International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979; Impact Factor: RJIF 5.72
Received: 28-06-2020; Accepted: 15-07-2020; Published: 23-07-2020
www.allsubjectjournal.com
Volume 7; Issue 8; 2020; Page No. 23-27

The dietary effect of brown seaweed meal to male and female Nile tilapia (*Oreochromis niloticus*)

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Abstract
A nutrition diet experiments were conducted to investigate the effects of dietary of supplemental brown seaweed meal on growth performance of male, female, and mixed-sex group of Nile tilapia, *Oreochromis niloticus*. Three different iso-proteinous (CP 32%) *diets* containing 0% (BSM 0), 4% (BSM 4), and 8% (BSM 8) level of brown seaweed meal were prepared and fed to replicate trials of individual males, individual females, and mixed sex tilapia. Male Nile tilapia groups also showed the best result of WG, FE, SGR, PER, and NPU came from fish fed by BSM 8. In case of experimental groups BSM 4, the results were significantly ($p<0.05$) greater than fish in BSM 0 but not different with fish in BSM 8 from Male and Mixed-sex group of rearing condition. In female Nile tilapia group, the result of WG, FE, SGR, PER, and NPU were not significantly ($p>0.05$) different within levels of feeding trial with range 1.07-1.52%/day and 60.40-89.46%, respectively. Nevertheless, the highest results of SGR and FE also were observed in BSM 8. Based on the result, BSM could be added in a fish diet up to 8%, and male tilapia are greater than female for production pupose.

Keywords: Seaweed, Sargassum, Nile tilapia, Sex ratio, Growth performance, die*t*

1. Introduction
Plants have a big proportion of dietary factors such as a source of amino acids and lipid content in aquaculture not only to increase the production but also to decrease costs [1]. Plant based ingredients in feed have been applied in some species of fish such as catfish [2, 3] or tilapia [4], but their utilization is still restricted because of the anti-nutritional factors, for example, protease inhibitors, phytates, glucosinolates, saponins, tannins, lectins, etc [5]. By the time, seaweeds have been investigated as the one of the potential plant-based feed ingredient for intensive aquaculture due to their high protein content and high production rate [6]. In Indonesia, seaweeds are available largely and being used for many industrial things.
The brown seaweed grows rapidly in coastal sea areas all over the world from temperate to tropical regions [7]. *Sargassum* is one of seaweed species with a good source of minerals, vitamins, carbohydrates and some amino acids [8, 9] and capable to increase the immune response [10]. The utilities of *Sargassum* as feed ingredient has been examined in Japanese flounder *Paralichthys olivaceus* [11], shrimp *Penaeus monodon* [10], rainbow trout *Oncorhynchus mykiss* [21], and Nile tilapia *Oreochromis niloticus* [6, 12].
Tilapia is one of the most popular culture fish in the world and become the 2nd important finfish species group cultured worldwide in terms of production, after carp. In 2015, global culture tilapia production reached 5.7 million tonnes. Tilapia, additionally, unlike most teleost, shows a very clear sex behavior. Males grow greater than females (Toguyeni, *et al*. 1999). Rearing mixed-sex of tilapia in the same pond might be led the overpopulation results. This high reproductive efficiency is related to early sexual maturation, prolific spawning, and fry care (toguyeni, *et al*. 1999). That sexual character is the strong reason for male tilapia mostly cultured for production purposes. Many researches have been done to control the reproduction and increase the growth performance of tilapia for commercial benefits, but the information about the growth response of both sex for new feeding treatments are needed to evaluate its influence on nutritional values of this species. Therefore, the present work has been carried out to investigate the effects of brown seaweeds (*Sargassum* spp.) on growth performance of male and female Nile tilapia.

2, Materials and Methods
2.1. Experimental Diets and Feeding Trials
Brown seaweed (identified as *Sargassum* spp.) was obtained fresh, among other seaweeds from the near-shore waters of The Tidung Island, Kepulauan Seribu, Indonesia. Sargassum sample were thoroughly washed with seawater and Epiphytes were removed from the thalli, dried in an oven at 40ºC for 48h, and a ground into a powder by hammer mill to result 1 kg finely ground sample. The proximate analysis were conducted using this sample (Table 1). Three diets were formulated to contain 32% of crude protein (CP), which fulfills the requirement of tilapia culture. The diets were designed iso-proteinous with three different levels of Brown Seaweed Meal (BSM) 0%, 4%, and 8%. Namely, BSM 0, BSM 4, and BSM 8. Details of diet formulation and proximate composition is shown in Table 2. All dietary ingredients were blended thoroughly, moisturized, pelleted with diameter of 3 mm, and dried at 60ºC for 24 h. Diets were stored at -4ºC until used. The test diets were randomly fed to apparent satiation three times daily, at 08.00; 12.00, and 17.00 h with a feeding rate 2-3% of BW.

