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Boosted Growth Performance, Mucosal and Serum Immunity, and Disease Resistance Nile Tilapia (*Oreochromis niloticus*) Fingerlings Using Corncob-Derived Xylooligosaccharide and *Lactobacillus plantarum* CR1T5

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Abstract
The present work, herein, studied the effects of corncob-derived xylooligosaccharides (CDXOS) and *Lactobacillus plantarum* CR1T5 (LP) integrated into fish diets (diet 1 (0—control), diet 2 (10 g kg^{-1} CDXOS), diet 3 (10^8 CFU g^{-1} *L. plantarum* CR1T5), diet 4 (10 g kg^{-1} CDXOS +10^8 CFU g^{-1} *L. plantarum* CR1T5)) on growth performance, innate immune parameters, and disease resistance of Nile tilapia (*Oreochromis niloticus*). Fingerlings, with average mean weight of 4.97 ± 0.04, were randomly distributed into 16 glass tanks (20 fish per tank) for 12 weeks. Growth performance, skin mucus, and serum immune parameters were evaluated at the conclusion of the experiment. Eight randomly selected fish were used for challenge test against *Streptococcus agalactiae*. The results indicated that fish fed CDXOS and LP had significantly improved final weight (FW), weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR). However, no significant difference in survival rate was observed between specimens fed the supplemented diets and the control. Regarding skin mucus, the dietary inclusion of CDXOS and LP significantly increased lysozyme and peroxidase activities compared with the control ($P < 0.05$). Similarly, significant increases in serum lysozyme, peroxidase, alternative complement, phagocytosis, and respiratory burst activities were observed in the fish fed the supplemented diets. However, no significant differences were found in these parameters between fish fed CDXOS and LP diets. For the challenge test, diet 4 produced a higher relative percentage of survival (RPS) and resistance to *S. agalactiae* than fish from the other experimental groups ($P < 0.05$). The results suggested that CDXOS and *L. plantarum* CR1T5 are viable considerations for potential feed-additive sources.

Keywords Xylooligosaccharides · Corncob · *Lactobacillus plantarum* CR1T5 · Nile tilapia · Immune response · *Streptococcus agalactiae* · Growth performance

Introduction

Due to the expansion of intensive aquaculture and increases in culture density, fish diseases have become a frequent dilemma [1–8]. The common way in which farmers deal with the outbreak of diseases in aquaculture is through antibiotics and/or chemotherapeutics [9, 10]. Nonetheless, their application, which is banned in some countries, may give rise to other

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problems, such as resistance to bacterial strains, environmental hazards, and difficulties with food safety [11, 12]. Of recent concern is the possible transfer of antimicrobial resistance genes and resistant bacteria from aquatic animals to humans, with side effects occurring in both humans and the aquatic environment [10]. These concerns have led to a search for natural strategies as alternatives to the use of antibiotics and chemotherapeutics in aquaculture [10, 13]. Among them, the inclusion of prebiotics and probiotics in farmed fish and shellfish diets has been assayed to enhance digestion, digestive enzymes, growth, and immune response [14–21].

A prebiotic is defined as a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host [22, 23]. It has well-established that prebiotics play a pivotal role in enhancing host's health and well-being, when by-products are fermented by favorable microbiota [24–26]. In this sense, agricultural by-products are potential fiber sources, which, when integrated as functional ingredients in food products, can inhibit diseases related to the alterations in intestinal microflora [27]. As they are often discarded as wastes, or burned in the field after each crop, the further use of these by-products will help in their proper disposal, as well as to generate employment and provide farmers with an additional income [28]. Global corn production is approximately 1 billion metric tons [29], resulting in huge amounts of corncobs. Corncobs have long been used as a component for growing several industrially important bacteria and fungi, and in the production of pharmaceuticals and nutraceutically important enzymes [28]. The major elements of corncob by-product are xylooligosaccharides (XOS), xylitol, and xylose [30, 31]. Of these, XOS has proved to be a potential prebiotic [32] and has been considered a potential functional ingredient in the diet. Several properties that play a vital role in improving human health have been attributed to XOS, immunomodulatory, antioxidative, antidiabetic, and anti-cancer activities, as well as the ability to stimulate the proliferation of colonic bifidobacteria, calcium absorption, and lipid metabolism [33–35].

