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Dietary inclusion of Orange peels derived pectin and *Lactobacillus plantarum* for Nile tilapia (*Oreochromis niloticus*) cultured under indoor biofloc systems

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ABSTRACT

A 12-week feeding trial was carried out to investigate possible effects of dietary orange peels derived pectin (OPDP) and *Lactobacillus plantarum* CR1T5 (LP) singularly or combined on innate immune response, disease resistance, and growth performance of Nile tilapia fingerlings under indoor biofloc system. The fingerlings were fed the following diets: diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP + 10^8 CFU g^{-1} *L. plantarum*). At the end of feeding trial, skin mucus parameters, serum immune parameters, and growth performance were measured. Ten randomly selected fish were used in a challenge test with *Streptococcus agalactiae*. The results indicated that supplementations of OPDP + LP or/and significantly ($P < .05$) increased growth performance, skin mucus and serum immunity responses. The highest values were revealed in fish fed both OPDP and LP vs. individual applications. However, no significant ($P > .05$) differences were observed between fish fed OPDP and LP. The challenge test revealed that the relative percent survival (RSP) in diet 2, diet 3, and diet 4 was 43.33%, 50.0%, and 70.0%, respectively. Among the supplemented groups, fish fed 10 g kg^{-1} OPDP + LP showed significant ($P < .05$) higher RPS and resistance to *S. agalactiae* than the other groups. The present results suggested that the combination of OPDP and LP could be considered as potential feed-additives for aquaculture farmed fish under indoor biofloc system.

1. Introduction

According to FAO (2016), the aquaculture industry is the fastest food producing sector, and has significantly contributed to provide high quality and affordable protein source worldwide (Koehn et al., 2017). Due to its fast growth rate and good flesh quality, Nile tilapia (*Oreochromis niloticus*) is one of the most farmed fish globally, and is produced in > 100 countries (Gu et al., 2017). The global production of tilapia are estimated to be 6.532 million metric tons in 2018 (GOVL, 2017) and is expected to reach 7.3 million metric tons by 2030 (Behera et al., 2018).

The increase in fish demand for human consumption has pushed the aquaculture industry toward intensified culture systems, but has increased the risk of infectious diseases including streptococcosis (Chen et al., 2012; Gallage et al., 2017). Besides, the drainage water from aquaculture activities has been considered as major obstacle for the development of the industry; with high content of organic matter, nitrogen and phosphorus, and can cause severe pollution and frequent harmful algal blooms in aquatic ecosystems (De Schryver and Verstraete, 2009; Mansour and Esteban, 2017; Piedrahita, 2003). Therefore, a sustainable treatment and culture system of tilapia aquaculture is of high importance to evaluate.

Prebiotic defined as a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host (Bindels et al., 2015). The beneficial effects in fish include enhanced growth and immunological response (e.g. Buentello et al., 2010; Li and Gatlin, 2005; Zhou et al., 2010), increased microvilli area of intestinal absorption (e.g. Zhou et al., 2010), and improved survival after challenges against pathogens (e.g. Buentello et al., 2010; Li and Gatlin, 2005). Among the prebiotics used, pectin derived from agricultural by-products, such as orange peels have

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been considered as a promising one (Ho et al., 2017). It is well known that pectin possesses numerous biological activities as a functional food substance with a wide range of pharmaceutical applications (Naqash et al., 2017). Pectin and its derivative have proved to be potential prebiotics with improved properties, by positively modulating the gut microbiota and for causing positive effects in the distal part of the colon (Gómez et al., 2016), compared to other prebiotics, such as fructooligosaccharide or galactooligosaccharide (Gómez et al., 2013). However, to our knowledge, there is no information available regarding application of orange peels derived pectin in aquaculture.

Probiotics are defined as "live microorganisms which when administered in adequate amount confer a health benefit on the host" (FAO/WHO, 2001). The beneficial effects of probiotics, include improvement of growth performance, enhancement of immune response, and increases disease resistance have been demonstrated in numerous studies (Abarike et al., 2018; Akhter et al., 2015; Dawood et al., 2018; Nayak, 2010; Pérez-Sánchez et al., 2018; Xia et al., 2018). Among the probiotics, *Lactobacillus plantarum* has been considered as promising, and numerous studies have reported stimulated effect by dietary inclusion of *L. plantarum* on immune response, enhanced growth performance, and improved disease resistance in several fish species (e.g. Feng et al., 2019; Li et al., 2018b; Van Doan et al., 2018a; Van Nguyen et al., 2019).

