Aquaculture 460 (2016) 59–68

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Dietary prebiotics and probiotics influence growth performance, nutrient digestibility and the expression of immune regulatory genes in snakehead (*Channa striata*) fingerlings

Mohammad Bodrul Munir [a,*], Roshada Hashim [b], Yam Hok Chai [d], Terence L. Marsh [c], Siti Azizah Mohd Nor [a]

[a] School of Biological Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia
[b] Faculty of Science and Technology, Universiti Sains Islam Malaysia, 71800 Bandar Baru Nilai, Negeri Sembilan, Malaysia
[c] Department of Microbiology and Molecular Genetics, Biomedical & Physical Sciences, Michigan State University, 567 Wilson Road, East Lansing, MI 48824, USA
[d] Faculty of Applied Sciences, UCSI University, 56000 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 19 March 2016
Accepted 21 March 2016
Available online 06 April 2016

Keywords:
Prebiotics
Probiotics
Growth performance
Nutrient digestibility
Immune regulatory genes and snakehead (*Channa striata*)

ABSTRACT

This study evaluated the effect of duration of feeding with selected prebiotics (β-glucan, GOS, MOS) and probiotics (*Saccharomyces cerevisiae*, *Lactobacillus acidophilus*) on growth performance, nutrient digestibility, the expression of immune regulatory genes and the capacity of *Channa striata* fingerlings to retain the benefits derived after the intake of these supplements with time. Fish on the supplemented diets resulted in better growth performance, nutrient digestibility and the expression of immune regulatory genes significantly ($P < 0.05$) outperforming those on the control diet; highest performance was found in fish fed with *L. acidophilus* supplement. The growth trends were lower in all prebiotics compared to fish on the probiotics supplemented diets. The results obtained from the present study showed that supplementation with *L. acidophilus* is best for growth and the expression of immune regulatory genes of *C. striata* fingerlings regardless of feeding duration unlike the prebiotic treatments whose early differences became insignificant by the time feeding was extended to 16 weeks.

1. Introduction

The snakehead, *Channa striata* (Bloch, 1793), is a carnivorous freshwater fish, which is widely distributed in Asia. It is a valuable food fish (Wee, 1982) known for its high protein content (Annasari *et al.*, 2012), low fat and minimal intramuscular spines and medicinal qualities, (Haniffa and Marimuthu, 2004) used traditionally to treat injuries and burns. Hence, in recent years the snakehead aquaculture industry has expanded and production yields have increased from 16 tons in 1998–2000 to 42 tons in 2010–12 (FAO, 2014). However, in common with other intensive aquaculture practices, snakehead culture has resulted in problems associated with the deterioration of water quality and the outbreak of diseases (FAO, 2012). For decades, disease in farms are managed through the widespread and often uncontrolled use of antibiotics, which in turn led to the advent of antimicrobial resistant pathogens, reduction in beneficial microbiota in the gastrointestinal (GI) ecosystem, including the accumulation of residual antibiotics in fish muscle making it unsuitable for human consumption (FAO, 2005). Therefore, the use of all sub-therapeutic antibiotics as growth-promoting agents was banned by the European Union in 2006 (Denev *et al.*, 2009) and efforts are focussed on exploring new strategies in feeding and health management in fish aquaculture practice (Balcâzar *et al.*, 2006). These include evaluating new dietary supplements which promote health and growth-promoting compounds such as prebiotics, probiotics, symbiotics, phytobiotics and other functional dietary supplements (Denev, 2008).

Probiotics and prebiotics are bioactive components (Kapka *et al.*, 2012) that provide not only nutrients, but microorganisms, oligosaccharides and polysaccharides, which enhance growth performance subsequently, increase fish production (Diana, 1997; Abdelghany and Ahmad, 2002). Probiotics are live microorganisms (Fuller, 1989) like bacteria (*L. acidophilus*, *Bacillus cereus*), or live yeast (*Saccharomyces cerevisiae*), which have been proven to have beneficial effects on fish growth by improving its intestinal microbial balance (Al-Dohail *et al.*, 2009; Dhanaraj *et al.*, 2010). In addition to promoting growth (Grisdale *et al.*, *2008*), prebiotics which are non-digestive feed ingredients (Gibsen and Roberfroid, 1995), also increase the metabolism of health-promoting bacteria like lactobacillus, bifidobacteria, nutrient digestibility and the expression of immune regulatory genes (Mohsen and Xin, 2015). The consumption of these oligosaccharides is reported to reduce β-glucuronidase and nitroreductase activities resulting in the enhancement of immunity and modulation of mucin production (Arturo *et al.*, 2010). Currently, prebiotics are increasingly used for fish disease management as it is reported to improve water quality (Denev, 2008). There is increasingly strong evidence that both prebiotics and probiotics are able to cause an up or down

* Corresponding author.
E-mail address: hsjewel730@yahoo.com (M.B. Munir).

http://dx.doi.org/10.1016/j.aquaculture.2016.03.041
0044-8486/

regulation of immune regulatory genes, particularly the innate immune regulatory system which is the first line of defense. In fish, attention has been focused on TGF-β1 gene and the nuclear factor kappa-light-chain-enhancer of activated β-cell or NF-κ B (Awad *et al.*, 2011). The present study was carried out to determine the influence of selected dietary prebiotics and probiotics on growth performance, feed utilization & body indices; nutrient digestibility & digestive enzyme activities of *C. striata* fingerlings and the duration of their effectiveness for a specified period of post-feeding without any supplementation. In addition, the response of the fish innate immune system with respect to the mRNA expression of the TGF-β1 and NF-κ B genes to the dietary intake of the selected probiotics and prebiotics was also investigated.

2. Methodology

2.1. Experimental fish and husbandry conditions

The study was conducted at the Aquaculture Research Complex in Universiti Sains Malaysia (USM), Penang, Malaysia. A total of 10,000 snakehead fries (1.5 cm) was purchased from the local fish farm and raised on Artemia cysts (OSI brand, USA) followed with tubiflex worms (purchased daily from the local aquarium shop) till they achieved 3 cm in length. The fish were then weaned to artificial feed by first feeding them with custard made of local fish meal and chicken egg yolk for a further 3 weeks before finally feeding them with a commercial sea bass pellet containing 43% crude protein and 6% crude lipid. The experimental fish (Av. wt. 22.40 g ± 0.06) were then stocked in 12 outdoor cement tanks (2 m × 1 m × 0.5 m) at the rate of 400 fish/tank. Water temperature and pH were recorded twice daily and ranged between 26.52 °C ± 0.31 to 28.27 °C ± 0.22, and 6.6 ± 0.08 to 7.3 ± 0.10 respectively.

2.2. Diet preparation

A non-supplemented control diet and five experimented diets supplemented with three prebiotics, 0.2% β-glucan (Macrogard(R)), 1% galacto-oligosaccharides (Vivinal(R) GOS syrup, Friesland Campina Domo, Netherland), 0.5% mannan-oligosaccharides (Alltech(R), Actigen 1, USA), and two probiotics, 1% live yeast (*S. cerevisiae*, Alltech(R), YEA-SACC 1026, USA) and 0.01% *L. acidophilus* powder (Sigma^R LBA-10^8 CFU) were formulated (Table 1). All diets contained 40% protein and 12% lipid. The six diets were prepared using a composite pelletizer (Model: KL5M, China) at Fisheries Research Institute, Pulau Sayak, Kedah and dried at 35 °C for 48 h and stored at −20 °C frozen temperature. The feed ingredients and proximate composition of diets (Table 1) were analyzed as described in AOAC (1997).

2.3. Feeding trials

The feeding trial was divided into two phases. Phase 1 involved feeding with the six experimental diets respectively, for 16 weeks followed by Phase 2, referred to as the post-feeding trial period, in which the fish in all treatments were fed the non-supplemented control diet for a further 8 weeks. This was done to evaluate the efficacy of prebiotics and probiotic intake in Phase 1. In both phases, fish were fed to satiation three times daily.

2.4. Growth performance

Fish weight was taken fortnightly in Phase 1 from the 4th week of the feeding treatment and weekly in Phase 2. During each sampling, three groups of twenty fish were collected randomly from each replicate tank and weighed. The relative growth (RG); the specific growth rate (SGR), survival rate (SR) and for feed utilization, the protein efficiency rate (PER), food conversion ratio (FCR) was calculated

Table 1
Feed ingredients and proximate composition of the formulated diet (g/kg, dry matter).

