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Dietary Prebiotics and Probiotics Influence the Growth Performance, Feed Utilisation, and Body Indices of Snakehead (*Channa striata*) Fingerlings

[1]Mohammad Bodrul Munir*, [1,3]Roshada Hashim, [4]Mohammad Suhaimee Abdul Manaf and [1,2]Siti Azizah Mohd Nor

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia
[2]Centre for Marine and Coastal Studies (CEMACS), Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia
[3]Faculty of Science, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia
[4]Fisheries Research Institute (FRI), Pulau Sayak, 08500 Kota Kuala Muda, Kedah, Malaysia

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Abstrak: Kajian ini menggunakan dua fasa percubaan pemakanan untuk menentukan pengaruh prebiotik dan probiotik yang terpilih ke atas pertumbuhan, pengambilan makanan, dan perubahan morfologi ke atas anak ikan haruan (*Channa striata*) dan juga kesan yang dialami dalam tempoh kajian tanpa penggunaan diet tambahan. Tiga kumpulan ikan (22.46 ± 0.17 g) dibesarkan menggunakan enam diet yang berbeza: tiga prebiotik (0.2% β-glucan, 1% galakto-oligosakarida [GOS], dan 0.5% mannan-oligosakarida [MOS]), dua probiotik (1% yis hidup [*Saccharomyces cerevisiae*] dan 0.01% serbuk *Lactobacillus acidophilus* [LBA]) dan satu diet kawalan (tanpa makanan tambahan); setiap rawatan dilakukan sebanyak tiga kali. Semua diet mengandungi 40% protein mentah dan 12% lipid mentah. Ikan-ikan ini diberi makan sebanyak tiga kali sehari. Tiada kematian ikan direkodkan semasa Fasa 1 dijalankan. Walau bagaimanapun, 14% kematian telah direkodkan semasa Fasa 2 untuk ikan-ikan prebiotik dan kawalan. Pada akhir Fasa 1, prestasi pertumbuhan dan penggunaan makanan adalah lebih tinggi ($p<0.05$) dalam ikan yang dirawat menggunakan LBA, diikuti oleh yis hidup, berbanding dengan diet yang lain. Pertumbuhan ikan dalam tiga diet prebiotik tidak jauh berbeza antara satu sama lain tetapi pertumbuhan ikan yang menggunakan diet kawalan sangat tinggi. Dalam Fasa 2 (fasa pemberian makanan), pertumbuhan ikan berterusan sehingga minggu ke-6 untuk diet berasaskan probiotik tetapi mendatar selepas empat minggu untuk ikan yang diberi makan diet prebiotik. Nisbah penukaran makanan (FCR) adalah lebih tinggi terhadap semua rawatan semasa tempoh memberi makan. Indeks hepatosomatik (HSI) tidak berbeza dengan ketara terhadap diet yang diuji. Indeks visceral somatik (VSI) dan intraperitoneal lemak (IPF) adalah paling tinggi dalam diet yang menggunakan LBA dan diet kawalan, masing-masing. Indeks badan berbeza secara ketara ($p<0.05$) di antara Fasa 1 dan 2. Kajian ini menunjukkan bahawa diet berasaskan probiotik mempunyai

*Corresponding author: hsjewel730@yahoo.com

pengaruh yang positif ke atas pertumbuhan, pengambilan makanan, dan kelangsungan hidup terhadap *C. striata* berbanding penggunaan diet makanan yang menggunakan prebiotik.

Kata kunci: Prebiotik, Probiotik, Prestasi Pertumbuhan, Ikan Haruan (*Channa striata)*

Abstract: This study used a two-phase feeding trial to determine the influence of selected dietary prebiotics and probiotics on growth performance, feed utilisation, and morphological changes in snakehead (*Channa striata*) fingerlings as well as the duration of these effects over a post-experimental period without supplementation. Triplicate groups of fish (22.46 ± 0.17 g) were raised on six different treatment diets: three prebiotics (0.2% β-glucan, 1% galacto-oligosaccharides [GOS], 0.5% mannan-oligosaccharides [MOS]), two probiotics (1% live yeast [*Saccharomyces cerevisiae*] and 0.01% *Lactobacillus acidophilus* [LBA] powder) and a control (unsupplemented) diet; there were three replicates for each treatment. All diets contained 40% crude protein and 12% crude lipid. Fish were fed to satiation three times daily. No mortalities were recorded during Phase 1; however, 14% mortality was documented in the control and prebiotic-amended fish during Phase 2. At the end of Phase 1, growth performance and feed utilisation were significantly higher ($p<0.05$) in the LBA-treated fish, followed by live yeast treatment, compared with all other diets tested. The performance of fish on the three prebiotic diets were not significantly different from one another but was significantly higher than the control diet. During Phase 2 (the post-feeding phase), fish growth continued until the 6th week for the probiotic-based diets but levelled off after four weeks for the fish fed the prebiotic diets. The feed conversion ratio (FCR) was higher in all treatments during the post-feeding period. The hepatosomatic index (HSI) did not differ significantly among the tested diets. The visceral somatic index (VSI) and intraperitoneal fat (IPF) were highest in the LBA-based diet and the control diet, respectively. The body indices were significantly different ($p<0.05$) between Phases 1 and 2. This study demonstrates that probiotic-based diets have a more positive influence on the growth, feed utilisation, and survival of *C. striata* fingerlings compared with supplementation with prebiotics.