Biofloc is technology has been widely applied in fish and shrimp farming; its outstanding feature is that they contain the mixture of bacteria, algae, and other detritus which would be available feed for the fishes of omnivorous feeding habits (Bossier and Ekasari, 2017; Crab et al., 2012; Daniel and Nageswari, 2017). Recently, several studies have revealed positive effect of the biofloc technology on water quality, growth, non-specific immunity, and disease prevention in fish (Ekasari et al., 2016; Kamilya et al., 2017; Li et al., 2018a; Mansour and Esteban, 2017). On the other hand, prebiotics and probiotics play similar roles in fish as biofloc (Akhter et al., 2015; Dawood and Koshio, 2016). Biofloc, prebiotics, and probiotics have been adopted by farmers in practical aquaculture; however, the occurrence of certain diseases is still common and results in lower survival rate of fish and shellfish at farms (Su et al., 2008). As diseases acquired by animals are often linked to specific pathogenic bacteria, the action of specific antagonistic/beneficial bacteria would favorably minimize the problems (Wang, 2007). Therefore, it has been hypothesized that addition of specific prebiotic and probiotic to the biofloc proliferate the bacterial population either in the water or animals' gut in order to suppress the potentially harmful pathogenic strains. Based on this hypothesis, recent studies have been attempted in these areas, and reports seem to suggest that addition of prebiotics and probiotics to the biofloc further improve water quality, animal growth, immunity, and survival rate of animals than that of biofloc (Ahmad et al., 2016; Dash et al., 2018; Doan et al., 2018; Rodrigues et al., 2018). A study on the influences of prebiotic and probiotic on biofloc based aquaculture system is novel and an integrative approach; however less explored. Thus, the aims of the present investigation were to evaluate the effects of orange peels derived pectin (OPDP) singular or combined with *L. plantarum* on skin mucus- and serum immune parameters, disease resistance against *Streptococcus agalactiae* and growth performance of Nile tilapia cultured under indoor biofloc condition.

2. Materials and methods

2.1. Preparation of orange peels derived pectin (OPDP)

OPDP was obtained from a local market, Chiang Mai province, Thailand. Upon arrival, orange peels were dried in oven at 60 °C for 48 h, then ground by using hammer mill, and filtered with the use of 40-mesh sieve, and stored at 4 °C until further use. Pectin was isolated from orange peels as described elsewhere (Prakash Maran et al. (2013) with some modifications. Briefly, 1 g of orange peel powder was placed in 250 mL Pyrex beaker, and thoroughly mixed with 16.9 mL of distilled water pH 1.4 (pH was adjusted by sulfuric acid). The beaker with solution was put in a microwave oven with 422 W of power and 169 s irradiation time, cooled down to room temperature (25 °C), and centrifuged at 4 °C for 5 min (10.000 rpm) in 50 mL tube. The supernatant was collected and precipitated with an equal volume of 95% (*v*/v) ethanol, and thereafter washed three times with 95% (v/v) ethanol to remove the mono and disaccharides and dried at 50 °C in the oven until achieving constant weighed.

2.2. Lactobacillus plantarum preparation

Lactobacillus plantarum CR1T5 derived from fermented rice was kindly provided by Dr. Saowanit Tongpim (Department of Microbiology, Faculty of Science, Khon Kaen University; Thailand). The administration dose of *L. plantarum* (10^8 CFU g^{-1}) used in the present study was selected based on previous investigations (Son et al. (2009); Giri et al. (2013). The *L. plantarum* supplemented diets were daily prepared according to the method of Irianto and Austin (2002a).

2.3. Diets preparation

The test diets used in the present investigation was adapted from Van Doan et al. (2018). Four experimental diets were prepared by incorporating OPDP and *Lactobacillus plantarum* CR1T5 in the basal diet as follows: diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP + 10^8 CFU g^{-1} *L. plantarum*) (Table 1). For pellets preparation, fine feedstuffs were thoroughly blended together, and thereafter soybean oil (5 mL^{-1}) and water (300 mL kg^{-1} feed) were added to produce stiff dough. It was then passed through extruder machine to produce pellets. The pellets were dried in an oven at 50 °C until the moisture content was approximately 10%, and thereafter stored in plastic bags at 4 °C until further use.

Table 1
The formulation and proximate composition of experimental diet (g kg^{-1}).

