

# Blending fishwaste and chicken manure

*by* Stenly Wullur 40

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**Submission date:** 21-Dec-2020 08:51AM (UTC+0700)

**Submission ID:** 1479847230

**File name:** Ogello\_et\_al\_2019\_Blending\_fishwaste\_and\_chicken\_manure.pdf (444.13K)

**Word count:** 5965

**Character count:** 30875

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To cite this article: E O Ogello *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **567** 012022

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## Blending fishwastes and chicken manure extract as low-cost and stable diet for mass culture of freshwater zooplankton, optimized for aquaculture

E O Ogello<sup>1</sup>, S Wullur<sup>2\*</sup>, A Hagiwara<sup>3</sup>

<sup>1</sup>Department of Fisheries and Natural Resources, Maseno University, P.O.Box Private Bag, Maseno, Kenya

<sup>2</sup>Ins Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado 95-115, Indonesia

<sup>3</sup>Graduate School of Fisheries and Environmental Sciences, Nagasaki University, 852-8521, Nagasaki, Japan

Corresponding author: [stenlywullur@unsrat.ac.id](mailto:stenlywullur@unsrat.ac.id)

**Abstract:** This study investigated the feasibility of fishwastes and chicken manure extract (CME) as cheap diet for mass culture of freshwater zooplankton. CME and fishwastes as well as carbon source were used to make fishwaste diets (FWD). Each diet was triplicated 3 days before inoculation with 5, 2 and 0.4 ind ml<sup>-1</sup> of rotifers, copepods and cladocerans, respectively in each tank. About 5ml of water was done daily, from which the zooplankters were counted. Harvesting was done at the first exponential growth phase by replacing 50% of the water and FWD. There was a significantly higher density of zooplankton and SGR in FWD<sub>B</sub> than FWD<sub>A</sub> and control tanks. The zooplankton obtained highest densities on day 7 as follows: rotifers: 100.6±14.8, 146.3±7.0, and 60.0±7.9 ind ml<sup>-1</sup> in FWD<sub>A</sub>, FWD<sub>B</sub> and the control tanks, respectively; the copepods: 8.0±11.1, 12.6±13.6 and 4.3±2.1 ind ml<sup>-1</sup> in FWD<sub>A</sub>, FWD<sub>B</sub> and control tanks, respectively; the cladocerans: 3.3 ± 6.0, 8.6 ± 8.7 and 3.6±2.5 ind ml<sup>-1</sup> in FWD<sub>A</sub>, FWD<sub>B</sub> and control tanks, respectively. The most abundant genera were *Brachionus* sp., *Cyclops* sp. and *Daphnia* sp. for the rotifers, copepods and cladocerans, respectively. This study offers an alternative to expensive on-site microalgal production toward a more cost-effective aquaculture.

### 1. Introduction

In the recent past, aquaculture activities have increased considerably in the tropical countries, leading to high demand for the zooplankton species such as rotifers, copepods, cladocerans and *Artemia* nauplii as suitable starter foods for fish larviculture [1]. The live foods are highly digestible, more palatable, contain essential nutrients and, preserve water quality unlike inert diets [2]. However, inconsistent supply of the live food resources continues to limit the intensive culture of economically valuable fishes. Naturally, the zooplankton communities form a significant component of aquatic ecosystems, and are the primary prey for most larval fishes and crustaceans. The zooplankters have relatively high reproductive rates, thus making them attractive for use in semi-intensive culture systems, which are popular in the developing countries.

Fresh microalgae are first choice diet for culturing various zooplankton species in hatcheries, for aquaculture [3]. However, the challenges of high density microalgal culture protocols have caused inconsistencies in the production of sufficient quantities of the zooplankton to match the increasing demand for the live foods in aquaculture. Alternatively, cheaper diets such as baker's yeast have been



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used but culture instabilities due to imbalances of vitamin B<sub>12</sub> and bacterial flora are common [4]. Other studies have also reported the use of waste-grown bacteria and/or synthetic medium grown-bacteria as rotifer diet [5][6] and dried algae [7]. Indeed, limited zooplankton production capacity often disrupts fish seedling programs in microalgae-based hatcheries. Organic manures especially chicken manure has always been used in zooplankton production. Studies have attributed the effects of the manures on pond productivity to the actions of different sex hormones present in them [8]. For example, egg-laying chicken excrete about 50 and 250 ng g<sup>-1</sup> dry manure day<sup>-1</sup> of 17β-estradiol and testosterone respectively [9]. There are evidences that such chemicals are used by zooplankton to regulate reproduction, induce predator defenses, and accomplish selective foraging [10][11][12]. In our previous study, application of 2.0 ml l<sup>-1</sup> of chicken manure extract (CME) increased the parthenogenetic reproduction of the freshwater rotifer, *Brachionus angularis* [13].

