The Use of Mannan-oligosaccharide (MOS) to the Growth and Survival Rate of Java Eel (Anguilla b. bicolor)

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Abstract. Growth and survival rate are the most crucial in commercial culture as well as feed quality, and it's pricing. This study aimed to obtain the effect of different dose of MOS to the growth and survival rate early big pencil size of shortfin eel *Anguilla b. bicolor*. Three replications with four treatments of feed containing 0, 0.1, 0.2, and 0.3 % doses of MOS (noted as T_0, T_1, T_2, and T_3) were administered during 84 days culture. The fish were fed with 2.2% wet basis d^{-1} of commercial shrimp feed combined with catfish feed. Initial density of approx. 5 kg seed stock of early big pencil eel in 0.4 m^3 recirculating water system. The result indicated that absolute, individual and biomass-specific growth rate (SGRi/b) have the similar patterns corresponds to the dose of MOS. The highest *SGRi* was T_3 (0.69±0.05% d^{-1}) followed by T_2 (0.60±0.03% d^{-1}), T_1 (0.46±0.04% d^{-1}) and T_0 (0.44±0.03% d^{-1}). Meanwhile the *SGRb* shows significantly different ($\alpha < 0.05$) between T_3 (0.66±0.05% d^{-1}) and T_2 (0.57±0.04% d^{-1}), but no significantly different ($\alpha > 0.05$) between T_1 (0.43±0.05% d^{-1}) and T_0 (0.40±0.02% d^{-1}), and both of T_3 and T_2 have significantly different ($\alpha < 0.01$) to T_1 and T_0. The best SR was in T_2 (98.0 %), followed by T_1 (97.5 %), T_3 (97.3 %) and T_3 (96.4 %). Hence, the MOS gave a positive impact on growth and slightly gave a different effect on survival rate.

1. Introduction

The first migratory fish population in nature were mainly either for foraging and their reproduction. *Anguilla* sp. as a carnivorous species have thousands kilometers and met with variability in habitat condition [2] and hence found the variation of preys. In this case, eels as predators [7] [12], become increasingly piscivorous by increasing body length [16]. During hunting, ells used their ability in maneuvering to caught the wild prey, i.e. crustacean and zooplankton [12], eggs and larvae, *Asellus* sp., Nymphs *Ephemeroptera, Gammarus pulex,* crayfish, and fish of Sculpin and Stickleback [7]. These feed intake mostly rich on nutrition and presumably as healthy food due to straight to catch and selected from nature.

Racing fish in aquaculture system normally used formulated feed, which forms as either pelleted, powder or pasted feed. This kind of feeds commonly have been handled with steams or either cooking, where the treatment aims to form in sizes or easy to digest. Contrarily, these formulated feeds face an

obstruction especially in their preservation, some can live longer, and some were not — these due either to rancidity of the lipid and other possibilities as a medium of bacterial and fungal. Patreska et al. [9] stated that the feed might be responsible for the decrease in fish accretion and the reason for the fish diseases occurrence. Hence they investigate the microbiological status of various feeds used in the fish diet. On the other hand, numbers and taxonomic composition of the bacterial populations often reflect those of the surrounding water [1]. Furthermore, Ebeneezar et al. [4] evaluate the quality of commercial fish feeds in India concerning microbiological parameters.

On the other hand, recently published on global outlook report entitled Fish to 2030, only small gains in capture production are feasible in a limited number of regions [8]. It is projected that any significant amount of additional fish production globally will come from aquaculture. Approximately half of the projected increase in aquaculture production, and thereby total fish production, is projected to take place in China alone. Meanwhile all of Asia combined will comprise almost 90 % of the growth in global fish production. In most regions of the world will contribute more than 100 % increase in the future production. Hereafter, aquaculture is more than offsetting actual decreases in capture production. At any rate, in any region with significant projected increases in production, the contribution of aquaculture is generally close to 100 percent in 2030 [8]. Due to this reasons, Indonesian aquaculture should take place in developing and make a significant contribution for global aquaculture, especially in Java eel production.

Mannan-oligosaccharides (MOS) believed as a probiotic widely used either for poultry [11] and aquaculture used. Immune stimulation on sea bass (*Dicentrarchus labrax*) conducted by Torrecillas et al [17], Chotikachinda et al [3] working with white shrimp *Litopenaeus vannamei*, Indariyah et al [6] testing MOS on Artemia production, while growth performance and survivability on climbing perch (*Anabas testudineus*) conducted by Singh et al [13]. Nevertheless study of MOS for catadromous species especially *Anguilla sp*. have not been conducted.