Ingredients	Diets (g kg^{-1})			
	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	270	270	270	270
Corn meal	200	200	200	200
Soybean meal	270	270	270	270
Wheat flour	60	60	60	60
Rice bran	150	150	150	150
OPDP[a]	0	10	0	10
Lactobacillus plantarum (CFU g^{-1})	0	0	10^8	10^8
Cellulose	30	20	30	20
Soybean oil	5	5	5	5
Premix[b]	10	10	10	10
Vitamin C[c]	5	5	5	5
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)				
Crude protein	319.36	320.10	319.36	320.10
Crude lipid	74.75	75.02	74.75	75.02
Fiber	52.48	52.48	52.48	52.48
Ash	106.68	105.41	106.68	105.41
Dry matter	817.80	829.10	817.80	829.10
GE (cal/g)[d]	4089	4091	4089	4091

[a] OPDP: Orange peels derived pectin.
[b] Vitamin and trace mineral mix supplemented as follows (IU kg^{-1} or g kg^{-1} diet): retinyl acetate 1,085,000 IU; cholecalciferol 217,000 IU; D, L-a-tocopherol acetate 0.5 g; thiamin nitrate 0.5 g; pyridoxine hydrochloride 0.5 g; niacin 3 g; folic 0.05 g; cyanocobalamin 10 g; Ca pantothenate 1 g kg^{-1}; inositol 0.5 g; zinc 1 g; copper 0.25 g; manganese 1.32 g; iodine 0.05 g; sodium 7.85 g.
[c] Vitamin C 98% 8 g.
[d] GE = gross energy.

2.4. Fish preparation and experimental design

Nile tilapia were brought from Chiang Mai Patana Farm, Chiang Mai, Thailand and stocked in cages (5 m in length x 5 m in width x2 m in height). The commercial pellets (CP 9950, Charoen Pokphand Group Co., Ltd., Thailand) were fed to the fish daily. Prior to the experiments, fish were transferred to a 1.000-L tank and fed the control diet for 2 weeks. Then, 10 fish were randomly caught and checked the health status by gills examination and internal organs observation under light microscope. Thereafter, 640 fish with an average weight of 5.92 ± 0.08 g were randomly distributed into 16 fiberglass tanks (300 *L*) assigned to four treatments, repeated in quadruplicate. The stocking density was 40 fish tank^{-1}, and fish were hand-fed ad libitum two times daily at 9:00 a.m. and 17:00 p.m.

2.5. Water quality management

The water quality parameters were checked at 08:30 a.m. and 16:30 p.m. Temperature, pH and dissolved oxygen (DO) were monitored using Multiparameter Waterproof Meter (HI98196, Hana Instruments, Romania). Total ammonia-nitrogen (TAN) was measured using an Ammonia Portable Photometers (HI96733, Hana Instruments, Romania). Biofloc volume was determined using an Imhoff cone as described elsewhere (Avnimelech and Kochba (2009).

2.6. Samples preparation and immunological analysis

2.6.1. Samples preparation

At 4, 8, and 12 weeks post-feeding, four fish were randomly sampled, lottery method, from each tank for immune response analysis. Skin mucus was collected following the method (Khodadadian Zou et al., 2016). Blood samples were collected and sera separated as described previously (Van Doan et al., 2016b; Van Doan et al., 2016a), and kept at -20 °C for further assays. Leukocytes from blood were separated using the protocol of Van Doan et al. (2018).

2.6.2. Immunological parameters

Lysozyme activity: Lysozyme activity was detected following the method of Parry et al. (1965) and the results are expressed as μg mL^{-1}.

Peroxidase activity: Determination of peroxidase was carried as previously described by (Doan et al., 2017).

Phagocytosis activity: The activity was measured based on the method of Yoshida and Kitao (1991) with some modifications as described by Van Doan et al. (2017).

Respiratory burst activity: The activity was identified following the protocol of Secombes (1990) with some modifications as described previously (Van Doan et al., 2017).

Alternative complement pathway activity: The alternative complement activity was determined following the protocol of Yanno (1992).

2.7. Growth performance

Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) were calculated after 8 weeks feeding on experimental diets using the formulae: WG = final weight (g) – initial weight (g); SGR (%) = 100 × (ln final weight – ln initial weight)/ Duration of experiment; FCR = feed offered (dried weight)/weight gain (wet weight); SR (%) = (final fish number/initial fish number) × 100.

2.8. Challenge test

The source of *S. agalactiae* and bacterial preparation are described by Van Doan et al. (2018b). At the end of feeding trial (12th week), 10 fish were randomly selected from each tank and intraperitoneally injected with 0.1 mL of 0.85% normal saline solution containing 10^7 CFU mL^{-1} of *S. agalactiae*. The dose of bacteria(10^7 CFU mL^{-1}) used in present study was selected based on previous study (Wang et al., 2016) During the challenge test, the dead fish from each tank were counted and removed immediately. The mortality (%) of fish in each treatment was calculated 15 days post-challenge, and relative percentage of survival (RPS) was computed based on equation by Amend (1981):

RPS = 100 - (test mortality/control mortality) ∗ 100.