On the other hand, fishwastes contain appreciable amount of recoverable essential bio-molecules including the n-3 series of the long chain polyunsaturated fatty acids (PUFAs) e.g. eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [14]. These elements are critical for growth and survival of the fish larvae feeding on the rotifers [15]. The fish wastes also provide excellent substrates for microbial growth, some of which have probiotic properties such as *Bacillus* sp. and lactic acid bacteria (LAB) [16]. Probiotics are known to enhance growth and immunity of rotifers and fish larvae either individually or in combination [17][18][19]. In this study, we hypothesized that a blend of the CME and the FWD can provide positive synergy to enhance growth and reproduction of freshwater zooplankton (i.e. rotifers, copepods and cladocerans), for aquaculture. The zooplankton obtain a substantial proportion of their carbon from detritus pathways via bacteria, and previous studies have reported the role of certain bacterial species in improving population growth and nutrition status of various zooplankton species [20][21].

Composing organic matter yields huge densities of bacterial cells under optimum carbon/nitrogen (C/N) ratio [22]. Bacteria, and by extension the microbial loop are known to play important roles as recycling pathways for C and N in the ecological food webs [23]. Usually, optimum C/N ratio ensures immobilization of inorganic nitrogen into huge bacterial proteins (biomass) and restores good water quality by removing toxic ammonia [24]. Heterotrophic production of bacteria using fish defecates for *in situ* production of zooplankton communities has been exploited in the polyculture of shrimp, catfish and tilapia [25] with adequate carbon source [26]. However, the feasibility of the combined CME and fishwastes for *in situ* zooplankton production is not yet clear. This study explored the applicability of the fishwastes and CME for mass culture of freshwater zooplankton communities in an outdoor tank culture system, optimized for aquaculture production.

## 2. Materials and Methods

### 2.1 Study area

This study was conducted at Kenya Marine & Fisheries Research Institute (KMFRRI), Kegati Aquaculture Research Centre, located at 00°42'S; 034°47'E. The inoculant zooplankton i.e. rotifers, cladocerans and copepods were obtained from one of the fish production ponds that was previously fertilized with diammonium phosphate (DAP) and urea. One litre of the pond water was randomly sampled and filtered through a kitchen sieve to remove physical debris before determining the zooplankton densities in 1 ml sub-samples from each filtrate using a graduated plate with lugol fixation under stereo microscope, at × 25 magnifications. The densities of the zooplankton were used to determine the quantity of pond water needed to inoculate the experimental tanks. Plankton net of pore size 45 μm was used to filter the pond water to concentrate the zooplankton.

### 2.2 Chicken manure extract (CME)

One kilogram of fermented chicken manure was boiled in 5 l of pond water for 40 - 50 min and then kept overnight at room temperature. The supernatant liquid was filtered off the sludge using nylon net of pore size 100μm and used as CME.

### 2.3 Fishwaste diet (FWD)

*Barbus altinialis* Linnaeus 1758, which is a low-valued bi-catch freshwater fish, were caught from river Mara, Kenya, using an electro-fisher (Smith-Root GPP USA) and transported to the laboratory in

a cooler box. The fish were gutted and the fish heads were crushed to make a diet as explained in our patent No. P00201609066 registered in Indonesia. We added 2 ml l<sup>-1</sup> of CME and 0.2 g l<sup>-1</sup> of maize flour carbon source into the tanks.

#### 2.4 Experimental design

The experiment was conducted in 9 asbestos tanks each containing 500 l of well water. Three diets i.e. FWD<sub>A</sub> (0.5 g l<sup>-1</sup> of fishwaste + 0.2 g l<sup>-1</sup> of maize flour), FWD<sub>B</sub> (FWD<sub>A</sub> + 2 ml l<sup>-1</sup> of CME) and control (2 ml l<sup>-1</sup> of CME only) were each triplicated in the culture tanks, 3 days prior to inoculation with a combination of 5, 2 and 0.4 ind ml<sup>-1</sup> of rotifers, copepods and cladocerans, respectively in each culture tank on day 0. Different plankton nets (mesh sizes 250, 500 and 1000 µm) were used to adjust the density of rotifers, copepods and cladocerans, respectively before inoculation in the experimental tanks. The tanks were covered with mosquito nets to keep off insects and birds. Every day, 5 ml of water was randomly sampled three times at the top 10 cm of each tank from which, the zooplankton were counted with lugol fixation. At the end of the first exponential phase, partial harvesting was done by replacing 50% of all the tank water with new well water and fresh FWD. The experiment lasted for 16 days.