	Control	β-glucan 0.2%	GOS 1%	MOS 0.5%	Live yeast 1%	*L. acidophils* 0.01%
Ingredients						
Danish fish meal[a]	534	534	534	534	534	534
Korean corn starch	340	340	340	340	340	340
Fish oil	5	5	5	5	5	5
Soyabean oil	60	60	60	60	60	60
Cellulose	11	9	1	6	1	10.9
CMC[b]	10	10	10	10	10	10
Vitamin mix[c]	20	20	20	20	20	20
Mineral mix[d]	20	20	20	20	20	20
Supplement	0	2	10	5	10	0.1
Proximate composition g/kg						
Moisture	81.9	52.2	63.1	71.9	96.5	92.76
Protein	410	407.3	409.4	406.8	409.1	409.7
Lipid	118.8	117.5	118.4	118	120.3	121.2
Ash	10.09	10.15	9.8	10.33	9.9	10.56
Fiber	123	123.2	123.2	121.8	121.8	120.6
NFE[e]	256.21	289.65	276.1	271.17	242.4	245.18
GE[f] (MJ/kg)	198.9	197.6	198.5	199.2	198.7	196.9

[a] Danish fish meal kg^{-1} = crude protein 746.6 and crude lipid 101.6.
[b] CMC = carboxymethyl cellulose.
[c] Vitamin mix kg^{-1} = rovimix 6288, Roche Vitamins Ltd. Switzerland; Vit $_A$ 50 million i.u., Vit $_D$ 310 million i.u., Vit $_E$ 130 g, Vit $_{B1}$ 10 g, Vit $_{B2}$ 25 g, Vit $_{B6}$ 16 g, Vit $_{B12}$ 100 mg, Biotin 500 mg, Pantothenic acid 56 g, Folic acid 8 g, Niacin 200 g, Anticake 20 g, Antioxidant 200 mg, Vit $_{K3}$ 10 g and Vit $_C$ 35 g.
[d] Vitamin mix kg^{-1} = calcium phosphate (monobasic) 397.65 g, Calcium lactate 327 g, Ferrous sulfate 25 g, Magnesium sulfate 137 g, Potassium chloride 50 g, Sodium chloride 60 g, Potassium iodide 150 mg, Copper sulfate 780 mg, Manganese oxide 800 mg, Cobalt carbonate 100 mg, Zinc oxide 1.5 g and Sodium selenite 20 g.
[e] NFE = nitrogen free extract (1000-{Moisture + Protein + Lipid + Ash + Fiber}).
[f] GE = gross energy measured using bomb calorimeter, Parr 1356 bomb calorie.

according to following formula (Busacker *et al.*, 1990; Ahmed *et al.*, 2002; Abdel-Tawwab *et al.*, 2008, USAID, 2011).

$$RG\ (\%) : (\{\text{Final weight} - \text{Initial Weight/initial weight}\} \times 100).$$

$$SGR\ (\%) : (\ \ln\text{final weight} - \ln\text{initial weight/nos.of days}) \times 100.$$

$$SR\ (\%) : \{(\text{Final Number of Fish/Initial Number of Fish}) \times 100\}.$$

$$PER : \{(\text{Final Weight} - \text{Initial Weight})/\text{Protein Intake}\}.$$

$$FCR : (\text{Total Feed Consumption/Weight Gain of Fish}).$$

The hepatosomatic index (HSI), visceral somatic index (VSI) and intraperitoneal fat (IPF) were determined by sacrificing nine fishes per replicate tank at the end of Phase 1 and Phase 2, respectively, using the following formula (Busacker *et al.*, 1990).

$$HSI\ (\%) : \{(\text{Liver Weight/Fish Weight}) \times 100\}.$$

$$VSI\ (\%) : \{(\text{Viscera Weight/Fish Weight}) \times 100\}.$$

$$IPF\ (\%) : \{(\text{IPF Weight/Fish Weight}) \times 100\}.$$

2.5. Relative protein digestibility assays

Relative Protein Digestibility (RPD) was determined in vitro using the pH drop method described by Lazo *et al.* (1998). Crude enzyme was extracted according to the modified method of Chisty (2005). At the end of Phase 1 and Phase 2, respectively, twenty seven fishes were randomly collected from each replicate tank after 4 h of feeding, sacrificed and placed on blocks of ice to collect the intestines. The protein suspension mixtures (6.25 mg/ml) were used to determine the relative protein digestibility (Saterlee *et al.*, 1979; Sharifah *et al.*, 2014). The protein concentration was measured by using Bradford (1976) with

Bovine Serum Albumin (BSA) as standard. Casein was used as standard. RPD was calculated as follows:

$$\text{Relative Protein Digestibility (RPD)} = \{(-\Delta\text{pH feedstuff})/(-\Delta\text{pH casein})\} \times 100.$$

2.6. Digestive enzyme assays

Protease, amylase and lipase enzyme activities were determined in digestive enzyme assays using the same crude enzyme samples extracted from the fish intestine during determination of the relative protein digestibility. The specific protease activities were determined using casein digestion method modified by Chong *et al.* (2002) as described by Walter (1984). The one unit of specific protease activity was defined as the amount of enzyme needed to release one micromole tyrosine per minute per milligram protein of the enzyme extract. The specific amylase activities were determined according to Worthington (1993) method described by Akter *et al.* (2015). It was measured by one unit of amylase activity was defined as the amount of micromoles maltose released per minute per milligram protein. The specific lipase enzyme was determined according to Bier (1955) method modified by Natalia *et al.* (2004) as described by Akter *et al.* (2015). It was done as the volume of 0.01 M NaOH required to neutralize the fatty acid release during the 4 h incubation period with the substrate and after correction with a blank. The protein concentration of extracted crude enzymes was measured by using Bradford (1976) with Bovine Serum Albumin (BSA) as standard. The digestive enzyme assay was performed at the end of 8 weeks and 16 weeks in the Phase 1; and at the end of Phase 2.

2.7. Gene expression

The present study evaluated the influence and the duration of effectiveness of prebiotics (β-glucan, GOS and MOS) and probiotics (yeast and *L. acidophilus*) on the expression of immune-regulatory genes (TGF-β1 and NF-k B) in snakehead (*C. striata*). The primer sequence of two genes was collected from the relevant literature (Hernandez *et al.*, 2013; Amy, 2011). Beta Actin (β-Actin) was used as reference gene Weizhang and Qionglin (2008). Conventional PCR was used to verify the correct amplification of two immune regulatory and housekeeping genes. All sequences (Table 2) were confirmed using NCBI nucleotide BLAST software (<http://blast.ncbi.nlm.nih.gov>).

RNA extraction and real time qPCR

The total RNA was extracted from the head kidney of the individual fish as this tissue is proven to have the highest mRNA for immune response (Uma *et al.*, 2015; Hernandez *et al.*, 2013; Feng *et al.*, 2009; Weizhang and Qionglin, 2008). Six fishes were randomly collected from each replicate tank making six groups and were sacrificed to collect the head kidney. All dissections were performed on blocks of ice and at 20 °C room temperature. The RNA was extracted using the protocol of easy spin™ (DNA free) Total RNA Extraction Kit (Cat No. 17221. iNtRON Biotechnology, Inc). The quality and quantity of purified RNA were checked using Nanodrop (Quawell, UV Spectrophotometer, Q3000, Taiwan) measuring at OD 260/280. Furthermore, the quality was checked by separation on an agarose gel, as described by Liu *et al.* (2002).

The expression of two immune regulatory genes (Table 3) was determined using real-time qPCR. The qPCR reaction was done in triplicate in a final volume of 20-μl by using the guidelines of manufacturer i.e. i-Green™ One Step qRT-PCR Kit (S) (Cat No. 25109. iNtRON Biotechnology, Inc.). The amplification was carried out with a systematic negative control (non-template control) by using BIO-RAD qPCR machine (Model CFX96™ Real-Time System).

The expression was normalized against β-Actin and presented as the relative expression compared with the non-treated control sample. Gel electrophoresis was used to verify the correct amplification. All the C_T-values were exported to the spread sheet format, and these were further analyzed for measuring the gene expressing using the relative quantification $2^{-\Delta\Delta C}{}_T$ method as stated by Schmittgen and Livak (2008).

2.8. Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA). Multiple comparisons were performed with Duncan's test to analyze the differences between treatment means to be significant at 95% confidence level using SPSS software. The study also performed the two way ANOVA ($P < 0.05$) to measure the effect of diet and time on growth performance, nutrient digestibility and expression of two immune regulatory genes.

Table 2
Detail feeding trial in the study.

