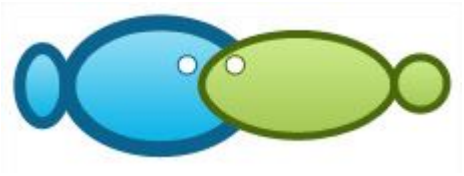

Types and abundance of plankton in the hybrid tilapia brackish water culture media enriched with mixed booster (plankton, aqua enzyme and amino liquid)

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Abstract. Nutrient in the water is an important factor to grow plankton and thus addition of booster may improve the plankton abundance. This research aims to understand the effects of fermented booster mixture addition toward plankton abundance. There were 5 treatments applied, Po (control); P_1 (0.075 mL L^{-1}); P_2 (0.45 mL L^{-1}); P_3 (0.825 mL L^{-1}) and P_4 (1.2 mL L^{-1}). The booster was added to hybrid tilapia (*Oreochromis aureus* x *O. niloticus*) culture media (circular tank, 80 L, brackish water 17 ppt). The fish (2.04-2.87 g body weight (BW), 5.2-5.43 cm total length (TL)) density was 40 fishes $tank^{-1}$, and they were fed on commercial pellet (38% protein content), 3 times a day, 5% of their biomass. The fish was reared for 70 days and the fermented booster that contains plankton booster (molasses, inositol, manganese, zinc, iron, copper, cobalt), aqua enzyme (*Bacillus subtilis, B. polymyxa, B. licheniformis* (9×10^{11} CFU L^{-1}), alpha protease, amylase, cellulose), and amino liquid (vitamine A, D3, E, K3, B1, B6, C, folic acid, L-lysine, arginine, glycine, cystine, methionine, leucine and serine) was added to the media once a week. Results showed that the addition of booster positively affected the water quality as well as the plankton abundance. During the experiment, temperature, pH, salinity, dissolved oxygen (DO), CO_2, ammonia, nitrate and nitrite in the water were able to support the life of plankton. The best result was provided by P2, the plankton abundance was the highest (5,107 cells L^{-1}) by the end of experiment. The type of plankton in all treatments applied was the same, namely Bacillariophyceae, Chlorophyceae, Charophyceae, Cyanophyceae, Chrysophyceae, Rotifera, Dinophycea and Euglenophyceae. The plankton life cycle was 32 days and there was no difference in the cycle length among the treatments. There were 22 types of plankton present in the stomach of the fish (5.3% of total feed).
Key Words: fish natural feed, *Oreochromis aureus*, *O. niloticus*, plankton booster, fish culture media.

Introduction. Feed has become a significant contributor to production of fish in intensive and extensive culture system. Under intensive culture, natural feed such as phytoplanktonic and zooplanktonic organisms is needed as artificial feeds are no match to live food organisms in terms of acceptance, nutritional and other factors. The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food for feeding fish fingerlings (Das et al 2018).

Plankton is a major source of nutrition for fish, which is widely present in fresh water, brackish water, sea water, and can show a rapid growth rate. Previous studies by Canini & Metillo (2017), Sahami et al (2017), Kadim et al (2018), and Das et al (2018) suggested that the presence of plankton could be a key indicator for current status of marine ecosystem, besides its significance as a part of food chain. Temporal changes in structure of phytoplankton community are important for metabolic activity of aquatic

system. Utojo (2015) stated that the need for existence of plankton in culture system is undeniable for some reasons. It can be beneficial to shade the ponds, enabling to induce fish more actively feed during the day time. In addition, phytoplankton is able to produce O_2 in water, while it is also a source of nutrients for fish, especially at larval stage. Furthermore, it also enables to reduce growth of mosses on the bottom of ponds. Interestingly, phytoplankton is meaningful in absorbing noxious compounds such as ammonia, nitrite and nitrate.

By using fish farm booster, plankton abundance could be enhanced by adding external ingredients, i.e. mixture of fermented rice bran and plankton booster (molasses, inositol, manganese, zinc, iron, copper, cobalt), aqua enzyme (*Bacillus subtilis*, *B. polymyxa*, *B. licheniformi*s, alpha protease, amylase, cellulose), and amino liquid (vitamin A, vitamin D_3, vitamin E, vitamin K_3, vitamin B_1, vitamin B_6, vitamin C, folic acid, L-lysine, arginine, glycine, cystine, methionine, leucine, serine). Sudarmadji & Booster Team (2014) discussed advantageous effects of fermented booster. First, the mixture accelerates the growth of seed since it contains superfluous quantity of hydrolyzed protein from degradation of protease, amylase and cellulose. Second, it serves as natural feed (phytoplankton and zooplankton), which can be source of nutrition for fish fingerling. Third, it helps decomposition process of organic matters by *Bacillus* sp., thus it improves the breakdown of uneaten feed metabolism wastes. Sumule et al (2017) stated that the use of probiotic in fish culture could be expected to upgrade water quality by breaking down feed debris and feces, as well as improving growth of phytoplankton due to increment of CO_2.