The percent relative abundance (% RA) of each zooplankton species was calculated as [mean abundance (MA) / total zooplankton taxa (N)] × 100. The population specific growth rate (SGR) ( $r$ ) was calculated as  $r = [\ln N_t - \ln N_0] / t$ , where,  $N_0$  = initial population density,  $N_t$  = population density after the time (t) and t = time (days) and the coefficient of variation (CV) of the mean SGR was calculated as standard deviation / mean SGR. For qualitative composition, the zooplankters were identified up to the genus level according to [27] while the zooplankton diversity was calculated using the Shannon-Weiner diversity index:  $H' = - \sum [(pi) * \ln (pi)]$ , where pi = number of individuals of species i / total number of samples [28].

#### 2.5 Water quality

Temperature (°C), dissolved oxygen (DO; mg l<sup>-1</sup>), pH and electrical conductivity (µS cm<sup>-1</sup>) were measured *in situ* using a multi-probe water checker (U-10 model, Horiba, Tokyo, Japan). The water turbidity (cm) was also measured *in situ* using a secchi disk. The water quality parameter measurements were taken daily at 1200 hours.

#### 2.6 Data analysis

The data was analyzed using R statistical software (version 3.2.1 of the R Foundation for Statistical Computing Platform © 2015). The Bartlett test was used to determine the homogeneity of variances. Two-way analysis of variance (ANOVA) was used to test the effect of the FWD and culture days on the water quality parameters and zooplankton densities. One-way ANOVA was used to test the effect of FWD on the zooplankton mean abundance, SGR and diversity ( $H'$ ). The Tukey's HSD Post Hoc test was performed to locate any significant differences at  $p < 0.05$ .

### 3. Results

#### 3.1 Water analysis

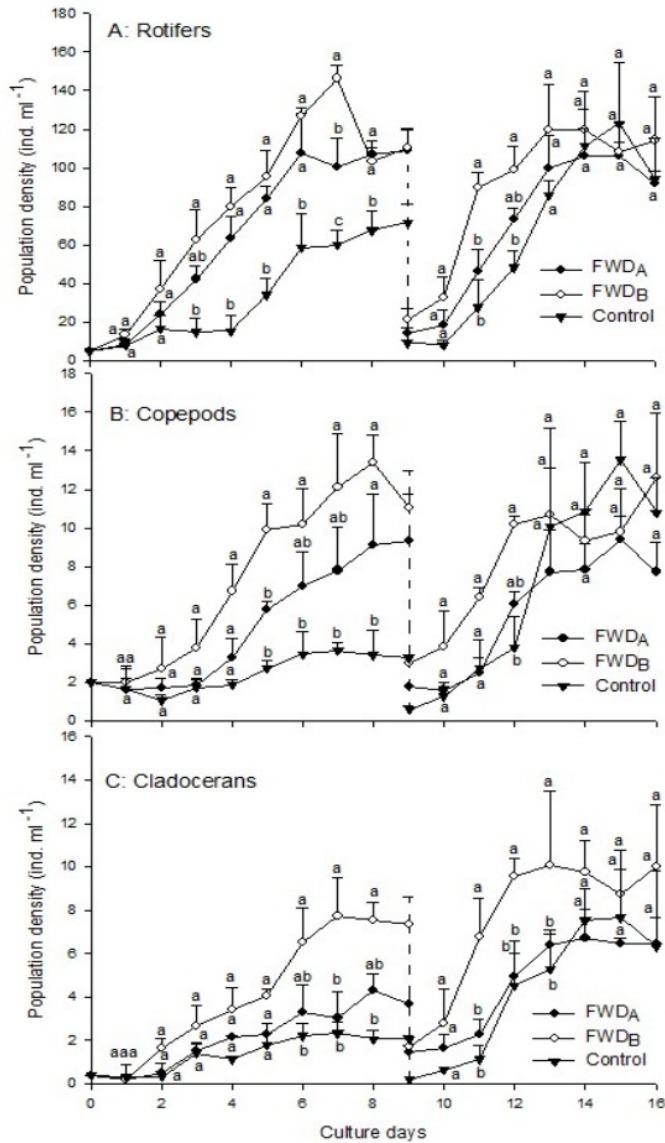
Measurements of the water quality parameters are presented in Table 1. The water temperature varied significantly during the culture days ( $F=2.31$ ,  $p=0.01$ ) but was not affected by the FWD ( $F=0.45$ ,  $p=0.63$ ). Similarly, conductivity varied significantly during the culture days ( $F=1.76$ ,  $p=0.04$ ) with no significant effects of FWD ( $F=0.56$ ,  $p=0.57$ ). The DO was significantly affected by the FWD ( $F=4906.74$ ,  $p<0.05$ ) and culture days ( $F=200.44$ ,  $p<0.05$ ), with higher DO being recorded in the control than in the FWD-treated tanks ( $p=0.00$ ). FWD<sub>A</sub> tanks had significantly higher DO than FWD<sub>B</sub> tanks ( $p=0.00$ ). Turbidity varied significantly within the culture days ( $F=2.83$ ,  $p<0.05$ ), and between the FWD ( $F=841.80$ ,  $p<0.05$ ), with higher turbidity being recorded in the FWD tanks than in the control tanks ( $p=0.00$ ). FWD<sub>B</sub> tanks were more turbid compared to FWD<sub>A</sub> tanks ( $p=0.00$ ). The pH was neither affected by the FWD ( $F=1.18$ ,  $p=0.31$ ) nor culture days ( $F=1.07$ ,  $p=0.38$ ).