Keywords: Prebiotics, Probiotics, Growth Performance, Snakehead (*Channa striata)*

INTRODUCTION

The striped snakehead, *Channa striata* (Bloch 1793), is a carnivorous freshwater fish that is widely distributed in Asia. It is a valuable food fish (Wee 1982) that contains high levels of protein (Annasari *et al*. 2012), high quality flesh, low fat, and fewer intermuscular bones as well as medicinal qualities (Haniffa & Marimuthu 2004); in particular, products such as fins and scales are a good source of albumin and are traditionally used to treat injuries and burns. Therefore, snakehead aquaculture has recently gained more attention and the production yield has increased from 16 tons in 1998–2000 to 42 tons in 2010–2012 (FAO 2012).

The continuing goal of new world aquaculture (FAO 2014) is to maximise the efficacy and optimise the profitability of fish production. As a result, global aquaculture is becoming more intensified. This may lead to increased fish yields and per capita fish production; however, it is also directly leading to a deterioration in water quality resulting in outbreaks of fish diseases (Bondad *et al*.

2005). Farmers usually control fish diseases by using antibiotics as feed supplements. The excessive use of antibiotics results in the development of antimicrobial-resistant pathogens, inhibits or kills the beneficial microbiota in the gastrointestinal (GI) system, and produces antibiotic residues in the fish body that are accumulated in fish products and may be harmful for human consumption (FAO 2005). The European Union banned the import of fish fed with antibiotic supplements in 2006. Subsequently, aquaculture scientists began to explore new strategies to replace the antibiotics used in the feeding and health management of fish in aquaculture (Balcâzar *et al*. 2006). These researchers have evaluated new dietary supplements (Diana 1997; Abdelghany & Ahmed 2002) such as dietary prebiotics, probiotics, symbiotics, phytobiotics, and other functional dietary supplements (Denev 2008).

The present study was conducted with a similar objective: to determine the influence of selective single doses of dietary prebiotics and probiotics on growth performance, feed utilisation, and body indices of *C. striata* fingerlings and the duration of these effects over a period of post-experimental feeding without supplementation. In general, dietary prebiotics are an undigested feed ingredient (Gibsen & Roberfroid 1995) that benefits fish by selectively stimulating growth (Grisdale-Helland *et al.* 2008; Talpur *et al.* 2014), whereas probiotics are live bacteria, cyanobacteria, microalgae, fungi, etc. (Fuller 1989) having beneficial effects on host growth by improving the intestinal balance of microbes (Al-Dohail *et al.* 2009; Dhanaraj *et al.* 2010).

MATERIALS AND METHOD

Experimental Animals and Husbandry Conditions

The study was conducted at the Aquaculture Research Complex of Universiti Sains Malaysia (USM), Pulau Pinang, Malaysia. This was a preliminary indoor study to determine the long term effect of dietary prebiotic and probiotic feed supplements on snakehead fingerling growth and health status. This paper evaluates only the effect of dietary prebiotics and probiotics on snakehead fingerling growth status. The study was conducted in two immediately consecutive phases. The first phase comprised 16 weeks, and the second phase comprised the subsequent 8 weeks for a total of 24 continuous weeks from the start of the experiment.

A total of 360 snakehead fry (3–4 in) were purchased from a local fish farm, reared for 4 weeks in two outdoor cement tanks (2 × 1 × 0.5 m) on a diet of commercial sea bass pellets containing 43% crude protein and 6% crude lipid, to acclimate the fish to the environment and reduce mortality. Water temperature and pH were recorded twice per day. The survival rate was approximately 80.5%. After 4 weeks, a total of 180 individual snakehead (*C. striata*) fingerlings (avg. wt. 22.46 ± 0.17 g) were raised on experimental diets (10 fish/tank and 3 tanks for each feeding trial plus a control) in 18 round plastic tanks (200 L).