Studies on diversity and abundance of plankton in fish farm booster are rather scarce. The addition of mixed booster fermentation to the culture media was able to trigger an increase in plankton abundance (Wulandari 2014), but the optimal dosage needed on brackish water was unknown. Therefore, this present work aimed to investigate the effects of fermented booster dose on production of plankton in tilapia culture under booster farming system using brackish water.

Material and Method. The research was conducted from March to June 2018 in the Breeding Laboratory and research ponds, Fishery Faculty, Riau University. There were 600 fingerlings of hybrid tilapia (*Oreochromis aureus* x *O. niloticus*) (weight 2.04-2.87 g, total length 5.2-5.43 cm) used in this study, obtained from the Balai Penelitian and Pemuliaan Ikan (Fish Breeding and Research Center) Sukamandi. The fish was then acclimatized in brackish water at 17 ppt (Simanjuntak et al 2018). Sea water was obtained from Bungus, Kabung Bay in West Sumatera. Experimental fish was reared at density of 1 fish/2 L in round container (60 cm in diameter, 45 cm in height) filled with 80 L of sea water at salinity of 17 ppt (Pasha 2015). Commercial pellet (production code FF-999, PT. Charoen Pokphand) was applied as experimental feed, with chemical composition as follows: protein 38%, fat ≥ 2%, crude fiber ≤ 3%, ash ≤ 13%, moisture ≤ 12%, and given at dose of 5% fish biomass, 3 times a day.

The plankton booster was composed of rice bran (0.5 kg), plankton booster (200 cc), aqua enzyme booster (10 g), and amino liquid booster (20 cc). These materials were mixed well and then fermented for 36 h (28.7-28.9°C, pH 6.2-7.1). Well fermented booster was characterized by soft texture, grey colored and fermented odor. The fermented booster was added to fish culture media once/7 days. The booster dosages were 0 mL L^{-1} (in P_0); 0.075 mL L^{-1} (in P_1); 0.45 mL L^{-1} (in P_2); 0.825 mL L^{-1} (in P_3), and 1.2 mL L^{-1} (in P_3). The plankton abundance was studied by sampling the plankton once/2 days. Three liters of media's water was filtered using a plankton net (25 µm mesh size).

Regarding to improvement of water quality, the following booster was used: blue copper (cupri sulphate, cethyl piridium chloride, cethyl trimethyl amonium bromide, surfactant) for sterilization; booster manstap (P_2O_5, KNO_3, SiO_2, and trace element Ca, Mg, Co, Cu, Fe, Mn, Se, Zinc at high dose) for increasing pH of the water; booster multi cells (*Bacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp. CFU) for decreasing ammonium level; mixture of fermented rice bran + plankton booster (molase, inositol, manganese, zinc, iron, copper, cobalt) + aquaenzyme (*B. subtilis*, *B. polymyxa*, *B. licheniformis*,

protease-alfa, amylase, cellulose) + amino liquid (vitamin A, vitamin D3, vitamin E, vitamin K_3, vitamin B_1, vitamin B_6, vitamin C, folic acid, L-lysine, arginine, glycin, cystine, methionine, leucin, serin, pro) in water, for rising plankton abundance. For improving non-specific immune response of the fish, the seed was submerged in booster fish immunovit (extract of *Echinacea* flower, vitamin C, vitamin B_1, vitamin B_2, vitamin B_6, biotin). Regarding the enhancement of fish feed quality, the following booster was used, i.e. booster grotop (vitamin B_1, B_2 and C, L-lysine, DL-methionine, enzyme protease), premix aquavita (vit A, vit D_3, vit K_3, vit E, vit B_1, vit B_2, vit B_6, vit B_{12}, vit C, Ca panthotenate, folic acid, biotin, inositol, nicotinamide, choline chloride, L-lysine, DL-methionine, Co, Cu, Mn, Se, Zn), and liquid amino in feed to alleviate feed conversion and raise growth of tilapia fish (Sudarmadji & Booster Team 2014).

Completely randomized design was arranged, consisting of 1 factor, 5 levels of treatment, 3 replicates (Steel & Torrie 1993). The factor used in this case was dose of fermented booster mixture used by Sudarmadji & Booster Team (2014), i.e. fermented rice bran 0.5 kg + plankton booster 200 cc + booster aquaenzyme 10 g + booster amino liquid 20 cc + water 10 L . In this recent research, one mL of fermented matter contained consortium of bacteria, such as *B. subtilis*, *B. megaterium* and *B. polimyxa* at density of 9 x 10^{11} CFU L^{-1}.

The effects of the treatments were evaluated on parameters such as the quantity of bacteria that was determined by method of McFarland referring to Whitman (2003) and Sutton (2011). Determination of plankton diversity referred to Mizuno (1979), Yamaji (1980), Gotoh (1986), Shamsudin (1990), and Yunfang (1995). Physicochemical features of the water were also observed, including temperature, pH, salinity, dissolved oxygen (DO), free carbondioxide (CO_2), ammonia (NH_3), nitrite and nitrate (referring to Alaerts & Santika (1984), and SNI (1990)).

