Optimum utilization of brewers' yeast (*Saccharomyces cerevisiae*) to replace local fish meal in juvenile Sea cucumber (*Holothuria scabra*) diets

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Abstract
The juvenile sea cucumber, *Holothuria scabra* was reared for 55 days in a re-circulatory tank system consisting of filtered seawater flowing at 2.5-3 L/min. One hundred and forty-four juveniles of an average weight of 0.88±0.03 g were randomly distributed in 12 tanks of 250 L. Sea cucumber were fed four diets containing different dietary concentrations of brewer's yeast (BY), *Saccharomyces cerevisiae* (BY_0: 0%; BY_{10}: 10%; BY_{30}: 30%; BY_{50}: 50 %) to replace the local fish meal (CM fish meal®). At the end of the experiment, the specific growth rate (SGR), weight gain (WG) and final body weight (FW) of juvenile sea cucumber fed with BY_{10} was significantly higher than those of juveniles fed BY_0 and BY_{50} ($p<0.05$) though, not significantly higher than those fed BY_{30} ($p>0.05$). The optimum replacement level of CM fish meal® with brewers' yeast was 13.75% for juvenile *H. scabra* by polynomial regression analysis of WG.

Keywords: Brewer's yeast, *Saccharomyces cerevisiae*, local fishmeal, juvenile sea cucumber, *Holothuria scabra*

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Introduction

Sea cucumber became a high-demanded commodity among all seafood varieties in the world after the new trade economy of China started in the 1980's (Hamel *et al.*, 2001). Many coastal nations lost their wild sea cucumber population due to overexploitation (Ajith Kumara *et al*., 2013; Battaglene *et al*., 1999; Hamel *et al*., 2001; Purcell *et al*., 2012). Hence, the larval production of *Holothuria scabra* increased gradually in commercial hatcheries and it cost a high market value. The species is naturally distributed in shallow coastal areas across the Indo-Pacific region. As the pioneer in the South Asian region, India started to produce *H. scabra* seeds while the wild stock was declining (James, 2004). The production of *H. scabra* juveniles in Sri Lankan hatcheries was initiated in 2011 (Kumara and Dissanayake, 2017). The processed sea cucumber production collected from wild and pen cultured in Sri Lanka ranged from 258 tons in 2005 to 248 tons in 2018, with several annual fluctuations (DOF, 2019). The highest export value was reported as 10.33 USD million in 2013, while the second-highest value reported was 7.65 USD million in 2018 (DOF, 2019).

In general, dried and powdered seaweed are used as feeds in sea cucumber hatcheries. Seasonality issues in the availability of seaweed, drying issues, and nutrient imbalances occur in feeding seaweed for sea cucumber juveniles. The predominant protein source in aqua feeds is still fishmeal (FM). The fish meal provides adequate levels of amino acids for aquaculture species. The negative impacts, such as imbalances in amino acid profile, reduced palatability, and availability of anti-nutritional factors, have been reported in using plant protein sources as alternative sources (NRC, 2011). Therefore, higher levels of plant protein sources to replace the fish meal are also impractical. However, soybean meal replaced 40% of fishmeal in sea cucumber, *Apostichopus japonicus* juvenile diets without affecting growth (Fan *et al*., 2010). In contrary, 60% FM replacement by soybean meal did not affect growth in sea cucumber, *Apostichopus japonicus* juveniles (Liao *et al*.,2015). Other alternative fish meal replacers in sea cucumber diets such as silkworm caterpillar meal (Sun *et al*., 2014) and alga *Spirulina* meal *(Tan et al*., 2009) were suggested to be good fish meal replacers in sea cucumber, *Apostichopus japonicus* juvenile diets without affecting growth.

Saccharomyces species are used in the brewing industry to produce beer from wort (Bourdichon *et al*., 2012). The fermented product in beer production called brewers' yeast (BY) has been studied as a dietary supplement and growth promoter for hybrid striped bass, *Morone chrysops*× *M. saxatilis* (Li and Gatlin, 2003) and hybrid of African catfish, *Clarias gariepinus* (Essa *et al*., 2011), as an immune stimulant for hybrid striped bass (Li & Gatlin, 2004), and as an FM replacer for sea bass, *Dicentrarchus labrax* juveniles (Oliva-Teles & Gonçalves, 2001), goldfish, *Carassius auratus* (Gumus, Aydin, & Kanyilmaz, 2016) and pacu, *Piaractus mesopotamicus* (Ozório *et al*., 2010). The studies on the utilization of brewers' yeast products suited for aqua feeds have been expanded up to compounds contained in brewers' yeast, *Saccharomyces cerevisiae*. For instance, glucan derived from *S. cerevisiae* (bakers' yeast) was injected to channel catfish, *Ictalurus punctatus* and improved disease tolerance against *Edwardsiella ictaluri* (Chen & Ainsworth,1992). However, partial substitution of dietary baker's yeast *S. cerevisiae* did not affect trout fish growth (Rumsey, Hughes, & Kinsella, 1990). Dietary addition of *S. cerevisiae in* rainbow trout, *Oncorhynchus mykiss* diets enhanced disease resistance against *Aeromonas salmonicida* (Siwicki, Anderson, & Rumsey, 1994) and improved growth and immunity with disease resistance against *Yersinia ruckeri* (Tukmechi *et al*., 2011).

