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BLENDING FISHWASTES AND CHICKEN MANURE EXTRACT AS A LOW-COST AND STABLE DIET FOR PLANKTONIC LIVE FOOD PRODUCTION

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Introduction

The global increase of aquaculture has expanded the demand for the zooplankton as live foods for larviculture. Although freshly cultured microalgae is the common diet for zooplankton, microalgal culture protocols are laborious and costly, thus limiting continuous production of sufficient zooplankton and sometimes disrupts larval fish production in the microalgae-based hatcheries (Lubzens et al., 1995). Alternatively, cheaper diet (e.g. baker's yeast) has been used as live food diet but culture instabilities due to bacterial flora imbalance are common. Other products (e.g. condensed microalgae and artificial diets such as Selco (Inve-Co. Ltd) are also commercially available but costly for most fish farmers, especially in developing countries and some parts of Asia, which are potential future leaders in marine larviculture production. Therefore, research studies are needed to develop cheap and stable microalgal replacement diets for profitable aquaculture. This study investigated the feasibility of a fishwaste diet (FWD) made of fishwastes (heads) and chicken manure extract (CME) at optimum carbon/nitrogen ratio as a cost-effective and stable diet for planktonic live food production.

Materials and methods

CME and FWD preparation
About 1kg of fermented chicken manure was boiled in 5 l of pondwater for 40-50min. The supernatant liquid was filtered and used as CME (Ogello and Hagiwara, 2015). The CME, minced heads (using meat mincer) of *Barbus altinalis* (Cyprinidae) and starch (maize flour) was used to make FWD treatments as follows: FWD_A ($0.5g.l^{-1}$ of fishwaste + $0.2g.l^{-1}$ of starch), FWD_B (FWD_A + $2ml.l^{-1}$ of CME), and control ($2ml.l^{-1}$ of CME only). Our FWD preparation protocol has so far been patented under the registration number P00201609066 in Indonesia.

Outdoor cultures

The outdoor experiments were conducted in 9 asbestos tanks each containing 500 l of underground water (plankton free) for 16 days in Kenya. The inoculant rotifers, copepods, and cladocerans were obtained from a freshwater fishpond which was previously fertilized with diammonium phosphate (DAP) and urea. Each treatment was triplicated 3 days before inoculation with 5, 2, and 0.4 $ind.ml^{-1}$ of rotifers, copepods and cladocerans, respectively in each tank. A random sample of 5ml of water was done daily in each tank, from which the zooplankters were counted under lugol fixation. Partial harvesting was done at every zooplankton exponential growth phase by replacing 50% of the water with fresh medium and FWD. The zooplankters were identified to the genus level and the specific growth rate (SGR) was calculated as $r = [\ln N_t - \ln N_0].t^{-1}$, where, N_o = initial population density, N_t = population density after time t (days). The coefficient of variation (CV%) was computed as standard deviation ÷ mean SGR × 100% to determine stability of the cultures.

Laboratory cultures

The laboratory experiment was conducted in Nagasaki University, Japan. Here, minced heads of the Chub mackerel *Scomber japonicus* and starch (wheat flour) was used to develop the FWD, and used to culture the euryhaline rotifer, *Brachionus rotundiformis* (SS-type) in 30-l tanks of seawater (22ppt) at 28±1°C for 18 days. The FWD treatments were: FWD_1 (fishwastes only), FWD_2 (FWD_1+ starch), and control (*Chlorella vulgaris* only). CME was not used in this experiment. The zooplankton population density was monitored daily in three 1-ml water samples, and SGR and CV% were calculated as mentioned previously.