Table 1: Chemical composition of dietary ingredients (% Dry Matter)

Ingredients	*Crude Protein*	*Crude Lipid*	*NFE***	*Ash*	*Crude Fiber*
FM*	47.80	6.50	17.70	26.60	1.40
SM*	44.30	6.21	41.76	5.37	2.36
BSM*	11.77	0.21	52.27	15.42	20.33
Tapioca	0.52	0.04	99.39	0.05	0.00
Rice Bran	8.91	3.31	63.91	19.16	4.71
CM*	10.79	5.79	79.72	1.75	1.95

*FM: Fish Meal; SM: Soybean Meal; BSM: Brown Seaweed Meal; CM: Corn Meal
**NFE supposedly represents the soluble carbohydrate of the feed, such as starch and sugar.

Calculate the nitrogen free extracts content by the following formula:
Nitrogen free extracts content (%) = 100 − {moisture content (%) + crude protein content (%) +ether extract content/crude lipid content (%) + crude fiber content (%) +crude ash content (%)}

Table 2: Percentage and proximate composition of the experimental diets containing different energy and BSM levels

Ingredients	*0%*	*4%*	*8%*
FM	32	32	32
SM	32	32	32
SBM	0	4	8
Tapioca	7	7	7
Rice Bran	13	9	5
CM	13	13	13
Fish Oil	1	1	1
Premix*	1.75	1.75	1.75
Choline Chloride	0.25	0.25	0.25
Nutrient Contents			
Crude Protein	32.07	32.18	32.30
Crude Lipid	6.25	6.13	6.01
Nitrogen-free extract	44.66	44.19	43.73
Ash	12.95	12.80	12.65
Crude fiber	2.07	2.69	3.32
Gross Energy (kJ g^{-1})[b]	274.53	272.77	271.00
E/P	8.56	8.48	8.39
*Containing antibiotic (zinc bacitracin), amino acid (methionine and lysine), 6 essential minerals and 12 essential vitamins.			

2.2. Animals and experimental design
The experimental fish, juvenile Nile tilapia *Oreochromis niloticus* were obtained from a sub stream of the Nikko River, then transported to an outdoor concrete tank of the Experimental Fish Tank Facility. Fish were acclimatized to the experimental condition for one month and fed with commercial diets (CP 30%). A total of 150 fish were randomly distributed and maintained in an indoor room with a 100 l volume polyethylene cylinder tank. Three types of experimental rearing condition; males, females, and a mixed sex group were assigned with three levels treatment of BSM and three replicates each. At the beginning of experiment, the fish were anesthetized with 0.01% ethyl m-aminobenzoate methansulfonate to weigh the initial body and the mean wet weights. The average body weight (BW) ±standard deviation (SD) for the male trials was 78.3±10.0 g, female trials was 71.4±5.1 g, and the mixed sex group trials was 17.3±1.8 g. Each trial group was separately reared 10 fish per tank for about three weeks fed by three levels of BSM. Throughout the feeding trial, water was maintained at temperature of 26°C, 12:12 LD photoperiod and supplied with 10 l/min of filtered and aerated water.
Fish were weighed and recorded every 2 weeks after a 24-h starvation period and at the end of experiment were weighed to attain the final body weight. Twenty g random meat sample from each group was collected, homogenized using food processor, and tested for proximate analyses of body composition.

2.3. Analytical procedures
Carcass samples from each experiment were homogenized and analyzed for proximate composition at the beginning and end of the feeding trial. Proximate analysis was conducted on all ingredients, test diets, and fish samples. Moisture, total lipid, crude protein, and ash content were all determined by standard Association of Official Analytical Chemist (AOAC) methodology.

2.4. Data analysis
Growth performance and feed utilization from this experiment were evaluated in terms of Weight Gain (WG, %), Feed Efficiency (FE%), Specific Growth Rate (SGR, % day-1), Protein Efficiency Ratio (PER g), and Net Protein Utilization (NPU %). The formulae used are as follows:

WG = 100 x [(final fish weight (g) - initial fish weight (g))/initial fish weight]
FE = [50 x (final weight–initial weight) x (initial number of fish+final number of fish)]/ dry weight of feed
SGR = 100 x [ln (final fish weight) – ln (initial fish weight)]/experimental days
PER = weight gain (g)/protein fed (g)
NPU = 100 x [(final body protein (g) - initial body protein (g)/dietary protein consumption (g)]

Prior to statistical analyses, all growth data were subjected to the Wilcoxon-Mann-Whitney test to determine if each parameter was significantly different using SPSS software.