Plankton abundance value is used to know the number of plankton in each water volume (cells L^{-1}). Plankton abundance (N) analysis was calculated using the formula of APHA (1989) as follows:

$$N = \frac{Oi}{Ov} \times \frac{Vr}{Vo} \times \frac{1}{Vs} \times \frac{n}{p}$$

where: N = number of cells per liter (cells L^{-1}); Oi = area of cover glass (mm^2); Op = area of a view space (mm^2); Vr = volume of filtered water (mL); Vo = volume of sample under the cover glass (mL); Vs = volume of filtered seawater sample (L); n = number of phytoplankton cells in entire view space (cells); p = number of spaces observed (mm^2).

Stomach content analysis was conducted by removing stomach of 3 fishes per treatment by the end of the experiment. The stomachs were fixed in 4% formaldehyde and the content was analysed and identified. The preponderance index was calculated based on Natarajan & Jhingran (1961), using this following formula:

$$PI\ (\%) = \frac{Vi \times Oi}{\sum Vi \times Oi} \times 100$$

where: PI = preponderance index; Vi = percentage of a type of food; Oi = occurrence of a type of food; $\sum VixOi$ = Vi x Oi.

Statistical analysis. Plankton abundance was statistically evaluated according to completely randomized design (Steel & Torrie 1993). The statistical analysis was performed in SPSS version 13.0, using F value test. Significant difference between means ($p < 0.05$) was then verified using Newman-Keuls test. In terms of plankton diversity, feed consumption, and water quality, they were analyzed using descriptive approach. Model for plankton abundance was analyzed using stepwise regression.

Results and Discussion

Physico-chemical water parameters. Results obtained in this research showed that the addition of fermented booster did not affect water temperature, pH, salinity, DO and CO_2 (Table 1). However, it affects the concentration of ammonia, nitrate and nitrite. The best nitrate concentration was in P2, it was 0.075 mg L^{-1} in the 1st day and increased to

0.590 mg L^{-1} in the end of the experiment (70th day). The lowest nitrite, however, was also obtained in P2, it was 0.284 mg L^{-1} by the end of experiment. This condition may be due to the presence of bacteria group that consisted of *B. subtilis*, *B. megaterium* and *B. polimyxa* (9 x 10^{11} CFU L^{-1}) in the water. Those bacteria may be able to boost the degradation of organic material originated from feces and fish feed remains and transform it into ammonia, nitrite and nitrate forms. Karigar & Rao (2011) stated that *Bacillus* sp. secreted extracellular enzymes such as protease, amylase and lipase able to foster degradation of organic components in aquatic ecosystem. In the treatment with lower dose of booster, the number of bacteria present was lower and thus the fermentation process may be slower. In P3 and P4, the booster doses were higher, but the nitrate concentration was lower than that of P2. The bacteria population in these treatments was high and it may cause the negative feedback effects, as the bacteria as well as plankton using nitrate as energy source and thus reduce the nitrate concentration in the water. Zhang et al (2016) stated that nitrate concentration is negatively correlated to microorganism population. Other water quality parameters such as temperature ranges (26.2-28.9°C), pH (6.5-8), DO (3.8-6.4 mg L^{-1}), CO_2 (2-4 mg L^{-1}) and ammonia (0.001-0.06 mg L^{-1}) were almost the same in all treatments.

Table 1

Parameters for water quality as treated with with 5 levels of fermented booster mixture

Parameters	*Treatments*				
	P_0	*P_1*	*P_2*	*P_3*	*P_4*
Temperature (°C)	26.7-28.9	26.2-28.9	26.2-28.2	26.4-28.7	26.2-27.9
pH	6.5-7.8	6.5-8	6.5-8	6.5-7.9	6.5-7.8
Salinity (ppt)	17-18	17-18	17-18	16-18	16-18
DO (mg L^{-1})	3.8-5.7	4.8-6.1	4.8-6.4	4.5-6.2	4.4-5.9
CO_2 (ppm)	2.66-4.00	2.00-3.33	2.00-2.66	2.22-2.66	2.00-3.33
Ammonia (mg L^{-1})	0.001-0.06	0.001-0.06	0.001-0.04	0.001-0.03	0.001-0.04
Nitrate (mg L^{-1})	0.035-0.268	0.064-0.395	0.075-0.590	0.05-0.287	0.035-0.209
Nitrite (mg L^{-1})	0.039-0.345	0.034-0.283	0.019-0.284	0.037-0.326	0.049-0.292

Note: dose of treatment. P_0 = 0 mL L^{-1}, P_1 = 0.075 mL L^{-1}, P_2 = 0.45 mL L^{-1}, P_3 = 0.825 mL L^{-1}, and P_4 = 1.2 mL L^{-1}.

Plankton diversity. Nitrate concentration in all treatments was suitable for plankton growing. The presence of 0.01-1 mg L^{-1} nitrate in the water was able to support the life of phytoplankton (Herawati et al 2020). Rumanti et al (2014) stated that nitrate influences plankton abundance at 77.4%, meanwhile Effendi (2003) argued that with 0.2 mg L^{-1} nitrate can be categorized as fertile, but nitrite should not be > 0.05 mg L^{-1} since it potentially exerts noxius effects on aquatic organisms. In short, the level of nitrite in this experiment still remains unsatisfied for plankton growth.