For the microbial analysis, Zobell marine agar 2216 (Difco™ Becton, Dickinson & Co. France) was used to make agar solution under sterile conditions and allowed to solidify, for plating. Some rotifer biomasses from each treatment were washed with distilled water (to remove external flora), dried using filter paper, homogenized, and serially diluted up to 10^{-6}. Then, 0.1ml of the diluted aliquots were seeded over the surface of solidified agar and incubated at 32°C for 48 hours. Similar dilutions were done for the culture medium from each treatment. Different bacterial colonies were aseptically isolated for biochemical identification according to the Bergey's manual (Holt et al., 1994). The rotifers were harvested, washed, dried and preserved at -80°C for total lipid analysis. Repeated analysis of variance (ANOVA) was used to determine the effects of the FWD on the zooplankton population growth and, the Tukey's HSD Post Hoc test were performed to locate any statistical differences at $p<0.05$.

Results and discussion

In the outdoor experiment, there was significantly higher densities of each zooplankton taxa and SGR in FWD_B than FWD_A and control tanks ($p<0.05$). On day

7, the zooplankton densities in FWD_A, FWD_B and the control tanks, respectively were as follows: rotifers: 100.6±14.8, 146.3±7.0, and 60.0±7.9 ind.ml^{-1}; the copepods: 8.0±11.1, 12.6±13.6, and 4.3±2.1 ind.ml^{-1}; the cladocerans: 3.3±6.0, 8.6±8.7 and 3.6±2.5 ind.ml^{-1}. The most abundant genera were *Brachionus* sp., *Cyclops* sp. and *Daphnia* sp. for rotifers, copepods, and cladocerans, respectively. Highest SGR (day^{-1}) were realized with FWD_B for rotifera (0.48±0.01), copepod (0.26±0.04) and cladocera (0.42±0.03). FWD did not affect the CV%, which were 13.74±7.73, 7.74±6.64, and 15.26±11.39 for FWD_A, FWD_B and control cultures, respectively. The CME provided growth hormones (Yang and Snell, 2010), and facilitated phytoplankton growth, while the fishwastes aided the proliferation of microbial flora, thus expanded zooplankton forage base in the FWD_B cultures.

Table I. The specific growth rate (SGR) as at day 4, 9, and 13 of the rotifer, *B. rotundiformis* cultured with the FWD and control diet and, CV (%) of the treatments. Partial harvesting (50%) was done on days 5, 10, and 14. Two-way ANOVA, Tukey HSD test, a>b; different superscripts each day indicate significant differences at $p<0.05$; $n=3$. For CV (%), One-way ANOVA, $p=0.43$, $n=3$.

Day	Treatments		
	FWD_1	**FWD_2**	**Control**
4	0.78 ± 0.04^{a}	0.81 ± 0.04^{a}	0.78 ± 0.04^{a}
9	0.60 ± 0.09^{ab}	0.69 ± 0.06^{a}	0.44 ± 0.02^{b}
13	0.61 ± 0.07^{ab}	0.76 ± 0.05^{a}	0.58 ± 0.07^{b}
CV (%)	11.43 ± 5.18^{a}	7.47 ± 1.68^{a}	8.01 ± 3.72^{a}

In the laboratory cultures, there were significantly higher rotifer population densities and SGR in FWD_2 than FWD_1 and control tanks ($p<0.05$). About 1200 rotifers.ml^{-1} was obtained in FWD_2 between days 8-12. The FWD did not affect the CV. The SGR at different culture stages and the CV is presented in Table I. The FWD-cultured rotifers ingested bacterial species such as *Pseudomonas* sp., *Bacillus* sp., *Thiocapsa* sp., and *Shewanella* sp., while the control-rotifers mostly ingested *Micrococcus* sp. About 0.35 and 0.39mg.g^{-1} of DHA and EPA, respectively, was obtained in the FWD-cultured rotifers and both were under detectable the limit in the control rotifers. High rotifer growth in the FWD cultures is attributed to the ingested probiotic bacteria species, which have been found to increase rotifer growth rates and densities (Yasuda and Taga, 1980; Hagiwara et al., 1994). The FWD appears to be a nutritionally rich microalgal replacement diet with essential fatty acids and probiotics that can be beneficial to the cultured fish larvae.

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