Phase	Period	Feeding treatments	Feeding management	Replicate number
Phase 1	0 to 8 weeks (initial)	Treatment 1	Fish fed with control diet	Each feeding treatment had 3 biological replicates 400 fishes/replicate
		Treatment 2	Fish fed with β glucan compared to control	
		Treatment 3	Fish fed with GOS compared to control	
		Treatment 4	Fish fed with MOS compared to control	
		Treatment 5	Fish fed with live yeast compared to control	
		Treatment 6	Fish fed with LBA compared to control	
Phase 1	8–16 weeks (middle and end of 1st phase)	Treatment 1	Fish fed with control diet	
		Treatment 2	Fish fed with β glucan compared to control	
		Treatment 3	Fish fed with GOS compared to control	
		Treatment 4	Fish fed with MOS compared to control	
		Treatment 5	Fish fed with live yeast compared to control	
		Treatment 6	Fish fed with LBA compared to control	
Phase 2	Following 8 weeks or continuous after 16 weeks to 24 weeks	Treatment 1	Fish in control tank fed with control (continue)	
		Treatment 2	Fish in β glucan feeding trials fed with control	
		Treatment 3	Fish in GOS feeding trials fed with control	
		Treatment 4	Fish in MOS feeding trials fed with control	
		Treatment 5	Fish in live yeast feeding trials fed with control	
		Treatment 6	Fish in LBA feeding trials fed with control	

β-glucan = beta glucan as prebiotics feed supplement.
GOS = glacto-oligosaccharides as prebiotics feed supplement.
MOS = manna-oligosaccharides as prebiotics feed supplement.
Live yeast = *Saccharomyces cerevisiae* as prebiotics feed supplement.
LBA = *Lactobacillus acidophilus* as prebiotics feed supplement.

Table 3
Primers (with accession number in NCBI) used for Real Time qPCR analysis.

Transcript	Primer sequence (5′–3′)	Fragment (bp)	Accession no.
TGF-β1-F	AACTACTGCATGGGGTCCTG	174	BC16236.1
TGF-β1-R	GGACAATTGCTCCACCTTGT		
NF-κ B-F	CTTCAGGTTCCGTTATGGGTGTGA	78	LN590714.1
NF-κ B-R	ATGACTTCCTGTTCTTCTCGCTGG		
β-Actin-F	CACTGTGCCCATCTACGAG	196	KC967219.1
β-Actin-R	CCATCTCCTGCTCGAAGTC		

3. Results

3.1. Growth performance

Supplementation with the prebiotics and probiotics tested improved growth performance, feed utilization and survival of *C. striata* fingerlings compared to the control. The values of growth performance, feed utilization and survival of *C. striata* fingerlings were all significantly improved relative to the control at 8 weeks and 16 weeks of supplementation, respectively (Table 4). Although the growth performance and the feed utilization decreased in the 2nd Phase of the study, values remained significantly higher than the control.

At the end of the Phase 1, fish maintained on the LBA supplemented diet had the significantly highest RG (400.45%) and the SGR (1.44) compared to all test diets. However, both the probiotics tested (LBA and Yeast) performed better than the prebiotics (β-glucan, MOS and GOS). The relative growth (RG) and the specific growth rate (SGR) among the fish fed the three prebiotics did not differ significantly ($P < 0.05$) at the end of the Phase 1. Although specific growth rate (SGR) trends in Phase 2 were similar to Phase 1 for all treatments tested, values were significantly lower. At the end of the Phase 2 (Fig. 1), highest RG (606.24%) and SGR (1.16) were observed in the LBA feeding trial, followed by yeast (RG = 528.44%; SGR = 1.09), β-glucan (RG = 367.91%; SGR = 0.92), MOS (RG = 342.12%; SGR = 0.88), GOS (RG = 302.50%; SGR = 0.83) and control (RG = 246.86%; SGR = 0.74). SGR values decreased depending on the type of supplemented feed intake. The specific growth rate of the fish fed with LBA supplemented diets dropped drastically 6 weeks into Phase 2 while that of fish on the yeast diet dropped earlier i.e. by the 5th week. Fish in all the 3 prebiotic fed groups could only maintain the specific growth rates up to the 4th week of Phase 2 (Fig. 1) and by the end of Phase 2, all values among the prebiotic treatments were not significantly different.

Feed conversion ratio (FCR) values after prebiotics and probiotics intake were lower significantly ($P < 0.05$) compared to the control in the 8th week of Phase 1; the values improved further by the end of Phase 1 for each respective treatment. In this period, fish fed with the LBA treatment recorded the lowest significant different value (1.24) followed by the live yeast (1.35), β-glucan (1.50), MOS (1.61) and GOS (1.62) treatments. By the end of Phase 2, all FCR values for each supplemented treatment increased significantly ($P < 0.05$) compared to values at the end of Phase 1 and at the 7th week of Phase 2. Fish fed with LBA supplemented diets showed the best FCR performance in the both phases. The trend in protein efficiency ratio (PER) values of fish fed with the prebiotics and probiotics feed supplements was the reverse of FCR values. The highest significant PER value was measured in LBA (1.99) at the end of Phase 1 followed by the yeast (1.82), β-glucan (1.64), MOS (1.53) and GOS (1.51); while at the end of Phase 2, the values of PER in all treatments decreased significantly ($P < 0.05$). Fish fed with the 3 prebiotics and the control did not show any significant differences at the end Phase 2 (Table 4).

The highest survival was observed in the fish fed with the LBA treatment (97.50%), followed by live yeast (96.75%), β-glucan (93.25%), MOS (92.63%) and GOS (91.25%) at the end of the Phase 1. No mortalities were recorded in the two probiotics supplemented treatments from week 4 of Phase 1 until the end of Phase 2 (Table 4). Visceral somatic index (VSI), hepatosomatic index (HSI) and intraperitoneal fat (IPF) also increased significantly ($P < 0.05$) at the end of Phase 1; but decreased at the end of Phase 2 (Fig. 2) for all treatments. Intake of the three prebiotics did not show any significant difference in the VSI values at the end of the Phase 1 and the values were lower than the two probiotics for the same period. However, by the end of Phase 2, the VSI values for both prebiotics and probiotics treatments did not differ significantly (Fig. 2). The value of HSI was significantly ($P < 0.05$) highest in the fish fed with the LBA at the end of Phase 1, followed by yeast, β-glucan, MOS, GOS and control. The HSI trend differed slightly in Phase 2 when the treated fish with the yeast had the highest significant HSI value followed by LBA and the other 3 prebiotics. At the end of the second Phase, the HSI values of all treated fish remained higher compared to the fish fed with the control since Phase 1. In both Phases, fish fed with the control diet had the highest significant ($P < 0.05$) IPF compared to the fish fed with the supplemented diets. The values of

Table 4
Growth performance, feed utilization and survival of *Channa striata* fingerlings.

Parameters/feeding phase		Control	β glucan	GOS	MOS	Live yeast	LBA
Initial weight in g	Initial	22.42 ± 0.07	22.38 ± 0.07	22.38 ± 0.07	22.41 ± 0.06	22.39 ± 0.05	22.42 ± 0.05
Weight gain in g	Phase 1_8W*	29.97 ± 0.1^{a}	39.12 ± 0.3^{c}	36.67 ± 0.8^{b}	38.14 ± 1.1^{c}	43.87 ± 1.4^{d}	48.06 ± 1.2^{e}
	Phase 1_16W*	49.09 ± 0.3^{a}	78.09 ± 2.2^{b}	68.95 ± 1.6^{b}	73.60 ± 1.9^{b}	101.27 ± 2.2^{c}	112.20 ± 2.2^{d}
	Phase 2_8W**	77.8 ± 0.6^{a}	104.7 ± 1.5^{d}	90.1 ± 4.4^{b}	99.1 ± 6.8^{c}	140.7 ± 3.1^{e}	158.34 ± 7.4^{f}
RG	Phase 1_8W*	33.71 ± 0.3^{a}	74.78 ± 0.9^{c}	63.89 ± 3.3^{b}	70.21 ± 4.8^{c}	95.95 ± 5.6^{d}	114.35 ± 5.4^{e}
	Phase 1_16W*	118.97 ± 0.9^{a}	248.91 ± 10.0^{b}	246.87 ± 9.3^{b}	240.40 ± 9.8^{b}	352.39 ± 9.7^{c}	400.45 ± 10.2^{d}
	Phase 2_8W**	246.86 ± 1.9^{a}	367.91 ± 5.5^{d}	302.5 ± 19.4^{b}	342.12 ± 30.1^{c}	528.44 ± 14.1^{e}	606.24 ± 32.6^{f}
SGR	Phase 1_8W*	0.52 ± 0.01^{a}	1.00 ± 0.01^{d}	0.88 ± 0.01^{b}	0.95 ± 1.0^{c}	1.20 ± 1^{e}	1.36 ± 0.01^{f}
	Phase 1_16W*	0.70 ± 0.01^{a}	1.12 ± 0.03^{b}	1.11 ± 0.02^{b}	1.09 ± 0.03^{b}	1.35 ± 0.02^{c}	1.44 ± 0.02^{d}
	Phase 2_8W**	0.74 ± 0.01^{a}	0.92 ± 0.01^{d}	0.83 ± 0.03^{b}	0.88 ± 0.4^{c}	1.09 ± 0.01^{e}	1.16 ± 0.03^{f}
FCR	Phase 1_8W*	2.06 ± 0.1^{d}	1.56 ± 0.02^{b}	1.78 ± 0.1^{c}	1.75 ± 1.0^{c}	1.57 ± 0.04^{b}	1.47 ± 0.03^{a}
	Phase 1_16W*	1.90 ± 0.01^{e}	1.50 ± 0.02^{c}	1.62 ± 0.07^{d}	1.61 ± 0.07^{d}	1.35 ± 0.09^{b}	1.24 ± 0.08^{a}
	Phase 2_8W**	1.78 ± 0.03^{c}	1.71 ± 0.02^{c}	1.75 ± 0.01^{c}	1.74 ± 0.01^{c}	1.60 ± 0.12^{b}	1.53 ± 0.03^{a}
PER	Phase 1_8W*	1.17 ± 0.05^{a}	1.60 ± 0.00^{de}	1.37 ± 0.08^{b}	1.40 ± 0.09^{b}	1.55 ± 0.05^{c}	1.65 ± 0.05^{d}
	Phase 1_16W**	1.29 ± 0.01^{a}	1.64 ± 0.02^{c}	1.51 ± 0.06^{b}	1.53 ± 0.07^{b}	1.82 ± 0.12^{d}	1.99 ± 0.14^{e}
	Phase 2_8W*	1.37 ± 0.02^{a}	1.44 ± 0.02^{a}	1.40 ± 0.01^{a}	1.41 ± 0.01^{a}	1.54 ± 0.12^{b}	1.60 ± 0.03^{c}
Survival %	Phase 1_8W*	92.12	95.75	93.38	93.88	96.75	97.50
	Phase 1_16W*	80.63	93.25	91.25	92.63	96.75	97.50
	Phase 2_8W**	80.00	88.75	86.50	87.50	96.75	97.50