3. Results
Results obtained for the growth performance and diet utilization from various level of BSM during the feeding trial from male, female, and mixed group are summarized in Table 3. All diets were readily accepted by fish, indicating no issues relating to palatability of algal supplemented diets. There was no mortality recorded during the 24-26 days feeding trial. The data indicate that dietary BSM in various levels inclusion significantly improved fish growth and diet utilization when compared with the control (0%) except in female group. The supplementation of BSM 8 in the diets of Male group gave the highest result of mean WG (57.45±0.25%), FE (127.11±14.39%), SGR (3.48±0.32%/day), PER (0.54±0.22), and NPU (15.17±0.83), this result were significantly different (P>0.05) with control (BSM 0) but no significant different with the fish fed by BSM 4.
Specifically, SGR and FE of male Nile tilapia rearing

condition treatment ranged 2.16-3.48%/day and 67.89-127.11%, respectively. Fish in the male Nile tilapia rearing condition treatment ranged 1.29-2.12%/day and 63.55-106.81%, respectively. Male Nile tilapia groups also showed the best result of WG, FE, SGR, PER, and NPU came from fish fed by BSM 8. In case of experimental groups BSM 4, the results were significantly ($p<0.05$) greater than fish in BSM 0 but not different with fish in BSM 8 from Male and Mixed-sex group of rearing condition.

Interestingly, different result revealed in female Nile tilapia group. The result of WG, FE, SGR, PER, and NPU were not significantly ($p>0.05$) different within levels of feeding trial with range 1.07-1.52%/day and 60.40-89.46%, respectively. Nevertheless, the highest results of SGR and FE also were observed in BSM 8. Comparing the male and female group fed by BSM, the BSM seemed gave a significant influence in male Nile tilapia group but not in female group. Overall, the best improvements in all parameters measured were found in BSM 8 dietary groups.

Table 3: Growth performance and nutrient utilization of *Oreochromis niloticus* fed by the experimental diets

Male Nile tilapia					
Treatments	***Weight Gain (%)***	***Feed Efficiency (%)***	***Specific Growth Rate (%fish/day)***	***Protein Efficiency Ratio (%)***	***Net Protein Utilization (%)***
BSM 0	30.82±9.96	63.55±12.61^{a}	1.39±0.37^{a}	0.85±0.41^{a}	15.75±0.07^{a}
BSM 4	42.30±14.49	87.02±18.73^{ab}	1.79±0.50^{ab}	1.12±0.57^{ab}	16.32±0.51^{ab}
BSM 8	48.96±19.63	106.81±20.85^{b}	2.12±0.58^{b}	1.37±0.72^{b}	17.10±0.41^{b}
Female Nile tilapia					
Treatments	*Weight Gain (%)*	*Feed Efficiency (%)*	*Specific Growth Rate (%fish/day)*	*Protein Efficiency Ratio (%)*	*Net Protein Utilization (%)*
BSM 0	18.57±7.74	60.40±16.65^{a}	1.07±0.25^{a}	0.58±0.24^{a}	12.41±0.66^{a}
BSM 4	21.03±10.41	68.57±25.18^{a}	1.19±0.38^{a}	0.65±0.32^{a}	12.94±0.15^{a}
BSM 8	22.37±8.45	89.46±14.29^{a}	1.52±0.19^{a}	0.72±0.26^{a}	13.10±0.39^{a}
Mixed sex of Nile tilapia					
Treatments	*Weight Gain (%)*	*Feed Efficiency (%)*	*Specific Growth Rate (%fish/day)*	*Protein Efficiency Ratio (%)*	*Net Protein Utilization (%)*
BSM 0	55.94±0.32	67.89±5.80^{a}	2.16±0.16^{a}	0.34±0.12^{a}	14.34±0.53^{a}
BSM 4	56.81±0.24	100.88±5.83^{ab}	2.94±0.15^{ab}	0.52±0.05^{ab}	14.92±0.92^{ab}
BSM 8	57.45±0.25	127.11±14.39^{b}	3.48±0.32^{b}	0.54±0.22^{b}	15.17±0.83^{b}

*Each value is the mean ± S.E.M. of data from replicate groups. Within a column, means with different letters indicate a significant difference ($P<0.05$).

4. Discussion

The utilization of brown seaweed as a possible feed ingredient for cultured fish has been demonstrated in recent year to increase the growth performance [4], including *Sargassum* [11, 12]. As far as its known, there is many obscure point about the consideration level of BSM as a feed ingredients in various sex of Nile tilapia.