During the research, the most common plankton obtained in all treatments were Bacillariophyceae and Chlorophyceae. Wijayanti (2011) reported that Bacillariophyceae and Chlorophyceae were easily found in a stable condition. These phytoplankton can grow well at 30-35°C and 20-30°C, while Cyanophyceae can survive at higher temperature (> 35°C) in comparison with Bacillariophyceae and Chlorophyceae. Dhahiyat et al (2003) reported that the optimum condition for plankton was found at pH of 6-9. Soedarsono et al (2013) reported that phytoplankton enabled to grow at salinity of 25, 30, and 35 ‰, i.e. *Microcystis* sp., *Oscillatoria* sp., *Anabaena* sp., *Lyngbya* sp., *Pleurosigma* sp., *Nitzschia* sp., *Navicula* sp., *Rhizosolenia* sp., and *Chlorella* sp., in which *Microcystis* sp. constitutes the most abundant organism. Some researchers argued that plankton could reach optimum growh at oxygen level of > 3 mg L^{-1}. Phytoplankton did not change with increasing CO_2 concentration, however increasing CO_2 concentration gave a negative effect to phytoplankton abundance. There was a changing on dominant species of phytoplankton with increasing of CO_2 concentration, which was dominated by diatoms (Rukminasari et al 2018). Raharjo et al (2016) reported that CO_2 level of 21-33 mg L^{-1} was still acceptabe for plankton growth with high degree of diversity. Organisms may have various response towards level of CO_2 in water, but it is known well that concentration of CO_2 at 15 mg L^{-1} can bring adverse impacts.

Table 2 shows the diversity and the quantity of plankton. In all treatments, there was almost no difference in the type of plankton presence. It indicates that the addition of the booster do not affects the type of plankton. The type of plankton growing in P0 was not different with the plankton growing in other treatments.

Table 2

Diversity and abundance of phytoplankton

No.	Taxa	Treatment (cells L^{-1})				
		P_0	P_1	P_2	P_3	P_4
A.	PHYTOPLANKTON					
I.	Phylum: Bacillariophyta					
I.1	Class: Bacillariophyceae					
1	*Asterionella* sp.	194	214	524	0	142
2	*Fragilaria* sp.	552	285	388	134	778
3	*Navicula* sp.	0	895	463	599	97
4	*Nitzschia sublinearis*	1,643	1,680	2,786	2,282	2,014
5	*Thalassiothrix* sp.	11,165	17,172	17,930	14,993	13,078
I.2.	Class: Coscinodiscophyceae					
1	*Rhizosolenia* sp.	8,796	11,324	15,240	10,171	10,209
2	*Triceratium* sp.	4,633	6,203	6,420	5,227	4,543
I.3.	Class: Mediophyceae					
1	*Isthmia* sp.	954	790	2,626	1,312	549
2	*Leptocylindrus* sp.	594	979	179	114	179
3	*Skeletonema* sp.	129	269	171	27	52
4	*Biddulphia* sp.	1,382	1,978	2,580	1,949	2,067
5	*Streptotheca* sp.	1,933	4,637	4,342	4,046	2,817
	Total	31,975	46,426	53,649	40,854	36,525
II.	Phylum: Chlorophyta					
II.1.	Class: Chlorophyceae					
1	*Oedogonium* sp.	5,669	10,215	13,595	9,072	10,698
2	*Scenedesmus* sp.	6,417	7,739	9,738	8,100	8,142
3	*Echinopshaerella* sp.	157	0	601	157	0
II.2.	Class: Trebouxiophyceae					
1	*Chodatella* sp.	4,928	6,447	10,392	10,856	8,448
2	*Actinastrum hantzschii*	10,770	13,419	19,913	8,687	9,002
3	*Scotiella* sp.	2,081	1,698	1,638	2,610	3,763
II.3.	Class: Ulvophyceae					
1	*Ulotrix* sp.	4,807	5,711	8,899	6,087	6,457
	Total	34,829	45,229	64,776	45,569	46,510
III.	Phylum: Charophyta	5,214	8,133	8,284	6,716	6,351
	Class: Charophyceae					
1	*Cosmarium* sp.	5,214	8,133	8,284	6,716	6,351
	Total	5,214	8,133	8,284	6,716	6,351
IV	Phylum: Cyanobacteria					
	Class: Cyanophyceae					
1	*Aphanothece* sp.	2,925	2,890	4,185	4,357	3,198
2	*Microcystis* sp.	1,611	3,169	2,982	2,324	2,667
3	*Tolypothrix tenus*	902	1,203	1,352	1,632	1,342
4	*Oscillatoria* sp.	2,001	2,252	2,153	2,000	2,277
5	*Synechocystis aquetilis*	2,237	4,873	5,036	3,814	5,446
6	*Lyngbya* sp.	1,777	1,655	1,337	1,491	1,607
7	*Calothrix fusca*	283	909	743	308	447
	Total	11,736	16,950	17,790	15,927	16,984
V.	Phylum: Ochrophyta					
	Class: Chrysophyceae					
1	*Hidrurus foetidus*	1,543	1,155	1,403	2,110	1,420
	Total	1,543	1,155	1,403	2,110	1,420
B.	ZOOPLANKTON					
VI.	Phylum: Ciliophora					
	Class: Oligotrichea					