Brewers' yeast grouped into the single-cell protein (SCP) category gives many advantages to fish feeds (Couttau & Lavens, 1989). Single-cell proteins like microalgae, yeast and bacteria are utilized in fish feeds considering their nutrients like proteins, pigments, B-vitamins, and carbohydrates (especially glucans) (Sanderson & Jolly, 1994;Tacon, 1995). B-glucan consisted in the yeast cell wall is an immunostimulant/ feed additive for aquaculture organisms (Sakai, 1999; Thanardkit *et al.*, 2002). Generally, microbial products or fractions like barley and brewers'yeast are utilized for the β-glucan extraction (NRC, 2011). Likewise, sea cucumber, *A. japonicus* juveniles fed diets containing dietary β-glucan showed an improved immune response and balance of intestinal microbiota (Yang *et al*., 2015).

Therefore, we aimed to find the optimum supplement level of brewers' yeast, *S. cerevisiae* by replacing a local FM in juvenile sea cucumber (*H. scabra*) diet to maximize their growth.

Materials and Methods

Experimental site

The experiment was carried out in a re-circulatory tank system of 12 fiberglass tanks of 250 L at the hatchery of the regional research center, National Aquatic Resources Research and Development Agency (NARA), Kalpitiya, Sri Lanka.

Experimental feeds

The main ingredients, a local fish meal/ CM fish meal® (CMFM) procured from Cool man fish meal factory, Pesalei and brewers' yeast provided by Lion Brewery (Ceylon) PLC, Biyagama were used with all other ingredients that purchased from the retail market, Pettah. Soybean meal, meat and bone meal, maize, poonac, rice polish and brewers' yeast were ground separately by a hammer mill and sieved with a 0.5 mm sieve. Four diets (BY_0: 0% brewers' yeast (BY); BY_{10}: 10% CMFM replaced by BY; BY_{30}: 30% CMFM replaced by BY; BY_{50}: 50 % CMFM replaced by BY) were formulated and prepared practical diets (Table 2). Then, prepared feeds were stored at – 20 °C until the commencement of the feed trial.

Table1. Proximate composition of dry brewers'yeast (*Saccharomyces cerevisiae*)

	Crude Protein%	Moisture%	Ash%	Fiber%	Fat%
CMFM	42.37	2.9	33.9	1.4	5.92
BY	39.3	6.0	6.5	4.3	0.7

Experimental Design

One hundred and forty-four sea cucumber juveniles produced in SL Aquatech International (Pvt.) Ltd., Chilaw averaging 0.88 ± 0.04 g size were placed in 12 fiberglass tanks of 250 L capacity at a stocking density of 12 juveniles per tank. The four experimental diets were randomly assigned in triplicated tanks.

Feeding trial

Prior to the commencement of the trial *H.scabra* juveniles were acclimated to the tank system and feeds for a week. The culture system was operated with filtered seawater for 55 days of the feeding trial. The re-circulatory system was operated 2 hrs per day at a 2.5-3 L/ min flow rate and water was exchanged three times per week. Salinity, dissolved oxygen (DO) and ionized ammonia NH_3+ were checked daily while the values were maintained 25-28ppt, 5.5-6 mg/ L and 0.07-0.10 mg/ L respectively. Feeding proceeded once a day at 4% of the body weight. Growth data were collected biweekly to adjust the feed amount.

