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The positive contributions of PowerLac™ supplementation to the production performance, feed utilization and disease resistance of Nile tilapia Oreochromis niloticus (L.)

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Abstract

The objective of this research was to determine the optimum dietary supplementation level of a probiotic (PowerLac™), for improving Nile tilapia *Oreochromis niloticus* growth performance and feed utilization, as well as enhanced protection against disease. For laboratory experimentation, a completely randomized experimental design, consisting of four treatments of a dietary probiotic (PowerLac™, containing *Lactobacillus lactis* D1813) supplementation at different levels (0.25, 0.5, 1.0 and 2.0 g kg^{-1}), against a control (0 g kg^{-1}), was performed for 8 weeks (of culture period). For field experiments, three treatments of different dietary probiotic supplementation levels (0.25, 0.5 and 1.0 g kg^{-1}), plus a control, were employed for 22 weeks (of culture period). Under controlled experimental conditions, in the first experiment, significantly higher fish final body weight, growth and lower food conversion ratio (FCR) were achieved by treatments with dietary probiotic supplementations of 0.25 and 0.5 g kg^{-1} ($P < 0.05$). In strong partial support of this, at field experimentation level, a dietary PowerLac™ supplementation of 0.5 g kg^{-1} showed the most pronounced results, as indicated by the higher growth, and protein and lipid retention, as well as lower FCR, and reduced mortality following the *Aeromonas hydrophila* challenge test ($P < 0.05$).

Keywords: dietary probiotics, PowerLac™, Nile tilapia, feed utilization, disease resistance, production

Introduction

Tilapia is one of the most important aquaculture species with the fastest supply growth at global level (Food and Agriculture Organization [FAO] 2014). In 2011, the world production of tilapias has reached about 7 million MT and is projected to increase by 30% in 2030 (FAO 2014). Despite the elevated global demand, the sustainability of tilapia production is still hampered by some factors including disease outbreak. For instance, Lusiastuti, Textor, Seeger, Akineden and Zschöck (2014) noted that with the culture intensification, the prevalence of *Streptococcus agalactiae* has been increasing and has caused mortality and morbidity in the cultured tilapia in various tilapia producing countries including Indonesia. More recent report by Dong, Nguyen, Le, Sangsuriya, Jitrakorn, Saksmerprome, Senapin and Rodkhum (2015) pointed out that disease outbreaks, caused by bacterial and viral pathogens infection, occurred annually in Nile tilapia farms along the Mekong river in Thailand and resulted in severe economic losses.

One of the strategies to control disease in aquaculture is the application of probiotics and immunostimulants. Probiotics in aquaculture is defined as live microbial feed supplement that beneficially affect the host animal by modifying the host's microbial community, improving feed nutrient utilization, enhancing the host's immune response against disease and improving the culture environmental quality (Verschuere, Rombaut, Sorgeloos & Verstraete 2000; Maeda, Shibata, Biswas,

Korenaga, Kono, Itami & Sakai 2014). Recently, Dash, Raman, Prasad, Makesh, Pradeep and Sen (2015) introduced a new concept of paraprobiotics in aquaculture, which is defined as non-viable microbial cells that are expected to be beneficial for the host. Studies showed that probiotics and/or paraprobiotics application could significantly control disease infection in various aquaculture species including tilapia (Pirarat, Kobayashi, Katagiri, Maita & Endo 2006; Wang, Tian, Yao & Li 2008; Selim & Reda 2015). Furthermore, probiotics and/or paraprobiotics can be incorporated in the aquaculture feed not only to enhance the fish resistance to disease infection and stress but also to improve the fish growth and feed efficiency (Ng, Kim, Romano, Koh & Yang 2014; Talpur, Munir, Mary & Hashim 2014; Iwashita, Nakandakare, Terhune, Wood & Ranzani-Paiva 2015; Selim & Reda 2015; Van Hai 2015).

PowerLac™ is a commercial probiotics consisting of heat-killed and lyophilized *Lactococcus lactis* strain D1813, which was isolated from the intestine of wild-captured Kuruma shrimp (*Marsupenaeus japonicus*) in Tachibana Bay, Nagasaki prefecture, Japan (Maeda *et al.* 2014). Previous studies showed that the use of this bacterium as a probiotic resulted in a significant increase in the transcript level of lysozyme in the hepatopancreas of Kuruma shrimp and a significant improvement in the *Vibrio penaecida* post-challenged survival (Maeda *et al.* 2014). The objective of this study is to determine the optimum dietary supplementation level of PowerLac™, which best improves Nile tilapia *Oreochromis niloticus* growth performance and feed utilization and enhances protection against disease infection.

Materials and method

Experimental design

The experiment consisted of one experiment in the laboratory and one in the field. For the former, a completely randomized experimental design with four treatment levels of PowerLac™ dietary supplementation (0.25, 0.50, 1.00 and 2.00 g kg^{-1}, later denoted as treatments L0.25, L0.5, L1.0 and L2.0 respectively) and a control (0 g kg^{-1}, treatment L0) in triplicate was performed for an 8-week period. Based on the results of the laboratory experiment, three different dietary probiotics mixtures (0.25, 0.50 and 1.00 g kg^{-1}, later denoted as treatments F0.25, F0.5 and F1.0 respectively) and one control (F0) in triplicate were employed in the field experiment with a culture period of 22 weeks.