On the other hand, probiotics are defined as "live microorganisms which, when administered in adequate amount, confer a health benefit on the host" [36, 37]. The beneficial effects of dietary probiotics consumption have been demonstrated in aquaculture, including the promotion of growth, stimulation of the immune response, and enhanced disease resistance [14, 38–41]. *Lactobacillus plantarum* belongs to the genus *Lactobacillus*, which plays an important role in the fish intestine. Strains belonging to this species are able to release antibacterial compounds which inhibit the growth of harmful microorganisms [42, 43]. Dietary supplementations of *L. plantarum* have been reported to stimulate the immune response, enhance growth performance, and metabolic functions, compete for adhesion and for nutrition, as well as improve disease resistance in several fish species [44–50].

Synbiotics are defined as "mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host" [51]. In the last decade, the application of synbiotics has been widely studied in fish and shellfish [15]. However, to the best of our knowledge, there are no previous studies regarding the use of corncob-derived xylooligosaccharide (CDXOS) and *Lactobacillus plantarum* on the growth performance, humoral immunity (both mucosal and seric), and disease resistance of Nile tilapia (*O. niloticus*); this situation has given rise to the present study.

Materials and Methods

Xylooligosaccharides Preparation

Raw Materials Preparation Corncobs obtained from the experiment farm of the Faculty of Agriculture, Chiang Mai University (Thailand), were oven dried at 60 °C for 2 days before being crushed by hammer mill, and then filtered using 100-μm mesh size sieve, and stored at 4 °C until use.

Xylan Extraction Xylan was isolated following the protocol of Chapla et al. [28] with some modifications as described in our previous publication [52]. The xylan obtained was used as substrate for enzymatic hydrolysis [53].

Enzymatic Hydrolysis XOS was obtained by enzymatic hydrolysis of xylan after thoroughly mixing with 0.01 M potassium phosphate buffer at pH 6.5 (15% *w/v*). Then, 100 U/g of substrate of crude xylanase from *Aspergillus niger* (supplied by ASIA STAR CO., LTD.) was added and the incubation was carried out at 55 °C for 24 h [54]. The incubated samples were collected and then centrifuged at 10,000 rpm for 10 min. The supernatant was gathered and freeze-dried at − 40 °C in a freeze dryer (FreezeZone® Plus Labconco, USA), and the powder was kept at − 20 °C until further use.

Experimental Design

Nile tilapia fingerlings were bought from the Chiang Mai Pattahana Farm, Chiang Mai, Thailand. Upon arrival, the fish were placed in 3000-L tanks and allowed to acclimatize for 2 weeks; after which, they were randomly distributed into 16 glass tanks (150 L), stocked at a density of 20 fish $tank^{-1}$. The fish were fed the experimental, twice a day (at 09.00 h and 17.00 h) to apparent satiation, for 12 weeks. Fifty percent of the water in each tank was exchanged daily to maintain water quality. The water quality parameters, temperature, pH, and dissolved oxygen, were monitored daily and maintained at

28.55 ± 0.81 °C, 7.90 ± 0.50, and 5.40 ± 0.35 mg l^{-1}, respectively.

Lactobacillus plantarum CR1T5 was kindly provided by Dr. Saowanit Tongpim, (Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand). A pure culture of *L. plantarum* CR1T5 was inoculated in MRS broth and incubated at 30 °C. After 15 h of incubation (30 °C), the bacterial cells were harvested, washed with 0.85% (*w*/*v*) NaCl, and resuspended in the same solution. The cell suspension density was adjusted spectrophotometrically to reach an optical density at 600 nm (OD600) of 0.2 to 1.8, using sterile 0.85% (*w*/*v*) NaCl. Various cell concentrations within the OD_{600} were observed, and thus a linear relationship between the viable cells, established through the spread-plate technique, and the OD_{600} was determined. The OD_{600} of each cell suspension was set up to a desired cell concentration (CFU mL^{-1}) for further feed formulation experiment.

