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Supplemental effects of shochu distillery by-product and some crude ingredients to improve the nutritional utilization of low fish meal diets for red sea bream, *Pagrus major*

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Abstract
A feeding trial was conducted to improve nutritional utilization of low fishmeal diets by supplementing shochu distillery by-product (SDBP) alone and with some crude ingredients (krill meal or KM and squid meal or SM). Six test diets were formulated, where diet 1 was fish meal (FM) based control diet and diets 2-5 were prepared by replacing same amount of fish meal protein. Triplicate groups of fish (average initial weight 3.35g) were randomly stocked in 18 100-L polycarbonate tanks at a stocking density of 15 fishes per tank. The test diets were hand delivered twice a day up to satiation level for 42 days. The average temperature of water during the whole period of experiment was 23-29℃. The results demonstrated that weight gain and specific growth rate was similar in FM based diet 1 and SDBP content diet 6 but other groups showed the significantly ($P<0.05$) lower value than diet 1. Hepato Somatic Index (HSI) also followed the similar trends. But no difference was found between SDBP alone and SDBP with crude ingredients incase of growth parameter. Feed intake (FI) was significantly higher in d1 group than other groups, but FI was showing the increasing tendency with the increase in the level of SDBP. Whole body proximate compositions were not markedly influenced by the dietary treatments. No difference was found to contain P and N in feed and whole body, but showing the increasing tendency incase of SDBP alone and SDBP with crude ingredients. Blood parameters were significantly influenced by SDBP than FM based protein group. Incase of oxidative stress, fish fed higher level of SDBP alone showed the significantly higher antioxidant effect than FM based control diet. Based on the overall performances of fish, it was demonstrated that it possible to improve utilization of low fishmeal diet by using SDBP alone or SDBP with krill and squid meal.

Keywords: Shochu distillery by-product ; Krill meal; Squid meal; Oxidative stress

Introduction
In commercial aquaculture production feed is the most important factor to successfully governing this sector. Among the feeds fishmeal is a dominant ingredient in diet formulation for most species, mainly for carnivorous fishes. As fishmeal is the first choice to develop aquaculture rapidly, so cost of fishmeal increasing constantly. But still fishmeal production is in constant level in the world. Therefore, feed industry operators and aquaculture industry operators are finding alternative sources both from plant and animal origin to make up the place instead of fishmeal and to secure a stable supply for commercial diets (Hardy, 2006)[1]. So to solve this problems fish nutritionist are paying their attention on feeding stimulants such as certain amino acids (taurine, glycine, arginine, glutamic acid and alanine, etc.) which can maintain the palatability or acceptability and feed intake with lower, higher or completely replacing the fish meal protein. These stimulants are naturally rich in marine organisms such as fish, squid, krill, shrimp, etc. (Garber, 2005; Smith *et al.,* 2005; Mai *et.*) [2]. Lots of research has already done by using this mixture of feeding stimulant in many species of fish such as sea bass, *Dicentrarchus labrax* (Dias *et al.,* 1997) [3], red drum, *Sciaenops ocellatus* (McGoogan and Galtin, 1997) [4], striped bass, *Morone saxatilis* (Papatryphon and Soares, 2000,2001) [5] and yellow tail, *Seriola quinqueradiata* (Kofuji *et al.,* 2006) [6]. But Kohbara *et al.,* (1989) [7] reported that natural feeding stimulants are better than the synthetic one because shortage of some effective components. To avoid this problem fish nutritionist are trying to use tissue

extracts of marine organisms such as fish (Hidaka *et al.,* 2000) [8], shrimp (Mearns *et al.,* 1987) [9], squid (Toften *et al.,* 2003 [10]; Kofuji *et al.,* 2006 [6]; Kader *et al.,* 2010) [11], krill (Kofuji *et al.,* 2006 [6]; Kader *et al.,* 2010 [11]), mussel (Tandler *et al.,* 1982) [12] and worms (Fuke *et al.,* 1981) [13] as feeding stimulants. In this experiment we used some crude ingredients such as squid and krill meal with shochu distillary by-product (SDBP). This SDBP itself a feeding stimulant because it contains growth promoting factor which not only improve the body weight but also improve the feed intake (Mahfudz *et al.,* 1996, 1997 [14]; Kamizono *et al.,* 2010 [15]). On the other hands it contains some functional ingredients such as polyphenol, vitamin E and C and it also rich in amino acids such as alanine, glycine, arginine, glutamine, lysine, asparagines, threonine and phenylalanine, etc. (Sudoh, 1975) [16]. Hayashi *et al.,* (2009) [17] and Ohtsuka *et al.,* (1998) [18] reported that SDBP is potential feed additive for land animals. In this experiment we tried to use SDBP as feed additive for marine fish, red sea bream (*Pagrus major*). In aquaculture production red sea bream (*Pagrus major*) is the second largest species in Japan (Koshio, 2002) [19] but its production still depends on the fishmeal protein mainly (Yokoyama, 2003) [20]. However, limited supply with increasing demand of fishmeal making pressure on feed manufacturers to reduce the fishmeal in red sea bream diets. To solve this problem we tried to use SDBP alone and SDBP with some crude ingredients by replacing the fishmeal or to improve the utilization low fishmeal diet for red sea bream.