Each value is the mean (±SD, n = 6). Superscripts in each row represent significant ($P < 0.05$) differences among the treatments tested

* Feeding treatments with supplementation and control diet, respectively.

** Treated fish fed with a control diet only for the next 8 weeks.

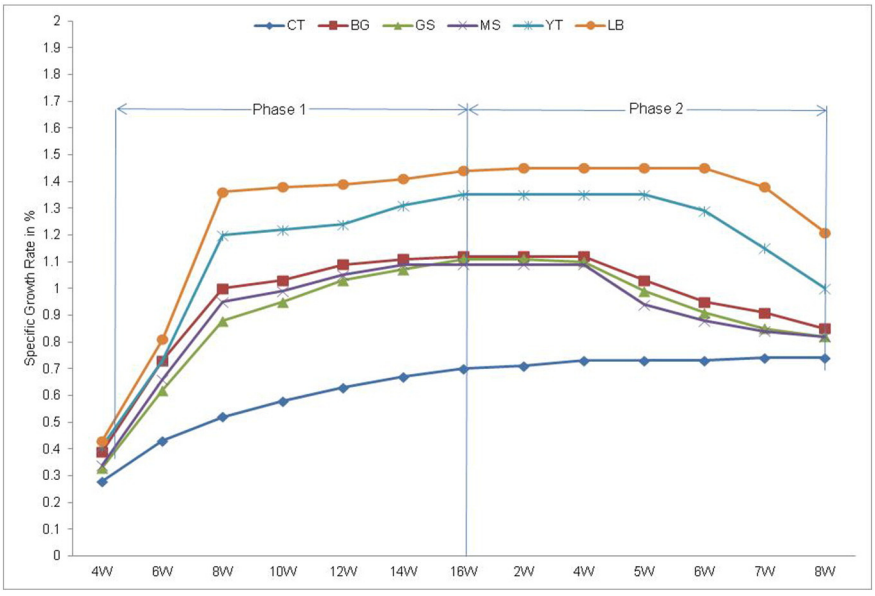

Fig. 1. Specific growth rate of *Channa striata* fingerlings. CT = control without any supplementation; BG = feed with β-glucan; GS = feed with glacto-oligosaccharides; MS = feed with mannan-oligosaccharides; YT = feed with live yeast; LB = feed with *Lactobacillus acidophilus*. Significance ($P < 0.05$) among the feeding trial with times.

IPF were significantly ($P < 0.05$) higher in fish fed with the probiotics compared to the fish fed with the prebiotics (Fig. 2).

3.2. Nutrient digestibility and digestive enzyme activities

The supplemented diets had a positive effect on the relative protein digestibility (RPD) of the test diets in *C. striata* fingerlings (Fig. 3). The values of RPD in Phase 1 and 2 were higher significantly ($P < 0.05$) in the fish fed with the probitics compared to fish fed with prebiotics, significantly highest in fish fed with LBA supplements. Like growth performance, the RPD values of the supplemented diets started to increase significantly from the 8th week of Phase 1, and it continued until the end of Phase 1. At the end of Phase 2, the values of RPD of all treated fish were significantly ($P < 0.05$) reduced; however the RPD values remained better compared to the fish fed with the control since the initial week of Phase 1. At all interval tested, fish fed the LBA supplemented diet the highest performance of RPD in both phases.

Amylase, protease and lipase specific activities were significantly higher in fish fed with the probiotics compared to those on the prebiotic supplemented diets in both phases (Table 5). In the first Phase, the

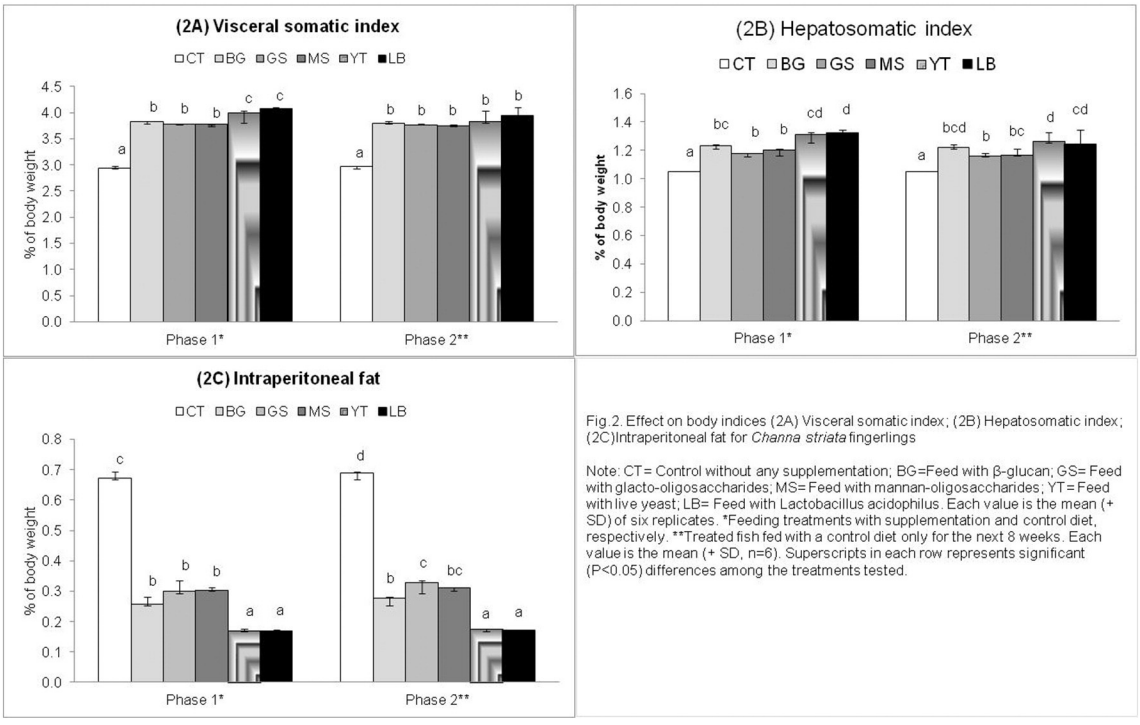

Fig. 2. Effect on body indices: (2A) visceral somatic index; (2B) hepatosomatic index; (2C) intraperitoneal fat for *Channa striata* fingerlings. Note: CT = control without *any* supplementation; BG = Feed with β-glucan; GS = Feed with glacto-oligosaccharides; MS = Feed with mannan-oligosaccharides; YT = Feed with live yeast; LB = Feed with Lactobacillus acidophilus. Each value is the mean (+SD) of six replicates. *Feeding treatments with supplementation and control diet, respectively. **Treated fish fed with a control diet only for the next 8 weeks. Each value is the mean (+SD, n = 6). Superscripts in each row represents significant ($P < 0.05$) differences among the treatments tested.

