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Potential of fungal exopolysaccharide as novel source for prebiotic supplement to broiler chicken diet

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ABSTRACT

The potential of using a fungal exopolysaccharide produced by *Ophiocordyceps dipterigena* BCC 2073, as a prebiotic in broiler chicken diet by supplementing the diet at different concentrations (0–120 g/25 kg feed) was evaluated over a period of 42 days. Intestinal microbial populations in faeces were identified. The effects of a combination of pre- and pro-biotic supplements were also studied by combining exopolysaccharide with *Lactobacillus acidophilus* BCC 13938. Total *Lactobacilli* sp. counts in faecal samples of chickens fed diet containing both exopolysaccharide and *L. acidophilus* BCC 13938 were 50–folds higher than the non prebiotic but probiotic supplemented groups. Total body weight, body weight increase, feed intake, feed conversion efficiency and growth rate of chickens on a diet supplemented with 150 g of *L. acidophilus* BCC 13938 plus 1.5 kg of water or 30 g of *O. dipterigena* BCC 2073 exopolysaccharide plus 150 g of *L. acidophilus* BCC 13938 plus 0.75 kg of water were higher than those fed other combinations of supplements. The microbial community of faecal samples from chickens supplementation with exopolysaccharide had reduced numbers of *E. coli, Staphylococcus* sp.*, Streptococcus* sp., *Enterococcus* sp. and increased numbers of *Lactobacilli* and *Bacilli*. The numbers of faecal pathogenic bacteria inversely correlated with total body weight, body weight increase, feed intake, feed conversion efficiency and growth rate. This is the first report on the benefit of fungal exopolysaccharide as a novel prebiotic dietary supplement to chicken.

Key words: Beta-glucan, Denaturing gradient gel-electrophoresis, Exopolysaccharide, *Ophiocordyceps dipterigena,* Prebiotic

Polysaccharides, the polymeric carbohydrate molecules, are commonly found as a major component in all living organisms, serving as a structural support and a source of storage of carbohydrate (starch and cellulose in plant cell walls), chitin (exoskeleton of insects and crustaceans, and of fungal cell walls), peptidoglycan (bacterial cell wall), pectin (plants), alginates (seaweed), dextran (bacteria and yeasts), and glycogen (animal cell). These polysaccharides can be classified, based on the structures of monosaccharide units, as homo- and heteropolysaccharide (Sutherland 1990).

Many microbial exopolysaccharides are commercially available, such as scleroglucan (Coviello *et al*. 2005), curdlan (Funami *et al*. 1998), pullulan (Shingel 2004), and xanthan (Yoo and Harcum 1999). They have wide range of applications in food, petroleum, pharmaceutical, chemical industries (Iyer *et al*. 2006, Vinarta *et al*. 2006) as well as emulsifiers, stabilizers, lubricants, film formers, and gelling, thickening and suspending agents (Sutherland 1990).

Prebiotics are usually oligosaccharides, which cannot be digested by the host but can stimulate growth of an essential consortium of (commensal) bacteria in the gastrointestinal tract (Lomax and Calder 2009, Saulnier *et al*. 2009). Oligosaccharides of manno-, fructo, galacto-, xylo-, and malto-oligosaccharides have beneficial prebiotic effects in humans and animals (Bruzzese *et al*. 2009, Falony *et al*. 2009, Hernot *et al*. 2009). However, uses of microbial exopolysaccharides as prebiotics have not been often reported due to limitation of mass production of these carbohydrates at a low economic cost (Kocharin *et al*. 2010, Prathumpai *et al*. 2013).