Based on the results of the present study as presented in Table 1, the crude protein (CP) of BSM in the present study was higher (11.77%) than previous studies about seaweed as a fish feed ingredient attained in *Sargassum fulvellum* 4.12% CP [11] *Sargassum* spp. 10.28% CP [12], and *Ulva rigida* 9.91% CP [14]. Beside protein, dietary lipids in aquaculture has an important role as a source of energy and essential fatty acids [15]. The optimum level of lipids could improve the growth rates, feed conversion ratios, nutrient utilization, and reduce the nitrogen excretion [16]. Crude lipid in the present study was 0.21%. This result is lower than previous results for seaweeds; 0.45% in *Sargassum vulgare* [17], 0.6% in *Ulva* sp. and 0.9% in *S. chordalis* [18]. The variation of results could be related to the seaweed species, seasons of sampling, location and growth conditions [19]. Mostly, seaweeds have a relatively low lipid content [20]. As far as its reported, seaweeds have been reported to contain high dietary fiber [20]. Crude fiber contents were higher (20.33%) than results previously reported 6.3% [21]; 10.25% [12] in *Sargassum* spp. The nitrogen-free extract (NFE) of BSM was calculated based on the proximate analysis (Table 1) which showed that BSM contained a high value of NFE (40.14%) and apparently represents the soluble carbohydrates.

The present study showed that the inclusion 8% of BSM can be added as the optimum results for WG, FE, SGR, PER, and NPU in males, females, or mixed group rearing condition. In male treatments, the highest SGR values ($p<0.05$) were observed from group fed by BSM 8%, also in female treatments (Table 3), although no significant differences for the female fish were found among treatments ($p>0.05$). Our finding showed the increasing of SM in diets could increase the growth performance. However, by adding more might give a negative impact to growth performance [6]. *Sargassum ilicifolium* could be used as a fish feed diets up to 10% but 7.5% inclusion in rainbow trout diet gave the significant difference of growth performance [22].

The nutritional values of BSM made the important result of growth performance in Nile tilapia, both in males and females. The present study the result showed in line with most of researches about tilapia, that the growth performance of males was higher than females. The response of fish to BSM diets seems to be sex-specific. Moreover, it was indicated by adding SM at level 8% of inclusion could give the best growth performance for male and mixed sex Nile tilapia. It seemed that males have a higher capacity for digesting and metabolizing nutrients. In the other hand, particularly the steroid and thyroid hormones might describe difference responses in nutrient metabolism [13]. Nevertheless, there are an interesting result, that mixed group tilapia has the highest result compared to two other treatments (Fig. 1). Various mechanisms might be the reason of this thing, which could be direct or indirect. In addition, Experiments using male and female by monitoring the fish individually under different experimental conditions is needed [13].

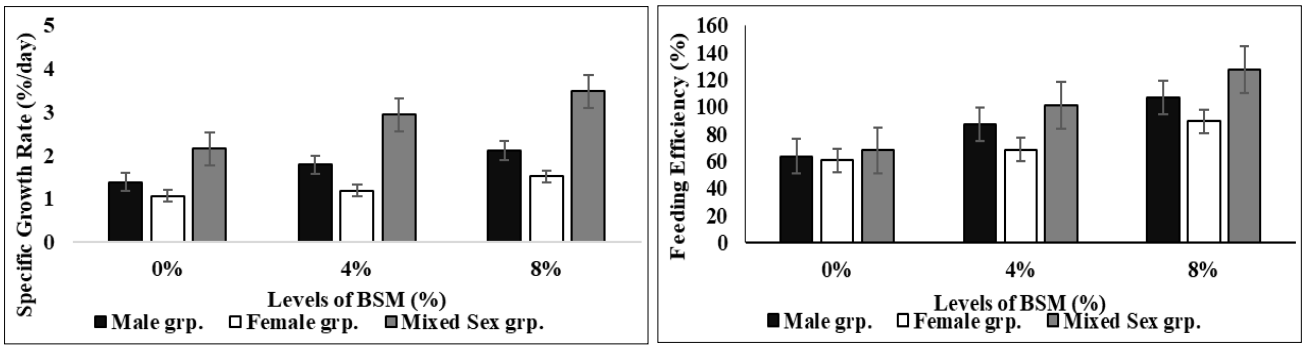

Fig 1: Graphics of growth performance result: a) Specific Growth Rate and b) Feeding Efficiency from Males, Females, and Mixed-sex group of Nile tilapia fed by BSM

5. Conclusions

In conclusion, the results of the present experiment clearly demonstrate that brown seaweed (identified as *Sargassum* sp.) could be an effective supplement for Nile tilapia at least at 4% without negative effects and 8% level inclusion gave the best results for growth performance for males tilapia and mixed-sex tilapia. Howeever, itl could show a different effect on the growth relating with sex-characteristic and interaction. Thus, the investigation for the further study about the optimum level of BSM should be examined.

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