The selected *L. plantarum* CR1T5 dose (10^8 colony forming units, cfu g^{-1}) replicated that of our previous studies [45, 55]. The *L. plantarum* CR1T5 added to the tested diets was prepared daily, following the protocol described by Irianto, Austin [56]. A basal diet [57] was supplemented with CDXOS and/or *L. plantarum* CR1T5 in which to prepare the experimental diets: 0 g kg^{-1} CDXOS and 0 *L. plantarum* CR1T5 (diet 1—control), 10 g kg^{-1} of CDXOS (diet 2), 10^8 cfu g^{-1} *L. plantarum* CR1T5 (diet 3), and 10 g $kg^{-1}$of CDXOS +10^8 cfu g^{-1} *L. plantarum* CR1T5 (diet 4) (Table 1).

Growth Performance

After 4-, 8-, and 12-week post feeding, the final weight, weight gain, specific growth rate, and feed conversion ratio were calculated according to the previously described formulae [48].

Sample Collection and Immune Response Analysis

Sample Collection

Within the same periods described above, four fish from each replication were used for innate immune response analysis, in which skin mucus was collected according to the method of Ross et al. [58]. Blood and serum were collected, as described in our previous studies [46, 59]. Leucocyte separation and collection from un-clotted blood was carried out according to Chung, Secombes [60] with modifications, as described in previous studies [46, 59].

Immune Parameters

***Lysozyme* Activity** The protocol described by Parry et al. [61] was followed for the determination of serum and mucus lysozyme activities, and expressed as μg ml^{-1}.

Peroxidase Activity Serum and mucus peroxidase activities were measured according to the methods of Quade, Roth [62] and Cordero et al. [63]. Briefly, 5 μL of serum or skin mucus was placed in flat bottomed, 96-well plates in triplicate. Then, 45 μL of Hank's balanced salt solution (HBSS), without Ca^{+2} or Mg^{+2}, and 100 μL of solution (40 ml of distilled water, 10 μL of H_2O_2 (30%—Sigma-Aldrich), and one tablet of 3,3′,5,5′-tetramethylbenzidine (TMB; Sigma-Aldrich)) were added. Fifty microliters of 2 M H_2SO_4 was added once the reaction color changed, and the optical density was read at 450 nm via a plate reader (Synergy H1, BioTek, USA). Standard samples with no serum or skin mucus were considered as blanks, a single unit was defined as the amount producing an absorbance change of 1, and the activity was expressed as units of (U) mg^{-1} serum or mucus.

Phagocytosis Activity The serum phagocytic activity was determined according to the method described by Yoshida and Kitao [64] with slight modifications, described in details in our previous studies [46, 59].

Respiratory Burst Activity The respiratory burst activity of the Nile tilapia peripheral blood leucocytes was determined through the suggestions of Secombes [65], with slight modifications, as described in detail in our previous studies [46, 59].

Alternative Complement Pathway Activity The serum alternative complement pathway activity (ACH50) was measured according to Yanno [66], as described in [46, 59].

Challenge Study

Streptococcus agalactiae source, preparation, and injection dose were as detailed in a previous publication [48]. Briefly, *S. agalactiae* was cultured in Tryptic Soy Broth and incubated at 37 °C for 24 h in a rotation shaker, at a speed of 110 rpm. The sub-culture was obtained from the stock, as follows: 5 mL of the stock solution was transferred into a 50-mL flask containing Tryptic Soy Broth, and incubated at 37 °C for 24 h. The sub-cultures within the present study were raised in duplicate, under similar conditions. Growth was evaluated by optical density of 560 nm and then confirmed by plate counting, in Tryptic Soy Agar.

After 12 weeks of feeding, eight randomly selected fish in each tank were injected intraperitoneally with 0.1 ml of 0.85% normal saline solution (NSS) containing 10^7 CFU ml^{-1} of *S. agalactiae* [67]. Dead fish from each tank were removed daily, and the mortality (%) in each treatment was computed 15 days, post-challenge. The relative percentage of survival (RPS) was then calculated through the following equation:

$$\mathrm{RPS} = 100 - (\text{test mortality}/\text{control mortality}) \times 100$$

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
CDXOS[1]	0	10	0	10
Lactobacillus plantarum (CFU g^{-1})	0	0	10^8	10^8
Cellulose	30	20	30	20
Soybean oil	2	2	2	2
Premix[2]	10	10	10	10
Vitamin C[3]	8	8	8	8
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)				
Crude protein	319.36	319.35	319.36	319.35
Crude lipid	71.75	71.75	71.75	71.75
Fiber	52.48	52.48	52.48	52.48
Ash	106.68	107.27	106.68	107.27
Dry matter	817.80	816.90	817.80	816.90
GE (Cal g^{-1})[4]	4066	4064	4066	4064

[1] *CDXOS*, xylooligosaccharides from corncobs

[2] 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

[3] Vitamin C 98% 5 g

[4] *GE*, gross energy

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The mean values were considered significantly different, when $P < 0.05$. All statistical analysis was conducted using SAS Computer Program [68].