Experimental Diets

In this study, five experimental diets along with a control (six diets total) were prepared at Fisheries Research Institute (FRI), Pulau Sayak, Kedah, and transported to the USM Aquaculture Complex in airtight polyethylene bags. The diets were maintained at –20°C. The five supplemented diets included three prebiotics (0.2% β-glucan [Macrogard® Louisville, KY, USA], 1% galacto-oligosaccharides [Vivinal® GOS syrup, Friesland Campina Domo, The Netherlands], 0.5% mannan-oligosaccharides [Alltech®, Actigen 1, USA]) and two probiotics (1% live yeast [*Saccharomyces cerevisiae*, Alltech®, Yea-Sacc 1026, USA] and 0.01% *Lactobacillus acidophilus* [LBA] powder [Sigma® LBA– 10^8 CFU]).

The control diet contained no feed supplements. All the prepared diets contained 40% crude protein and 12% crude lipid. The feed ingredients and proximate composition of the diets (Table 1) were analysed using the Association of Official Analytical Chemists (AOAC) methodologies (AOAC 1997).

Feeding Trial

Only one feeding trial was conducted consisting of two phases. The first phase comprised 16 weeks with dietary prebiotics or probiotics followed by another 8 weeks of the control diet during the second phase. Three replicate groups of fish were raised on treatment diets along with the control in 18 indoor tanks (200 L capacity) and were fed to satiation three times daily. Water temperature and pH were measured twice daily (early morning and late afternoon); although these two parameters did not change significantly (because of the indoor, closed, non-circulating, continuously aerated water environment), it was important to document the cleanliness of the aquaculture tank.

Growth Performance

Fish weights were taken every two weeks during Phase 1 beginning at the 4th week of the feeding treatments and weekly during Phase 2. Each feeding treatment had 3 replicates and each replicate contained 10 *C. striata* fingerlings. Prior to weighing each fish, the water in each tank was lowered gradually and the fish were then collected using a soft scoop net and were temporarily held in another covered container. Each fish was individually removed with a small soft towel, dried using tissues, and the weight and length were recorded; fish were subsequently released to their respective tanks, which were filled with clean new water. To analyse growth performance, the conditional factor (CF), relative growth (RG), specific growth rate (SGR), and survival rate (SR) were determined using the formulae described by Austreng (1978), Busacker *et al*. (1990), and Ahmad *et al*. (2002). Moreover, the protein efficiency rate (PER) and food conversion ratio (FCR) were calculated to measure the growth efficiency of the test feeds using the following formulae (Abdel Tawwab *et al*. 2008; United States Agency for International Development [USAID] 2011):

CF (%): (Final weight [g] / L^3 [cm]) × 100
RG (%): (Final weight – initial weight) / Initial weight) × 100
SGR (%): (Final weight – initial weight / Nos. of days) × 100
SR (%): (Final number of fish / Initial number of fish) × 100
PER: Final weight-initial weight / Protein intake
FCR: Total feed consumption / Weight gain of fish

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

Ingredient	Control	β-glucan 0.2%	GOS 1%	MOS 0.5%	Live yeast 1%	*L. acidophilus* 0.01%
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
Soybean oil	60	60	60	60	60	60
Cellulose	11	8	1	6	1	10.9
CMC[b]	10	10	10	10	10	10
Vitamins mix[c]	20	20	20	20	20	20
Minerals mix[d]	20	20	20	20	20	20
Supplement	0	2	10	5	10	0.1
Proximate composition (g/kg)	**Control**	**β-glucan 0.2%**	**GOS 1%**	**MOS 0.5%**	**Live yeast 1%**	***L. acidophilus* 0.01%**
Moisture	81.9	52.2	63.1	71.9	96.5	92.8
Protein	410.0	407.3	409.4	406.8	409.1	409.7
Lipid	118.8	117.5	118.4	118.0	120.3	121.2
Ash	10.1	10.2	9.8	10.3	9.9	10.6
Fibre	123.0	123.2	123.2	121.8	121.8	120.6
NFE[e]	256.2	289.6	276.1	271.2	242.4	245.1
GE[f] (MJ/kg)	198.9	197.6	198.5	199.2	198.7	196.9

Notes: [a]Danish fish meal (kg^{-1}) = crude protein 746.6 g and crude lipid 101.6 g; [b]CMC = carboxymethyl cellulose; [c]Vitamin mix (kg^{-1}) = Rovimix 6288 (Roche Vitamins Ltd., Switzerland: Vit $_A$ 50 million IU, Vit $_D$ 310 million IU, 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, antioxident 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 sulphate 25 g, magnesium sulphate 137 g, potassium chloride 50 g, sodium chloride 60 gm, potassium iodide 150 mg, copper sulphate 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+fibre}); [f]GE = gross energy; measured using bomb calorimeter (Parr 1356 bomb calorimeter).