2.9. Statistical analysis

The obtained data were analyzed using a SAS Computer Program (SAS, 2003) for least significant differences among the treatments where the Duncan's Multiple Range Test was used. Mean values were considered significantly different at $P < .05$. Data are presented as means ± standard deviation.

3. Results

3.1. Water quality parameters

No significant ($P > .05$) differences in water quality parameters among experimental treatments were revealed. Temperature was maintained at 29 ± 0.51 °C, and dissolve oxygen was kept above 6.5 ± 0.14 mg L^{-1}. pH varied from 7.85 ± 0.19, and total ammonia was in range of 0.79 ± 0.11 mg L^{-1}. The biofloc volume was 8.20 ± 0.48 mL.

3.2. Innate immune response

After 12 weeks of feeding, supplementations of OPDP + *Lactobacillus plantarum* CR1T5 or/and resulted in a significant ($P < .05$) increase in skin mucus lysozyme activity (SMLA) and skin mucus peroxidase activity (SMPA) compared to the control group (Table 2). The highest values of these parameters were recorded for fish fed both OPDP + *L. plantarum* supplementations. Nonetheless, no

Table 2
Skin mucus lysozyme and peroxidase activities of *O. niloticus* after 12 weeks feeding with experimental diets (mean ± S.E., $n = 4$): diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP + 10^8 CFU g^{-1} *L. plantarum*). Different letter in a row denotes significant difference ($P < .05$).

		Diet 1	Diet 2	Diet 3	Diet 4
4 weeks	SMLA	0.59 ± 0.05^{c}	1.07 ± 0.10^{b}	1.22 ± 0.07^{ab}	1.40 ± 0.05^{a}
	SMPA	0.05 ± 0.006^{c}	0.08 ± 0.005^{b}	0.09 ± 0.007^{b}	0.12 ± 0.005^{a}
8 weeks	SMLA	1.25 ± 0.08^{c}	2.00 ± 0.13^{b}	2.23 ± 0.15^{b}	2.60 ± 0.05^{a}
	SMPA	0.09 ± 0.005^{c}	0.12 ± 0.005^{b}	0.13 ± 0.008^{b}	0.17 ± 0.008^{a}
12 weeks	SMLA	1.86 ± 0.08^{c}	2.52 ± 0.18^{b}	2.63 ± 0.16^{b}	3.36 ± 0.16^{a}
	SMPA	1.15 ± 0.01^{c}	0.20 ± 0.009^{b}	0.23 ± 0.02^{b}	0.33 ± 0.02^{a}

SMLA (μg mL^{-1}) = Skin mucus lysozyme activity.
SMPA (μg mL^{-1}) = Skin mucus peroxidase activity.

Table 3
Serum immunity of *O. niloticus* after 12 weeks of feeding with experimental diets contain different diets (mean ± S.E., n = 4): diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP + 10^8 CFU g^{-1} *L. plantarum*). Different letter in a row denotes significant difference ($P < .05$).

		Diet 1	Diet 2	Diet 3	Diet 4
4 weeks	SL	4.71 ± 0.30^c	6.84 ± 0.41^b	7.78 ± 0.40^b	9.15 ± 0.20^a
	SP	0.09 ± 0.006^c	0.15 ± 0.01^b	0.17 ± 0.02^b	0.23 ± 0.009^a
	ACH50	113.86^b ± 3.98^c	139.26 ± 5.03^b	146.70 ± 6.49^b	172.80 ± 5.17^a
	PI	1.11 ± 0.03^c	1.23 ± 0.01^b	1.26 ± 0.02^b	1.44 ± 0.04^a
	RB	0.05 ± 0.006^c	0.07 ± 0.005^b	0.08 ± 0.007^b	0.12 ± 0.004^a
8 weeks	SL	7.68 ± 0.27^c	10.68 ± 0.86^b	11.18 ± 0.73^b	14.09 ± 0.44^a
	SP	0.15 ± 0.009^c	0.21 ± 0.006^b	0.23 ± 0.02^b	0.31 ± 0.006^a
	ACH50	136.53 ± 4.59^c	173.99 ± 8.29^b	185.08 ± 8.42^b	216.03 ± 6.88^a
	PI	1.39 ± 0.04^c	1.94 ± 0.10^b	2.00 ± 0.07^b	2.52 ± 0.09^a
	RB	0.10 ± 0.01^c	0.14 ± 0.01^b	0.16 ± 0.01^b	0.20 ± 0.01^a
12 weeks	SL	8.92 ± 0.35^c	12.34 ± 0.87^b	13.09 ± 0.77^b	17.62 ± 0.47^a
	SP	0.20 ± 0.01^c	0.26 ± 0.008^b	0.28 ± 0.02^b	0.34 ± 0.03^a
	ACH50	190.11 ± 5.01^c	237.91 ± 7.51^b	257.94 ± 12.93^b	326.94 ± 16.04^a
	PI	1.86 ± 0.09^c	2.42 ± 0.13^b	2.64 ± 0.11^b	3.44 ± 0.10^a
	RB	0.16 ± 0.01^c	0.23 ± 0.01^b	0.25 ± 0.01^b	0.34 ± 0.01^a