34  
 Tab 22. The mean values of water quality parameters  $\pm$  SD in the culture tanks for 16 days; Values with different superscript in each row are significantly different at  $p<0.05$ ; Two-way ANOVA; Tukey HSD test,  $a>b>c$ ,  $n=48$ .

Water quality parameters	Treatments			
	Before experiment	FWD <sub>A</sub>	FWD <sub>B</sub>	Control
Temperature ( $^{\circ}$ C)	25.1 $\pm$ 1.0	25.6 $\pm$ 1.0 <sup>a</sup>	25.7 $\pm$ 1.0 <sup>a</sup>	25.8 $\pm$ 1.2 <sup>a</sup>
Conductivity ( $\mu$ S $\text{cm}^{-1}$ )	113.7 $\pm$ 2.2	115.9 $\pm$ 2.8 <sup>a</sup>	116.2 $\pm$ 2.4 <sup>a</sup>	115.7 $\pm$ 2.1 <sup>a</sup>
Dissolved oxygen ( $\text{mg l}^{-1}$ )	5.73 $\pm$ 0.19	3.94 $\pm$ 0.77 <sup>b</sup>	3.84 $\pm$ 0.68 <sup>c</sup>	5.61 $\pm$ 0.21 <sup>a</sup>
pH	7.51 $\pm$ 0.01	6.63 $\pm$ 0.03 <sup>a</sup>	6.65 $\pm$ 0.04 <sup>a</sup>	6.66 $\pm$ 0.12 <sup>a</sup>
Turbidity (cm)	39.6 $\pm$ 1.5	35.7 $\pm$ 1.3 <sup>c</sup>	23.4 $\pm$ 3.1 <sup>a</sup>	27.6 $\pm$ 3.1 <sup>b</sup>