The fungal exopolysaccharide, beta-glucan, of *Ophiocordyceps dipterigena* BCC 2073 is water soluble but insoluble in dimethyl sulfoxide (DMSO). Its structure is composed of a (1, 3)-ß -D-glucan backbone and contains arabinose, mannose, galactose and glucose (Madla *et al*. 2005, Methacanon *et al*. 2005). It is biocompatible, non-cytotoxic and a strong inducer of interleukin-8 (IL-8), a cytokine responsible for enhancing wound healing process (Madla *et al.* 2005, Methacanon *et al*. 2005). As prebiotics are normally produced from plants, this exopolysaccharide has the potential to be used a novel prebiotic supplement in

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animal feed, but *in vitro* and *in vivo* studies on its prebiotics properties are needed. Kocharin *et al*. (2010) reported mass production of exopolysaccharides of *O. dipterigena* BCC 2073. This raises the prospect of a high enough production yield of *O. dipterigina* BCC 2073 exopolysaccharide to be feasible as a prebiotic in animal feeds.

This study reports the impact of use of *O. dipterigena* BCC 2073 exopolysaccharide as a prebiotic supplement in broiler chicken diet on the growth and commensal microbial populations in faecal samples. Additionally, the effects of combining the prebiotic exopolysaccharide with a probiotic (*Lactobacillus acidophilus* BCC 13938) were assessed.

MATERIALS AND METHODS

Preparation of exopolysaccharide and probiotic additives: O. dipterigina BCC 2073 was cultivated in a 10 L fermentor using 8 L working volume containing 60 g/L glucose, 14 g/L malt extract, 0.5 g/L KH_2PO_4, 0.2 g/L K_2HPO_4, 0.2 g/L $MgSO_4$. $7H_2O$, 0.14 g/L $MnSO_4$. H_2O, 1 mL/L vitamin solution and 1 mL/L trace element solution. The culture was incubated for 5 days at 25°C, with agitation at 300 rpm, aeration at 1 vvm, and no regulation of pH. The culture broth was centrifuged at 10,000 *g* for 10 min at 4°C and fungal mycelium removed. The supernatant containing exopolysaccharide was collected for feed supplement (Kocharin *et al*. 2010).

L. acidophilus BCC 13938 was cultivated in a 5 L fermentor using a 4 L working medium containing 20 g/L glucose, 5 g/L casein, 5 g/L peptone, 10 g/L meat extract, 5 g/L yeast extract, 1 g/L Tween-80, 2 g/L K_2HPO_4, 5 g/L CH_3COONa, 2 g/L $(NH_4)_2$citrate, 0.2 g/L $MgSO_4 \cdot 7H_2O$ and 0.05 g/L $MnSO_4 \cdot H_2O$. The culture was incubated for 48 h at 37 ºC, with agitation at 150 rpm and no aeration or pH control. The culture was centrifuged at 12,000 *g* for 10 min at 37°C and cells were collected and viability determined using plate count on De Man, Rogosa and Sharpe (MRS) agar medium (Jung *et al*. 2008).

Broiler chicken feeding: Male and female broiler chickens (525; Betagro, Bangkok, Thailand) were reared from 1 to 42 days. Chickens were randomly allocated to 7 dietary regimens (3 pens of 25 chickens per regimen) with initial body weight of 47.56±0.42 g. A 2–phase feeding program was performed: first phase diet from day 1 to 21 and second phase diet from day 22 to 42. Basal diet (ME 2,900 kcal/kg) (Jung *et al*. 2008) was composed of fish powder, defatted soybean meal, sorghum powder, defatted rice bran, molasses, vegetable oil, calcium carbonate, dicalcium phosphate, salts, vitamins, minerals, amino acids, and choline chloride (providing 21% protein, 3% fat, 5% fiber and 13% moisture content). First phase (ME 2,900 kcal/kg) feed contained 21% (w/w) crude protein and second phase (ME 2,900 kcal/kg) contained 17% (w/w), with fat and fiber content kept at 3 and 5% (w/w) respectively. Chickens feeds were supplemented with *O. dipterigena* BCC 2073 exopolysaccharide (by fine spraying of exopolysaccharide solution to basal feed powder) and *Lactobacillus acidophilus* BCC 13938 as follows: T1 (control) group received a supplement (per 25 kg feed) of T_1, 0.15 kg of corn starch + 1.5 kg of water; T2, T1 supplement + 30 g of exopolysaccharide; T3, 0.15 kg of corn starch + 120 g of exopolysaccharide; T4, 150 g of *L. acidophilus* BCC 13938 + 1.5 kg of water; T5, 30 g of exopolysaccharide + 150 g of *L. acidophilus* BCC 13938 + 0.75 kg of water; T6, 120 g of exopolysaccharide + 150 g of *L. acidophilus* BCC 13938; and T7, T1 supplement + 0.75 kg of glucose:iulin 24:1. Viability of *L. acidophilus* BCC 13938 was $3x10^{11}$ CFU·g^{-1}. At 1, 7, 14, 21, 28, 35 and 42 days of age, body weight, daily feed intake (FI), growth rate and feed conversion ratio (FCR) of the 25 chickens in each pen were determined. FI was calculated from the formula:

FI = Total feed intake/(Growth period (days) × Total number of birds)
Growth rate was calculated from the formula:
Growth rate = Body weight increase/Growth period (days)
FCR was calculated from the formula:
FCR = FI × 100/Body weight increase

Total feed intakes of broiler chickens during 42 days of their growth are shown in Table 1.

Table 1. Total feed intake of broiler chicken during 42 days of growth

Day	Feed intake g/bird/week	Bird numbers/ treatment	Feed intake of 3 pens/ treatment (kg)
7	175	75	13.125
14	450	75	33.75
21	650	75	48.75
28	900	75	67.5
35	1200	75	90
42	1500	75	112.5

25 chickens/pen.

Determination of microorganisms in chicken faeces

On day 7 and 42, faeces from 10 randomly selected chickens from each pen were collected in fecal collected pen and freeze-dried; 1 g was mixed with 9 mL of phosphate-buffer solution pH 7.4 (PBS) and samples 10-fold serially diluted to $1:10^{-4}$. Total viable bacteria counts were determined by plating on compact dry total count medium at 35°C for 48 h; lactic acid bacteria by plating on MRS agar for 48 h at 37°C; *Coliform* and *Escherichia coli* by plating on compact dry selective medium for *E. coli* and coliform bacteria at 35°C for 24 h; *Salmonella* spp. by plating on compact dry selective medium for *Salmonella* at 41°C for 24 h; and *Vibrio* spp. by plating on compact dry selective medium for *Vibrio* at 37°C for 24 h (Jung *et al*. 2008).

The changes in intestinal microbiota during broiler chicken growth was determined by analyzing 16S rDNA from fecal samples using PCR denaturing gradient gel-electrophoresis (DGGE). On day 1, 7 and 42, 2 birds from each of 21 pens were randomly selected for faecal sample collection as described above and DNA was extracted from

250 mg of freeze dried-faecal samples using DNA stool kit according to the manufacturer's protocol. Universal primers, 357F (5'TACGGGAGGCAGCAG 3') and 518R (5'ATTACCGCG GCTGCTGG 3') were used to amplify the V3 variable region of 16S rDNA. An additional GC clamp (5'CGCCCGCCGCGCGCGGCGGGCGGGGCGG GGGCACGGGGG 3') was also added to primer 357F to generate primer 357F-GC. The reaction mixture (50 µL) contained 0.25 µM each primer, 200 µM each dNTP, 2.5 U *Taq* polymerase and 3 µL of DNA. Thermocycling conditions (conducted using an I cycler apparatus) were as follows: 94 °C for 5 min; 35 cycles of 94°C for 30 sec, 56°C for 30 sec, and 68°C for 40 sec; and a final step at 68°C for 10 min.

Amplicons were separated by DGGE in a DCODE system on 8% (v/v) polyacrylamide (37.5:1 acrylamide:bisacrylamide) gels (200 by 200 by 1 mm) containing a 40% to 60% gradient of urea-formamide in Tris-acetate-EDTA (TAE) buffer (50X TAE is composed of 2 M Tris pH 8.0, 1 M acetic acid and 50 mM EDTA). The 40% gradient solution contained 20 mL of acrylamide-bisacrylamide, 2 mL of 50X TAE, 16 mL of formamide, and 18.8 g urea; and the 60% gradient solution contained 20 mL of acrylamide-bisacrylamide, 2 ml of 50X TAE, 24 mL of formamide, and 25.2 g urea. A 17 mL aliquot of each solution was mixed with 153 µL of 10% (w/v) ammonium persulfate and 15.3 µL of tetramethylethylenediamine. Gels were established using a 475 gradient delivery system. Electrophoresis was performed at 85 V in 1X TAE buffer at a constant temperature of 60°C for 16 h. Gels were stained with ethidium bromide, washed with ultrapure water, and recorded under UV light using a Gel Doc 2000 system equipped with Quantity One software (Salazar *et al.* 2009). Bands were excised from the gels, sequenced (1st Base sequencing) and compared with those deposited in GenBank database using NCBI.