Experimental diets

Experimental diets were prepared using local feed ingredients (Table 1). The pelleting and drying processes of the diet were performed at a temperature of 70–80°C. Proximate composition of experimental diets is presented in Table 1.

Experimental setup

For the laboratory experiment, 15 tanks (100 × 50 × 50 cm) filled with 200 L of dechlorinated freshwater were prepared indoor (12 h per 12 h photoperiod), each with an individual recirculating and heating system. Nile tilapia with an average body weight 11.07 ± 0.07 g (previously acclimatized to laboratory condition for 2 weeks) were randomly stocked at a density of 20 fish $tank^{-1}$ (80 fish m^{-3}). For the field experiment, 12 concrete outdoor tanks (3 × 2 × 0.7 m) were filled with 3 m^3 of dechlorinated water. Nile tilapia with an average body weight 13.7 ± 0.2 g was randomly stocked at a density of 50 fish $pond^{-1}$ (17 fish m^{-3}). In both indoor and outdoor, feeding was provided to satiation three times daily at 07.00 h, 13.00 h and 17.00 h. Feeding satiation was determined according to visual observation with an approximate feeding period of 5 min per tank. To monitor fish survival and growth, biomass sampling was performed every 2 weeks (laboratory) and 4 weeks (field experiment), starting from day 15 to day 29 respectively.

Water quality in each culture unit in laboratory experiment was maintained by individual recirculating system. Water quality monitoring was performed by daily measurement of water temperature and dissolved oxygen, as well as weekly measurement of total ammonia nitrogen (TAN, APHA 1998) and pH. Water quality *in situ* measurements and water sample collection in both laboratory and field experiments were conducted at 07.00. Water quality parameters, both in laboratory and field experiments, were within acceptable ranges for optimal growth of tilapia. Faecal materials in the aquaria or the tanks were siphoned out together with daily water replacement at about 5–10% of the total water volume.

Table 1 Composition of experimental diets

	L0 (0 g kg^{-1})	L0.25 (0.25 g kg^{-1})	L0.5 (0.50 g kg^{-1})	L1.0 (1.00 g kg^{-1})	L2.0 (2.00 g kg^{-1})
Feed ingredients (%)					
Meat bone meal	9.00	9.00	9.00	9.00	9.00
Soybean meal	33.50	33.50	33.50	33.50	33.50
Corn gluten meal	8.00	8.00	8.00	8.00	8.00
Pollard	39.20	39.18	39.15	39.10	39.00
Palm kernel oil	3.00	3.00	3.00	3.00	3.00
Fish Oil	0.60	0.60	0.60	0.60	0.60
$CaHPO_4$	2.00	2.00	2.00	2.00	2.00
Cassava meal	2.00	2.00	2.00	2.00	2.00
Polymethyl carbamide	0.20	0.20	0.20	0.20	0.20
Vitamin and mineral premix*	2.50	2.50	2.50	2.50	2.50
PowerLac™	0.00	0.025	0.05	0.10	0.20
Proximate composition					
Protein (%)	31.75	30.93	31.69	30.64	31.13
Lipid (%)	7.56	7.74	7.54	7.61	7.79
Fibre (%)	8.29	7.33	6.27	6.4	6.61
Ash (%0	15.56	15.56	15.96	15.95	16.06
NFE** (%)	36.85	38.45	38.54	39.4	38.41
Energy (KcalGE kg^{-1})	4304	4351	4379	4390	4381

*Vitamin and mineral premix composition: Retinol (A) 900 IU kg^{-1}; ascorbic acid (C) 200 mg kg^{-1}; cholecalciferol (D) 200 IU kg^{-1}; menadione (K3) 10.0 mg kg^{-1}; d/l α-tocopherol (E) 100 mg kg^{-1}; choline 1000 mg kg^{-1}; inositol 100 mg kg^{-1}; thiamine (B1) 15 mg kg^{-1}; riboflavin (B2) 20 mg kg^{-1}; pyridoxine (B6) 15 mg kg^{-1}; d-pantothenic acid (B5) 50 mg kg^{-1}; nicotinic acid 75 mg kg^{-1}; biotin 0.5 mg kg^{-1}; cyanocobalamin (B12) 0.05 mg kg^{-1}; folic acid 5 mg kg^{-1}; Co (as $CoCl_2{\cdot}6H_2O$) 0.5 mg kg^{-1}; Cu (as $CuSO_4{\cdot}5H_2O$) 5 mg kg^{-1}; Fe (as $FeSO_4{\cdot}7H_2O$) 50 mg kg^{-1}; I (as KI) 4 mg kg^{-1}; Cr (as $CrCl_3{\cdot}6H_2O$) 0.1 mg kg^{-1}; Mg (as $MgSO_4{\cdot}7H_2O$) 150 mg kg^{-1}; Mn (as $MnSO_4{\cdot}H_2O$) 25 mg kg^{-1}; Se (as $NaSeO_3$) 0.1 mg kg^{-1} and Zn (as $ZnSO_4{\cdot}7H_2O$) 100 mg kg^{-1}.
**NFE, nitrogen-free extract.