Therefore, the present study was conducted to investigate the effect and efficacy of SDBP alone and with some crude ingredients incase of growth performance, feed utilization, oxidative condition, health condition and macro-nutrients contents of the whole body.

Materials and Methods

Experimental system

Juveniles of red sea bream 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 3.35g） 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 1.5l/ min was maintained throughout the experimental period (42 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 (42 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 keep at -20℃ for body chemical composition. Using heparinized syringes, blood was collected from the caudal vein of five in each replicate tank and polled. Plasma samples were obtained by centrifugation at 3000 ×g for 15 minutes using a high speed refrigerated micro-centrifuge (MX-160; Tomy Tech USA Inc., Tokyo, Japan) kept at -80℃. Fish were dissected for liver and muscle and weighed and store in -80^{0}c for measuring the hepato-somatic 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 (Hayashi *et al.,* 2009[) [17].

Six isonitrogenous (more than 44% crude protein), isolipidic (10-11% total lipid) diets were formulated; where diet 1 was a 100% fishmeal (FM) based control diet (D1). Diets 2 to 5 were prepared as follows by replacing FM protein with SDBP 2% or D2; SDBP 2% and KS meal (Krill+Squid meal) or D3; SDBP 16% or D4; SDBP 16% and KS meal (Krill+Squid meal) or D5 and SDBP 20% or D6. 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 and 1.9 mm and pellets was oven (DK 400 Yamato Scientific, Tokyo, Japan) dried for 2 h at 60℃. The diets were stored in a cold room.

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 (AOAC, 1990) [21].

Amino acid analysis

Amino acid analysis of diets samples was analyzed using high performance liquid chromatography (HPLC, Shimadzu Corp.) according to Teshima *et al.,* 1986 [22]. 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 and oxidative stress

To measure the health condition we used plasma. So plasma chemical parameters were measured spectrophotometrically with an automated analyzer (SPOTCHEM^{TM} 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.*(2002) [23].

Total phosphorus and nitrogen

Concentration of P in diet and whole body was determined spectrophotometric ally. Approximately 0.2 g sample was weighed into Kjeldhal flask on a paper n free and added 5 ml HNO_3 (60-62%) for each 0.1 g sample. A standard sample

was also prepared in another similar flask by putting 3 ml P standard solution (P1000) together with 10 ml HNO_3. Flask with 10 ml HNO_3 is as blank. All flasks were digested in acid digestion unit until yellowish brown fume changes to completely white fume (4-6 h). After white fume appeared and solution becomes clear, digestion was continued until solution remained 1 ml. Then digestion solution cooled to room temperature, 4 ml of perchloric acid ($HCLO_3$) 60% was added and continued the digestion. The step was terminated after intensive white fume appeared (30-60 minutes). After cooling down, digested solution was transferred to 50 ml volumetric flask with pure water by washing the flask at least 4 times and shaken by hand. From there approximately 0.2 ml (200µl) sample solution was taken by pipette into 50 ml graduated color comparison glass tube. The volume of solution to be sampled depends also on the P concentration of the sample. Buffer solution of 5 ml, 1 ml ammonium molybdate solution and 1 ml ascorbic acid solution were added to the glass tube, respectively. Glass tube was then mixed with touch mixer. After mixing, samples were messed up to 25 ml with pure water and covered by stopper, then put into water bath for incubation at 35-40℃ for 30 minutes. After 30 minutes later cooling down the samples and mixed again before reading with spectrophotometer at 750 nm wave length. Total N in diet and whole fish was determined by Kjeldhal method.