Data analysis: Statistical analysis was performed using Duncan's new multiple range test (DMRT) of Statistical Analysis System (SAS) software program. P-value < 0.05 is considered significant.

RESULTS AND DISCUSSION

Ophiocordyceps dipterigena BCC 2073 exopolysaccharide was used as a prebiotic at different concentrations in broiler chicken feed. There were no significant differences in broiler chicken weights at different ages (from 1 to 42 days) among the 7 dietary groups (Table 2). At day 42, the average broiler chicken body weight was

Table 2. Body weight at different ages of broiler chickens fed with different diets.

Bird age (days)	Weight (g)							*P*-value
	T1	T2	T3	T4	T5	T6	T7	
1	47.60±2.21	47.87±2.45	48.00±2.51	47.73±2.43	47.73±2.43	46.80±2.18	47.20±2.19	0.5285^{NS}
7	171.60±9.16	169.60±8.78	170.39±8.94	164.07±7.95	161.47±7.52	163.47±7.78	164.93±8.01	0.0897^{NS}
14	410.93±4.71	400.40±4.02	409.47±4.52	398.73±3.87	397.97±3.90	392.40±3.46	395.33±3.54	0.5447^{NS}
21	830.53±5.84	799.73±4.84	806.63±5.45	822.03±5.75	821.27±5.70	751.07±4.81	798.27±5.13	0.0726^{NS}
28	1302.80±8.87	1261.60±7.87	1283.67±7.98	1333.10±10.12	1316.41±9.95	1205.60±5.36	1219.36±5.98	0.0578^{NS}
35	1818.80±7.31	1793.07±6.58	1804.38±6.85	1833.33±8.68	1889.41±9.21	1734.27±5.97	1721.78±5.64	0.3031^{NS}
42	2360.13±2.43	2389.68±3.87	2410.48±5.78	2499.34±8.54	2483.78±7.59	2333.94±2.35	2333.94±2.33	0.4873^{NS}

T1, 0.15 kg of corn starch + 1.5 kg of water; T2, T1+ 30 g of exopolysaccharide; T3, 0.15 kg of corn starch + 120 g of exopolysaccharide; T4, 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5, 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6, 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7, T1+ 0.75 kg (glucose:inulin 24:1).

NS= P>0.05

Table 3. Feed intake at different ages of broiler chickens fed with different diets.

Bird age (days)	Feed intake (g/bird.day)							*P*-value
	T1	T2	T3	T4	T5	T6	T7	
7	$24.32±0.81^{A}$	$24.34±0.72^{A}$	$24.76±0.82^{A}$	$23.49±0.78^{AB}$	$22.32±0.69^{B}$	$24.19±0.71^{A}$	$24.13±0.71^{A}$	0.0159^{*}
14	53.20±2.35	51.16±2.23	53.28±2.41	54.34±2.54	50.29±1.98	51.77±2.21	50.02±1.87	0.2742^{NS}
21	92.46±7.85	89.92±7.42	89.66±7.35	90.32±7.46	92.12±7.68	87.96±6.54	88.82±6.86	0.4912^{NS}
28	$122.63±4.65^{AB}$	$126.53±5.52^{A}$	$131.06±6.25^{A}$	$129.95±6.02^{A}$	$123.72±4.78^{AB}$	$130.93±6.15^{A}$	$113.63±4.02^{B}$	0.0326^{*}
35	149.20±5.24	161.56±4.21	159.46±3.58	162.65±2.58	159.57±6.57	155.79±2.15	146.84±3.21	0.4321^{NS}
42	179.68±5.24	180.91±4.21	187.14±3.54	194.36±5.28	185.41±8.24	184.57±7.14	182.38±5.16	0.7382^{NS}