Zootechnical parameters

Sampling was performed weekly to monitor fish growth and survival. Specific growth rates were calculated according to Huisman (1987). The feed conversion ratio was calculated by dividing the total amount of feed given in each replicate by the total fish biomass gain. Protein and lipid retention were determined according to the formula: (protein or lipid in final fish biomass wet weight minus protein or lipid in initial fish biomass wet weight)/protein or lipid in total feed × 100%.

Physicochemical analyses

Proximate composition of the experimental diets and fish were determined according to the procedures described in Takeuchi (1988). RNA/DNA ratios in liver, and digestive enzyme activity (amylase, trypsin and pepsin) in the fish intestine, were measured on the final day of the laboratory experiment. RNA and DNA extraction was performed using extraction kits ISOGEN (Nippon Gene, Toyama, Japan) and Puregene (Qiagen, Hilden, Germany) respectively. The concentrations were subsequently measured using DNA/RNA Quant. Protein concentration in the intestine for digestive enzyme calculation was determined according to Bradford (1976), whereas amylase and pepsin activities were analysed according to Worthington (1993), and trypsin activity was determined according to Borlongan (1990).

Haematological analyses

Blood samples were taken from the caudal arch of anaesthetized fish (150 mg L^{-1} tricaine methanesulfate) using a 25-gauge needle and a 3-mL heparinized syringe. For the laboratory experiment, blood samples were collected to measure glucose concentration, total erythrocyte, total leucocyte, haemoglobin, haematocrit and phagocytic index at the end of the experiment. For the field experiment, blood samplings were performed prior and after the challenge test to measure total erythrocyte, total leucocyte, haemoglobin, haematocrit and phagocytic index, all of which were determined according to Blaxhall and Daisley (1973), except for blood glucose which was measured according to Dubowski (1962).

Challenge test

To evaluate fish resistance to disease infection, a challenge test using the pathogenic *Aeromonas hydrophila* was performed on the final day of the field experiment. Prior to the challenge test, a preliminary experiment was performed to determine the LD_{50} (lethal dose) of the pathogenic bacteria. For the challenge test, 10 healthy fish were selected from each replicate tank and transferred into another tank previously filled with dechlorinated freshwater. *Aeromonas hydrophila* was cultured for 24 h in a tryptic soy broth medium and diluted to 10^6 cell mL^{-1}, using phosphate buffer saline (PBS). The fish were anaesthetized using tricaine methanesulfonate and intramuscularly injected with 1 mL of the bacteria suspension. As a negative control, 10 healthy fish were collected from treatment F0 (0 g kg^{-1}) and injected with 1 mL of PBS. The challenge test was performed for 2 weeks, and blood samples were subsequently collected to measure blood parameters and respiratory burst.

Statistical analyses

All data were statistically evaluated using analyses of variance (ANOVA), and any significant difference between treatments was subsequently determined by post hoc Duncan's test. Statistical analyses were performed by using the statistical program SPSS version 13.0 (IBM, New York, USA).

Results

Laboratory experiment

Growth and feeding performance

Growth and feeding performance of Nile tilapia from the laboratory experiment are presented in Table 2. There was no difference in fish survival, which was 100% for all treatments. Significantly higher fish final body weight and growth, and significantly lower feed conversion ratio were shown by treatments L0.25 and L0.5 ($P < 0.05$).

Digestive enzymes activity and RNA/DNA ratio in liver

The addition of PowerLac™ to Nile tilapia diet did not affect the activity of amylase and trypsin in the fish digestive tract (Fig. 1). As for pepsin activity, the fish fed with L2.0 diet showed considerably lower activity compared with other treatments in this experiment. The RNA/DNA ratio in fish liver at the end of the laboratory experimentation presented in Fig. 2 showed no significant differences between treatments.

Blood parameters

There were no significant differences observed in total erythrocyte, total leucocyte, haemoglobin (Hb) and haematocrit amongst treatments (Table 3). However, treatments L0.5 and L1.0 showed significantly higher phagocytic indexes. Furthermore, treatment L0.5 also showed the highest blood glucose concentration compared with other treatments in this experiment.

Field experiment

Growth and feeding performance

The growth and feeding performance of Nile tilapia in the field experiment confirm the results from laboratory-scale experiment, where treatment F0.5 showed the highest values, and the differences were significant ($P < 0.05$) in comparison to other treatments (Table 4).