### 3.2 Zooplankton population dynamics

41  
 The population density of each zooplankton taxa was significantly affected by the FWD, culture days and the interaction between them ( $p<0.05$ ). There was significantly higher rotifer density in FWD<sub>A</sub> than in control tanks from day 4 - 6 (Tukey HSD,  $p<0.05$ ), and higher density in FWD<sub>B</sub> than in control tanks from day 3 - 7 and 11 - 12 ( $p<0.05$ ). Meanwhile, there was significantly higher rotifer density in FWD<sub>B</sub> than in FWD<sub>A</sub> tanks on day 7 and 11 ( $p<0.05$ ). The rotifer densities were 100.6 $\pm$ 14.8, 146.3 $\pm$ 7.0, and 60.0 $\pm$ 7.9 ind  $\text{ml}^{-1}$  in FWD<sub>A</sub>, FWD<sub>B</sub> and the control tanks, respectively on day 7 (Figure 1). The population density of copepods was significantly higher in FWD<sub>A</sub> than in control tanks on day 5 and 8 ( $p<0.05$ ), and higher in FWD<sub>B</sub> than in control tanks from day 5 - 8 and 12 ( $p<0.05$ ). There was no significant difference between the FWD tanks ( $p>0.05$ ). The copepods' densities were 7.8 $\pm$ 2.5, 12.1 $\pm$ 2.7 and 3.6 $\pm$ 0.26 ind  $\text{ml}^{-1}$  in FWD<sub>A</sub>, FWD<sub>B</sub> and control tanks, respectively on day 7 (Figure 1). For the cladocerans, there was no significant difference in the population density between FWD<sub>A</sub> and control tanks ( $p=0.08$ ). However, there was significantly higher density in FWD<sub>B</sub> than in control tanks from day 6 - 8 and 11 - 13 ( $p<0.05$ ), and higher density in FWD<sub>B</sub> than in FWD<sub>A</sub> tanks on day 7 and 11-13 ( $p<0.05$ ). The densities of the cladocerans were 3.1 $\pm$ 1.2, 7.7 $\pm$ 1.7 and 2.3 $\pm$ 0.5 ind  $\text{ml}^{-1}$  in FWD<sub>A</sub>, FWD<sub>B</sub> and control tanks, respectively on day 7 (Figure 1). The summary of the composition of zooplankton taxa, mean and percent abundance is presented in Table 2. The most abundant genera were *Brachionus* sp., *Cyclops* sp. and *Daphnia* sp. for rotifers, copepods and cladocerans, respectively.



**Figure 1:** The population density curves of each zooplankton taxa. Each plot and vertical bar represents mean  $\pm$  SD. Half of the culture water was replaced with new media on day 9 in each treatment as shown by the dotted lines. Different superscripts on each day represent significantly different mean population densities at  $p < 0.05$ ; Two-way ANOVA, Tukey HSD test;  $n=3$ ,  $a > b > c$

The SGR of each zooplankton taxa was significantly affected by the FWD (One-way ANOVA,  $p < 0.05$ ) with higher SGR for the rotifers in FWD<sub>B</sub> than in FWD<sub>A</sub> ( $p = 0.05$ ) and control tanks ( $p = 0.00$ ), and higher SGR in FWD<sub>A</sub> than in control tanks ( $p = 0.00$ ) (Table 3). The SGR for the copepods was significantly higher in the two FWD than in the control tanks ( $p < 0.05$ ), but not significantly different between the FWD tanks ( $p = 1$ ). There was significantly higher SGR for the cladocerans in FWD<sub>B</sub> than in FWD<sub>A</sub> ( $p = 0.02$ ) and control tanks ( $p < 0.05$ ), but not significantly different between FWD and control tanks ( $p = 0.66$ ) (Table 5-2.4). The FWD did not affect the diversity ( $H'$ ) of the rotifers (One-way ANOVA;  $F = 0.03$ ,  $p = 0.93$ ), copepods (One-way ANOVA;  $F = 0.09$ ,  $p = 0.91$ ) and cladocerans (Kruskal-Wallis test;  $\chi^2 = 1.67$ ,  $df = 2$ ,  $p = 0.43$ ) (Table 3). Similarly, there was no significant difference in coefficient of variation (CV) among the treatments (One-way ANOVA,  $F = 1.97$ ,  $p = 0.22$ ) (Table 3).

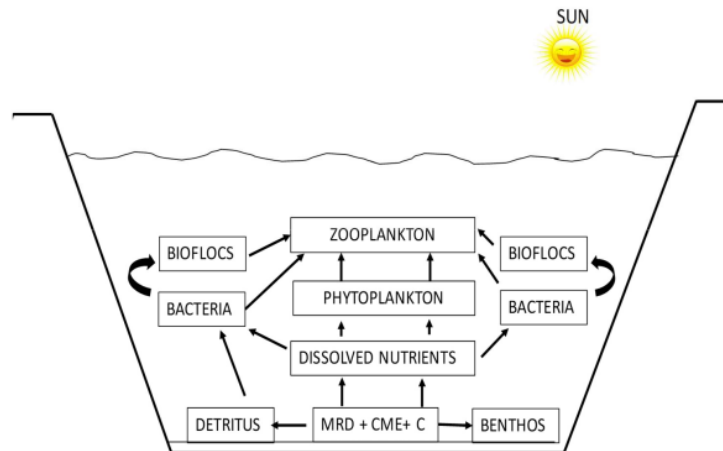
**Table 2:** Zooplankton taxa composition, mean abundance (MA) and percent relative abundance (% RA) of the zooplankton species in each treatment at day 16; the values are mean ind/ml  $\pm$  SD. Different superscripts in a row denote significant differences for each zooplankton species at  $p < 0.05$ , One-way ANOVA, Tukey HSD test,  $n = 6$ ,  $a > b > c$ ; -, absent.