In addition, as a shortage species [16], *A. b. bicolor* also had already been noted in the red list of IUCN. These reasons and other reasons above, tell the fact that the world facing decreased in fish capture production due either to overfishing, global warming or decreased in environmental quality. While the prospect of Indonesian aquaculture is also facing obstacles in imported feed stuff, preservation in feed for a longer used. The concern with MOS which can be used widely and can be performed as natural feed intake aims in understanding MOS for widely use in Aquaculture need to be conducted. Hence the use of mannan-oligosaccharides for the eel growth and survival rate can hopefully be healing of the problems.

2. Research Method

Early big pencil size (approx. 20 gr) of eel *Anguilla bicolor bicolor* were obtained from seed collectors in Cilacap (Central Java). Each treatment used biomass of fish seeds approx. 15 kg divided into three replications with basic recirculating system conducted during the previous study [15] [16]. Hence, approx 5 kg fish were kept in 400 l water volume with normal diurnal temperatures (26 to 28° C), pH (7.1 to 7.5) and dissolved oxygen (4 to 4.2 ppm). Two kinds of commercial feeds (powder and pelleted mixture into pasted feed) was given by 2.2 % d^{-1} [15] in wet basis and gave in twice a day: the feed used with the base of protein content 39.67 and 34.11 %, and the water used as three parts of 7 parts of the total food given [16].

Mannan-oligosaccharides made from the outer cell wall of yeast *Saccharomyces cerevisiae* known as Bio-MOS® was obtained from Alltech Indonesia. The MOS was a mixture with powdered and pelleted feed stirred homogenously and added the warm water to be pasted feed as a feed given assembled with biomass of eel weight [16] and sampling were taken in every three weeks. Hence these weight samplings were used as a base of food and percent of MOS given. The MOS used in these treatments were 0, 0.1, 0.2 and 0.3 % of feed given known as T_0, T_1, T_2, and T_3 respectively.

Absolute and specific growth of individual and biomass of fish per tank in a group of treatments (T_0, T_1, T_2, and T_3) was calculated during 84 days observation. Absolute growth calculated by the difference of weight at *t* time divided by initial weight in percent and specific growth rate using

different normal logarithmic of weight formula conducted by Taufiq-Spj et al [16]. Survival rate was evaluated during 84 days observation and to ensure the percent of feed given from the biomass, death rate was noted in every three weeks. Statistical analysis uses a completely random design and analysis co-variance with four treatments and three replications. Absolute and specific growth (individual and biomass) were analyzed by using generalized linear model repeated measurement ANOVA continued by post hoc test using multiple comparisons based on observed means.

3. Result and Discussion

Mannan-oligosaccharide apparently gives a good impact in the growth of *A. b. bicolor*, the growth increase by increasing the percent of MOS given. Regarding survival rate, these treatments did not give a different impact for the cultured organism. However the death rate shows much lower than previous study when not using the Bio-MOS®. Feed rate tends to be stable when administered in the recirculating water system with a medium aeration, hence not many uneaten food excesses. Nevertheless the feed rate itself accompanied by, i.e. the condition of an organism, the texture of feed [15], temperature and other water quality, but it was correlated to the degree of protein content [16].

3.1. Absolute growth

In order to get figure whether the culture had been efficiently conducted or not, the farmers normally calculated an absolute growth of species culture [15]. Weight gain and absolute growth either for individual and biomass increased by increasing MOS concentrations (Table 1). The weight gain has a similar pattern among treatments, where during 84 days observation the cultured organisms have grown differently either individual or biomass by the time. ANOVA analysis of those growth measured during four time measurements, the grown show significantly different by the time ($\alpha = 0 < 0.05$). Nevertheless T_1 have no significantly different to T_0 ($\alpha = 0 > 0.05$), and both T_3 and T_2 have significantly different to T_1 and T_0 ($\alpha = 0 < 0.01$).

Table 1. Initial individual and biomass weight (I_0 and B_0), individual and biomass weight gain (IWG and BWG), and percent individual and absolute biomass growth (IAg and BAg) during 84 days culture.