Table 2 Growth and feed utilization of Nile tilapia *Oreochromis* sp. fed with a practical diet supplemented with different levels of PowerLac™ (0 g kg^{-1}, 0.25 g kg^{-1}, 0.50 g kg^{-1}, 1.00 g kg^{-1} and 2.00 g kg^{-1}) at laboratory experiment. Different superscript letters following mean values within the same row indicate significant differences ($P < 0.05$)

Parameter	L0 (0 g kg^{-1})	L0.25 (0.25 g kg^{-1})	L0.5 (0.50 g kg^{-1})	L1.0 (1.00 g kg^{-1})	L2.0 (2.00 g kg^{-1})
Survival (%)	100 ± 0^a	100 ± 0^a	100 ± 0^a	100 ± 0^a	100 ± 0^a
Final body weight (g)	43 ± 1^a	47 ± 1^b	47 ± 1^b	42 ± 1^a	42 ± 0^a
SGR[1] (% per day)	2.92 ± 0.04^a	3.05 ± 0.10^b	$3.03 \pm 0.05a^b$	2.89 ± 0.08^a	2.89 ± 0.08^a
Total feed (kg)	1.28 ± 0.003^a	1.28 ± 0.001^a	1.27 ± 0.013^a	1.26 ± 0.015^a	1.22 ± 0.005^b
FCR[2]	2.01 ± 0.05^a	1.87 ± 0.13^b	1.79 ± 0.03^b	2.03 ± 0.11^a	1.95 ± 0.01^a
Protein retention (%)	26 ± 1^a	31 ± 4^a	30 ± 2^a	26 ± 3^a	28 ± 2^a
Lipid retention (%)	44 ± 5^a	47 ± 7^a	55 ± 6^a	48 ± 9^a	56 ± 13^a

SGR, specific growth rate; FCR, feed conversion ratio.

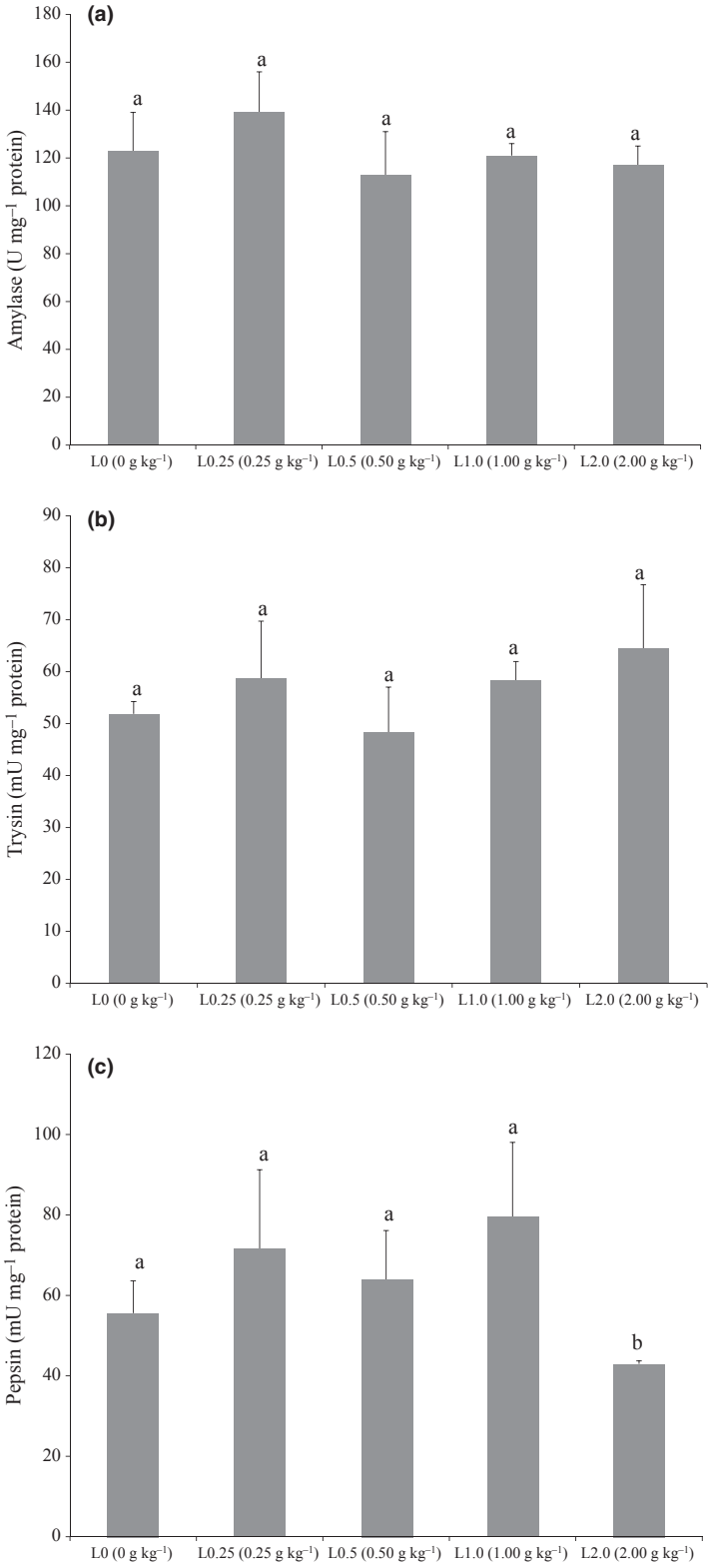

Figure 1 Digestive enzyme activity: (a) amylase, (b) trypsin and (c) pepsin, in the intestine of Nile tilapia *Oreochromis* sp. fed with a practical diet containing different PowerLac supplementation levels (0 g kg^{-1}; 0.25 g kg^{-1}; 0.50 g kg^{-1}; 1.00 g kg^{-1} and 2.00 g kg^{-1}). Different letters in each bar indicate significant difference ($P < 0.05$).