SL = Serum lysozyme activity (μg mL^{-1}); SP = Serum peroxidase activity (μg mL^{-1}); ACH50 = Alternative complement activity (units mL^{-1}); PI = Phagocytosis activity (bead $cell^{-1}$); RB = Respiratory burst activity.

significant ($P > .05$) difference was revealed between diet 2 and diet 3 supplemented groups (Table 2).

Serum lysozyme (SL) activity was significantly ($P < .05$) higher in supplemented groups vs. control fed fish (Table 3). The highest value was recorded in fish fed both OPDP and *L. plantarum* compared to the individual applications. No significant ($P > .05$) difference was observed between fish fed OPDP and *L. plantarum* singularly (Table 3). Similarly, alternative complement (ACH50) activity and phagocytosis (PI) activity were significant higher ($P < .05$) in the supplemented groups, when compared to control fed fish (Table 3). The highest values were displayed in fish fed dietary both OPDP + *L. plantarum* compared to individual applications (Table 3). No significant ($P > .05$) differences in these parameters were observed in fish fed OPDP or *L. plantarum* alone (Table 3). With regard to serum peroxidase (SP) activity, fish fed supplemented diets showed significant ($P < .05$) higher SP when compared to the control. However, no significant ($P > .05$) difference was observed between diet 2 and diet 3. Likewise, significant ($P < .05$) difference in respiratory burst (RB) activity was observed in fish fed supplemented diets, when compared to the control after 12 weeks of feeding (Table 3).

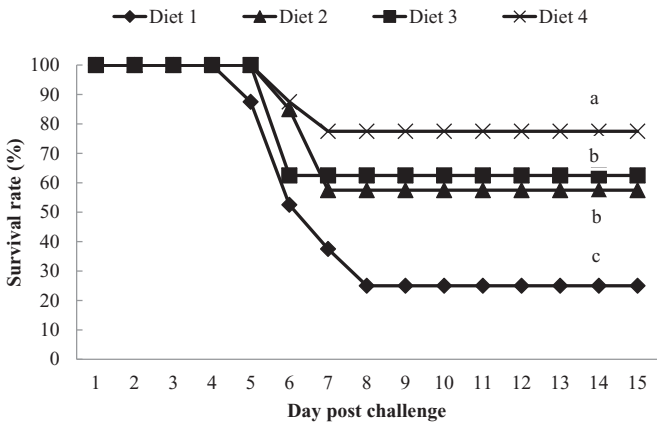

Fig. 1. Survival rate of tilapia, *O. niloticus* fed with experimental diets (mean ± S.E., $n = 40$): diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP + 10^8 CFU g^{-1} *L. plantarum*). Different letter in a row denotes significant difference ($P < .05$).

3.3. Challenge test

Compared to the control treatment (25% survival), the survival rate of fish fed the OPDP and *Lactobacillus plantarum* CR1T5 diets were significantly ($P < .05$) higher; by 57.5% (diet 2), 62.25% (diet 3), and 77.5% (diet 4) (Fig. 1). Typical symptoms of *Streptococcus* infection included darkness skin, exophthalmia, pair-fins basal haemorrhage, and pale liver. The relative percent survival (RSP) was 43.33%, 50%, and 70% in diet 2, diet 3, and diet 4, respectively. Among the supplemented groups, the combination of 10 g kg^{-1} OPDP and 10^8 CFU g^{-1} *L. plantarum* showed significantly ($P < .05$) higher RPS and highest resistance to *S. agalactiae* compared with the other groups.

3.4. Growth performance

After 4, 8, and 12 weeks of feeding, fish fed the supplemented diets showed a significant ($P < .05$) increase in specific growth rate (SGR), weight gain (WG), final weight (FW) compared to control fed fish (Table 4). The highest SGR and WG values were revealed in fish fed both dietary OPDP and *Lactobacillus plantarum* CR1T5. However, no significant ($P > .05$) differences in these parameters were observed by feeding fish OPDP or *L. plantarum* alone. The FCR was significantly ($P < .05$) lower in fish fed 10 g kg^{-1} OPDP + 10^8 CFU g^{-1} *L. plantarum* than in the other treatment groups, while highest ($P < .05$) FCR values were noticed in the control group. In contrast, survival rate of the fish showed no significant ($P > .05$) differences between the experimental groups.