The hepatosomatic index (HSI), visceral somatic index (VSI), and intraperitoneal fat (IPF) were determined by sacrificing three fish per replicate tank in each feeding treatment at the end of Phase 1 and Phase 2 using the following formulae (Busacker *et al*. 1990):

HSI (%): (Liver weight / Fish weight) × 100
VSI (%): (Viscera weight / Fish weight) × 100
IPF (%): (IPF weight / Fish weight) × 100

Fish muscle from the 6 feeding treatments was collected in small universal bottles covered with aluminium foil to determine the proximate composition. The aluminium foil covers were punched and held continuously at –70ºC to –75ºC for 24 hours. The freeze-dried muscles were removed and analysed for proximate composition according to the AOAC (1997) guidelines.

Data Analysis

The results were analysed using SPSS (version 18). A one-way ANOVA (analysis of variance) was used to compare the data on growth performance, feed utilisation and body indices between the two phases. Multiple comparisons were analysed with Duncan's test to assess the differences between treatment means at a 95% confidence level.

RESULTS

The inclusion of dietary prebiotics and probiotics (Table 2) resulted in a significant (p<0.05) change in the growth of *C. striata* fingerlings between the two phases. The growth performance was significantly increased in the feeding treatments during the first phase (Table 2) but decreased significantly (p<0.05) at different points during the second phase. Growth was significantly higher in both phases for fish fed the LBA diet. The SGR for the 3 prebiotic treatments did not differ significantly from the live yeast treatment (probiotic) during the first phase but decreased significantly by the end of second phase (Fig. 1). Prebiotic and probiotic feed supplements significantly increased the SGR of *C. striata* fingerlings (Fig. 1) during the first phase, but the SGR decreased gradually for all prebiotic fish after 4 weeks in the second phase, when no feed supplement was used; live yeast and LBA treatments decreased after the 6th and 7th weeks, respectively (Fig. 1). In both phases, the SGR of the LBA treatment was significantly higher than the live yeast treatment (Fig. 1).

This study found that feeding probiotics, particularly LBA, resulted in significantly higher feed utilisation efficiency. The FCR and PER were significantly (p<0.05) affected by the inclusion of dietary prebiotics and probiotics (Table 2). In the first phase of the experiment, the lowest FCR was obtained in the LBA feeding treatments followed by the β-glucan treatment; however, the FCR values of all treatments had increased by the end of the post-feeding phase (Table 2). Similarly, after 16 weeks, the PER was highest in the LBA feeding treatments followed by β-glucan and GOS treatments; however, during the post-feeding trial, the PER was significantly higher in both probiotic treatments compared with the 3 prebiotic treatments (Table 2).

In all feeding treatments, 100% survival was maintained until the end of the first phase; however, by the end of second phase, survival had declined to 90% in the control and β-glucan treatments and 88% and 82% in the MOS and

GOS treatments, respectively. Overall mortality was 14% at the end of the second phase. No mortality was recorded for the probiotic feeding treatments in either phase (Table 2).

The condition factor was also affected by the dietary supplements (Fig. 2). The greatest change was found in the MOS treatment at end of first phase followed by the live yeast, β-glucan, GOS, and LBA treatments and the control, whereas no significant difference was found between any prebiotic and the control during the post-supplementation feeding period or at the end of the second phase. In the second period, a highly significant difference was observed for both probiotic feed supplements (Fig. 2). This study did not find any significant (p<0.05) differences in HSI, VSI, or IPF between the first and second phases, but a decrease in Phase 2.

The proximate composition of fish muscle (Table 3) was significantly changed by the inclusion of dietary prebiotics and probiotics. The tested diets showed a significant increase in the crude protein content; the highest levels were found in the LBA-based diet followed by the 3 prebiotics and live yeast (probiotic) treatments compared with the control during at the end of 16 weeks. In contrast, there was an observed decrease in the crude lipid content; the LBA-based diet produced the lowest crude lipid in the fish muscle followed by the live yeast and the 3 prebiotic treatments (Table 3). In both phases, the fish muscle contained a low ash content, but significantly differed from the control diet.