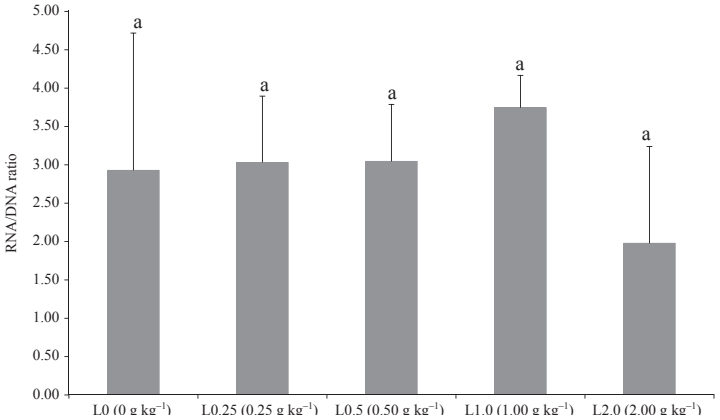

Figure 2 RNA/DNA ratio in the liver of Nile tilapia *Oreochromis* sp. fed with practical diet containing different PowerLac supplementation levels (0 g kg^{-1}; 0.25 g kg^{-1}; 0.50 g kg^{-1}; 1.00 g kg^{-1} and 2.00 g kg^{-1}). Different letters in each bar indicate significant difference ($P < 0.05$).

Table 3 Blood profile and glucose content of Nile tilapia *Oreochromis niloticus* fed with a practical diet supplemented with different levels of probiotics (0, 0.25, 0.50, 1.00 and 2.00 g kg^{-1}). Different superscript letters following mean values within the same row indicate significant differences ($P < 0.05$)

Parameter	L0 (0 g kg^{-1})	L0.25 (0.25 g kg^{-1})	L0.5 (0.50 g kg^{-1})	L1.0 (1.00 g kg^{-1})	L2.0 (2.00 g kg^{-1})
Blood glucose (mg mL^{-1})	0.45 ± 0.04^{a}	0.42 ± 0.02^{a}	0.67 ± 0.10^{b}	0.52 ± 0.01^{ab}	0.62 ± 0.03^{b}
Total erythrocyte ($\times 10^6$ cell mm^{-3})	3.79 ± 0.29^{a}	4.77 ± 0.49^{a}	4.08 ± 0.68^{a}	4.19 ± 0.27^{a}	4.23 ± 0.12^{a}
Total leucocyte ($\times 10^6$ cell mL^{-1})	2.10 ± 0.39^{a}	2.30 ± 0.18^{a}	2.73 ± 0.23^{a}	1.99 ± 0.04^{a}	2.25 ± 0.21^{a}
Haemoglobin (g%)	7.43 ± 1.78^{a}	7.13 ± 1.81^{a}	6.67 ± 1.47^{a}	6.47 ± 1.17^{a}	6.47 ± 0.31^{a}
Haematocrit (%)	26.48 ± 3.58^{a}	33.05 ± 5.03^{a}	28.95 ± 4.05^{a}	34.26 ± 6.99^{a}	24.74 ± 2.89^{a}
Phagocytic index (%)	18.96 ± 0.90^{a}	24.78 ± 3.06^{ab}	26.21 ± 2.38^{b}	31.52 ± 4.77^{b}	17.29 ± 2.81^{a}

Table 4 Growth and feeding performance of Nile tilapia *Oreochromis niloticus* fed with a practical diet containing different PowerLac™ supplementation levels (0, 0.25, 0.50 and 1.00 g kg^{-1}) at field experiment. Different superscript letters following the mean value within the same parameter row indicate significant differences

Parameter	F0 (0 g kg^{-1})	F0.25 (0.25 g kg^{-1})	F0.5 (0.50 g kg^{-1})	F1.0 (1.00 g kg^{-1})
Initial weight (g)	13.8 ± 0.2	13.5 ± 0.1	13.8 ± 0.2	13.6 ± 0.2
Final biomass (kg)	8.07 ± 0.7^{a}	10.02 ± 0.74^{b}	12.03 ± 0.61^{c}	9.50 ± 0.15^{b}
Survival (%)	91 ± 1^{a}	97 ± 3^{b}	99 ± 1^{b}	99 ± 1^{b}
SGR (% per day)	1.69 ± 0.06^{a}	1.81 ± 0.06^{b}	1.90 ± 0.02^{c}	1.75 ± 0.01^{ab}
FCR	3.50 ± 0.29^{a}	2.78 ± 0.21^{b}	2.31 ± 0.12^{c}	2.94 ± 0.05^{b}
Protein retention (%)	16.25 ± 1.34^{a}	19.62 ± 1.54^{bc}	23.34 ± 1.25^{c}	19.76 ± 0.33^{b}
Lipid retention (%)	17.91 ± 1.41^{a}	39.42 ± 2.95^{c}	48.99 ± 2.52^{d}	31.79 ± 0.5^{b}

SGR, specific growth rate; FCR, feed conversion ratio.