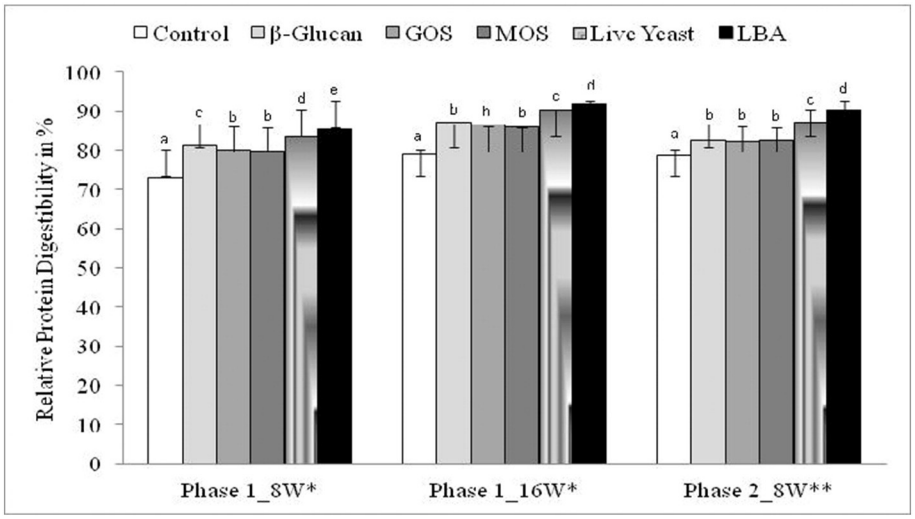

Fig. 3. Effect of prebiotics and probiotics on relative protein digestibility. *Feeding treatments with supplementation and control diet, respectively. **Treated fish fed with a control diet only for the next 8 weeks. Each value is the mean (+SD, n = 6). Superscripts in each row represents significant (P < 0.05) differences among the treatments tested.

highest amylase (2.79 U/mg), protease (0.90 U/mg) and lipase (10.69 U/mg) activities were in the LBA treatment, followed by yeast, β-glucan, MOS and GOS treatments (Table 5), this trend remained unchanged by the end of Phase 2. Among the three enzyme activities, the lipase activity was found highest compared to the amylase and protease enzyme activities. At the end of the 2nd Phase, the trend among the treatment remained unchanged, but values were significantly (P < 0.05) lower than those in Phase 1 (Table 5).

3.3. Expression of immune regulatory genes

The expression of immune-regulatory genes was significantly (P < 0.05) up-regulated in the fish fed with the supplemented diets (Fig. 4 and Fig. 5). At the end of Phase 1, the expression of transforming growth factor beta 1 (TGF β1) was significantly (P < 0.05) higher in the fish fed with the probiotics compared to the fish fed with the prebiotics (Fig. 4). The LBA supplemented diet was found to have the highest significant (P < 0.05) up-regulation of the TGF β1 gene at the end of Phase 1, followed by the yeast, β glucan, MOS and GOS. There was no significant (P > 0.05) difference in the up-regulation of this gene between the fish fed with the MOS and GOS at the end of Phase 1 (Fig. 4). The performance trend of TGF β1 expression remained unchanged at the end of Phase 2 except the values were lower compared to Phase 1.

Similarly, the nuclear factor kappa beta (NF κ B) was also up-regulated in the supplemented feeding trials (Fig. 5) at the end of Phases 1 and 2. Although the pattern of expression in Phase 2 was similar to Phase 1, the relative expression of NF κ B gene was lower in Phase 2. The fish fed with LBA supplementation resulted the highest NF κ B gene expression in the both Phases, followed by yeast, β glucan, MOS and GOS. There was no significant difference (P < 0.05 / P > 0.05) among the three prebiotics on the NF κ B gene expression in both Phases, although values were lower than the Phase 2.

Overall, the two-way ANOVA result confirmed that both diet and time significantly (P < 0.05) influenced growth performance, feed utilization, nutrient digestibility and the expression of immune regulatory gene; but supplemented diets played a dominant role (Table 6) compared to time. The interaction between diet and time did not influence significantly amylase activities ($F = 0.86$, $p = 0.573$, $r^2 = 0.993$), lipase activities ($F = 1.30$, $p = 0.244$, $r^2 = 0.993$), and the expression of TGF β1 ($F = 2.22$, $p = 0.063$, $r^2 = 0.993$) and NF κ B ($F = 0.45$, $p = 0.807$, $r^2 = 0.993$) genes.

4. Discussion

This study is a follow up to a previous study by Talpur *et al.* (2014) who determined the growth response and blood parameters against *Aeromonas hydrophila* infection in *C. striata* fingerlings fed the same probiotics and prebiotics but for 8 weeks. Generally both the probiotics tested performed better than the prebiotics and with the exception of MOS and GOS, the results for the same feeding duration are consistent with Talpur *et al.* (2014). The better growth performance of fish fed the MOS supplemented diet over that of GOS observed here could be due to the higher inclusion level of 0.5% MOS instead of 0.2% used previously. It is well established that intake of both probiotics and prebiotics cause the production of bioactive microbial metabolites such as vitamins, bioactive peptides, organic acids or fatty acids during fermentation (Stanton *et al.*, 2005). These in turn enhance overall nutrient digestion in the gut and subsequently improve growth rates. Generally,

Table 5
Digestive enzymes (amylase, protease and lipase) activities of *Channa striata* fingerlings.

Phase_period	Enzymes	Control	β-glucan	GOS	MOS	Live yeast	LBA
Phase 1_8W*	Amylase (U/mg)	1.77 ± 0.06^{a}	2.22 ± 0.16^{d}	2.06 ± 0.10^{b}	2.12 ± 0.04^{c}	2.50 ± 0.11^{e}	2.58 ± 0.05^{f}
	Protease (U/mg)	0.63 ± 0.12^{a}	0.75 ± 0.13^{d}	0.69 ± 0.20^{b}	0.72 ± 0.17^{c}	0.78 ± 0.12^{c}	0.80 ± 0.14^{e}
	Lipase (U/mg)	6.19 ± 0.42^{a}	8.16 ± 0.13^{b}	7.49 ± 0.61^{b}	7.70 ± 0.37^{b}	9.29 ± 0.31^{c}	9.68 ± 0.14^{c}
Phase 1_16W*	Amylase (U/mg)	2.09 ± 0.03^{a}	2.49 ± 0.05^{c}	2.32 ± 0.12^{b}	2.34 ± 0.14^{b}	2.68 ± 0.02^{d}	2.79 ± 0.13^{e}
	Protease (U/mg)	0.70 ± 0.06^{a}	0.86 ± 0.19^{c}	0.82 ± 0.17^{b}	0.83 ± 0.29^{b}	0.87 ± 0.09^{c}	0.90 ± 0.13^{d}
	Lipase (U/mg)	7.09 ± 0.16^{a}	9.54 ± 0.73^{bc}	9.03 ± 0.32^{b}	9.25 ± 0.49^{b}	9.91 ± 0.29^{c}	10.69 ± 0.53^{d}
Phase 2_8W**	Amylase (U/mg)	2.09 ± 0.11^{a}	2.46 ± 0.09^{c}	2.24 ± 0.19^{ab}	2.28 ± 0.80^{bc}	2.66 ± 0.19^{d}	2.79 ± 1.25^{d}
	Protease (U/mg)	0.70 ± 0.11^{a}	0.84 ± 0.25^{c}	$0.78 \pm 0.0.07^{b}$	0.80 ± 0.12^{b}	0.87 ± 0.10^{cd}	0.88 ± 0.09^{d}
	Lipase (U/mg)	7.02 ± 0.21^{a}	9.27 ± 0.70^{bc}	$8.86 \pm 0.0.63^{b}$	9.17 ± 0.62^{bc}	9.72 ± 0.51^{cd}	10.16 ± 0.40^{d}

Each value is the mean (±SD, n = 6). Superscripts in each row represent significant (P < 0.05) differences among the treatments tested.
* Feeding treatments with supplementation and control diet, respectively.
** Treated fish fed with a control diet only for the next 8 weeks.

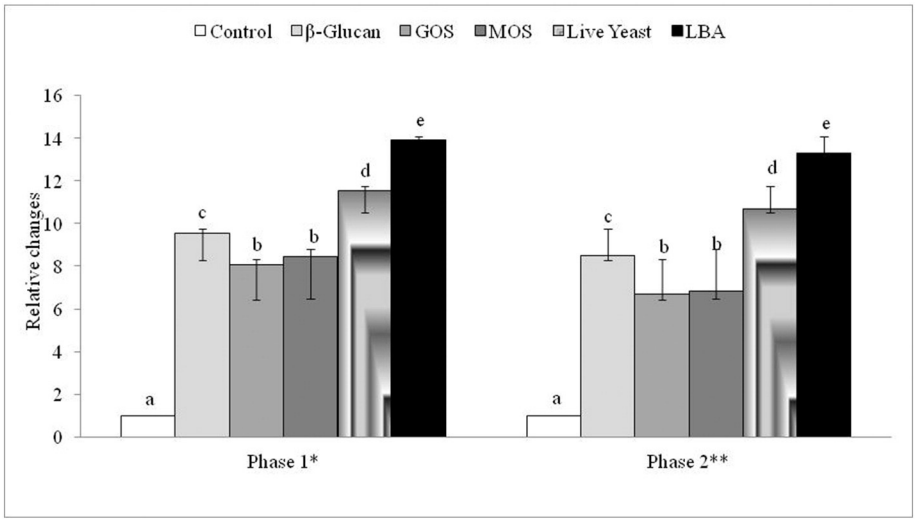

Fig. 4. Effect of a single dose of selective prebiotics and probiotics on head kidney expression of TGF-β1 mRNA transcripts. *Feeding treatments with supplementation and control diet, respectively. **Treated fish fed with a control diet only for the next 8 weeks. Each value is the mean (+SD, n = 6). Superscripts in each row represents significant ($P < 0.05$) differences among the treatments tested.