Results

Growth Performance

Statistically significant increases were recorded for the specific growth rate (SGR), weight gain (WG), and final weight (FW) within each supplemented diet, for 4, 8, and 12 weeks, as compared to the control group ($P < 0.05$; Table 2). Fish fed diet 4, a combination of CDXOS and *L. plantarum* CR1T5 (LP), had the highest FW, WG, and SGR values (Table 2), yet had the lowest feed conversion ratio (FCR). The highest FCR was found in fish from the control group (diet 1) ($P < 0.05$). However, there were no significant differences in any of the parameters in fish fed diet 2 or 3 ($P > 0.05$; Table 2). There were also no effect on the survival rate detected among fish receiving either the control, or any of the supplemented diets (Table 2).

Innate Immune Parameters

Dietary supplementations of CDXOS (diet 2), LP (diet 3), and the combination of CDXOS and LP (diet 4) each significantly enhanced skin mucus lysozyme and peroxidase activities (SMPA), compared with the control group, after each of the three feeding periods ($P < 0.05$; Table 3). The highest values were found in fish fed diet 4, followed by fish fed diet 3, and diet 2. However, no significant differences were detected in mucus parameters among fish fed the control and the supplemented diets after 4 weeks, or between fish fed diet 2 and 3 ($P > 0.05$; Table 3).

Differences were observed in serum lysozyme activity between control and the supplemented groups (Table 4), in which diets 2, 3, and 4 produced higher serum lysozyme activity than that of the control diet ($P < 0.05$; Table 4). The

Table 2 Growth performances and feed utilization (mean ± SE) of the Nile tilapia fed different diets: diet 1 (0—control), diet 2 (10 g kg^{-1} CDXOS), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} CDXOS +10^8 CFU g^{-1} *L. plantarum*). Different letter in a row denote significant difference ($P < 0.05$)

	Diet 1	Diet 2	Diet 3	Diet 4
IW (g)	4.91 ± 0.09	4.94 ± 0.08	4.96 ± 0.07	5.05 ± 0.04
FW (g)				
4 weeks	13.11 ± 0.74^b	17.06 ± 0.84^a	17.19 ± 0.95^a	18.68 ± 0.56^a
8 weeks	23.33 ± 0.64^c	27.40 ± 0.92^b	27.80 ± 0.91^b	32.00 ± 1.02^a
12 weeks	47.71 ± 1.10^c	50.86 ± 0.82^b	51.84 ± 0.52^b	57.36 ± 0.99^a
WG (g)				
4 weeks	8.20 ± 0.77^b	12.13 ± 0.81^a	12.23 ± 0.94^a	13.63 ± 0.58^a
8 weeks	18.41 ± 0.63^c	22.46 ± 0.89^b	22.84 ± 0.85^b	26.95 ± 1.04^a
12 weeks	42.80 ± 1.04^c	45.93 ± 0.74^b	46.88 ± 0.47^b	52.31 ± 1.03^a
SGR				
4 weeks	3.26 ± 0.21^b	4.12 ± 0.15^a	4.13 ± 0.18^a	4.36 ± 0.11^a
8 weeks	2.60 ± 0.05^c	2.85 ± 0.05^b	2.87 ± 0.03^b	3.08 ± 0.06^a
12 weeks	2.53 ± 0.02^c	2.59 ± 0.01^b	2.61 ± 0.01^b	2.70 ± 0.03^a
FCR				
4 weeks	1.44 ± 0.02^a	1.37 ± 0.01^b	1.36 ± 0.01^b	1.34 ± 0.01^b
8 weeks	1.52 ± 0.008^a	1.47 ± 0.01^b	1.46 ± 0.01^c	1.42 ± 0.009^b
12 weeks	1.62 ± 0.009^a	1.55 ± 0.01^b	1.56 ± 0.01^c	1.50 ± 0.006^b
SR (%)	98	99	99	99