4. Discussion

Recently, two studies evaluated prebiotics and probiotics applications in fish and crustacean systems with biofloc, in order to investigate whether they could be included separately (Kathia et al., 2017; Rodrigues et al., 2018). However, as only one study has been performed to identify the beneficial effects of prebiotics and probiotics on biofloc based aquaculture (Daniel and Nageswari, 2017), the aims of the present study were to evaluate effects of orange peels derived pectin (OPDP) singular or combined with *Lactobacillus plantarum* CR1T5 on skin mucus- and serum immune parameters, disease resistance against *S. agalactiae* and growth performance of Nile tilapia cultured under indoor biofloc condition.

The present study revealed that dietary administration of OPDP significantly enhanced skin mucus and serum immunity, disease resistance, and growth performance of Nile tilapia cultured under biofloc system. Similarly, dietary administration of mannoprotein derived from

Table 4
Growth performances and feed utilization (mean ± SE) of the Nile tilapia fed different diets: diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP + 10^8 CFU g^{-1} *L. plantarum*). Different letter in a row denotes significant difference ($P < .05$).

	Diet 1	Diet 2	Diet 3	Diet 4
IW (g)	5.88 ± 0.54	5.95 ± 0.32	5.94 ± 0.26	5.90 ± 0.24
FW (g)				
4 weeks	18.57 ± 0.89^{b}	19.59 ± 0.48^{ab}	20.14 ± 0.56^{ab}	21.01 ± 0.47^{a}
8 weeks	37.75 ± 1.00^{c}	42.30 ± 1.04^{b}	43.34 ± 0.83^{b}	46.73 ± 0.58^{a}
12 weeks	66.19 ± 3.21^{c}	74.00 ± 1.93^{b}	75.26 ± 1.69^{b}	82.86 ± 1.69^{a}
WG (g)				
4 weeks	12.68 ± 0.62^{b}	13.64 ± 0.32^{ab}	14.20 ± 0.44^{a}	15.11 ± 0.38^{a}
8 weeks	31.87 ± 0.80^{c}	36.36 ± 0.91^{b}	37.40 ± 0.79^{b}	40.83 ± 0.58^{a}
12 weeks	60.30 ± 2.97^{c}	68.06 ± 1.80^{b}	69.28 ± 1.63^{b}	76.96 ± 1.71^{a}
SGR				
4 weeks	3.83 ± 0.03^{c}	3.98 ± 0.01^{b}	4.07 ± 0.04^{b}	4.23 ± 0.05^{a}
8 weeks	3.10 ± 0.05^{c}	3.27 ± 0.03^{b}	3.31 ± 0.04^{b}	3.45 ± 0.04^{a}
12 weeks	2.69 ± 0.02^{c}	2.80 ± 0.02^{b}	2.82 ± 0.02^{b}	2.94 ± 0.03^{a}
FCR				
4 weeks	1.49 ± 0.01^{a}	1.44 ± 0.01^{a}	1.42 ± 0.01^{b}	1.38 ± 0.01^{b}
8 weeks	1.54 ± 0.01^{a}	1.49 ± 0.006^{a}	1.47 ± 0.008^{b}	1.43 ± 0.007^{c}
12 weeks	1.58 ± 0.006^{c}	1.53 ± 0.007^{b}	1.54 ± 0.005^{b}	1.51 ± 0.005^{a}
SR (%)	98	99	99	99

Data assigned with different letter denote significant difference in a row ($P < .05$).