studies on freshwater fishes such as *Pangasianodon hypophthalmus* (Akter *et al.*, 2015), Asian common carp (Dhanaraj *et al.*, 2010) and *Clarias gariepinus* (Al-Dohail *et al.*, 2009) have shown that probiotics perform better than prebiotics and this has been attributed to the direct influence of the live microorganisms in the probiotics (Paola and Dariel, 2014; Ringo and Galewoupe, 1998) on the gastrointestinal wall which causes a more enhanced fermentation rate (Gill, 1998) in the lumen versus the indirect mechanism of action of prebiotics. In the case of *L. acidophilus* supplementation, the direct increase of its population in the gut could have replaced not only the pathogenic bacteria but they also produced nutrients and stimulated the release the digestive enzymes (Cüneyt *et al.*, 2008). This is supported by the higher gut digestive enzyme activities in fish fed with the LBA supplemented diets (Table 5) compared to those on live yeast and the better relative protein digestibility (Fig. 3) in the LBA treated fish. In contrast, the influence of live yeast appears more time dependent for yeast colonies to be formed on the intestinal wall (Waché *et al.*, 2006; Vázquez-Juárez *et al.*, 1997, Andlid *et al.*, 1998; Vázquez-Juárez *et al.*, 1994) and for the gut to mature. Studies in rainbow trout (Aubin *et al.*, 2005a; Gatesoupe *et al.*, 2005a; Waché *et al.*, 2006; Andlid *et al.*, 1995) have shown that maximal colonization of live yeast in the gut occurs during the first month of continued intake and can continue up to 5 months.

Structural differences among the prebiotics, β-glucan being an unbranched homopolysaccharide structure while both MOS and GOS are branched heteropolysaccharides, may have caused the different responses observed among the prebiotics tested. Based on the higher amylase specific activity, the former appeared more easily digested in the gut compared to the latter two prebiotics, after 8 weeks and subsequently resulting in a significantly better protein digestibility in the β-glucan treatment as gut fermentation matures. It is also evident from this study that these differences occurred only in the early stages of prebiotic intake because by Week-16, amylase specific activities in the MOS and GOS treatments were no longer significantly different and that differences with the β-glucan treatment although significant, was smaller than at Week-8. Indeed, relative protein digestibility among these three feed supplements were no longer significantly different during this period. As expected, growth trends in Phase 1 of the study are also reflected in the FCR and PER values similar to observations in hybrid striped bass (Li and Gatlin, 2005), rainbow trout (Staykov *et al.*, 2007), European sea bass (Torrecillas *et al.*, 2007) and red drum (Zhou *et al.*,

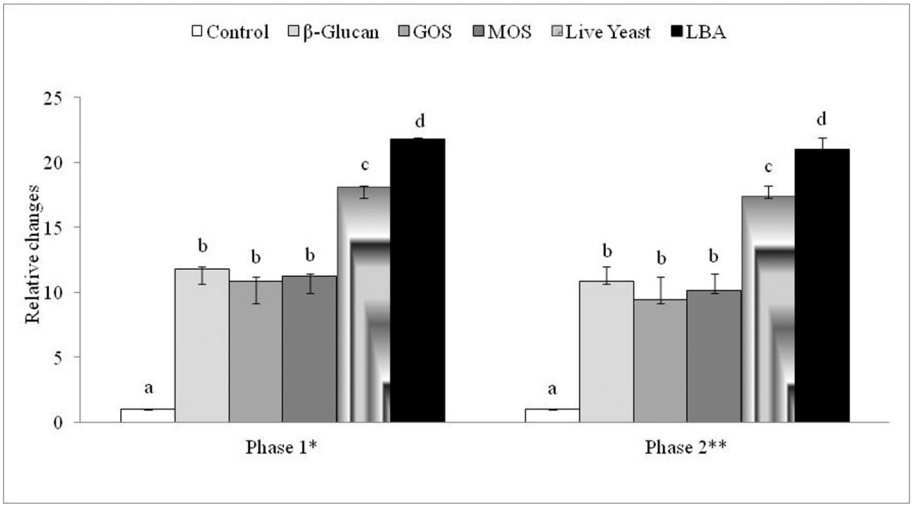

Fig. 5. Effect of a single dose of selective prebiotics and probiotics on head kidney of NF-k B mRNA transcripts. *Feeding treatments with supplementation and control diet, respectively. **Treated fish fed with a control diet only for the next 8 weeks. Each value is the mean (+SD, n = 6). Superscripts in each row represents significant ($P < 0.05$) differences among the treatments tested.

Table 6
Two-way ANOVA analysis showing the F and *P* values (the mean difference is significant at the P < 0.05) depending on diet and time.

Factor		GG	RG	SGR	FCR	PER	RPD	Amylase	Protease	Lipase	TGF β1	NF k B
Diet	F-value	770.04	784.04	1190.59	137.89	115.99	373.05	151.71	214.11	88.04	486.27	400.25
	P-value	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Time	F-value	5339.55	5445.71	460.34	72.02	79.20	420.02	73.26	276.00	49.15	34.36	7.74
	P-value	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.007*
Interaction	F-value	115.32	117.43	45.47	11.40	11.34	5.84	**0.86**	2.81	**1.30**	**2.22**	**0.46**
	P-value	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	***0.573***	*0.004*	***0.244***	***0.063***	***0.807***

GG = growth gain; RG = relative growth; SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency rate; RPD = relative protein digestibility; TGF β1 = transforming growth factor beta; NF k B = nuclear factor kappa-B. Individual feeding trial (diet), rearing weeks (time) and both were influenced significantly (P < 0.05) higher except both feed and time did not influence significantly for Amylase, Lipase and Immune regulatory genes (presented in bold).

2010) and fish survival. Hence, this study suggests that prolonged feeding up to 16 weeks with prebiotics had a positive effect on nutrient digestibility and growth performance, but this advantage is lost once prebiotic supplementation is removed.

Visceral somatic index (VSI), hepatosomatic index (HSI) and intraperitoneal fat (IPF) indicates food value (Keri *et al*., 2014) and feeding prebiotics and probiotics had a positive influence on these body indices. In this study, VSI and HSI were significantly higher in fish fed with dietary prebiotics and probiotics compared to fish fed the control diet, similar to Ahmad *et al*. (2012) and Gümüs and Ikiz (2009). On the other hand, IPF was higher in fish fed with the control diet compared to all the supplemented diets, probable due to a more efficient lipid digestion and assimilation in the diets containing prebiotics and probiotics.

The efficacy after dietary intake of probiotics and prebiotics was investigated by extending the feeding trial at the end of Phase 1 for another 8 weeks using only the control diets (Phase 2) and monitoring the specific growth rates. Growth performance remained higher than the initial weeks of Phase 1 and continued until Week-4 for the prebiotic treatments while that live yeast and LBA dipped after Week-5 and Week-6, respectively. Similarly, all other growth and feed utilization parameters paralleled the specific growth rate trends for all treatments.

The association between dietary probiotic and prebiotic with the immune system is well documented (Hernandez *et al*., 2013). However, although Zduñczyk and Pareek (2009) showed that they have the capacity to modify gene expression and modulate fish immune system (Montero *et al*., 2008), the molecular mechanisms involved upon dietary intake of prebiotics and probiotics remains poorly understood. The present research evaluated two immune regulatory genes, the transforming growth factor (TGF) β1 and nuclear factor (NF) k B, in relation to dietary prebiotics and probiotics. The data demonstrated that the expression of these genes, altered significantly in fish fed the experimental diets containing prebiotics and probiotic compared to the group of fish fed the control diet. The overall order of expression of TGF β1 gene expression was LBA > Yeast > β-glucan > MOS ≥ GOS, while that of NF κ B gene was LBA > Yeast > β-glucan ≥ MOS ≥ GOS with the control being significantly lowest at the end of Phase 1. These are similar to the performance trend of growth described earlier. These results provide further support to the nutrigenomic principle that nutrients in the formulated feed are dietary signals which can be detected by a cellular sensor system, influencing gene and the protein expression, and subsequently produce metabolites (Müller and Kersten, 2003) which enhance the quality of the mRNA of immune regulatory genes such as transforming growth factor (TGF) β1, interleukin (IL)-1, interleukin (IL)-8, interleukin (IL)-10, nuclear factor (NF) κ B (Miyazaki *et al*., 1997; Letterio and Roberts, 1998; Gilmore, 2006). The metabolites produced are also reported to act as biological response modifiers (Dallard *et al*., 2007; Bhon, 1995), immunomodulatory (Chanpul *et al*., 2012; Novak and Vetvicka, 2009) as well as have immunostimulant roles (Meena *et al*., 2013) which enhance the production of cytokins to stimulate the NK- cells, B-cells and T-cells in preparation for pathogenic infection (Bunselmeyer and Buddendick, 2010). Although up-regulation of the TGF β1 and NF κ B genes continued in Phase 2 when the fish were no longer fed the supplemented diets, the significantly lower values observed in this phase indicate that sustaining the benefits attained upon ingestion of the probiotics and prebiotics becomes time dependant once supplementation ceases.