IW initial weigh $fish^{-1}$, *FW* final weight $fish^{-1}$, *SGR* specific growth rate $fish^{-1}$, *FCR* feed conversion ratio, *SR* survival rate

highest serum lysozyme activity (SL) was detected in fish fed diet 4, whereas no significant difference was observed between the CDXOS (diet 2) and LP (diet 3) supplemented diets ($P > 0.05$; Table 4). Similarly, alternative complement activity (ACH50), and phagocytosis activity (PI) increased in fish fed the supplemented diets, compared with the control; the highest values being generated by diet 4 (Table 4). No significant differences were detected in the values of fish fed the CDXOS (diet 2) and the LP (diet 3) diets, regarding seric lysozyme, complement activity, and phagocytosis ($P > 0.05$; Table 4). Higher levels of serum peroxidase activity resulted in fish fed the supplemented diets, versus those fed the control diet, though no significant differences were observed among the supplemented diets. Lastly, significant differences in respiratory burst activity were detected between fish fed supplemented diets and the control diet, but only at 4 weeks (Table 4).

Challenge Test

The challenge test using *S. agalactiae* was carried out after 12 weeks of feeding, and the survival rate was recorded over the following 15 days. Dead fish exhibited a loss of appetite, darkness, exophthalmia, fins basal hemorrhage, and pale liver, which are typical symptoms of *Streptococcus* infection. The results show that the survival rates of fish fed CDXOS (diet 2), 56.25%; LP (diet 3), 59.38%; and the combination of CDXOS + LP (diet 4), 71.88%, were significantly higher ($P < 0.05$) than that recorded for fish fed the control diet 31.25% (Fig. 1). Among the supplemented groups, fish fed diet 4 showed

Table 3 Skin mucus lysozyme and peroxidase activities of *O. niloticus* after 4, 8, and 12 weeks fed different diets (mean ± SE, $n = 4$): diet 1 (0—control), diet 2 (10 g kg^{-1} CDXOS), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} CDXOS +10^8 CFU g^{-1} *L. plantarum*). Different letters in a row denote significant difference ($P < 0.05$)

		Diet 1	Diet 2	Diet 3	Diet 4
4 weeks	SMLA	2.03 ± 0.38^a	2.13 ± 0.27^a	2.23 ± 0.33^a	2.49 ± 0.46^a
	SMPA	0.04 ± 0.004^c	0.06 ± 0.005^b	0.07 ± 0.006^b	0.11 ± 0.004^a
8 weeks	SMLA	3.38 ± 0.38^c	4.86 ± 0.09^b	4.87 ± 0.30^b	5.97 ± 0.10^a
	SMPA	0.09 ± 0.006^c	0.13 ± 0.01^b	0.14 ± 0.006^b	0.17 ± 0.01^a
12 weeks	SMLA	4.81 ± 0.27^c	6.06 ± 0.10^b	6.17 ± 0.21^b	7.48 ± 0.20^a
	SMPA	0.12 ± 0.008^c	0.17 ± 0.009^b	0.17 ± 0.02^b	0.27 ± 0.01^a

SMLA ($\mu g\ ml^{-1}$), skin mucus lysozyme activity; *SMPA* ($\mu g\ ml^{-1}$), skin mucus peroxidase activity

Table 4 Serum immunity of *O. niloticus* after 4, 8, and 12 weeks of feeding with different diets (mean ± SE, $n = 4$): diet 1 (0—control), diet 2 (10 g kg^{-1} CDXOS), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} CDXOS +10^8 CFU g^{-1} *L. plantarum*). Different letters in a row denote significant difference ($P < 0.05$)