1	*Leprotintinnus* sp.	8,876	11,787	13,232	10,125	11,283
2	*Tintinnopsis platensis*	293	604	514	124	296
3	*Tintinnopsis* sp.	2,794	4,625	3,784	3,334	2,708
	Total	11,963	17,016	17,530	13,583	14,287
VII.	Phylum: Dinoflagellata					
	Class: Dinophyceae					
1	*Peridinium* sp.	3,685	3,625	4,734	3,301	3,692
2	*Ceratium* sp.	601	1,683	1,521	1,017	803
	Total	4,286	5,308	6,255	4,318	4,495
VIII.	Phylum: Euglenozoa					
	Class: Euglenophyceae					
1	*Euglena oxyrus*	663	1,044	2,756	1,335	437
	Total	663	1,044	2,756	1,335	437
IX.	Phylum: Protozoa					
	Class: Spirotrichea					
1	*Tintinnidium* sp.	4,481	6,133	10,259	5,523	6,298
	Total	4,481	6,133	10,259	5,523	6,298
X.	Phylum: Rotifera					
	Class: Eurotatoria					
1	*Proales simplex wang*	736	1,071	1,367	914	485
	Total	736	1,071	1,367	914	485
	Abundance	107,420	148,469	183,850	136,848	133,791
	Average	2,984	4,124	5,107	3,801	3,716

The results showed 28 types of phytoplankton and 8 types of zooplankton classified into 10 phyla and 14 classes. These plankton groups are also commonly found in brackish water, particularly Bacillariophyta or diatoms (12 types). Diatoms refer to phytoplankton dominantly present in either brackish water or sea water, which may link to characteristics of the water acceptable to their growth. Zakiyyah et al (2016) found that diversity of plankton was dependent on their response towards environmental changes, as well as temperature, salinity, and DO. Yuliana (2007) and Utojo (2015) reported that phytoplankton in either brackish and sea water could exist in various area; they also showed a crucial role in food web, especially Bacillariophyta, Cyanophyta and Chlorophyta. Canini & Metillo (2017) explained that diatoms (Bacillariophyta) is considered as phytoplankton that massively existed in sea water with salinity of 21.3-31.7 part per thousand. The superfluous quantity of Bacillariophyta in brackish and sea water is closely related to their reproductive performance, compared to Dinoflagellata and other classes of phytoplankton (Zakiyyah et al 2016).

Among the plankton presence, Chlorophyta shows the highest abundance (Figure 1). The addition of the booster increase the nitrate concentration and it may serve as main component to support the life of Chlorophyta. The highest abundance of Chlorophyta was in P_2 (72,638 cells L^{-1}). Similar results was obtained by Andriyani et al (2014) who stated that the abundance of Chlorophyta in the Nile tilapia culture media that was enriched with fermented cassava peel flour was higher than the control, it was 8,217-15,750 cells L^{-1} and 6,682 cells L^{-1} respectively. Nitrate concentration in that research was 1.258-1.868 mg L^{-1}. Eventhough the nitrate concentration in this previous study was lower than that of the media with fermented cassava peel flour addition, the abundance of Chlorophyta was higher, indicates that the booster used in this study is more effective to grow the plankton.