In conclusion, the results obtained from the present study have shown that supplementation with LBA is best for growth and the expression of immune regulatory genes of *C. striata* fingerlings regardless of feeding duration unlike the prebiotic treatments whose early differences no longer became significant by the time feeding was extended to 16 weeks. There was no significant difference in the performance of the different probiotics after 16 weeks, however the prebiotics supplemented fish still performed significantly better than the control.

Acknowledgement

This research was supported under the Malaysian Government Fundamental Research Grant Scheme (Ref: 203/PBIOLOGI/6711308) as well as the USM Global Fellowship (USM.IPS/USMGF (02/13). The authors would like to acknowledge the contribution of the Fisheries Research Institute, Pulau Sayak, Kedah for feed preparation facilities, AllTech(R) for providing the Bioactin and Yaa-Sac, and to FriedlandCampina Domo(R) for Vivinal GOS Syrup and Bio-Origin for Macroguard(R) β-glucan.

References

Abdelghany, A.E., Ahmad, M.H., 2002. Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. Aquac. Res. 33, 415–423.

Abdel-Tawwab, M., Abdel-Rahman, A., Ismael, N., 2008. Evaluation of commercial live baker's yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia *Oreochromis niloticus* challenge in situ with *Aeromonas hydrophila*. Aquaculture 280, 185–189.

Ahmad, M., Qureshi, T.A., Singh, A.B., 2012. Effect of dietary protein, lipid and carbohydrate contents on the viscera composition and organ indices of *Cyprinus carpio communis* fingerlings. Afr. J. Biotechnol. 11 (33), 8361–8366.

Ahmed, M., Shalaby, A., Khattab, Y. a.-T., 2002. Effects of 17 alpha-methyltestosterone on growth performance and some physiological changes of Nile Tilapia fingerlings (Oreochromis niloticus L). Egypt. J. Aquat. Biol. Fish 4 (4), 295–311.

Akter, N. Mst, Sutriana, A., Talpur, A.D., Hashim, R., 2015. Dietary supplementation with mannan oligosaccharide influences growth, digestive enzymes, gut morphology, and microbiota in juvenile striped catfish *Pangasianodon hypophthalmus*. Aquac. Int. http://dx.doi.org/10.1007/s10499-015-9913-8.

Al-Dohail, M.A., Hashim, R., Aliyu, P.M., 2009. Effects of the probiotics, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African catfish (*Clarias gariepinus*, Burchell 1822) fingerling. Aquac. Res. 40, 1542–1652.

Amy, H., 2011. Identification and Investigation of Conserved Innate Immune Response Response Genes Between Zebrafish and Human. Chapter 3. pp. 40–65 A Master thesis of North Caroline State University.

Annasari, M., M.Aris, W., Yohanes, K., 2012. IEESE International Journal of Science and Technology (IJSTE) 1 (2, 1–8), 2252–5297 (ISSN).

AOAC or Association of Official Analytical Chemists, 1997. Animal Feed Official Methods of Analysis. 16th ed. Arlington, VA, USA (30 pp).

Andlid, T., Vazquez-Juarez, R., Gustafsson, L., 1998. Yeasts isolated from the intestine of rainbow trout adhere to and grow in intestinal mucus. Mol. Mar. Biol. Biotechnol. 7 (2), 115–126.

Andlid, T., Juarez, R.V., Gustafsson, L., 1995. Yeast colonizing the intestine of rainbow trout (*Salmo-gairdneri*) and turbot (*Scophtalmus-maximus*). Microb. Ecol. 30 (3), 321–334.

Arturo, A., Maria, R.M.L., Virginia, C., Victor, C., 2010. Assessment of prebiotics and probiotics: an overview Chapter 2 Bioactive Foods In Promoting Health: Prebiotics And Probiotics, pp. 3–41.

Aubin, J., Gatesoupe, F.J., Labbé, L., Lebrun, L., 2005a. Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac. Res. 36, 758–767.

Awad, E., Mitchell, W.J., Austin, B., 2011. Effecet of dietary supplements on cytokines gene expression in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 34, 629–634.

Balcâzar, J.L., De Blas, I., Ruiz-Zazuela, I., Cunningham, D., Vandrell, D., Muzquiz, J.L., 2006. The role of probiotics in aquaculture. Vet. Microbiol. 114, 173–186.

Bhon, J.A., 1995. (1–3)-β-D-glucan as biological response modifiers: a review of structure-function activity relationships. Carbohydr. Polym. 28, 13–14.

Bier, M., 1955. Lipases. RCOOR′ + H_2O = RCOOH + R′OH. Methods Enzymol. 1, 627–642.

Bloch, M.E., 1793. Naturgeschichte der Ausländischen fische, 7: Berlin. Morino & Co., Germany 144 p.

Bradford, M.M., 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protei-dye binding. Anal. Biochem. 72, 248–254.

Bunselmeyer, Britta, Buddendick, Kirsten, 2010. Probiotics and prebiotics-prevention and therapy in atopic eczema. Bioactive Foods in Promoting Health: Probiotics and Prebiotics, pp. 279–292.

Busacker, G.P., Adeleman, I.R., Goolish, E.M., 1990. Growth. In: Schreck, C.B., Moyle, P.B. (Eds.), Methods for Fish Biology. American Fisheries Society, Bethesda, Maryland, USA, pp. 363–387.

Chanpul, E., Reitsma, M., Kleinjans, L., Mes, J.J., Savelkoul, H.F., Wichers, H.J., 2012. B-glucans are involved in immune modulation of THP-1 macrophages. Mol. Nutr. Food Res. 56 (5), 822–833.

Chisty, A.H., 2005. Partial Protease Characterization, In Vitro Protein Digestibility and Utilization of Diatery Soyabean in Graint Fresh Water Prawn, *Macrobrachium rosenbergii* A PhD Thesis under School of Biological Sciences at University Sains Malayasia (USM), Penang, Malaysia p. 78.

Chong, A.S.C., Hashim, R., Chow-Yang, L., Ali, A.B., 2002. Partial characterization and activities of proteases from the digestive tract of discus fish (*Symphysodon aequifasciata*). Aquaculture 203, 321–333.

Cüneyt, S., Deniz, Ç., Okan, K.H., Şahin, S., Kürşat, F., Özge, O., Hakan, K., 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280 (1–4), 140–145.

Dallard, B.E., Ruffino, V., Heffel, S., Calvinho, L.F., 2007. Effect of a biological response modifier on expression of growth factors and cellular proliferation at drying off. J. Dairy Sci. 90 (5), 2229–2240.

Denev, S., Staykov, Y., Moutafchieva, R., Beev, G., 2009. Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture. Aquac. Res. 1, 1–29.

Denev, S.A., 2008. Ecological Alternatives of Antibiotic Growth Promoters in the Animal Husbandary and Aquaculture DSc. Thesis. Department of Biotechemistry Microbiology Trakia University Stara Zagora, Bulgaria, p. 294.

Dhanaraj, M., Haniffa, M.A., Arun, S., Jesu, A.A., Muthu, R.C., Seetharaman, S., Arthimanju, R., 2010. Effect of probiotics on growth performance of koi carp (*Cyprinus carpio*). J. Appl. Aquac. 22, 202–209.

Diana, J., 1997. Feeding strategies. In: Egna, H.S., Boyd, C.E. (Eds.), Dynamics of Pond Aquaculture. CRC Press, Boca Raton, Florida, pp. 245–262.

FAO, 2005. Responsible use of antibiotics in aquaculture. In: PH, Serrano (Ed.), Food and Agriculture Organization Technical Paper 469. FAO, Rome, Italy, p. 98.

FAO, 2012. The State of World Fisheries and Aquaculture 2012. Food and Agriculture Organization, Rome 223 pp.

FAO, 2014. The State of World Fisheries and Aquaculture 2014. Food and Agriculture Organization, Rome 223 pp.

Feng, C.Y., Johnson, S.C., Hori, T.S., Rise, M., Hall, J.R., Gamperl, A.K., Hubert, S., Kimball, J., Bowman, S., Rise, M.L., 2009. Identification and analysis of differentially expressed genes in immune tisues of Atlantic cod stimulated with formalin. Killed, a typical. *Aeromonas salmonicida*. Physiol. Genomics 37, 149–163.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66, 365–378.