Zooplankton taxa (ind/ml)	Treatments					
	FWD <sub>A</sub>		FWD <sub>B</sub>		Control	
	MA	% RA	MA	% RA	MA	% RA
<b>Rotifers</b>						
<i>Brachionus</i> sp	38.6 $\pm$ 8.6 <sup>ab</sup>	25.3	51.8 $\pm$ 12.1 <sup>a</sup>	25.5	33.6 $\pm$ 3.7 <sup>b</sup>	23.7
<i>Filinia</i> sp	-	-	9.6 $\pm$ 1.2	4.9	-	-
<i>Lecanea</i> sp	13.8 $\pm$ 4.6 <sup>a</sup>	9.1	10.6 $\pm$ 6.1 <sup>a</sup>	4.6	12.0 $\pm$ 3.2 <sup>a</sup>	8.4
<i>Keratella</i> sp	17.3 $\pm$ 3.0 <sup>a</sup>	11.0	12.2 $\pm$ 2.9 <sup>b</sup>	5.4	7.5 $\pm$ 2.8 <sup>c</sup>	5.6
<i>Asplanchna</i> sp	19.0 $\pm$ 2.2 <sup>b</sup>	12.3	25.5 $\pm$ 6.2 <sup>a</sup>	12.7	24.3 $\pm$ 2.1 <sup>ab</sup>	16.7
Others	4.6 $\pm$ 3.0	3.2	5.5 $\pm$ 2.2	2.9	3.3 $\pm$ 2.1	2.1
<b>Copepods</b>						
<i>Cyclops</i> sp	16.5 $\pm$ 2.6 <sup>b</sup>	11.0	21.3 $\pm$ 2.8 <sup>a</sup>	10.3	18.3 $\pm$ 1.6 <sup>ab</sup>	12.6
<i>Diaptomus</i> sp	10.8 $\pm$ 3.4 <sup>b</sup>	7.1	20.1 $\pm$ 5.7 <sup>a</sup>	9.8	14.5 $\pm$ 2.0 <sup>ab</sup>	10.5
<b>Cladocerans</b>						
<i>Diaphanosoma</i> sp	9.3 $\pm$ 2.2	5.8	16.3 $\pm$ 3.2	7.1	-	-
<i>Daphnia</i> sp	15.6 $\pm$ 3.7 <sup>a</sup>	10.4	16.0 $\pm$ 3.1 <sup>a</sup>	7.8	15.0 $\pm$ 5.1 <sup>a</sup>	10.5
<i>Moina</i> sp	7.6 $\pm$ 1.8 <sup>a</sup>	5.2	10.0 $\pm$ 7.1 <sup>a</sup>	4.9	13.6 $\pm$ 3.7 <sup>a</sup>	9.8
<i>Ceriodapnia</i> sp	-	-	4.5 $\pm$ 2.8	2.5	-	-
<b>Total (N)</b>	<b>154</b>		<b>204</b>		<b>143</b>	



**Table 3:** Specific growth rate (SGR) and diversity ( $H'$ ) of each zooplankton taxa in every treatment, and the coefficient of variation (CV) of each treatment. The values are mean  $\pm$  SD. One-way ANOVA, Tukey HSD test, different letters in each row represent significant differences at  $p < 0.05$ ;  $a > b > c$

		Treatments		
Parameters	Zooplankton taxa	FWD <sub>A</sub>	FWD <sub>B</sub>	Control
SGR ( $\text{day}^{-1}$ ) ( $n=3$ )	Rotifera	$0.42 \pm 0.02^b$	$0.48 \pm 0.01^a$	$0.35 \pm 0.02^c$
	Copepoda	$0.42 \pm 0.04^a$	$0.48 \pm 0.03^a$	$0.32 \pm 0.02^b$
	cladocera	$0.51 \pm 0.06^b$	$0.65 \pm 0.03^a$	$0.48 \pm 0.03^b$
Diversity ( $H'$ ) ( $n=6$ )	Rotifera	$1.44 \pm 0.14^a$	$1.51 \pm 0.21^a$	$1.36 \pm 0.14^a$
	Copepoda	$0.67 \pm 0.05^a$	$0.69 \pm 0.01^a$	$0.68 \pm 0.02^a$
	cladocera	$1.05 \pm 0.17^a$	$1.30 \pm 0.06^a$	$0.69 \pm 0.20^a$
CV ( $n=3$ )		$0.09 \pm 0.03^a$	$0.05 \pm 0.02^a$	$0.06 \pm 0.01^a$