Equation of growth performance parameter

The following equations were followed

Weight gain (%) = (final weight ˍ initial weight) ×100/initial weight

Specific growth rate (SGR %, day^{-1}) = (LN (final weight) - LN (initial weight)/duration) ×100

Survival (%) = 100 × (final no. of fish/ initial 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)

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± standard deviations

Results

Test diet analysis

All diets contained similar crude protein, total lipid 10-11% and crude ash 8-9%. The dietary contents of free amino acids were shown in table 2. The content of total free amino acids was highest in D6 group and other SDBP alone and SDBP with crude ingredients groups showed the increasing tendency when we increased the level of SDBP. Most of the free amino acids contents were higher SDBP alone and SDBP with crude ingredients group than fishmeal based control group except taurine. Taurine is higher in fishmeal based control group than other groups.

Table 1: Composition of experimental diets (% of dry matter)

Ingredients	D1	D2	D3	D4	D5	D6
Fish meal (BF)[1]	60	30	30	30	30	30
Soybean meal[2]	0	44	44	42	42	39.5
SDBP[3]	0	2	2	16	16	20
Krill meal (Com)4	0	0	0.5	0	0.5	0
Squid meal (Com)5	0	0	0.5	0	0.5	0
Wheat flour[6]	15.5	3	3	0.5	0.5	0
CMC	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[7]	2	2	2	2	2	2
Mineral mix[8]	2	2	2	2	2	2
L-lysine	0.5	0.5	0.5	0.5	0.5	0.5
DL-methionine	0.5	0.5	0.5	0.5	0.5	0.5
Fish oil[9]	4	4.5	4.5	4.5	4.5	4.5
α-cellulose	15	11	10	1.5	0.5	0.5
Total	100	100	100	100	100	100

[1] Nippon Suisan, Tokyo, Japan
[2] J-Oil Mills, Kanagawa, Japan
[3] Shochu Distillery By-product (SDBP) obtained from Faculty of Agriculture, Kagoshima University
[4] Nippon Suisan Co. Ltd., Tokyo, Japan
[5] Nippon Suisan Co. Ltd., Tokyo, Japan
[6] Riken A-three, Tokyo, Japan
[7] According to Yokoyama *et al*. (2006) [20]. with slight modification
[8] According to Kader *et al*. (2010) [11].
[9] Riken Vitamin, Tokyo, Japan

Here

D1: Fish meal based control diet
D2: SDBP 2%
D3: SDBP 2%+Krill meal+Squid meal
D4: SDBP 16%
D5: SDBP 16%+Krill meal+Squid meal
D6: SDBP 20%

Table 2: Free amino acid contents of experimental diets (FAA g/100g dry sample) [1]

	Dietary groups					
Amino acids	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Indispensable						
Arginine	0.25	0.28	0.27	0.31	0.32	0.33
Histidine	0.38	0.27	0.27	0.30	0.31	0.32
Isoleucine	0.05	0.05	0.06	0.08	0.08	0.07
Leucine	0.09	0.06	0.07	0.12	0.12	0.13
Lysine	0.27	0.25	0.24	0.23	0.25	0.24
Methionine	0.69	0.76	0.85	0.89	0.94	0.87
Phenylalanine	0.18	0.17	0.18	0.21	0.23	0.23
Threonine	0.05	0.04	0.04	0.06	0.06	0.07
Tryptophan	0.80	0.80	0.80	0.95	0.97	1.02
Valine	0.13	0.11	0.12	0.14	0.15	0.15
Dispensable						
Taurine	1.28	0.68	0.71	0.84	0.86	0.93
Aspartic acid	0.02	0.05	0.05	0.09	0.10	0.11
Glutamic acid	0.10	0.09	0.10	0.14	0.16	0.15
Serine	0.03	0.02	0.02	0.04	0.04	0.05
Proline	0.05	0.03	0.04	0.10	0.10	0.12
Glycine	0.05	0.03	0.03	0.07	0.07	0.08
Alanine	0.16	0.10	0.11	0.21	0.22	0.25
Tyrosine	0.17	0.18	0.20	0.18	0.20	0.19
∑FAA	4.75	3.96	4.16	4.96	5.19	5.31

[1]Values are mean of triplicate samples.
Here
D1: Fish meal based control diet
D2: SDBP 2%
D3: SDBP 2%+Krill meal+Squid meal
D4: SDBP 16%
D5: SDBP 16%+Krill meal+Squid meal
D6: SDBP 20%

Survival and growth performance

Growth performance and feed utilization of the fish are given table 3. Survival (%) of fish did not differ significantly ($P>0.05$) among treatments. Body weight gain (g), weight gain (%) and SGR were significantly lower ($P<0.05$) in D2, D3, D4, D5 group than fishmeal based control (D1) group. There was no significant difference between same level of SDBP alone and SDBP with crude ingredients. But high level of SDBP i.e. D6 and D1 were showing the similar trend. HSI also followed the similar trend like body weight gain. Feed intake was significantly higher in D1 group than other groups but FER did not differ significantly among the groups. PER was significantly decreased in D4 group than D1 group.