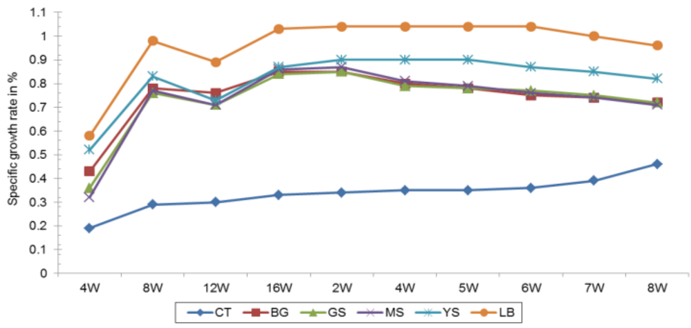

Figure 1: Specific growth rates of *C. striata* fingerlings (by week) during the two phases of the study.

Notes: CT = control diet without supplementation; BG = supplementation with β-glucan; GS = supplementation with galacto-oligosaccharides; MS = supplementation with mannan-oligosacharides; YS = supplementation with live yeast (*S. cerevisiae*); LB = supplementation with *L. acidophilus.*

DISCUSSION

The results obtained in the present study revealed that supplementation with dietary prebiotics and probiotics had a strong effect on growth performance in *C. striata* fingerlings. In the first phase, the ranking of performance for the supplemented diets was LBA>live yeast>β-glucan>MOS>GOS (Table 3); the

Table 2: Growth performance, feed utilisation and survival of *C. striata* fingerlings.

Parameter		Control	β-glucan	GOS	MOS	Live yeast	LBA
Initial weight (g)	Initial	22.34± 0.05	22.45± 0.17	22.57± 0.13	22.30± 0.21	22.57± 0.13	22.47± 0.16
Weight gain (g)	Phase 1	32.21± 0.55^{a}	58.15± 0.32^{b}	58.18± 0.27^{b}	58.64± 0.36^{bc}	59.56± 0.57^{c}	71.39± 0.89^{d}
	Phase 2	48.00± 0.10^{a}	75.77± 0.61^{c}	76.20± 0.30^{c}	73.43± 0.65^{b}	89.40± 0.70^{d}	112.90± 0.65^{e}
RG (%)	Phase 1	44.16± 2.16^{a}	159.00± 0.97^{bc}	157.76± 2.18^{b}	163.00± 2.10^{cd}	163.91± 4.07^{c}	217.68± 2.83^{d}
	Phase 2	114.86± 0.37^{a}	237.44± 0.15^{c}	237.57± 1.00^{c}	229.30± 0.90^{b}	296.10± 2.71^{d}	402.38± 1.86^{e}
SGR (%)	Phase 1	0.33± 0.01^{a}	0.85± 0.03^{bc}	0.84± 0.01^{b}	0.86± 0.07^{c}	0.87± 0.01^{c}	1.03± 0.01^{d}
	Phase 2	0.46± 0.00^{a}	0.72± 0.00^{c}	0.72± 0.00^{c}	0.71± 0.00^{b}	0.82± 0.00^{d}	0.96± 0.00^{e}
FCR	Phase 1	1.90± 0.17^{d}	1.63± 0.06^{b}	1.80± 0.00^{cd}	1.73± 0.06^{bc}	1.64± 0.006^{b}	1.43± 0.06^{a}
	Phase 2	1.79± 0.00^{d}	1.76± 0.00^{c}	1.89± 0.00^{f}	1.82± 0.00^{e}	1.80± 0.00^{cd}	1.56± 0.01^{a}
PER	Phase 1	1.28± 0.10^{a}	1.50± 0.08^{c}	1.33± 0.01^{ab}	1.42± 0.01^{bc}	1.50± 0.04^{c}	1.71± 0.04^{d}
	Phase 2	1.40± 0.00^{b}	1.40± 0.00^{b}	1.30± 0.00^{a}	1.30± 0.02^{a}	1.38± 0.16^{ab}	1.56± 0.06^{d}
Survival	Phase 1	100%	100%	100%	100%	100%	100%
	Phase 2	90%	90%	82%	88%	100%	100%

Notes: Each column represents different feeding treatments. All values are mean ± SD obtained from three replicate groups (n = 3). Data with different superscripts in the same row indicate significant differences (p<0.05) among the feeding treatments. RG = relative growth; SGR = specific growth rate; FCR = feed conversion rate; PER = protein efficiency rate; β-glucan = beta glucan; GOS = galacto-oligosaccharides; MOS = manna-oligosaccharides; live yeast = *S. cerevisiae*; LBA = *L. acidophilus*.