Table 2. Composition of experimental feeds and their proximate composition (means ± SE)

Ingredient	BY_0	BY_{10}	BY_{30}	BY_{50}
CMFM®[1]	20	18	14	10
Brewers'yeast[2]	0	2	7	10
Soybean meal[3]	20	20	20	20
Meat and bone meal[3]	5	5	5	5
Maize[3]	15	15	15	15
Poonac[3]	5	5	5	5
Rice polish[3]	5	5	5	5
Wheat flour[3]	26	26	25	26
Fish oil[3]	0.5	0.5	0.5	0.5
Vitamin mineral premix[3,4]	3	3	3	3
DL-Methionine[3]	0.3	0.3	0.3	0.3
L-lysine[3]	0.2	0.2	0.2	0.2
Proximate composition (DM basis)				
Moisture	9.32±0.35	9.21±0.79	8.82±0.01	9.01±0.11
CP	29.89±1.01	29.67±0.97	29.94±0.25	29.12±0.24
Ash	16.53±0.51	14.57±0.29	13.52±0.16	11.92±0.02
Crude Fat	3.17±0.24	2.97±0.27	2.64±0.32	2.31±0.15
Crude Fiber	2.75±0.14	2.86±0.11	3.08±0.10	3.30±0.09
NFE	38.34±0.23	40.72±0.49	42.6±0.20	44.34±0.13

[1] Cool Man fish meal® (CMFM) was procured from the cool man fish meal factory, Thaleimannar road, Pesalei, Sri Lanka.

[2] Brewers' yeast (BY) was provided by Lion Brewery (Ceylon) PLC, Colombo road, Biyagama, Sri Lanka.

[3] All the other ingredients were purchased from the retail market, Pettah, Sri Lanka.

[4] Contains (as mg/kg in diets): Vitamin A, 9000IU; Vit K, 2 mg; Vit E, 5 mg; Vit B1, 2 mg; Vit B2, 3.6 mg; Vit B6, 1 mg; Vit B12, 10 mg; Vit D3, 2000 IU; Chlorine chloride,150 mg; Mn,60 mg; Zn, 50 mg; Fe, 25 mg; Niacinamide, 16 mg; I,5.5 mg; Cu,5 mg; Calcium pantothenate,4 mg; Folic acid,0.5 mg; CO,0.1 mg.

Statistical Analysis

Growth performance data were subjected to one-way analysis of variance (ANOVA) tests at $p<0.05$ level of significance. If there is a significant difference, the treatments were compared by Tukey's test at $p<0.05$ using SAS 9.4 program.

Results and Discussion

During feeding trial, *H. scabra* juveniles accepted all the experimental feeds well. At the end of the experiment, a significantly higher specific growth rate (SGR), weight gain (WG) and final body weight (FW) showed by the juvenile sea cucumber fed BY_{10} followed by BY_{30} compared to juveniles fed BY_0 and BY_{50} ($p<0.05$). However, there were no significant differences in the growth performances of juveniles fed BY_{30} and BY_{10} ($p>0.05$) (Table 3).

For the last decades, the attention for brewers' yeast to be used in aquafeeds has increased. As it is a byproduct in the beer industry (Bourdichon *et al*., 2012) which consisting of protein, vitamin B complex and β-glucan (Sanderson & Jolly, 1994;Tacon, 1995), brewers' yeast has been suggested utilizing in aquafeeds as a feed additive and fish meal replacer. In our experiment, the dietary addition of brewers' yeast improved the growth of *H. scabra* juveniles. So far, the utilization of dietary brewers' yeast, *S.cerevisiae* in sea cucumber *H. scabra* juvenile diets has not been reported. Benefits of using brewers' yeast in the diets of other cultured species like freshwater prawn

Macrobrachium rosenbergii post larvae (Prasad *et al*.,2013) and finfish like sea bass *Dicentrarchus labrax* juveniles (Oliva-Teles & Gonçalves, 2001), hybrid striped bass *Morone chrysops* × *M. saxatilis* (Li & Gatlin, 2003),(Li & Gatlin, 2004), hybrid of African catfish *Clarias gariepinus* (Essa *et al*.,2011), pacu *Piaractus mesopotamicus* (Ozório *et al.*, 2010) and goldfish *Carassius auratus* (Gumus *et al*., 2016) were reported. In the study, 10% dietary addition of brewers' yeast enhanced growth performance results; though not significantly better than 30% brewers' yeast. Ozório *et al*. (2010) stated that brewers' yeast could replace 50% FM in pacu *Piaractus mesopotamicus* diets without affecting growth.