Table 3: Whole body proximate analysis (%) of juvenile red sea bream fed test diets for 42 days [1]

Composition[2]	**Initial**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Moisture	75.78	70.20±1.66	72.72±0.27	72.9±0.62	70.75±1.55	70.73±0.40	70.9±0.45
Crude protein	16.38	15.67±0.87	16.88±0.44	17.08±1.3	16.47±0.85	16.59±0.48	16.7±0.03
Total lipid	3.99	7.26±1.16	5.8±0.65	6.35±0.36	7.19±0.94	6.98±0.71	6.56±0.33
Ash	4.47	4.65±0.42	4.72±0.28	4.85±0.11	4.95±0.42	4.86±0.01	5.05±0.12

[1]Values are means±SD of triplicate groups.
[2]Values are expressed as wet weight basis.
Here
D1: Fish meal based control diet
D2: SDBP 2%
D3: SDBP 2%+Krill meal+Squid meal
D4: SDBP 16%
D5: SDBP 16%+Krill meal+Squid meal
D6: SDBP 20%

Whole body composition

Table 4 represents the whole body proximate analysis of fish. In comparison with the fishmeal 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 the feeding trial. But crude protein and crude ash content was higher in treatment groups than control.

Table 4: Growth parameters and feed utilization in red sea bream fed test diets for 42 days

	Dietary Groups					
Parameters measured	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Initial weight (g)	3.37±0.07	3.35±0.07	3.34±0.07	3.32±0.08	3.32±0.15	3.30±0.20
Weight gain (g/56 days)	26.74±0.54 b	17.23±1.89 a	17.67±1.29 a	20.43±1.93 a	21.12±1.24 a	22.55±1.00ab
Weight gain (%)	794±21 b	514±46 a	529±28 a	615±66 a	636±11 a	684±11 ab
SGR (% day^{-1})[1]	5.22±0.06 d	4.32±0.17 a	4.38±0.11 ab	4.68±0.22 bc	4.75±0.03c	4.90±0.03 cd
Total FI (g/42 days)	1462±2.55 d	1014±6.15 a	1044±15.29a	1190±10.97 b	1213±11.84b	1270±10.97c
FER[2]	0.82±0.01	0.73±0.01	0.68±0.07	0.69±0.16	0.77±0.05	0.77±0.03
PER[3]	2.03±0.03 b	1.83±0.02 ab	1.59±0.16 ab	1.55±0.36 a	1.67±0.11 ab	1.67±0.06 ab
HSI[4]	2.52±0.34 c	1.64±0.10a	1.62±0.12 a	1.85±0.09 ab	1.76±0.11 ab	2.22±0.23 bc
Survival (%)	100	91.11	86.67	84.44	93.33	83.33

Values are means±SD of triplicate groups. Values in each row with same letters are not significantly different (P>0.05). Absence of letters indicates no significant difference among the treatments.

[1] SGR: specific growth rate = 100 x (ln final weight–ln initial weight)/days

[2] Feed efficiency ratio = total live weight gain (g)/total dry feed intake (g)

[3] Protein efficiency ratio = live weight gain (g)/dry protein intake (g)

[4]HSI: hepatosomatic index (%) = wt of liver/ wt of fish×100

Here
D1: Fishmeal based control diet
D2: SDBP 2%
D3: SDBP 2%+Krill meal+Squid meal
D4: SDBP 16%
D5: SDBP 16%+Krill meal+Squid meal
D6: SDBP 20%

Blood parameter and oxidative condition

Table 5 summarizes the blood parameters and oxidative status of red sea bream after 42 days feeding trial. Plasma blood urea nitrogen (BUN), glucose (GLU), glutamyl oxaloacetic transaminase (GOT), glutamyl pyruvic transaminace (GPT) did not show significant difference but tended to be increased in the replacement groups. Total protein was significantly lower in D2 group than D1 and D6 group. But total cholesterol (T-cho) and triglyceride (TG) was significantly lower in D2 group and D3, D4 group respectively. Significantly higher levels of BAP were detected in D4 and D6 dietary groups than fishmeal based control group, while such variations were not found in d-ROM among different treatments.