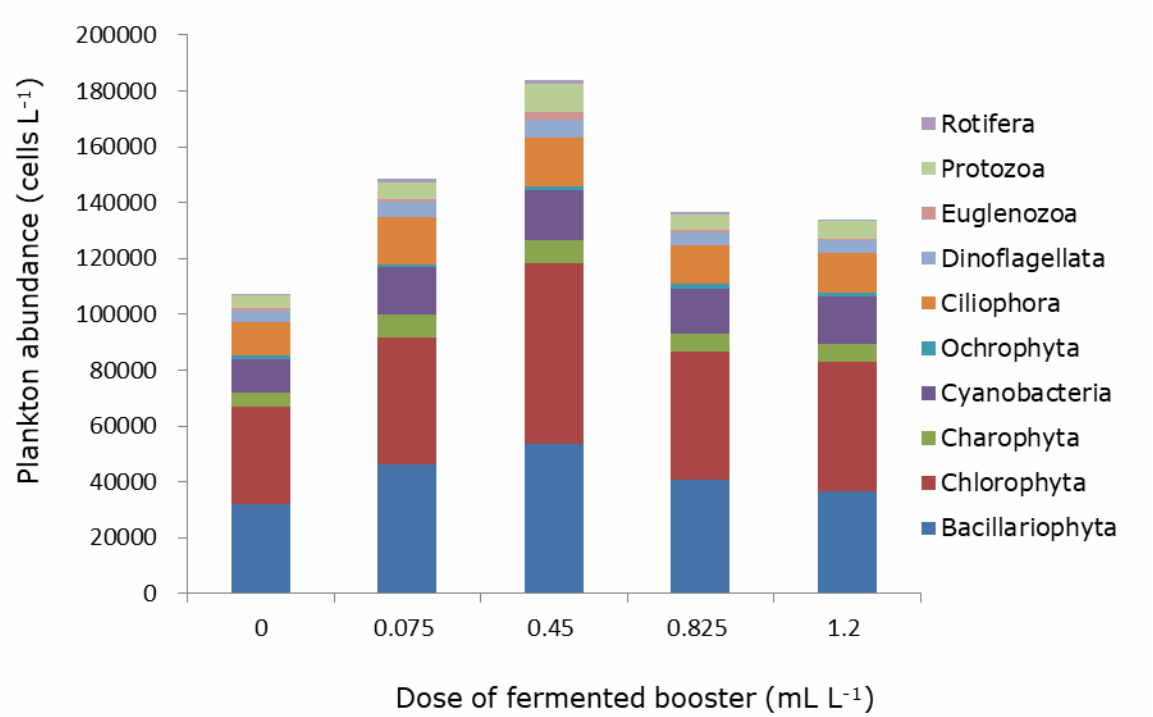

Figure 1. Plankton abundance based on class.

The least common plankton was Euglenozoa. This plankton is able to use organic matter as nutrition source and the addition of fermented booster that degrades organic matters may negatively affect that zooplankton. As the organic matter decrease, the Euglenozoa growth may also decrease. Van Vuuren (2006) stated that *Euglena* commonly inhabit fresh water as well as brackish water that is rich in organic matter. The lowness of *Euglena* abundance in this research indicates that the organic matter in the media has been degraded and thus the water quality is improved.

Stomach content analysis. Study on stomach content analysis indicates that tilapia is able to consume plankton available in the media. The stomach content consisted of 94.70% fish feed pellets and 5.30% plankton (Table 3). These data are in accordance with Tresna (2012), who reported that one of nutritional source for tilapia was phytoplankton, while the fish enabled to show rapid adaptive action towards changes in feed resource.

The most abundant phytoplankton type present in the media was Chlorophyta. This type of plankton has higher opportunity to be swallowed by the fish. However, stomach content analysis data shown that the highest number of plankton presence in the stomach was Bacillaryophyta. The cell wall of Chlorophyta consists of cellulose that can be digested by cellulase enzyme. Thorp & Covich (2010) stated that cellulose hydrolytic enzyme is present in the digestive tract of herbivorous aquatic organisms. In contrast the cell wall of Bacillaryophyta consists of silica that can not be broken by the digestive enzyme and as a consequence the Bacillariophyta cells in the digestive tract of the fish were intact.

Table 3

Types of plankton present in stomach of tilapia fish

No.	*Types of plankton*	*Index of preponderance (%)*
	I. Bacillariophyta	
1	*Fragilaria* sp.	0.6050
2	*Isthmia* sp.	0.7722
3	*Leptocylindrus* sp.	0.0231
4	*Navicula* sp.	0.7285
5	*Nitzchia* sp.	0.0117
6	*Rhizosolenia* sp.	0.0301
7	*Skeletonema* sp.	0.3274
8	*Thalassiothrix* sp.	0.0230
9	*Triceratium* sp.	0.0015
	Subtotal	2.5225
	II. Chlorophyta	
10	*Chodatella* sp.	0.0043
11	*Oedogonium* sp.	0.0053
12	*Scenedesmus* sp.	0.0007
13	*Scotiella* sp.	0.0031
	Subtotal	0.3199
	III. Charophyta	
14	*Cosmarium* sp.	0.3065
	Subtotal	0.3065
	IV. Cyanobacteria	
15	*Aphanothece* sp.	0.0109
16	*Calothrix fusca*	0.0017
17	*Lyngbia* sp.	0.0003
18	*Microcystis* sp.	2.1512
19	*Oscillatoria* sp.	0.2650
	Subtotal	2.4291
	V. Dinoflagellata	
20	*Ceratium* sp.	0.0109
21	*Peridinium* sp.	0.0100
	Subtotal	0.0209
	VI. Euglenozoa	
22	*Euglena* sp.	0.0117
	Subtotal	0.0117
	VI. Fish meal (pellet)	94.70
	Total	100.00

Plankton abundance. As depicted in Figure 2, statistical analysis showed that dose of fermented booster caused significant effects on plankton abundance ($p < 0.05$), whereas P_2 was significantly different compared to other treatments according to Newman Keuls test. The highness of plankton abundance in P_2 may be caused by the highness of nitrate and the lowness of nitrite concentrations, while the ammonia concentration in that treatment was fair and suitable for the life of phytoplankton. Conversely, the dose at P_3 and P_4 treatments tended to be excessive, enabling to intervene diffusion and osmosis of plant cells. This is in line with result of Miksen (2018), finding that extra fertilizer could exert lethal effects on plants, associated with diffusion and osmosis activity. Fertilizer can create a hypertonic environment, leading to withdrawal of water from cells. As a consequence, the plant experienced loss of turgor tension, then causing cellular plasmolysis. Ultimately, this reaction leads to degradation of cell wall. Over fertilizing with high-quality fertilizers can also lead to plant root burn due to an overabundance of soluble salts in the soil.