Gatesoupe, F.J., Aubin, J., Quentel, C., Labbé, L., (2005a). Ofimer probiotic study on rainbow trout. IV. The settlement of intestinal microbiota in rainbow trout (*Oncorhynchus mykiss*) fry submitted to probiotic treatment. In: Hendry, C.I., Van Stappen, G., Wille, M., Sorgeloos, P. (Eds.), Larvi 2005, 4th Fish and Shellfish Larviculture Symposium, 5–8 10 September 2005, Ghent University, Gent, Belgium. EAS Special Publication, vol. 36. European Aquaculture Society, Oostende, Belgium, pp. 180–183.

Gibsen, G.R., Roberfroid, M.B., 1995. Dietary Modulation of the Human Colonic Microbiota Introducing the Concept of Prebiotics.

Gilmore, T.D., 2006. Introduction to NF-κ B: players, pathways, perspectives. Oncogene 25 (51), 6680–6684. http://dx.doi.org/10.1038/sj.onc.1209954. PMID 17072321.

Gill, H.S., 1998. Stimulation of the immune system by lactic cultures. Int. Dairy J. 8, 535–544.

Grisdale, H.B., Helland, S.J., Gatlin, D.M., 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture 283, 163–167.

Gümüs, E., Ikiz, R., 2009. Effect of dietary levels of lipid and carbohydrate on growth performance, chemical contents and digestibility in rainbow trout, *Oncorhynchus mykiss*. Pak. Vet. J. 29 (2), 59–63.

Hernandez, J.A., Roman, D., Hooft, J., Cofre, C., Cepeda, V., Vidal, R., 2013. Growth performance and expression of immune-regulatory genes in rainbow trout (*Oncorhynchus mykiss*) juveniles fed extruded diets with varying levels of lupin (*Lupinus albus*), peas (*Pisum sativum*) and rapeseed (*Brassica napus*). Aquac. Nutr. 2013 (19), 321–332.

Haniffa, M.A., Marimuthu, K., 2004. Seed production and culture of snakehead. Infofish Int. 2, 16–18.

Kapka, S.L., Niedźwiecka, J., Wojtyła, A., Kruszewski, M., 2012. Probiotics and prebiotics as a bioactive component of functional food (article in Polish. Pediatr. Endocrinol. Diabetes Metab. 18 (2), 79–83.

Keri, A.I., Aziz, B.A., Abol-Munafi, A.B., 2014. The selection of viscerosomatic and hepatosomatic indices for the measurement and analysis of *Oreochromis niloticus* condition fed with varying dietary maltose levels. International Journal of Fauna and Biological Studies 1 (3), 18–20.

Lazo, J.P., Romaire, R.P., Reigh, R.C., 1998. Evaluation of three in vitro enzyme assays for estimating protein digestibility in the Pacific white shrimp *Penaeus vannamei*. J. World Aquacult. Soc. 29, 441–450.

Letterio, J.J., Roberts, A.B., 1998. "Regulation of immune responses by TGF-beta". Annu. Rev. Immunol. 16, 137–161. http://dx.doi.org/10.1146/annurev.immunol.16.1.137. PMID 9597127.

Li, P., Gatlin, D.M.I.I.I., 2005. Evaluation of the prebiotic grobiotic-A and brewers yeast as dietary supplements for subadult hybrid striped bass (*Morone chrysops*) challenged in situ with *Mycobacterium marinum*. Aquaculture 248, 197–205.

Liu, Y., Jovanovic, B., Pins, M., Lee, C., Bergan, R.C., 2002. Over expression of endoglin in human prostate cancer suppresses cell detachment, migration and invasion. Oncogene 21, 8272–8281.

Meena, D.K., Pronob, D., Shailesh, K., Mandal, S.C., Prusty, A.K., Singh, S.K., Akhtar, M.S., Behera, B.K., Kundan, K., 2013. Beta-glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiol. Biochem. 39 (3), 431–457.

Miyazaki, T., Ise, M., Seo, H., Niwa, T., 1997. Indoxyl sulfate increases the gene expressions of TGF-beta 1, TIMP-1 and pro-alpha 1(I) collagen in uremic rat kidneys. Kidney Int. Suppl. 62, S15–S22.

Mohsen, P., and Xin, Z., (2015). Prebiotics and gut microbiota in chickens. FEMS Microbiol. Lett. http://dx.doi.org/10.1093/femsle/fnv122 fnv122.

Montero, D., Grasso, V., Izquierd, M.S., Gang, R., Rea, F., Tort, L., Caballero, M.J., Acosta, F., 2008. Total substitution of fish oil by vegetable oils in gilthead sea bream (*Sparus aurata*) diets: effects on hepatic Mx expression and some immune parameters. Fish Shellfish Immunol. 24, 147–155.

Müller, M., Kersten, S., 2003. Nutrigenomics: goals and strategies. Nat. Rev. Genet. 4, 315–322.

Natalia, Y., Hashim, R., Ali, A., Chong, A., 2004. Characterization of digestive enzymes in a carnivores ornamental fish, the Asian bony tongue *Scleropages formosus* (Osteoglossidae). Aquaculture 233, 305–320.

Novak, M., Vetvicka, V., 2009. Glucans as biological response modifiers. Endocr Metab Immune Disord Drug Targets 9, 67–75.

Paola, N. and Dariel, TR., (2014). Use of Yeasts as Probiotics in Fish Aquaculture. Chapter 5: *In Sustainable Aquaculture Techniques*., pp 136–172.

Ringo, E., Galewoupe, F.J., 1998. Lactic acid bacteria in fish: a review. Aquaculture 16, 177–203.

Sharifah, R., Mohammed, A.P., Roshada, H., 2014. In vivo and in vitro protein digestibility in juvenile bagrid catfish *Mystus nemurus* (Cuvier and Valenciennes 1840) fed soybean meal-based diets. Aquac. Res. 1–10.

Saterlee, L.D., Marshall, H.F., Tennyson, J.M., 1979. Measuring protein quality. J. Am. Oil Chem. Soc. 56, 103–109.

Schmittgen, D.T., Livak, J.K., 2008. Analyzing Real-Time PCR Data by the Comparative C_T Method. Nature Publishing Group, pp. 1101–1108 (http://www.nature.com/natureprotocols. vol. 3. No. 6).

Stanton, C., Rose, R.P., Fitzgeraid, G.F., Van, S.D., 2005. Fermented functional foods based on probiotics and their biogenic metabolites. Curr. Opin. Biotechnol. 16 (2), 198–203.

Staykov, Y., Spring, P., Denev, S., Sweetman, J., 2007. Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquac. Int. 15, 153–161.

Talpur, A.D., Munir, M.B., Marry, A., Hashim, R., 2014. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. Aquaculture Journal 426–427, 14–20.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L., Izquierdo, M.S., 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrachus labrax*) fed mannan oligosaccharides. Fish Shellfish Immunol. 23, 969–981.

Uma, A., Rebecca, G., Jawahar, R.K., Jesu, A., 2015. Regulation of tissue specific gene expression of toll-like receptor 18 (TLR18) mRNA in freshwater shark *Pangasius pangasius* experimentally induced with poly I:C. Journal of Environment and Biotechnology Research 1 (1), 18–22.

USAID., (2011). Feed Conversion Ratio-Technical Bulletin# 07. www.CambodiaHARVEST.org/www.fintrac.com.

Vázquez-Juárez, R., Andlid, T., Gustafsson, L., 1994. Cell surface hydrophobicity and its relation to adhesion of yeasts isolated from fish gut. Colloids Surf. B: Biointerfaces 2 (1–3), 199–209.

Vázquez-Juárez, R., Andlid, T., Gustafsson, L., 1997. Adhesion of yeast isolated from fish gut to crude intestinal mucus of rainbow trout, *Salmo gairdneri*. Mol. Mar. Biol. Biotechnol. 6 (1), 64–71.

Waché, Y., Auffray, F., Gatesoupe, F.J., Zambonino, J., Gayet, V., Labbé, L., Quentel, C., 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss*, fry. Aquaculture 258 (1–4), 470–478.

Walter, H.E., 1984. Proteinases: Methods With Haemoglobin, Casein and Azocoll as Substrate. Verlag Chemie, Weinheim, pp. 270–277.

Wee, K.L., (1982). Snakehead—their biology and culture. In: Recent Advances in Aquaculture (Muir R. ed) pp. 181–213. Westview, Boulder, CO.

Weizhang, J., Qionglin, G., 2008. Gene structures and promoter characteristics of interferon regulatory factor 1 (IRF-1), IRF-2 and IRF-7 from snakehead *Channa argus*. Mol. Immunol. 45 (2008), 2419–2428.
Worthington, V., 1993. Worthington Enzyme Manual. Worthington Biochemical Corporation, New Jersey, USA.
Zduñczyk, Z., Pareek, C.S., 2009. Application of nutrigenomics tools in animal feeding and nutritional research research. J. Anim. Feed Sci. 18, 3–16.
Zhou, Q.C., Buentello, J.A., Gatlin, D.M., 2010. Effect of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture 309, 253–257.