Table 5: Blood parameters and oxidative condition of juvenile red sea bream fed test diets for 56 days

Parameters	**Dietary groups**					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
Glu(mg/dl)[1]	57.67±6.11	56.67±1.15	59.33±4.73	59.33±6.03	60.67±5.13	65.5±12.02
T-cho (mg/dl)[2]	272±31b	201±10 a	206±33 a	208±23 a	215±21a	231±16 ab
BUN (mg/dl)[3]	5±0	5.33±0.58	6±1	5.67±0.58	5.33±0.58	5±0
T-Bil (mg/dl)[4]	0.2±0	0.23±0.06	0.2±0	0.23±0.06	0.23±0.06	0.23±0.06
GOT (IU/L)[5]	51±25.46	77±19.80	87.5±3.54	53±5.66	68.5±13.44	105.5±26.16
GPT (Iu/L)[6]	10±0	14.67±4.62	10±0	14.67±8.08	14±6.93	16.33±7.09
T-Pro (g/dl)[7]	4.9±0.26 bc	3.97±0.06 a	4.27±0.40 ab	4.4±0.17 ab	4.4±0.40 ab	5.33±0.38 c
TG (mg/dl)[8]	476±33 b	240±4 ab	209±13 a	205±117 a	416±27 ab	379±92 ab
Oxidative stress parameters						
d-ROMs test (U.Carr)[9]	99±6	88±7	135±42	132±4	135±33	97±20
BAP test (µMol l^{-1})[10]	2589±285 a	2919±59 ab	2716±172 a	3405±232 b	3171±102 ab	3414±158 b

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.

[1]GLU: glucose, 2T-cho: total cholesterol, [3]BUN: blood urea nitrogen, [4]T-bil: total bilirubin, [5]GOT: glutamyl oxaloacetic transaminase, [6]GPT: glutamic -pyruvate transaminase, [7]T-pro: total protein, [8]TG: triglyceride, [9]d-ROMs: reactive oxygen metabolites, [10]BAP: biological antioxidant potential.

Here
D1: Fish meal based control diet
D2: SDBP 2%
D3: SDBP 2%+Krill meal+Squid meal
D4: SDBP 16%
D5: SDBP 16%+Krill meal+Squid meal
D6: SDBP 20%

Phosphorus (P) and nitrogen (N) content in feed and whole body

Table 6 represents the feed and whole body P and N content. P and N content of feed and whole body did not differ significantly among the Treatments, but tended to be increased in the replacement groups.

Table 6: Nitrogen and phosphorus content of feed and fish (%)

	D1	D2	D3	D4	D5	D6
Feed						
Nitrogen (%)	6.48±0.04	6.41±0.03	6.85±0.03	7.1±0.02	7.38±0.12	7.35±0.06
Phosphorus (%)	2.74±0.15	2.33±0.06	2.71±0.14	3.57±0.12	3.18±0.18	3.05±0.39
Fish						
Nitrogen (%)	8.8±0.58	9.53±0.18	9.41±0.43	9.02±0.49	9.09±0.23	9.18±0.01
Phosphorus (%)	5.11±0.33	5.14±0.0.21	5.2±0..17	5.4±0.33	5.44±0.59	5.45±0.41

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

Here
D1: Fishmeal based control diet
D2: SDBP 2%
D3: SDBP 2%+Krill meal+Squid meal
D4: SDBP 16%
D5: SDBP 16%+Krill meal+Squid meal
D6: SDBP 20%

Discussion

In recent years, a significant amount of research has been conducted to replace the fishmeal protein by different plant sources or different by-product or different crude ingredients. But this replacement results are highly variable for different species of fish. So, in the present study supplemental effects and efficacy of SDBP alone and SDBP with krill meal and squid meal in diets were evaluated not only on growth performance but also feed utilization, health condition, oxidative condition and macro nutrients content of the whole body.