unsupplemented (control) diet showed the lowest performance. This performance trend clearly demonstrated that there were attributes of the supplemented diets that enhanced the growth performance of *C. striata*. Watson and Preedy (2010) stated that dietary prebiotics and probiotics are functional bioactive foods that promote the growth and health of living organisms. Both types of supplements (prebiotics and probiotics) typically directly modulate the endogenous flora in the gastrointestinal tract by producing enzymes or influencing enzyme activity. The primary role of the digestive tract is to break down foodstuffs into smaller molecules compatible with absorption across the epithelial border of the gastrointestinal tract (Merrifield *et al*. 2011) with the aid of the digestive enzymes. The secretion of digestive enzymes can be enhanced in the intestines of fish by the intake of dietary prebiotics and probiotics. Numerous studies have demonstrated that dietary prebiotics and probiotics are initially responsible for modulating the favourable intestinal microflora that play a major role during the secretion of digestive enzymes, specially amylase (Xu *et al*. 2003; Yanbo & Zirong 2006; Essa *et al*. 2010; Askarian *et al*. 2011; Sang *et al*. 2011; Wu *et al*. 2014).

The present study showed improved performance in LBA-treated fish compared with the other probiotic (live yeast), probably due to their different modes of action in the gastrointestinal tract. Feeding a diet supplemented with *L. acidophilus* increases the population of *Lactobacillus sp.* and thus not only replaces pathogenic bacteria but also produces nutrients and stimulates the release of more digestive enzymes resulting in an enhanced, more rapid digestion process (Cüneyt *et al*. 2008). The ingestion of live yeast, on the other hand, involves the maturation of the gut via the formation of yeast colonies. The ability of yeast to colonise is thought to be related to cell surface hydrophobicity, which helps the live yeast strains grow on the intestinal mucous (Waché *et al.* 2006). This mode of action appeared to influence the growth performance of *C. striata* fingerlings supplemented with dietary prebiotics and probiotics in the present study. The mode of action in the gastrointestinal tract of the dietary prebiotics tested in this study was indirect. It is probable that the probiotics, which contain live bacteria or fungi (Fuller 1989), have a probioactive role (i.e., bioactivity originating from a combination of the food matrix and bacteria) in the gastrointestinal wall resulting in an enhanced rate of fermentation in the colon (Gill, 1998). Growth performance in response to the ingestion of dietary prebiotics showed differences that were probably related to structural differences. The β-glucan tested in this study has an unbranched homopolysaccharide structure, whereas the other two feed supplements, MOS and GOS, had a branched heteropolysaccharide structure. The unbranched homopolysaccharides are polymers of a single monosaccharide such as glucose; whereas the branched heteropolysaccharides contain different monosaccharides linked by glycosidic bonds in nature (Chanpul *et al*. 2012). Although these structural differences potentially influence the efficacy of the three prebiotics, the results of the present study did not show significant differences among them. The probable reason for this result is that β-glucan, which is an active prebiotic proven to modify biological responses, is a soluble carbohydrate (Bhon & BeMiller 1995) obtained from the cell walls of live yeast (*S. cerevisiae*), whereas galacto-oligosaccharides (GOS) and mannan-oligosaccharides (MOS) contain oligosaccharide carbohydrates with low molecular weights and degrees of polymerisation (Roberfold & Slavin 2000; Sanders *et al.* 2005). Overall, the results obtained from the first phase of this study revealed a positive effect of dietary prebiotics and probiotics as feed supplements for *C. striata* fingerlings. The survival data from the present study showed results similar to those on growth performance. This result is consistent with a previous study by Talpur *et al.* (2014), who used a selective single dose of dietary prebiotics and probiotics as feed supplements in a study on *C. striata* fingerlings. Similar results were observed in the African catfish, *Clarias garepinus* (Al-Dohail *et al.* 2009), *Cyprinus carpio* (Dhanaraj *et al*. 2010), a hybrid striped bass (Li & Gatlin 2005), rainbow trout (Staykov *et al*. 2007), European sea bass (Torrecillas *et al*. 2007), and red drum, *Sciaenops acellatus* (Zhou *et al*. 2010). Similar to the results for growth performance in Phase 1, the feed utilisation and body indices of *C. striata* were also positively affected by the inclusion of dietary prebiotics and probiotics (Table 3). All the diets tested reduced the FCR to less than 2, including the control diet, probably due to the 40% protein and 12% lipid content. The bioactive attributes of dietary prebiotics and probiotics accelerated a reduction in FCR, which indicates that the tested diets were economically viable. In addition, the inclusion of dietary prebiotics and probiotics increased the protein efficiency rate, which was a positive result because PER helps to reduce the FCR (USAID 2011). Fish fed with LBA performed significantly better, followed by live yeast, which as a beneficial fungi is another probiotic. The tested LBA and fungi may lead to greater activity in the gastrointestinal tract (Marteau *et al*. 1993) resulting in an increase in the protein efficiency rate and a reduction in the FCR. In contrast, the three tested dietary prebiotic feeding supplements facilitated the beneficial bacteria; by nature they are very similar to low-digestibility carbohydrates and influence the osmotic pressure in the gastrointestinal tract under fermentation (Roberfold & Slavin 2000), enhancing endogenous bacteria such as *Bacillus* and intestinal gas production associated with greater digestive activity. Therefore, they led to a decrease in the FCR and an increase in the PER.