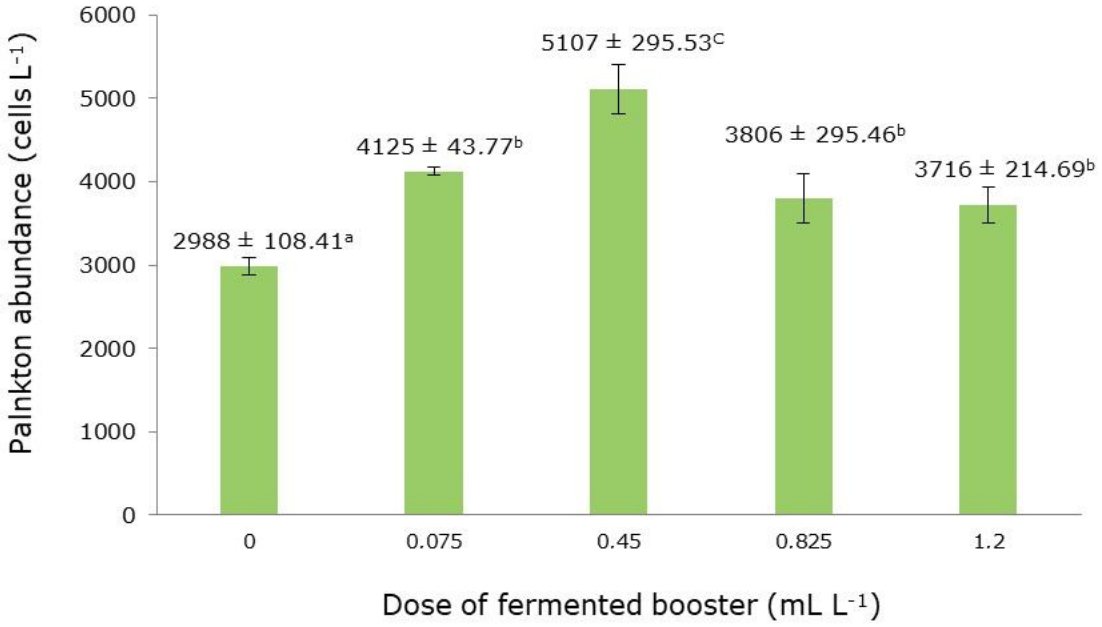

Figure 2. The average abundance of plankton as treated with 5 levels of fermented booster mixture (means with different superscripts (a, b, c) were significantly different ($p < 0.05$).

The plankton abundance found in P_0 treatment is the lowest. This is clearly due to absence of nutritional supply from booster; therefore, the nutrients are inadequate for growth of plankton. Chislock et al (2013) and Anyinkeng (2016) reported that supply of nutrition was positively correlated with phytoplankton abundance, but negatively correlated with diversity of phytoplankton. Muharram (2006) reported that quantity and communal structure of phytoplankton relied mostly on physical and chemical factors, i.e. availability of key nutrients, as well as their capability to utilize these components.

Additionally, the presence of bacteria group, that consisted of *B. subtilis*, *B. megaterium* and *B. polimyxa*, is a foremost factor responsible for accelerating formation of nutrients in culture medium. Hatmanti (2000) asserted that diversity of bacteria might improve performance of particular species. Combined species of *B. subtilis* is reported capable of producing protease that accounted for hydrolyzing feed debris and feces. *B. polymixa* enable to compose polymixin responsible for maintaining population of plankton, while *B. licheniformis* is able to produce a special compound responsible for retardation of unwanted bacteria *(Vibrio* sp.), thereby improving water quality.

The plankton life cycle. The abundance of phytoplankton was fluctuated throughout the research. Figure 3 shows that the initial abundance of the phytoplankton in all treatments was almost the same. The plankton abundance started to be various in the 2nd day. In all treatments as well as in control, the highest phytoplankton abundance was achieved in the 12th day and slightly fluctuated during the next 20 days. In the 32nd day the plankton abundance dropped into around 3,000 cells L^{-1}. Similar cycle was present in the next 30 days. During the first 6 days, the plankton was in growth and reproductive phases. During the 12th – 26th days, plankton was in quiescence phase and it means that there were neither reproduction activities nor increasing number of plankton. Within the next 6 days plankton was in death phase and achieved the lowest point by the 32nd day. The plankton was then starting a new cycle, it was increased during the 32nd – 42nd days, almost constant within 42nd – 58th days and dropped by the 64th day. Plankton abundance in all treatments applied as well as in control was showing similar life cycle pattern, it was 32 days cycle^{-1}. Von Dassow & Montresor (2011) asserted that a species can alternate in its life cycle between four distinct major phases: growth, reproduction, quiescence and cell death at day 15 in absence of nutrients. The longer life cycle of plankton in this research may be due to the constant availability of nitrate in the media, as the booster was added to the media once a week in order to retain the nitrate

concentration in the media. It seems that the fluctuation of phytoplankton abundance in all treatments was related to the availability of nitrate in the media that affects the life cycle of the cells. Knorr (2019) stated that phytoplankton require water, carbon dioxide and a variety of other nutrients from the water including nitrogen and phosphorous. The most important are phosphorous and nitrogen that are essential for survival and reproduction. Phytoplankton cannot continue growing when there is a lack of one or more nutrient components.