Trmnt	I_0 (g)	I_{84} (g)	IWG (g)	IAg (%)	B_0 (kg)	B_{84} (kg)	BWG (kg)	BAg (%)
$T_{0.1}$	20.97	31.32	10.35	49.35	4.992	7.111	2.119	42.45
$T_{0.2}$	20.72	29.90	9.19	44.34	4.910	6.878	1.968	40.08
$T_{0.3}$	21.16	30.11	8.95	42.28	5.036	6.924	1.888	37,50
Mean±SD			9.49±0.75	45.32±3.63			1.992±0.012	40.01±2.47
$T_{1.1}$	22.49	32.37	9.88	43.92	5.016	7.024	2.009	40.05
$T_{1.2}$	21.86	31.53	9.66	44.19	4.985	6.999	2.014	40.40
$T_{1.3}$	21.13	32.40	11.27	53.35	4.924	7.388	2.465	50.06
Mean±SD			10.27±0.87	47.15±5.37			2.162±0.262	43.50±5.68
$T_{2.1}$	21.35	34.32	12.97	60.72	4.954	7.756	2.802	56.56
$T_{2.2}$	21.34	35.67	14.33	67.13	4.973	8.097	3.124	62.83
$T_{2.3}$	21.11	35.57	14.46	68.48	4.961	8.287	3.326	67.05
Mean±SD			13.92±0.83	65.44±4.15			3.084±0.264	62.15±5.28
$T_{3.1}$	21.90	39.60	17.70	80.79	5.082	8.950	3.868	76.11
$T_{3.2}$	21.16	39.29	18.12	85.64	4.973	8.957	3.984	80.11

$T_{3.3}$	21.66	36.85	15.19	70.10	4.939	8.181	3.241	65.63
Mean±SD			17.00±1.59	78.84±7.95			3.698±0.399	73.95±7.48

Highest weight gain and absolute growth were attained with MOS concentration of 3 ‰ (T_3) and the lowest without MOS given (T_0) either for individual and biomass. In using MOS, [14] used two ‰ of MOS for racing rainbow trout (*Onchorhyncus mykiss*) found 13.7 % (in cages) and 9.97% (in raceway) have higher absolute growth than without MOS during six weeks culture period (42 days). Using European sea bass (*Dicentrarcus labrax*) with the same concentration of 0.2 % MOS, [17] found 11.98 % also higher in growth than control during 36 days and higher during 42 days of feeding. In comparison, with a similar concentration of MOS and a similar period of culture (42 days), this study shows the absolute growth at T_2 (0.2 % of MOS) was 30.6 % higher than controls (19.5 %). Hence the different about 11.1 % was a little bit lower compare to trout cultured in cages but higher than in raceway.

Nevertheless, in term of absolute growth, the trout and sea bass seems to have a better growth compare to eels of this study. From this point of few, the trout and probably the sea bass not so piscivorous as *A. b. bicolor,* hence formulated feed seems more appropriate to trout and bass than to eels. Another reason, eels seem to have a slower growth compared to other fin fishes species especially in aquaculture.

In term of MOS given, Ringo et al [10] and Singh et al [13] stated that a prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth, and/or the activity of one or a limited number of bacteria in the colon. This statement will give a big question, especially in growth mechanism. The MOS is originated from the yeast cell wall (*Saccharomyces cerevisiae*) has gained more prominent attention, mainly due to its ability to bind the threadlike *fimbriae* on pathogenic bacteria preventing them from attaching to the gut wall. Thereby averting their stabilization and the resulting colonization either multiplication, up to the disease level. Hence it had been showed to be a most capable solution for antibiotic-free diets, as well as furnishing effective support for digestion and immunity in poultry [11]. Several investigations confirmed that using MOS as a feed supplement in poultry diets allowed birds to achieve a similar trend as when they were fed a diet enriched with antibiotic growth promoters. In addition, it is also thought that it plays a role as an antioxidant, helping with mineral retention, improving bone mineralization and subsequently the overall improvement in the performance of culture organism [11].

3.2. Specific growth rate

Normal logarithmic of individual and biomass-specific growth rate shows the pattern of growth significantly different by time ($\alpha = 0 < 0.05$). However, the growth pattern was fluctuate in every treatment (Table 2) during measurements every 21 days. The rate of growth seems high in the first measurement (day 21st) and slower in the second measurement (42nd). The treatment without MOS (T_0) and 0.1 % MOS (T_1) seems consistently increased at the third (63rd) and fourth (84th) measurements, but T_2 (0.2 %) and T_3 (0.3 %) had a slower growth at fourth compare to third measurement (Table 2). This phenomenon presumably due to a small amount of MOS given, hence difficult to get homogenous compound in pasted feed to compare to diluted in liquid form. The other reasons, it can be in sizes or individual competition for feeding, the bigger sizes will eat more than smaller size of eels. The MOS itself as prebiotic sugar prevents digestive enzymes in the stomach and small intestine from breaking them down. This enables commensal organisms in the large intestine to directly utilize prebiotic sugars. Furthermore the MOS has been shown to act as pathogen binders to remove pathogen out of the gastrointestinal tract whereas prebiotic may promote the growth of beneficial bacteria in the colon [13]. Hereafter, the higher MOS intake will promote the growth of eel.