**Figure 2:** Schematic flow of nutrients from FWD, CME, detritus and carbon source (C) to biotic communities in the tank culture system. FWD = fishwaste diet; CME = chicken manure extract

**4. Discussion**

In larviculture, management of zooplankton on forage base is a critical phase for successful transition of fish larvae to fingerling stage. The dynamic characteristics of zooplankton populations have led researchers to apply different techniques to produce high densities of desired zooplankton species until fish are either harvested, or able to consume inert feeds. Despite significant progress in zooplankton cultivation techniques, cost-effective and stable protocols for producing sufficient quantities of the desired zooplankton species without algae are scanty. High density algal production is expensive and laborious, thus becoming a burden for most hatcheries. This study demonstrated the feasibility of FWD made by blending CME and fishwastes for culturing zooplankton species in an outdoor mass culture system.

Despite changes in DO, conductivity and pH during the experimentation period (Table 1), the values remained within the acceptable limits for freshwater pond aquaculture [29]. More than half of zooplankton species in every sample consisted of rotifers (Table 2). Rotifers, especially the *Brachionus* spp. are normally the first zooplankters to reach large numbers in newly colonized

habitats, thus taking competitive advantage [30,31]. With the shortest lifespan of 5 - 12 days, rotifers reach peak reproductive levels much earlier (about 3.5 days) than other zooplankters [32]. Copepods and cladocerans have similar life span (about 50 days) but have different peak reproductive periods which take about 24 and 15 days to reach, for copepods and cladocerans, respectively [33]. The copepods only reproduce sexually and therefore require longer periods to increase their population [32]. In addition, overcrowding decreases fecundity of the calanoid copepods and the reproduction of the harpacticoid copepod, e.g. *Tigriopus japonicus* is more successful when mixed-cultured with rotifers, perhaps due to inter- and intra-specific interactions [34].

Addition of CME to the fish wastes caused significant increase in SGR and population densities of the zooplankton species and therefore, appears as an improvement to the FWD. Decomposing fish tissues are known to provide substrates for the proliferation of diverse microbial flora, some of which have probiotic properties [16]. The CME probably facilitated phytoplankton growth in the tanks, thus expanding forage base (i.e. bacteria and phytoplankton) for the zooplankton growth and reproduction. This demonstrated the importance of the synergy of FWD and CME for zooplankton growth and reproduction. Chicken manure is also an excellent substrate for probiotic bacteria [12,35], and contains growth promoting compounds e.g. 17 $\alpha$  and  $\beta$ -estradiol that can positively influence zooplankton reproduction history [9] either individually or in combination [18]. The efficacy of CME in enhancing the production of *Diaphanosoma celebensis* and *Tigriopus japonicus* has been reported where up to 14 ind ml<sup>-1</sup> was produced within 12 days [36]. In our previous study, 2 ml l<sup>-1</sup> of CME enhanced parthenogenetic reproduction of *B. angularis* significantly [13]. It is hypothesized that omnivorous zooplankton species feed on detritus-bacteria complex in the absence of live prey. Therefore, fungi and bacteria associated with decaying organic substances could supplement phytoplankton foraging with essential proteins, lipids and vitamins to cause high growth effects for zooplankton. For this reason, phytoplankton alone do not necessarily increase zooplankton populations, thus explaining the relatively low zooplankton density in the control experiment. In this study, the zooplankton population densities increased to new peaks comparable to the previous ones after the first harvest, suggesting that the system can be self-sustaining, perhaps with frequent harvesting to reduce organic loads. Generally, the lower CV of the treatments suggests stability of the FWD technology. With application of FWD<sub>B</sub>, it is possible to obtain about 150, 12, and 8 ind ml<sup>-1</sup> of rotifers, copepods and cladocerans, respectively, on weekly basis. Comparatively, [37] obtained about 76 rotifers ml<sup>-1</sup> within 5 days using conventional chicken manure methods in ponds and tanks, respectively. The results of the present study demonstrate the superiority of FWD technique compared to the normal traditional live food culture methods using chicken manure.