		Diet 1	Diet 2	Diet 3	Diet 4
4 weeks	SL	4.62 ± 0.31^{c}	6.34 ± 0.33^{b}	6.72 ± 0.34^{b}	7.96 ± 0.15^{a}
	SP	0.12 ± 0.009^{b}	0.17 ± 0.006^{a}	0.18 ± 0.006^{a}	0.19 ± 0.005^{a}
	ACH50	139.36 ± 5.77^{c}	162.39 ± 2.62^{b}	161.76 ± 4.11^{b}	182.99 ± 2.86^{a}
	PI	1.53 ± 0.04^{b}	2.43 ± 0.18^{a}	2.45 ± 0.07^{a}	2.59 ± 0.04^{a}
	RB	0.05 ± 0.005^{b}	0.07 ± 0.008^{a}	0.08 ± 0.008^{a}	0.09 ± 0.009^{a}
8 weeks	SL	7.22 ± 0.22^{c}	8.93 ± 0.16^{b}	9.02 ± 0.34^{b}	10.91 ± 0.46^{a}
	SP	0.18 ± 0.007^{b}	0.23 ± 0.01^{a}	0.24 ± 0.01^{a}	0.25 ± 0.008^{a}
	ACH50	179.53 ± 9.05^{c}	224.10 ± 9.82^{b}	228.48 ± 10.18^{b}	277.20 ± 9.69^{a}
	PI	2.06 ± 0.11^{b}	2.76 ± 0.11^{a}	2.66 ± 0.05^{a}	2.81 ± 0.08^{a}
	RB	0.13 ± 0.01^{a}	0.14 ± 0.01^{a}	0.14 ± 0.01^{a}	0.14 ± 0.02^{a}
12 weeks	SL	$8.96 \pm 0.0.41^{c}$	11.81 ± 1.22^{b}	12.09 ± 1.21^{b}	17.41 ± 0.34^{a}
	SP	0.22 ± 0.003^{b}	0.28 ± 0.02^{a}	0.30 ± 0.03^{a}	0.31 ± 0.02^{a}
	ACH50	224.98 ± 10.97^{c}	282.33 ± 5.36^{b}	280.50 ± 9.15^{b}	350.24 ± 14.19^{a}
	PI	2.45 ± 0.09^{c}	3.12 ± 0.10^{b}	3.03 ± 0.05^{b}	3.68 ± 0.12^{a}
	RB	0.17 ± 0.01^{a}	0.18 ± 0.01^{a}	0.18 ± 0.02^{a}	0.19 ± 0.02^{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 (OD655)

significantly higher RPS than those within other groups, as well as the greatest resistance to *S. agalactiae* (Fig. 1).

Discussion

Because of today's growing restrictions on the use of antibiotics as growth promoters, safe and natural feed additives are being enthusiastically investigated as alternatives to enhance growth performance and to protect against diseases [69]. A wide range of feed additives, like probiotics and prebiotics, which distribute have positive effects upon the host, have been applied in aquaculture, in which to control diseases, promote growth, and enhance the host's immune response [70]. Such functional feed additives have gained great attention for their beneficial effects in enhancing production and the well-being of farmed fish, as well as for increasing overall resistance to diseases [71].

Dietary supplementation of corncob-derived xylooligosaccharides (CDXOS) and *L. plantarum* CR1T5 (LP), either alone or combined, produced significantly positive effects on final weight, weight gain, specific growth rate, and feed conversion ratio of Nile tilapia. To the best of our knowledge, this is the first investigation that has demonstrated such positive effect on growth performance of Nile tilapia. In line with the present study, the positive effects of XOS, *Lactobacillus plantarum* CR1T5, and other synbiotics were previously reported in European sea bass (*Dicentrarchus labrax*) [72, 73], blunt snout bream (*Megalobrama amblycephala*) [74], sea cucumber (*Apostichopus japonicus*) [75, 76], Nile tilapia (*Oreochromis niloticus*) [44–46, 48, 77],

