| ±0.84 | ±0.91 | ±1.46 | ±0.56 |
| 1.04 | 1.05 | 1.20 | 1.29 |
| $\pm 0.09^{a}$ | $\pm 0.04^{a}$ | $\pm 0.06^{b}$ | $\pm 0.04^{b}$ |
| 2.60 | 2.55 | 2.77 | 2.87 |
| $\pm 0.23^{ab}$ | $\pm 0.10^{a}$ | $\pm 0.15^{ab}$ | $\pm 0.09^{b}$ |
| 96 | 96 | 98 | 94 |
| | 973 ± 167 4.22 ± 0.29 4.81 ± 0.84 1.04 $\pm 0.09^{a}$ 2.60 $\pm 0.23^{ab}$ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ |

^TValues are means \pm SD of 4 replicate groups. Same letters are not significantly different (P>0.05).

²SGR: Specific growth rate=100x(ln final weight-ln initial weight)/days

 ${}^{3}FER$: Feed efficiency ratio=total live weight gain (g)/total dry feed intake (g)

⁴PER: Protein efficiency ratio=total live weight gain (g)/total dry protein intake (g)

Table 4. Whole body proximate analysis (%) in juvenile Japanese flounder fed test diets for 56 days¹

| | Dietary groups | | | |
|-------------------|----------------|--------|--------|--------|
| Comp ² | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| Moisture | 76.4 | 76.5 | 75.2 | 76.3 |
| | ±0.76 | ±0.47 | ±2.39 | ±0.14 |
| СР | 16.5 | 16.7 | 16.6 | 16.2 |
| | ±0.42 | ±0.55 | ±0.33 | ±0.21 |
| TL | 2.65 | 2.86 | 2.99 | 3.15 |
| | ±0.02 | ±0.42 | ±0.35 | ±0.06 |
| CA | 3.34 | 3.38 | 3.46 | 3.33 |
| | ±0.04 | ±0.12 | ±0.01 | ±0.10 |

¹Values are means±SD of triplicate groups.

²Values are expressed as wet weight basis. Com: Composition; CP: Crude protein; TL: Total lipid; CA: Crude ash

4. Discussion

In consideration of sustainable aquafeeds, the development of functional aquafeeds is one of the most important strategies. Since a very significant ingredient such as fishmeal (FM) has a very limited use, the optimal alternative protein sources should be found very urgently. On the other hand, possible alternatives are not perfectly accepted by cultured fish species due to several negative natures of those protein sources. Therefore, it will be very impacted to enhance the nutritive value of aquafeeds which contain low dietary FM or non-FM by supplementing functional compounds such as SDBP. In this regard, this study is the first challenge for Japanese flounder by using SDBP that was originally processed in Kagoshima University.

Since the mortality of fish fed the test diets was

very low, dietary SDBP supplementation would not be toxic to the fish used. It was reported that SDBP used in this trial contains a growth promoting factor for broiler, which is identified as a novel compound such as butoxybutyl alcohol (Mahfudz et al. 1996b, 1997).

Table 5. Oxidative condition (TBARS nmolMDA/g) of juvenile Japanese flounder fed test diets for 56 days¹

| Groups | Mus TBARS ² | Liv TBARS ² |
|---------|------------------------|------------------------|
| Diet 1 | 19.44±0.06 | 23.56±2.75 |
| Diet 2 | 15.27±2.46 | 38.34±0.16 |
| Diet 3 | 15.12±1.35 | 27.72±13.42 |
| Diet 4 | 16.01±0.66 | 33.78±0.34 |
| 1 * * 1 | | T 1 |

¹Values are means±SD of triplicate groups. There were no significant differences among all groups. ²TBARS: Thiobarbituric acid reactive substances, Mus: Muscle;

²TBARS: Thiobarbituric acid reactive substances, Mus: Muscle; Liv: Liver

It was further confirmed that this compound is active for broiler chicken (Kamizono et al. 2010). In case of broiler lower level of SDBP is more effective on body weight gain than the higher level, suggesting that a higher level of SDBP might contain growth inhibiting factor for broiler chickens (Kamizono et al. 2010, Mahfudz et al. 1996a).