In each phase, the abundance of plankton in P_2 was higher than those of other treatments and it may be due to the highest availability of nitrate and the lowest concentration of nitrite in the media.

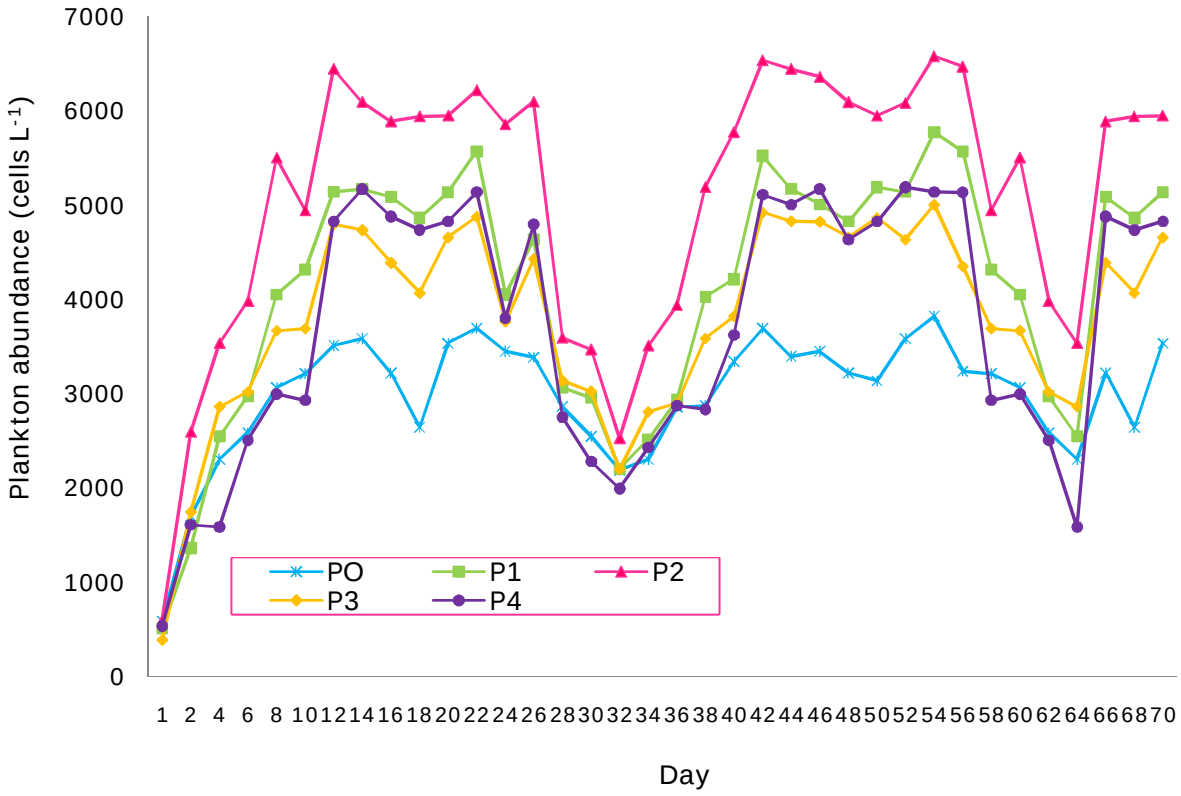

Figure 3. Peak points of plankton abundance as treated with with 5 levels of fermented booster mixture.

Stepwise regression model between plankton abundance and water quality. Statistical analysis shows that regression model connecting between plankton abundance and water quality (temperature, pH, DO, salinity, CO_2, ammonia, nitrate and nitrite) is presented as follows:

$$Y = 17{,}870.50 + 2{,}755.45\ X_1 + 4{,}745.12\ X_2 - 1{,}067.15\ X_3$$

where: Y = plankton abundance, X_1 = DO, X_2 = level of nitrate, X_3 = temperature. The r and R^2 values were 0.957 and 0.915 respectively.

The model suggested that plankton abundance was closely related (r > 50%) and positively related to level of DO and nitrate, but it was negatively related to the temperature. In terms of R^2, contribution of each variable to plankton abundance was at 91.5%.

Conclusions. The addition of booster in the tilapia culture media positively affects the water quality as well as plankton abundance. During the experiment, water quality parameters such as temperature, pH, salinity, DO, CO_2, ammonia, nitrite and nitrate

were in good condition and suitable to support the life of plankton. In all treatments applied, plankton abundances were higher than that of the control. P2 (0.45 mL L^{-1} booster addition) provided the best results, and achieved 5,107 cells L^{-1} by the end of experiment. The types of plankton present in the media were Bacillariophyceae, Chlorophyceae, Charophyceae, Cyanophyceae, Chrysophyceae, Rotifera, Dinophycea and Euglenophyceae. Stomach content analysis of the fish shown that there were 22 species of plankton present in the stomach of the fish, provided 5.30% of total feed consumed. Result of a Stepwise Regression showed that the combination of temperature, DO, and nitrate concentration in the water contributed 91.5% to plankton abundance.

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