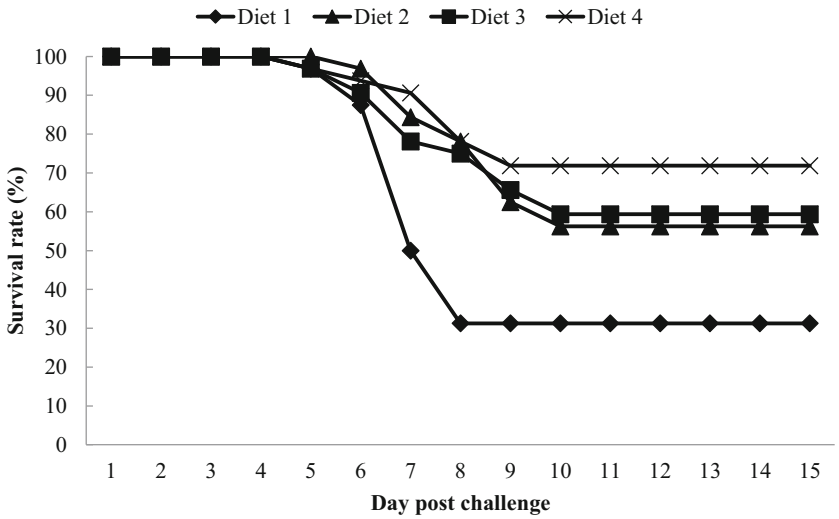

Fig. 1 Survival rate of tilapia, *O. niloticus* fed different concentrations of dietary CDXOS and *L. plantarum* ($n = 8$, mean ± SD): diet 1 (control), diet 2 (10 g kg^{-1} CDXOS), diet 3 (10^8 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} CDXOS +10^8 CFU g^{-1} *L. plantarum*) during 15 days post-challenge with *S. agalactiae*

angelfish (*Pterophyllum scalare*) [78], snakehead (*Channa striata*) [79, 80], rockfish (*Sebastes schlegeli*) [81], Asian sea bass (*Lates calcalifer*) [82], and major carp (*Cirrhinus mrigala*) [83]. In contrast, Abid et al. [84] reported that a *P. acidilactici* and scFOS supplemented diet had no effect on the growth performance of Atlantic salmon. Similarly, probiotic or prebiotic supplemented diets had no effect on the growth or survival of Totoaba (*Totoaba macdonaldi*) [85]. The discrepancies in these findings may attributable to differences in species, experimental design, XOS form, and the method of administration [86, 87], as well as sampling strategy. Indeed, reports have confirmed that the effects of probiotics on fish depend on the dose and duration of the treatment, and the source, as well as on the species in question [88]. It has been further reported that the inclusion of pre- and probiotics in feed is associated with improved health status, improved prebiotic digestion, and an increase in probiotic survival and colonization, compared with individual pre- or probiotic applications [89–92]. These effects were most probably mediated by short-chain fatty acids, as by-products of fermentation of probiotic strains in the existence of prebiotics [81, 93]. Yu et al. [94] reported that acetate was the dominant short-chain fatty acid found to result from the fermentation process between xylooligosaccharides from corn cobs and *L. plantarum*. In addition to short-chain fatty acids, the dietary consumption of both pro- and prebiotics resulted in the formation of bioactive microbial metabolites, such as vitamins and biological peptides [95]. These, in turn, improved nutrient digestion and absorption in the host intestine and consequently had a positive effect on growth. As previously reported, this may be attributable to the favorable effects of XOS that help normalize gut microbiota, and enhance the gut digestive and absorptive capabilities of fish [96], and may have led to the improvement of feed utilization in the present study.

The innate immune system of farmed fish is considered a crucial defense system, which provides protection against opportunistic pathogens [97]. The present investigation indicated that the dietary inclusion of prebiotics, probiotics, and a synbiotic resulted in an increase in serum lysozyme, serum peroxidase, ACH50, phagocytic, and respiratory activities. Also, the synbiotic diet led to higher non-specific immune parameters than in the other groups. To the best of our knowledge, there exists no information regarding the effects of CDXOS, *Lactobacillus plantarum* CR1T5, or their combination on the innate immunological parameters of Nile tilapia. Nonetheless, in agreement with present results, Hoseinifar et al. [98] revealed that serum ACH50 and lysozyme activities significantly increased in rainbow trout fingerlings fed the pre-, pro-, and synbiotic diets. Recently, Kumar et al. [83] showed that the dietary inclusion of mannanoligosaccharide (MOS) and *Bacillus subtilis* as a synbiotic significantly stimulated the innate immunological parameters in major carp (*Cirrhinus mrigala*). Similarly, a significant improvement in the innate immune response was observed in rockfish (*Sebastes schlegeli*), fed *Pediococcus acidilactici*, galactooligosaccharide, and synbiotic additives [81]; snakehead (*Channa striata*), fed the prebiotics GOS and MOS in combination with *Saccharomyces cerevisiae* and *L. acidophilus* [80]; and Asian sea bass (*Lates calcalifer*) fed low molecular weight sodium alginate and *Pediococcus acidilactici* [82]. However, the dietary inclusion of *Bacillus subtilis* and chitosan [91] as well as *B. subtilis* and FOS [90] failed to stimulate immune parameters of cobia (*Rachycentron canadum*) and yellow croaker (*Larimichthys crocea*), respectively. The discrepancies of these findings may be due partly to species-specific, fish ages, and/or pre- or probiotic doses and types [99].