Haematological parameters

It can be seen in Fig. 3 (pre-challenge) that feeding the fish with diets supplemented with 0.50 g and 1.0 g of PowerLac™ per kg of feed for 22 weeks in the field experiment resulted in significantly higher total erythrocytes than the control and the 0.25 g kg^{-1} supplementation level. Likewise, these treatments also resulted in higher total leucocytes, only this time the 0.50 g kg^{-1} was not significantly different with the control and the 0.25 g kg^{-1} treatment. Following the challenge test, the fish fed with PowerLac™ supplemented diets showed significantly higher total leucocytes than the control. Phagocytic indexes in the fish in PowerLac™ treatments were also higher than that in the control; however, a significant difference was only observed in treatment F1.0.

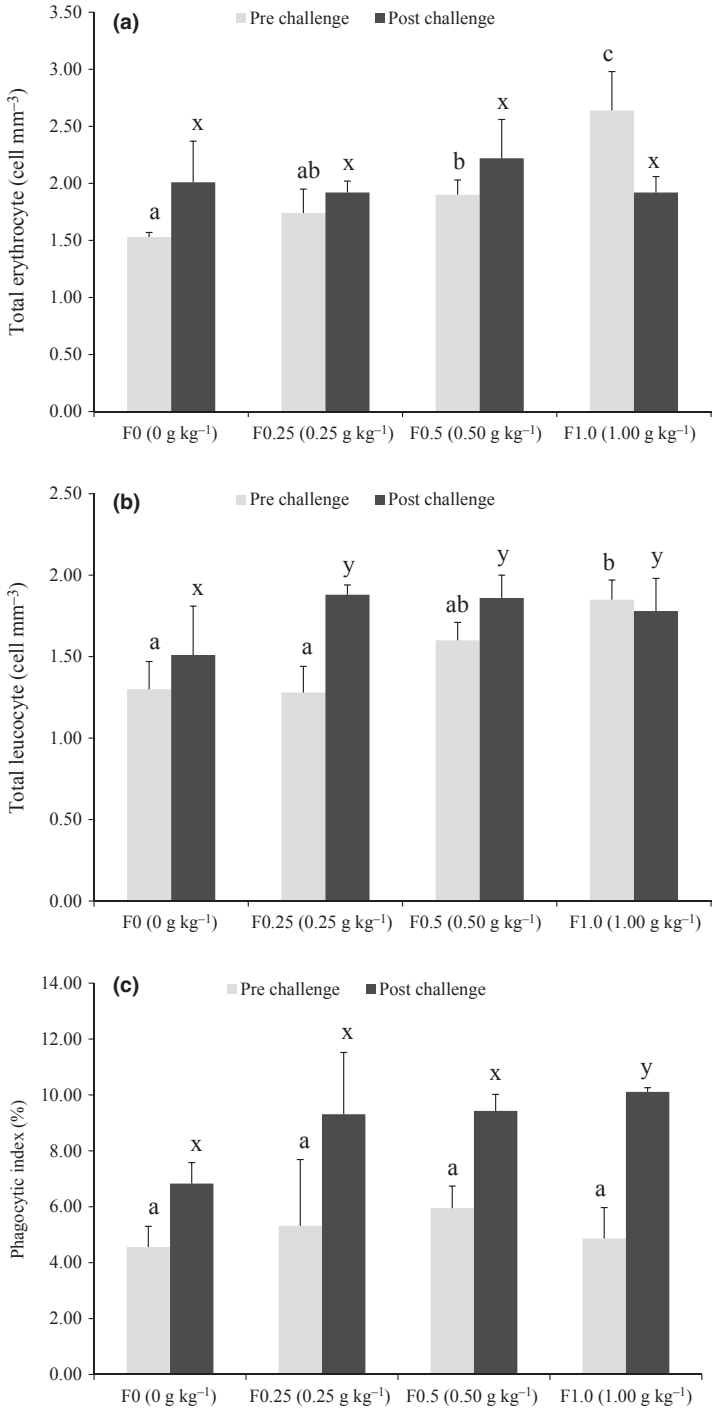

Figure 3 (a) Total erythrocytes (cell mm^{-3}), (b) total leucocytes (cell mm^{-3}) and (c) phagocytic index (%) of Nile tilapia (*Oreochromis niloticus*) fed with a practical diet containing different PowerLac™ supplementation levels (0 g kg^{-1}; 0.25 g kg^{-1}; 0.50 g kg^{-1} and 1.00 g kg^{-1}), before and after infection with *Aeromonas hydrophila* (10^6 cell mL^{-1}, 2 weeks). Different letters in the same colour bar indicate significant difference ($P < 0.05$).