Table 2. Mean values of individual and biomass-specific growth rate (% d^{-1}, SGRi and SGRb), measured every three weeks, during 84 days culture.

Treatment/ replication	SGRi d_{21} (% d^{-1})	SGRi d_{42} (% d^{-1})	SGRi d_{63} (% d^{-1})	SGRi d_{84} (% d^{-1})	SGRb d_{21} (% d^{-1})	SGRb d_{42} (%/d^{-1})	SGRb d_{63} (% d^{-1})	SGRb d_{84} (% d^{-1})
$T_{0.1}$	0.61	0.15	0.56	0.58	0.57	0.07	0.50	0.54
$T_{0.2}$	0.63	0.19	0.35	0.56	0.61	0.15	0.31	0.52
$T_{0.3}$	0.43	0.52	0.39	0.34	0.39	0.46	0.37	0.29
Mean±SD	0.56±0.11	0.29±0.20	0.44±0.11	0.49±0.14	0.52±0.12	0.23±0.20	0.40±0.10	0.45±0.14
$T_{1.1}$	0.43	0.24	0.51	0.56	0.39	0.22	0.48	0.52
$T_{1.2}$	0.45	0.51	0.29	0.48	0.41	0.47	0.27	0.46
$T_{1.3}$	0.88	0.34	0.32	0.50	0.84	0.31	0.30	0.47
Mean±SD	0.59±0.26	0.36±0.14	0.37±0.11	0.51±0.04	0.55±0.26	0.33±0.13	0.35±0.11	0.48±0.03
$T_{2.1}$	0.92	0.44	0.55	0.35	0.90	0.36	0.55	0.33
$T_{2.2}$	0.79	0.47	0.75	0.44	0.77	0.45	0.71	0.39
$T_{2.3}$	0.91	0.28	0.83	0.47	0.91	0.26	0.83	0.45
Mean±SD	0.87±0.08	0.40±0.10	0.71±0.15	0.42±0.06	0.86±0.08	0.36±0.10	0.69±0.14	0.39±0.06
$T_{3.1}$	0.91	0.58	0,76	0.56	0.87	0.54	0.74	0.54
$T_{3.2}$	0.92	0.83	0,68	0.53	0.90	0.79	0.68	0.44
$T_{3.3}$	0.92	0.59	0,74	0.27	0.92	0.53	0.72	0.23
Mean±SD	0,92±0,00	0.67±0.14	0,73±0,04	0.45±0.16	0.90±0.03	0.62±0.15	0.71±0.03	0.40±0.16

The biggest growth rate found in first measurements (three weeks culture) either in individual and biomass for all treatments. From the group samplings, slowest growth found at second sampling (day 42^{nd}), where individual and biomass were 0.29±0.20 and 0.23±0.20 at treatment 0 (T_0), 0.36±0.14 and 0.33±0.13 at T_1 as well as 0.40±0.10 and 0.36±0.10 % d^{-1}. While, T_3 shows the slowest (0.45±0.16 and 0.40±0.16 % d^{-1}) growth rate at fourth sampling (day 84^{th}) (Table 2). For this reasons, at first measurement, specimens of eels have a similar in feeding conditions after their acclimation. Hence they grew exponentially, then they had to be adjusted during second three weeks of culture and lowering the grew for T_0, T_1, and T_2. However, additional feed at T_3 with 0.3 % of MOS, gave a good impact in accelerating growth until 63 days culture and slower after that (Table 2).

As can be seen in Figure 1., the highest specific growth rate either for individual and biomass of *A. b. bicolor* were attained at treatments 3 of 3 ppt of MOS given, followed by T_2, T_1, and T_0 during 84 days culture. No, significantly different ($\alpha = 0 > 0.05$) between T_1 and T_0 and both of T_3 and T_2 have significantly different ($\alpha = 0 < 0.01$) to T_1 and T_0, while between T_3 and T_2 have different in degree of 0.05. This continuous growth may correlated to the number of hepatocytes activation, where the group of clothing factor will be respond for the synthesis of lipoprotein and glycoprotein. Torrecillas et al. [17] proofed that higher MOS given to sea bass resulted qualitatively in a regular-shaped morphology of the hepatocytes around sinusoidal spaces and a reduction on the lipid vacuolization of the cytoplasm.