Table 3. Growth performances of *H. scabra* juveniles fed four different feeds (means ± SE) (n=3)[1]

	Diets			
	BY_0	BY_{10}	BY_{30}	BY_{50}
Initial weight (g)	0.86±0.00	0.86±0.00	0.91±0.01	0.89±0.00
Final weight (g)	1.63±0.15^b	2.39±0.12^a	1.97±0.09^{ab}	1.73±0.15^b
WG (%)[2]	81.03±16.82^b	165.61±12.88^a	119.08±9.71^{ab}	92.30±17.14^b
SGR(%day^{-1})[3]	1.06±0.17^b	1.77±0.09^a	1.42±0.08^{ab}	1.17±0.17^b
Survival (%)[4]	58.33±16.67	52.78±2.78	63.89±7.35	55.56±10.02

[1]Values in each column with different superscripts are significantly different (p<0.05).

[2]Weight gain $= \left[\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}}\right] \text{x}100$

[3]Specific growth rate (SGR) = $\frac{\text{(Ln)final weight} - \text{(Ln)initial weight)}}{\text{days}}$x 100

[4]Survival = $\frac{\text{No.of juveniles at the start} - \text{No.of juveniles at the end}}{\text{No.of juveniles at the start}}$x 100

The optimum replacement level of CMFM with brewers' yeast for juvenile sea cucumber, *H. scabra* was 13.5% by the polynomial analysis of WG (Fig. 1).

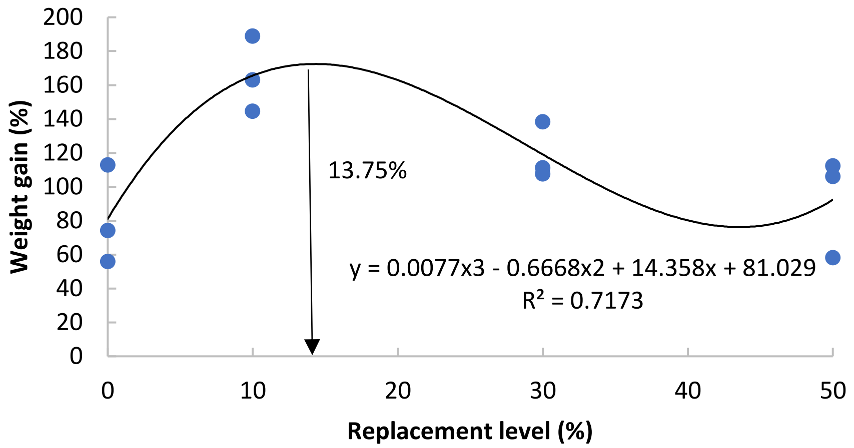

Fig. 1. Optimum replacement level of CMFM by brewers' yeast on weight gain of sea cucumber, *Holothuria scabra* juveniles fed 55 days.

Moreover, in goldfish *Carassius auratus* diets, brewers' yeast was able to replace 35% FM without adverse effects on growth (Gumus *et al*., 2016). Oliva-Teles & Gonçalves (2001) mentioned that brewers' yeast could replace 50% of FM in *Dicentrarchus labrax* juvenile diets with no effect on growth performances. Furthermore, 30% of dietary brewers' yeast in *Dicentrarchus labrax* diets enhanced protein utilization and feed efficiency. Likewise, the total nitrogen in trout diets can replace over 50% with bakers' yeast *S. cerevisiae* nitrogen without changing the growth (Rumsey *et al*., 1990). However, FM replacement with brewers' yeast at low levels in fish feeds showed best performances while no significant effect up to 45-50% replacement levels. In this study, 50% brewers' yeast replacement level significantly affected growth. Rumsey *et al*.(1990) mentioned that fish would not perform well, if brewers' yeast is the only protein source, because brewers' yeast is deficient in essential amino acids, vitamins, minerals, and nucleic acids. So high supplement levels of brewers' yeast in fish diets reduces growth and feed intake of fish (Gumus *et al*., 2016; Ozório *et al*., 2010).

The fish meal provides essential amino acids adequately in aquafeeds (NRC, 2011). Therefore, while partial replacement of fish meal by brewers' yeast increases growth performances, excessive levels could decrease their performance. For instance, alga Spirulina meal replaced 100% fish meal in sea cucumber, *Apostichopus japonicus* juvenile diets without affecting growth. Even though, sea cucumber juveniles showed better growth performances at 25% fish meal replacement level with alga Spirulina meal (Tan *et al*., 2009).

Conclusion

The optimum replacement percentage of CMFM® with brewers' yeast was 13.5% in juvenile *H. scabra* diets by the polynomial regression analysis of weight gain**.** Brewer's yeast could be a partial dietary replacer for high-value fishmeal in juvenile sea cucumber diets for maximizing growth and reducing production cost of sea cucumber feed in future.

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