yeast cell walls of *Saccharomyces cerevisiae* significantly improved survival rate, width and perimeter of intestinal villi, superoxide anion production after infection with *Vibrio parahaemolyticus* of Pacific white shrimp (*Litopenaeus vannamei*) cultured under indoor biofloc system (Rodrigues et al., 2018). The noticeable increase of growth performance, immune response, and disease resistance of Nile tilapia fingerlings revealed in the present study, may be due to the immunomodulatory effect of OPDP and biofloc technology. Previously, it has been reported that pectin; a soluble dietary fiber exerts physiological effects on the alimentary tract, by reducing glucose absorption (Grundy et al., 2016) and delaying gastric emptying (Schwartz et al., 1982). Pectin and its derivative have also been proposed as an excellent candidate for new-generation of prebiotics (Ho et al., 2017; Naqash et al., 2017). Furthermore, oral administration of pectin–derived acidic oligosaccharides revealed modulation of the gut microbiota and fecal short chain fatty acid (SCFA) production in mice (Bernard et al., 2015). Moreover, the immune improving properties of lemon pectin have been reported in an animal study, showing that supplementation prevented the induction of oral tolerance to OVA in rats, which was preceded by enhanced protein antigen penetration to the blood and activation of macrophages (Khramova et al., 2009). In a previous study, a lemon derived pectic-type polysaccharide was orally administered to mice, causing increased secretion of GM-CSF and IL-6 from Peyer's patches, indicating immune cell activation (Suh et al. (2013). More recently, lemon pectin has been demonstrated as an immunostimulatory fiber prebiotic; able to stimulate TLR and T84 intestinal epithelial cell barrier function (Vogt et al., 2016). Regarding improved growth performance, this may be a result of gut microbiota modulation by increasing the population level of beneficial bacteria and subsequent improvement of digestive function. However, this controversial hypothesis merits further investigation. On the other hand, Ho et al. (2017) showed that the gut microbiota are capable of fermenting pectin and revealed the potential of pectin as a novel prebiotic. In addition, beneficial effects on gut microbiota and digestive enzyme activities such amylase, lipase, and protease (Dawood and Koshio, 2016; Eshaghzadeh et al., 2015; Hoseinifar et al., 2016a; Kühlwein et al., 2014), liver enzyme activities (Hoseinifar et al., 2015a; Zhang et al., 2013), as well as enhanced appetite, production of vitamins, breakdown of indigestible components as well as improving gut morphology (Hoseinifar et al., 2015b; Irianto and Austin, 2002b) have been reported following prebiotic administration.

The results of present study indicated that dietary administration of *Lactobacillus plantarum* CR1T5 significantly increased growth performance, mucosal and serum immunity, as well as disease resistance against *S. agalactiae* of Nile tilapia. In agreement with present study, significant increase growth performance, immune response, and disease resistance with the addition of probiotics into biofloc system were observed in common carp, *Cyprinus carpio* (Sartika et al., 2012); Chinese shrimp, *Fennerpenaeus chinensis* (Kim et al., 2015); African catfish, *Clarias gariepinus* (Hapsari, 2016); *L. vannamei* (Ferreira et al., 2017; Hu et al., 2017; Krummenauer et al., 2014); freshwater prawn, *Macrobrachium rosenbergii* (Miao et al., 2017), and common carp, *Cyprinus carpio* (Dash et al., 2018). However, in the study of De Paiva et al. (2016), no significant differences were revealed when a commercial probiotic (*Bacillus* spp. and *Lactobacillus* sp.) was added in biofloc system of shrimp. These results could be assigned to anaerobiosis and deplections that can exist at pond bottom (De Paiva et al., 2016), and could influence the action of probiotic. Another factor that could affect was the commercial probiotic concentration (2.2×10^8 UFC g^{-1}), vs. recommended probiotic concentration in another study (1.0×10^9 UFC g^{-1}) (Aguilera-Rivera et al., 2014). Considering these results, biofloc development together with dietary addition of single or combination of probiotics may be a favorable approach for improving the physiological status of animal. This is probably due to the supplemented probiotics vs. the other bacteria to minimize the pathogenic load in the fish. The presence of bacteria in the biofloc or supplemented probiotics can exhibit the mitigating effects on pathogenic bacteria in the fish. This can ensure improvement in the non-specific immunity of the host, as it is well-documented in numerous studies that the immune system is non-specifically modulated by probiotics (Gatesoupe et al., 2010; Hoseinifar et al., 2015a; Lazado and Caipang, 2014; Llewellyn et al., 2014; Nayak, 2010). Moreover, adhesion and colonization of probiotics in fish intestines are necessary to enhance the immune response (Ausubel, 2005). Interaction between probiotic cells and immune systems are through microbe associated molecular patterns (MAMPs) consisting of specific cell wall polysaccharides (CPs), peptidoglycan (PGN), lipoprotein anchors, and lipoteichoic acids (Hosoi et al., 2003). Probiotic cells or components of immune system can interact with MAMPs by pattern recognition receptor (PRR) such as Toll like receptors (TLRs), C type receptor (CLRs), and nucleotide oligomerigation domain (NOD) like receptors (NLRs) (Bron et al., 2012; Kleerebezem et al., 2010; Lebeer et al., 2010).