Challenge test

Nile tilapia survival rates following the challenge test with *Aeromonas hydrophila* in PowerLac™ treatments were significantly higher ($P < 0.05$) than that of the control (F0) and were comparable to the negative control (Fig. 4).

Discussion

This study examined the effectiveness of heat-killed probiotics (PowerLac™) incorporated in the diet, in enhancing the growth, feed utilization and disease resistance of Nile tilapia, at both labora-

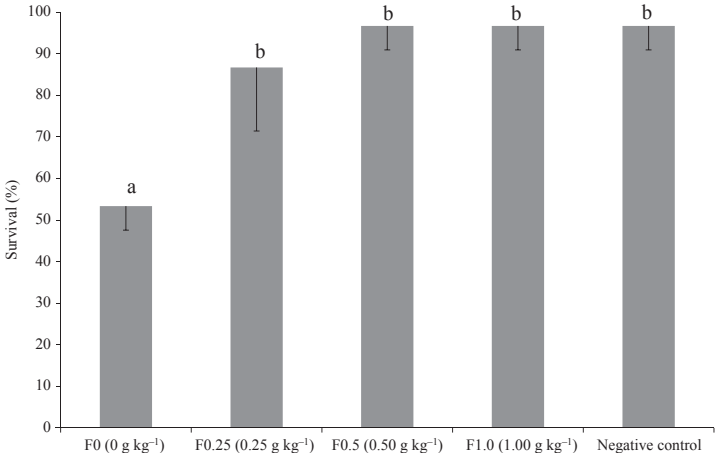

Figure 4 Survival of Nile tilapia (*Oreochromis niloticus*) fed with a practical diet containing different PowerLac™ supplementation levels (0 g kg^{-1}; 0.25 g kg^{-1}; 0.50 g kg^{-1} and 1.00 g kg^{-1}) and post-challenged with *Aeromonas hydrophila* (10^6 cell mL^{-1}, 2 weeks). Different letters in each bar indicate significant difference ($P < 0.05$).

tory and field scales. The results evidently indicated that the application of dietary supplementation of PowerLac™ in Nile tilapia grow-out culture bring about favourable effects in the growth and feeding performance that support the benefits of probiotics application reported in previous studies (Zhou, Tian, Wang & Li, 2010; Mohapatra, Chakraborty, Prusty, Das, Paniprasad & Mohanta 2012; Heo, Kim, Kim, Bai & Kong 2013; Maeda *et al.* 2014). However, it is important to note that the effect of heat-killed probiotics on the growth performance of the host could be different amongst aquaculture species (Dash *et al.* 2015; Dawood, Koshio, Ishikawa & Yokoyama 2015a,b). Positive effects of heat-killed probionts on the host growth and feed utilization were shown in grouper *Epinephelus coioides* (Yan, Xia, Yang, Hoseinifar & Sun 2015), red sea bream (Dawood *et al.* 2015a,b), amberjack (Dawood, Koshio, Ishikawa & Yokoyama 2015c) and sea cucumber (Yang, Han, Ren, Jiang, Wang & Zhang 2015). Whereas Dash *et al.* (2015) and Mohapatra *et al.* (2012) reported that dietary application of heat-killed probiotics did not affect the growth of prawn and rohu respectively.

The beneficial effects of probiotics application strongly depend on the supplementation levels, whereby the levels of 0.25 g kg^{-1} feed and 0.50 g kg^{-1} feed provided the best results in fish growth and FCR, both in laboratory and field experiments. Dose-dependent response against probiotics supplementation has been reported previously (among others, El-Dakar, Shalaby & Saoud 2007; Li, Tan & Mai 2009), and this could vary according to the probiotics type and species (Mohapatra *et al.* 2012). For instance, Hoseinifar, Mirvaghefi and Merrifield (2011) noted that 2% dietary supplementation of inactive brewer's yeast resulted in significantly improved growth performance of juvenile beluga than those in the control and 1% supplementation level. On the other hand, Abdel-Tawwab, Abdel-Rahman and Ismael (2008) reported that dietary supplementation of live baker's yeast of more than 0.5 g kg^{-1} significantly improved Nile tilapia weight gain, SGR, FCR and protein utilization. Furthermore, Dawood *et al.* (2015a,b) suggested that dietary supplementation of heat-killed *Lactobacillus plantarum* at 0.1–2 g kg^{-1} of feed significantly enhanced the growth and feeding performance of red sea bream compared with the control.

The advantageous effects of PowerLac™ on the growth and feed conversion ratio of Nile tilapia might be explained by the established positive role of probiotics vis-a-vis the targeted species, including the improvement on feed digestibility (Dawood *et al.* 2015a,b), and nutrient uptake and utilization by means of (1) contribution of digestive enzymes (Yanbo & Zirong 2006; Suzer, Çoban, Kamaci, Saka, Firat, Otgucuoğlu & Küçüksari 2008; Yang *et al.* 2015), (2) modulation of intestinal microbiota (Yang, Xia, Ye, Zou & Sun 2014), (3) contribution on the development of digestive tract morphology (an increase in microvilli) that allows higher surface area for nutrient uptake (Frouël, Le Bihan, Serpentini, Lebel, Koueta & Nicolas 2008; Sáenz de Rodrigáñez, Díaz-Rosales, Chabrillón, Smidt, Arijo, León-Rubio, Alarcon, Balebona, Moriñigo, Cara & Moyano 2009), and (4) stimulation of enzyme activity related to nutrient utilization, such as those involved in nutrient absorption in the intestinal brush border (alkaline

phosphatase and leucine aminopeptidase) (Sáenz de Rodrigáñez *et al.* 2009; Panigrahi, Kiron, Satoh & Watanabe 2010).