The mucosal immune system of fish consists of a unique array of specific and innate immune cells, which include lymphocytes, mast cells, macrophages, and granulocytes. It also contains molecules complement proteins, immunoglobulins, lysozyme, proteases, esterases, and antimicrobial peptides with antibacterial, anti-viral, and anti-fungal activities [100–103]. The present findings indicate that the dietary inclusion of prebiotic, probiotic, and synbiotic significantly increased skin mucus lysozyme and peroxidase activities in Nile tilapia. The fish that were fed the synbiotic diet obtained higher skin mucus immune parameters than the fish fed both components individually. The results were similar to previous findings involving the supplementation of heat-killed *L. plantarum* and β-glucan in red sea bream (*Pagrus major*) [104], *Pediococcus acidilactici* and galactooligosaccharide in common carp (*Cyprinus carpio*) [105], *Cordyceps militaris spent mushroom substrate and Lactobacillus plantarum in Nile tilapia* (*Oreochromis niloticus*) [45], *Pediococcus acidilactici* and GOS in rainbow trout and rock fish [81], and low molecular weight sodium alginate and *P. acidilactici* in Asian sea bass (*Lates calcalifer*) [82]. The significant improvement observed in the immune response of fish in the present study may be attributed to the effects of CDXOS and *L. plantarum* CR1T5. Yu et al. [94] demonstrated that a combination of CDXOS and *L. plantarum* in an in vivo model could increase the number of lactobacilli and bifidobacteria in mouse feces, and reduce the viability of *Enterococcus*, *Enterobacter*, and *Clostridi*a spp. Additionally, an in vitro antioxidant assay indicated that CDXOS fermented with *L. plantarum* possessed significant 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis, and superoxide anion radical-scavenging activities. However, the combination effects of CDXOS and *L. plantarum* CR1T5 merit further investigation.

It is well-known that nutritional manipulation is a useful means to enhance disease resistance in fish [106]. Synbiotic therapy is also regarded as an effective method of disease prevention [107]. The results of the present study showed that Nile tilapia fed prebiotic, probiotic, and synbiotic diets

significantly increased resistance to *Streptococcus agalactiae*, of which the highest resistance was detected in fish fed the synbiotic diet. The benefit for the fish's immune system provided by *CDXOS* and *L. plantarum* CR1T5 was demonstrated through the increased resistance to *S. agalactiae* infection in Nile tilapia through these supplements. Similar results, in which dietary supplementation with pre-, pro-, and synbiotic significantly increased disease resistance, were observed in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), against *Aeromonas hydrophilla* [108]; sea cucumber (*Apostichopus japonicus*), against *Vibrio splendidus* [76]; major carp (*Cirrhinus mrigala*), against *Aeromonas hydrophila* [83]; rockfish (*Sebastes schlegeli*), against *Edwardsiella tarda* [81]; and European sea bass (*D. labrax*), against *V. anguillarum* [73]. These significant increases in disease resistance may be due to the combined effects of CDXOS and *L. plantarum* CR1T5. A recent study showed that cell-free *L. plantarum* supernatant added to the corncob XOS presented strong antibacterial activities against *Shigella flexneri* and *E. coli*, compared with the activity of *Staphylococcus aureus* and *Salmonella typhimurium*. Culturing *L. plantarum* in MRS broth in the presence of XOS revealed inhibition zones. This indicated that the antagonistic activities of this strain originate from alternative or simultaneous acid and hydrogen peroxide (H_2O_2) inhibition. These results suggest that XOS could stimulate the proliferation of favorable microbiota and increase the production of the antimicrobial substances in the cell-free supernatant, which would include organic acids, H_2O_2, bacteriocins, and low molecular mass peptides [94, 109]. In conclusion, data from the present study suggest that CDXOS and *Lactobacillus plantarum* CR1T5, applied singularly or in combination, can be used as functional feed additives for better growth performance and *S. agalactiae* resistance in tilapia aquaculture.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval The study was performed in accordance with the guidelines on use of animals for scientific purposes (Chiang Mai University).

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