The FWD technology embodies a complex microbial control system that leads to degradation of waste materials, facilitates the recycling of nutrients and proliferation of microflora that flourishes under optimum C/N ratio. The FWD technology represents a biofloc microcosm where the decomposing fish tissues produce diverse microbial flora, which also form zooplankton diet. The system also attracts opportunistic benthic community that feed on the detritus and bioflocs. In addition, the dissolved nutrients from FWD, in presence of sunlight, facilitate primary production of phytoplankton that is grazed upon by the zooplankters. The complex food web that exists in FWD aided culture system is summarized in Figure 2.

Initiatives geared towards developing nutrition strategies such as bioflocs and periphyton that maximizes the contribution of natural and supplemental feeds in culture facilities would expand aquaculture production. Even though FWD can be used as cheap and stable diet for zooplankton production, the success of the FWD technology could be limited by presence of pathogenic microbes, which may affect the cultured animals. Copepods produced extensively may cause mass mortalities in cultured fishes through transmission of viruses and parasites.

In terms of production costs, successful microalgal culture in conventional larviculture systems basically depends on availability of optimal parameters such as nutrients, light, pH range, aeration, temperature and salinity. In addition, high ratio of algal biomass to target species (usually about 5-10:1) elevates the cost of microalgae production [38]. Average, mono-specific algal cultures cost up to USD 120-200 kg<sup>-1</sup> DW, where labour accounts for 50-85%, pumping; 4-24%, nutrients; 4-20% and mixing; 5-8% of the total production costs [38]. Consequently, high microalgae production costs

influence the zooplankton production costs. For example, the estimated cost of rotifer mass production using large scale batches is USD 4.5/ million rotifers where feeds, live algae and yeast count for 72, 50 and 22% of the production costs, respectively [38]. Comparatively, the FWD is significantly cheaper because fishwaste, which is the main ingredient in the diet, can be obtained for free. In addition, the efficacy of the FWD can be improved by addition of cheap carbon source. In general, the estimated cost of rotifer production in 500 l culture tanks using FWD is approximately USD 0.01/ million rotifers.

FWD medium can be used as an enrichment emulsion for low-quality live food diets such as Baker's yeast. Other home-made emulsion products have been produced using fish oil and egg yolk as cheap ways of enriching rotifer cultures, but these products are prone to oxidation and have short shelf life that limits their application in aquaculture. The bacteria-held PUFAs (in FWD) are more protected against oxidation, and provide a variety of other natural nutrients that meet the species-specific nutritional requirements of the rotifers, and their predators [39]. The FWD technology is a universal innovation that can be applied for both marine and freshwater live food production. Bioflocs are important in aquaculture, and has been used to improve growth rate of rotifers [40] and cultivable fishes [41]. FWD appears to be a major leap toward making pre-planning of fish seedling production in aquaculture facilities feasible.

## 5. Conclusion

To eliminate microalgae from aquaculture production chain, FWD is a promising alternative. FWD combined with CME produced about 150, 12, and 8 ind ml<sup>-1</sup> of rotifers, copepods and cladocerans, respectively, on weekly basis in outdoor culture tanks. FWD production protocol is cheap and costs about USD 0.01/ million rotifers. Therefore, FWD is convenient for profitable aquaculture production particularly in the regions without sophisticated infrastructural investments for high dense microalgal production. However, further studies should document a comprehensive account of the microbial flora associated with the FWD, including their successive colonization overtime. There is need to test the suitability of other environmental wastes e.g. from livestock abattoirs for live food production and the suitability of the FWD-fed zooplankton species for larviculture of the local fish species. The FWD offers an opportunity to 1) reduce environmental pollution sources by reusing poorly discarded fishwastes; 2) reduce or eliminate direct dependence on the immediately cultured or the expensive on-site microalgae production and, 3) to lower the cost of zooplankton enrichment, thus making it convenient for aquaculture production, especially in the less developed countries, where malnutrition is prevalent.

## Acknowledgements

This study was partly supported by JSPS KAKENHI grant No. JP17H03862 and JSPS, Asia-Africa No. 24380108 to Atsushi Hagiwara and by The Ministry of Research, Technology and Higher Education of Indonesia (Kemenristekdikti) under PPUPT research grant to SW. Erick Ochieng Ogello is grateful to the Ministry of Education, Culture, Sports, Science and Technology for the MEXT PhD. scholarship. We thank our anonymous reviewers for strengthening the scientific merit of our manuscript.

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