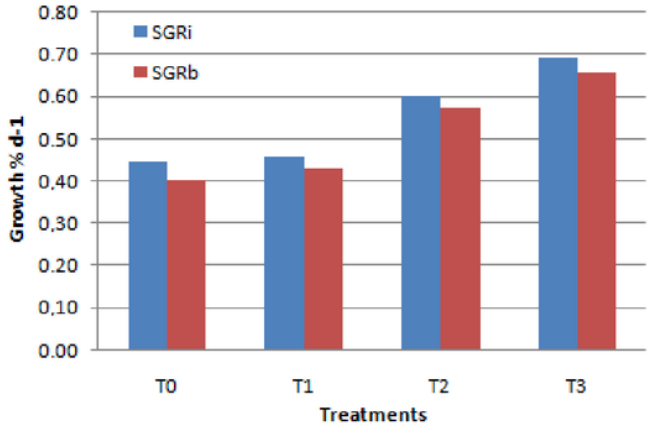

Figure 1. Mean values of individual and biomass-specific growth rate (% d^{-1}, SGRi, and SGRb) in a different dose of treatments (0, 1, 2 and 3 ‰) during 84 days culture.

3.3. Survival rate

The survival rate performance shows high during 84 days culture, all of the treatments have SR more than 96 % (Figure 2). This good performance as supported by a short study that in the normal water quality of tap water, Java eel *A. b. bicolor* have a very high ability in survival rate (SR). It was noted (in different part of study) that the glass eel have ability to survive more than 35 days and more than 40 days for the small pencil sizes of eel without feed given (Taufiq-Spj, unpublish data).

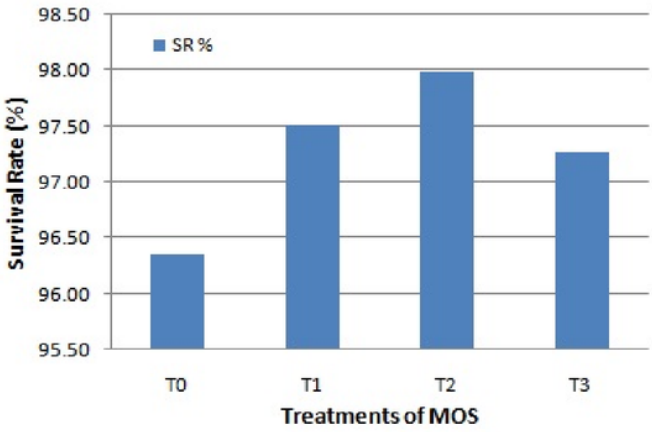

Figure 2. Mean value of survival rate (%) of *A. b. bicolor* treated by using a different dose of MOS (0, 1, 2 and 3 ‰) during 84 days culture.

The best SR found in treatment 0.2 % of MOS (T_2) given followed by T_1, T_3, and T_0 (Figure 2). Even though no significantly different among treatments ($\alpha = 0 > 0.05$), but it seems all treatments using MOS have higher SR compared to control. This phenomenon may be related to immune response. Chotikachinda et al [3] found no difference in the growth of white shrimp *Litopenaeus*

vannamei fed with a dietary supplement of the inactive yeast cell wall with a dose of 0, 1 and 2 ppt. However, the treatments improved in immune response indicated by increasing total hemocyte counts, granular hemocyte count, and bacterial clearance compare to control. The study in poultry and swine diets, the MOS has been shown to affect gut health by pathogen adsorption and immune modulation [11]. Moreover, in the previous study conducted by Fischer et al [5] also used aqua-MOS for *Litopenaeus vannamei*, the result shows that 2 and 4 ppt of MOS gave a good impact in the immune index of shrimp. The immune response indicated by increasing hemogram, plasm antibacterial activity and hemocyte respiratory burst in shrimp [5].

4. Conclusion

The different dose of MOS gave different effect on absolute and specific growth but not any different for a survival rate of *A. b. bicolor*. The best growth found in dietary containing 0.3 % MOS, but 0.2 % of MOS gave slightly higher in the survival rate of *A. b. bicolor* fed during 84 days culture.

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