The present study also revealed that the inclusion of dietary prebiotics and probiotics led to maintenance of the condition factor during growth, which reflects the nutritional status of an individual fish (Schreck & Moyle 1990). The proximate composition analysis indicated that the fish muscle in this study had a high protein content, but low fat and ash content. *C. striata* is a freshwater fish that typically contains high protein (Annasari *et al*. 2012) and low fat. In this study, the inclusion of dietary prebiotics and probiotics led to an increased crude protein and lower lipid content compared with the control, which may be beneficial for a food fish (Wee 1982).

The addition of the post-feeding trial (Phase 2), in which the treated fish were fed with an unsupplemented (control) diet for a period of time after the experiment, provides a complete study on the effects of dietary prebiotics and probiotics on fish growth performance. This is the first such post-feeding trial conducted to date in the field of fish nutrition research. The SGR showed a clear difference between Phase 1 and Phase 2 in the present study. In the post-feeding phase, it appears that the bioactive role continues for 7 weeks for the LBA treatment, 6 weeks for the live yeast treatment (Fig. 1), and 4 weeks for the 3 prebiotics tested in this study. The probable reason for this is the effect of residues stored in the gastrointestinal tract. In Phase 1, when the fish were fed the supplemented diets, they may not have used all of the nutrients derived from these diets for growth purposes; 16 weeks of continuous supplemented feeding during Phase 1 may have resulted in the deposition of supplemented diets as residue that might be available during Phase 2, when the treated fish were fed only the control diet. This hypothesis is consistent with the higher SGR of supplemented diets compared with the control diets provided after Phase 1 (Fig. 1). The residual effects of supplementation of fish in Phase 2 (post-feeding trial) was reflected in the higher FCR and the lower PER. It can be argued that fish require a similar level of energy to maintain growth in both phases, but that replacing the supplemented diets with the control (unsupplemented diets) could not supply sufficient energy to maintain the growth performance. Therefore, the growth performance of supplemented fish decreased over time in Phase 2. This is consistent with difference in survival of fish observed between Phase 1 and Phase 2. Nevertheless, there were no significant morphological changes (HSI, VSI, and IPF) in fish between these two phases, probably because there was no biological effect before supplementation was stopped.

In conclusion, the results obtained from the present study established the efficacy of supplemented diets. Fish growth, low FCR, and high PER with low fat demonstrated that fish feed formulated with dietary prebiotics and probiotics had a positive effect, particularly supplementation with 0.01% (10^8 CFU) LBA powder, which led to the highest fish growth with a low FCR and high PER. However, this was a preliminary study; this phenomenon needs to be studied in depth considering other parameters such as nutrient digestibility, blood parameters, gut microflora, innate immune response status, etc. for *C. striata* fingerlings.

Table 3: Proximate composition of body muscle between Phase 1 and Phase 2.