Previous studies suggested that probiotics could increase digestive enzyme activity in the digestive tract of fish and shrimp, which may be attributable to the secretion of exogenous enzymes by the bacteria or to the stimulation of endogenous digestive enzymes by the host (Yanbo & Zirong 2006; Wang 2007; Suzer *et al.* 2008; López, Soto, Escamilla, Ibarra, Ochoa, Drawbridge & Peres 2014). Our results, however, showed no significant differences in the fish digestive enzyme activities between treatments. Similar results were observed in Mohapatra *et al.* (2012) who reported that dietary supplementation of heat-killed probiotics did not enhance the digestive enzyme activities in rohu fingerling.

The significant increase in blood glucose was observed in the fish fed with diets supplemented with PowerLac™ at more than 0.25 g kg^{-1} feed. The increase in blood glucose may imply that a greater circulation of energy supply was available in the fish fed with these experimental diets. Panigrahi *et al.* (2010) reported that dietary heat-killed *Lactobacillus rhamnosus* supplementation resulted in significant elevations in plasma protein, triglycerides and alkaline phosphatase activity in rainbow trout after 20 days of feeding. However, the authors suggested that viable probionts had more pronounced influence on these biochemical processes in rainbow trout blood than the heat-killed one.

RNA/DNA ratio is an important indicator to evaluate the growth potential of a fish following feeding treatment (Tanaka, Gwak, Tanaka, Sawada, Okada, Miyashita & Kumai 2007; Abidi & Khan 2009; Zehra & Khan 2013), and positive effects of dietary probiotics on this parameter were reported in previous studies (Bandyopadhyay & Mohapatra 2009; Gonçalves, Maita, Futami, Endo & Katagiri 2011). RNA/DNA ratio may indicate the level of protein synthesis and is considered to be sensitive to essential nutrient levels in the fish body (Weber, Higgins, Carlson & Janz 2003; Abidi & Khan 2009). The RNA/DNA ratios in this study, however, were not affected by the dietary probiotics treatment.

The haematological parameters and the results of challenge test clearly indicated the immunomodulation effect of the dietary probiotic in this study. Positive roles of probiotics in host immune system activation and protection against disease infection have been examined in previous studies. The mechanism by which probiotics stimulate both innate and adaptive immune system responses has been well documented and includes increasing phagocytic and respiratory burst activity (Pirarat *et al.* 2006), promoting antibacterial activity (Aly, Ahmed, Ghareeb & Mohamed 2008) and stimulating complement activity (Panigrahi, Kiron, Puankaew, Kobayashi, Satoh & Sugita 2005; Wang *et al.* 2008). Certain probiotics can increase the number of erythrocytes, granulocytes, macrophages and lymphocytes in different fish and actively stimulate the proliferation of B-lymphocytes and elevate the level of immunoglobulin (Nayak 2010). Probiotics interact with immune cells, such as mononuclear phagocytic cells (monocytes, macrophages) and polymorphonuclear leucocytes (neutrophils), and natural killer (NK) cells to enhance immune responses (Nayak 2010; Selim & Reda 2015). A recent report by Selim and Reda (2015) suggested that the administration of dietary *Bacillus amyloliquefaciens* on Nile tilapia modulated the production of interleukin-1 and tumour necrosis factor-alpha the biomarkers of immune regulators that activate lymphocytes, macrophages and NK cells. This finding might explain the increase of the phagocytic index in the fish for most probiotics treatments, which clearly indicates the enhancement of innate immunity for these particular treatments in this study. Previous investigation of shrimp demonstrated that the application of the same strain of probiotic bacteria used in this study resulted in higher expression of some immune genes, including crustin, lysozyme, anti-lipopolysaccharide factor, superoxide dismutase and TLR1 (Maeda *et al.* 2014).

The improvements in fish growth in the treatments with probiotics supplementations in this study clearly suggest the production enhancement potential available from the utilization of this product. The data from the field experiment showed that by using 0.5 g of PowerLac™ per kg of feed, about 49% higher yield could be achieved. This production increase implies additional income, which is by far higher than the extra cost required for PowerLac™ supplementation (0.9 cent kg^{-1} of feed). In this regard, the application of PowerLac™ in tilapia production could be considered to be profitable. In addition, the administration of PowerLac™ also modulated higher fish immune response indicating a better protection against disease during the fish grow-out production.

Conclusion

The results of this study showed that a supplementation level of 0.5 g PowerLac™ kg^{-1} feed could improve the growth and feeding performance of Nile tilapia, as well as their immunity to disease infection.

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