In this experiment, all fish were affected by disease except control or diet 1 group. As a result growth was significantly decreased in D2, D3, D4 and D5 group than D1. But D6 group showed the similar trend like D1 group. SGR and HSI also showed the similar trend like growth performance. Total feed intake was significantly lower in SDBP alone and SDBP with crude ingredients. But Kader *et al.,* (2010) [11] reported that KM and SM significantly improved FI of fish and Mahfudz *et al.,* (1996, 1997) [14] reported that growth promoting factor of SDBP not only improve the growth but also improve the feed intake. In our previous study feed intake was similar between the fishmeal based control and SDBP based diet (unpublished data). But here we got totally different result this is might be caused of disease. Though the D6 group showed the significantly lower feed intake than D1 group, but body weight gain was similar like D1 group. This is might be the caused of growth promoting factor of SDBP (Mahfudz *et al.,* 1996, 1997) [14]. FER was not significantly affected by any groups but PER was significantly affected by D4 group than D1 group. Though the fish was affected by disease but whole body proximate composition did not differ significantly among the groups.

Free amino acids contents of the diet was showing increasing tendency when we increased the level of SDBP. Though the SDBP group showed the increasing tendency but feed intake was significantly lower in SDBP alone and SDBP with crude ingredients groups. This is might be the cause of disease

Blood parameters are reliable indicator to know overall health condition as well as welfare of fish. Total bilirubin, GOT and GPT were not significantly differing among the groups and value was considered to be within normal range of juvenile red sea bream compared with previous findings (Kader *et al.,* 2010) [11]. So we could say liver and kidneys are functioning as well they should be in each group. Higher level of total cholesterol was found in fish fed fishmeal based control group than the other groups and D2 showed the significantly lower value than fishmeal based control group. We know that this shochu distillery by-product is fermented alcoholic by product. During fermentation they used *Aspergillus species*. This *A. sp.* has effect on lowering the cholesterol level in broiler (Ahmed *et al.,* 2010) [24]. Because mechanism of cholesterol lowering effect of *Aspergillus* could be related to an inhibitor of 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Hajjaj *et al.,* 2005[25]; Ahamed, 2010) [24]. This inhibitor, called statin, which is extracted from fungus and use to inhibit the rate-limiting step in cholesterol synthesis (Hajjaj *et al*. 2005) [25]. This is might be the cause to decrease the total cholesterol level in plasma by SDBP than fishmeal based control diet. But why significantly lower value was found in D2 group only we don't know this phenomenon. So, further analysis is needed to clarify this phenomenon. Triglycerides also higher in fish fed fishmeal based control group than other groups and it was significantly lower in diet 3 and diet 4 groups than control group. As SDBP groups showed the lower triglyceride level than control group this might be the prebiotic effects of SDBP. On the other hands it also contains polyunsaturated fatty acids (PUFA), which helps to inhibit the activity of fatty acid synthase. So, this is might be the cause markedly decreased the triglyceride level. Total protein content was significantly higher in D6 group and significantly lower in d2 group. Actually SDBP increases the protein content of cultured cells because of growth promoting factor (Mahfudz *et al.,* 1997) [14].

Oxidative stress represents an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. So increased the level of reactive oxygen species or decreased the efficacy of antioxidant system is health risk emarging factor. To measure the oxidative condition of fish recently in our lab we are using the free radical analytical system (FRAS4) assessing the derivatives of d-ROM and BAP test (Kader *et al.,* 2011) [11]. Based on Kader *et al.,* (2011) [11] values obtained from the present study are within the ranges obtained in previous studies of red sea bream. The present study indicated that BAP value in D4 and D6 group was significantly higher than fish meal based control group, while similar levels of d-ROM were maintained among the treatments. The present studies result also supported the report of Mosa sanzida *et al*. 2011[26] on broiler chicken. Though the fish was affected by disease but SDBP group showed the higher antioxidant effect this is might be the cause of the antioxidant effect of SDBP (Sultana *et al.,* 2011) [26]. So this results clearly indicated that SDBP

has antioxidant effect not only in broiler chicken but also in red sea bream.

Macronutrient contents (phosphorus and nitrogen) in feed and whole body did not differ significantly among the treatments. But SDBP content groups showed the increasing tendency. So from the result we could say SDBP fed groups have lower chance to excrete phosphorus and nitrogen in feaces and water respectively. This results proved that SDBP has no negative effect on environment. But further analysis is needed.

The present study demonstrated that fish meal protein could be replaced by higher level of SDBP alone without any negative effects on growth and feed utilization, oxidative condition and blood parameters. But SDBP alone and SDBP with crude ingredients effect almost same in all parameters. However, this study is still preliminary on the effect and efficacy of SDBP alone and with krill meal and squid meal for fish; further analysis is required to clarify the effect and efficacy of SDBP alone and with krill meal and squid meal on fish growth, health performance for sustainable aquaculture.

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