Table 6. Main fatty acids (% of total fatty acid) of neutral lipid fraction in liver of juvenile Japanese flounder fed experimental diets for 56 days¹

| nounder fed experimental alets for 50 days | | | | | |
|--|-----------------------|------------------|-----------|--|--|
| Groups | LNA ² | EPA ³ | DHA^4 | | |
| Diet 1 | $0.66{\pm}0.05^{a}$ | 9.36±0.07 | 4.92±0.22 | | |
| Diet 2 | $0.87{\pm}0.04^{b}$ | 8.74±0.21 | 5.12±0.78 | | |
| Diet 3 | $0.94{\pm}0.00^{b}$ | 9.27±0.33 | 5.58±0.23 | | |
| Diet 4 | $0.99 {\pm} 0.00^{b}$ | 9.49±0.06 | 5.89±0.11 | | |

¹Values are means \pm SD of duplicate groups. Absence of letters are not significantly different (P>0.05)

²LNA: Linolenic acid

³EPA: Eicosapentaenoic acid

⁴DHA: Docosahaxaenoic acid

On the other hand, the body weight gain seemed to increase with increased dietary level of SDBP in the present study. The discrepancy might be due to the different physiological conditions between farm animals and aquatic animals. It was found that SDBP increased the feed intake in commercial broiler chicken due to the appetite stimulating factors in SDBP in addition to growth stimulating factor, resulting in the improvement of feed conversion ratio (Mahfudz et al. 1996a, 1996b). Bartov (1992) suggested that growth promoter improved growth rate as well as feed efficiency of broiler chicks because of their energy sparing effect, which in turn, increased dietary ME.

Efficiency for the utilization of soybean proteins, which is one of most popular FM alternative, varies among different fish species, leading to the number of challenges associated with soybean products. There are several negative factors in soybean proteins such as lower level of sulfur amino acids like methionine, less palatability, lower digestibility and the presence of antinutritional factors. Supplementation of lysine and methionine to compensate for the deficiency of indispensable amino acids and some others amino acids (e.g., glycine, alanine and taurine, etc) as attractants is beneficial in recovering amino acid balance and palatability in the use of high soybean protein based diets (Chatzifotis et al. 2008, Fuke et al. 1981, Kader et al. 2010, Takagi et al. 2001, Venou et al. 2006). In this trial, FI was similar among the groups. In case of broiler, the feed intake was improved by applying the diet containing SDBP compared to the diet without SDBP supplementation (Mahfudz et al. 1997). The different responses to the diets with SDBP between flounders and broilers are not fully understood yet, although it may be due to the species different, further study would need to be conducted in this area.

FER were significantly improved by feeding higher level of SDBP compared to those of the control and lowest SDBP groups. Likewise, PER also significantly increased in 14% SDBP group. Therefore, it can be concluded that flounders utilized SDBP very efficiently. It would be assumed that one of the reasons for the insignificance in growth data in the present study was due to the wide variation of standard error. However, it is important to mention the fact that there was a clear trend on growth data, in which the values increased with increased dietary SDBP. Accordingly, although there was no statistical significance on growth data, FER and PER were found to be significant.

Oxidative stress is the level of oxidative damage in a cell, tissue, or organ, caused by the reactive oxygen species. As SDBP contained large amounts of polyphenol and α -tocopherol (Sanzida et al. 2011), oxidative stress could be suppressed by SDBP. Though there was no significant difference between control and SDBP groups, TBARS values of SDBP groups showed the decreasing tendency in muscle. On the other hand, the values of liver TBARS varied. The reason for the phenomenon is not known at the moment. Lipid peroxidation mainly initiates in cellular membrane, where polyunsaturated fatty acids are highly contained. In case of broiler chickens plasma and muscle TBARS significantly decreased with feeding the diets with SDBP (Sanzida et al. 2011). It has been reported that α -tocopherol significantly ameliorated lipid peroxidation in glucocorticoid administrated rats (Ohtsuka et al. 1998) and chickens

(Taniguchi et al. 1999). Moreover, Eid et al. (2003) reported that tea polyphenols reduced glucocorticoid induced oxidative stress in chicken. The response between fish and land animals might not be the same.

Although significant difference was not detected, there was a trend that crude lipid contents of fish whole body in SDBP groups increased with increased dietary SDBP levels. Furthermore, fatty acid contents in liver were affected by dietary supplementation of SDBP. It was also found that SDBP contained α -linolenic acid (Yohanes et al. 2010). In the present study, liver linolenic acid significantly increased when fish were fed the diets with SDBP, and DHA was shown to be an increasing tendency with increased dietary SDBP. Based on the report of Yohanes et al. (2010) and Yamasaki et al. (2006), SDBP might have significant amount of DHA and linolenic acid, reflecting higher liver linolenic and docosahexaenoic fatty acids contents. Since the detailed mechanisms of the reactions are not fully understood, further study will be needed.

5. Conclusion

In conclusion, this study demonstrated that SDBP could be suitable and promising candidate as a dietary supplement to develop the functional aquafeeds for marine fish species concerning the sustainable aquaculture.

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