It is been demonstrated that *Lactobacillus plantarum* CR1T5 produce antimicrobial substances like plantaricin that are actively manifested against certain pathogens, such as *Pseudomonas putida*, *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* (Liu et al., 2016; Wen et al., 2016). Furthermore, a study have indicated that cell wall components of *L. plantarum*; surface-bound proteins, peptidoglycans and lipoteichoic acid, play vital roles in the prevention and treatment of intestinal inflammatory diseases (Baik et al., 2015). Additionally, lipoteichoic acid isolated from *L. plantarum* has proved to exert antipathogenic effects (Gao et al., 2016) as well as *L. plantarum* modify the intestinal microbiota, and thereby providing protection against pathogenic microorganisms (Balcázar et al., 2007).

Dietary administration of synbiotic, combination of pro – and prebiotics, has recently gained interest in aquaculture (Dawood and Koshio, 2016; Hoseinifar et al., 2016b; Ringø and Song, 2016). In accordance with the present study, synergistic actions were previously reported in European sea bass, *Dicentrarchus labrax* (Torrecillas et al., 2018); blunt snout bream, *Megalobrama amblycephala* (Abasubong et al., 2018); Indian major carp, *Cirrhinus mrigala* (Kumar et al., 2018); red tilapia, *Oreochromis niloticus* (Sewaka et al., 2019); olive flounder, *Paralichthys olivacerus* (Hasan et al., 2018); Japanese eel, *Anguilla japonica* (Lee et al., 2018); sea cucumber, *Apostichopus japonicus* (Li et al.,

2018b; Wang et al., 2017); snakehead, *Channa striata* (Munir et al., 2018); rockfish, *Sebastes schlegeli* (Rahimnejad et al., 2018), and Asian sea bass, *Lates calcalifer* (Ashouri et al., 2018). In contrast, Abid et al. (2013) reported that *P. acidilactici* and scFOS supplemented diet had no effects on Atlantic salmon (*Salmo salar*) growth performance. Likewise, there were no effects of the probiotic or prebiotic supplemented diets on growth or survival of totoaba, *Totoaba macdonaldi* (González-Félix et al., 2018). The discrepancies in these findings may be as result of differences in species, experimental design, prebiotic, and administration regime (Hoseinifar et al., 2010; Hoseinifar et al., 2013). Dietary inclusion of pre- and probiotic has been revealed to elevate health status, improved prebiotic digestion, or the increase in survival and probiotic's colonization in comparison to the individual pre- or probiotic application (Ai et al., 2011; Cerezuela et al., 2012; Geng et al., 2011; Ye et al., 2011). These effects seem likely mediated by SCFAs by-products of fermentation of the probiotic strains in the presence of prebiotics (Hoseinifar et al., 2017; Rahimnejad et al., 2018). In addition, dietary consumption of both pro- and prebiotics resulted in the formation of bioactive microbial metabolites, such as vitamins and biological peptides (Stanton et al., 2005), and these may improve the nutrient digestion and absorption in the host's intestine, and consequently increase its growth and health status of fish.

Bioflocs may contribute to the supply of essential nutrients and digestive enzymes either through the stimulation of endogenous production or by microbial secretion (Anand et al., 2014; Xu and Pan, 2012), and the enhancement of nutrient bioavailability facilitates higher nutrient assimilation. As a protein source, bioflocs could be considered as a good protein source for shrimp and a useful protein source for tilapia (Ekasari et al., 2014; Ekasari et al., 2015). Bioflocs also contain various bioactive compounds including essential amino acids, carotenoids, free amino acids and chlorophylls (Ju et al., 2008), trace minerals (Tacon et al., 2002), and vitamin C (Crab et al., 2012) which are known to have positive effects on aquaculture animals including enhancement of antioxidant status, growth, reproduction, and immune response. In addition, bioflocs offers MAMPs (microbial associated molecular patterns), which may be recognized as immunostimulants, resulting in higher resistance to diseases (Ekasari et al., 2014; Ekasari et al., 2015). Interestingly, when biofloc technology was applied in tilapia broodstock culture system, it enhanced the immunological status contributing to the improvement of the larvae robustness against diseases and environmental stress test (Ekasari et al., 2016). In biofloc systems, aquaculture animals may also benefit the pathogen pressure. Some previous studies revealed that the presence of potentially pathogenic bacteria could be reduced in biofloc systems (Crab et al., 2010; Zhao et al., 2012). It has been suggested that the reduction of *V. harveyi* population in biofloc environment might be related to the disruption of *V. harveyi* cell-to-cell communication, known as an important factor in determining the pathogenicity of this particular bacterium (Crab et al., 2010).

The conclusions of the present study are; dietary supplementation of OPDP and *L. plantarum* boost the immune response, growth and confers protection against *Streptococcus* infection in Nile tilapia fingerlings.

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