Parameter		Control	β-Glucan	GOS	MOS	Live yeast	LBA
Moisture (%)	Phase 1	5.24± 0.12^{d}	4.37± 0.40^{c}	4.52± 0.29^{c}	4.57± 0.22^{c}	3.57± 0.38^{b}	1.69± 0.29^{a}
	Phase 2	2.52± 0.45^{a}	3.70± 0.40^{c}	2.38± 0.02^{b}	2.73± 0.28^{ab}	2.57± 0.42^{a}	3.29± 0.44^{bc}
Crude protein (%)	Phase 1	81.13± 0.54^{a}	86.80± 0.71^{b}	86.56± 0.37^{b}	85.92± 0.36^{b}	86.19± 0.41^{b}	90.53± 0.57^{c}
	Phase 2	85.39± 0.25^{ab}	84.45± 0.38^{a}	86.12± 0.11^{b}	86.15± 0.66^{b}	85.13± 0.40^{ab}	85.92± 0.97^{b}
Crude lipid (%)	Phase 1	6.92± 0.07^{d}	5.49± 0.10^{bc}	5.52± 0.01^{c}	5.61± 0.09^{c}	5.36± 0.04^{ab}	5.25± 0.12^{a}
	Phase 2	6.61± 0.22^{c}	6.43± 0.50^{bc}	6.05± 0.07^{bc}	5.27± 0.12^{a}	6.28± 0.45^{bc}	5.88± 0.04^{b}
Ash (%)	Phase 1	5.34± 0.08^{f}	2.19± 0.08^{b}	2.59± 0.27^{c}	3.04± 0.09^{d}	4.09± 0.04^{e}	1.59± 0.32^{a}
	Phase 2	5.07± 0.41	5.01± 0.06	5.18± 0.20	5.40± 0.55	5.63± 0.49^{b}	4.64± 0.53^{a}

Notes: Each column represents different feeding treatments. All values are mean ± SD obtained from three replicate groups (n = 3). Data with different superscripts in the same row indicate significant differences (p<0.05) among the feeding treatments. B-glucan = beta glucan; GOS = galacto-oligosaccharides; MOS = manna-oligosaccharides; Live yeast = *S. cerevisiae*; LBA = *L. acidophilus*.

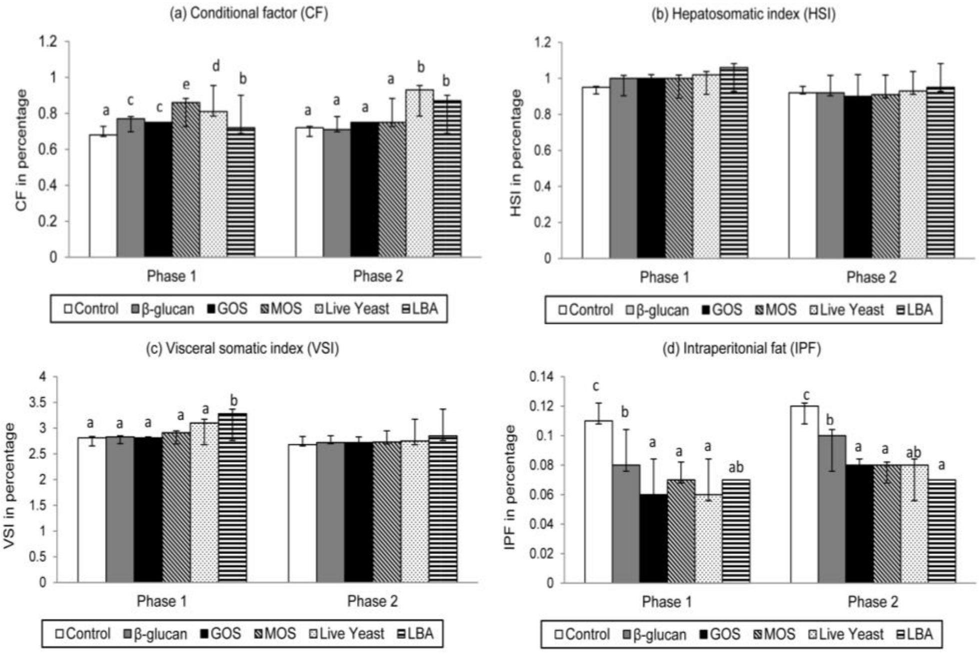

Figure 2: Effect of dietary prebiotics and probiotics on body indices in *C. striata* fingerlings during different phases.

Notes: All values are mean + SD obtained from three replicates groups (n = 3). The superscripts indicates significant difference (p<0.05) among the feeding treatments. B-glucan = beta glucan; GOS = galacto-oligosaccharides; MOS = manna-oligosaccharides; live yeast = *S. cerevisiae*; LBA = *L. acidophilus*.
CT = control diet without supplementation; BG = diet with β-glucan supplement; GS = diet with GOS supplement; MS = diet with MOS supplement; YS = diet with live yeast supplement; LB = diet with *L. acidophilus* supplement. Phase 1 = during feed supplementation; Phase 2 = treated fish fed with control diet.

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