T1, 0.15 kg of corn starch + 1.5 kg of water; T2, T1+ 30 g of exopolysaccharide; T3, 0.15 kg of corn starch + 120 g of exopolysaccharide; T4, 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5, 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6, 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7, T1+ 0.75 kg (glucose:inulin 24:1). ^{A,B,C,D}:Mean values with different superscript within a row differ at P<0.05;^{NS}, No statistically significant difference at P>0.05;*, with statistically significant difference at P<0.05.

2401.61±67.58 g. FI at 7 days was significantly different among all 7 treatment groups (Table 3) and FI at 7 and 28 days correlated with body weight gain (Table 4). Broiler chicken body weight gains among all groups were significantly different at 21 and 28 days ($P<0.05$) (Table 4). Growth rate of broiler chickens at ages of 21 and 28 days was significantly different at $P<0.05$ (Table 5). These results correlated with those of FI and body weight increase. In addition, growth rate of broiler chickens at ages 42 days in feed group T4 was the highest compared with chickens at the same ages in the other feed groups. FCR of broiler chickens in all feed group were not significantly different (Table 6). Total body weight, body weight increase, FI, FCR, and growth rate of broiler chickens in T4 (150 g of *L. acidophilus* BCC 13938 + 1.5 kg of water) and T5 (30 g of *O. dipterigena* BCC 2073 exopolysaccharide + 150 g of *L. acidophilus* BCC 13938 + 0.75 kg of water) feed groups gave the best results in comparison with the other groups,

Table 4. Chicken body weight gain at different ages of broiler chickens fed with different diets

Bird age (days)	Body weight gain (g/bird)							*P*-value
	T1	T2	T3	T4	T5	T6	T7	
7	124.00±2.21	121.73±2.02	122.39±2.19	116.33±1.89	113.73±1.78	116.67±1.84	117.73±1.91	0.1109^{NS}
14	239.33±3.24	230.80±3.25	239.08±3.56	234.66±4.25	236.51±2.65	228.93±2.48	230.40±2.15	0.7658^{NS}
21	$419.60±4.15^{A}$	$399.33±4.02^{A}$	$397.17±5.01^{A}$	$423.30±2.45^{A}$	$423.29±2.10^{A}$	$358.67±2.10^{B}$	$402.93±2.49^{A}$	0.0142^{*}
28	$472.27±0.69^{AB}$	$461.87±1.98^{AB}$	$477.04±2.15^{AB}$	$511.07±0.98^{A}$	$495.14±2.56^{A}$	$454.53±4.41^{AB}$	$421.09±3.23^{B}$	0.0434^{*}
35	516.00±2.12	531.47±2.01	520.71±4.02	550.24±3.12	573.00±2.15	528.67±3.14	502.42±4.10	0.7332^{NS}
42	541.33±1.12	596.61±2.15	606.11±1.10	616.01±0.58	594.37±0.98	593.43±0.79	612.16±1.25	0.6767^{NS}

T1, 0.15 kg of corn starch + 1.5 kg of water; T2, T1+ 30 g of exopolysaccharide; T3, 0.15 kg of corn starch + 120 g of exopolysaccharide; T4, 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5, 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6, 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7, T1+ 0.75 kg (glucose:inulin 24:1). ^{A,B,C,D}:Mean values with different superscript within a row differ at $P<0.05$; ^{NS}, No statistically significant difference at $P>0.05$;*, with statistically significant difference at $P<0.05$.

Table 5. Growth rate at different ages of broiler chickens fed with different diets

Bird age (days)	Growth rate (g/bird-day)							*P*-value
	T1	T2	T3	T4	T5	T6	T7	
7	17.71±0.21	17.39±1.23	17.48±1.20	16.62±1.02	16.25±0.56	16.67±0.35	16.82±0.87	0.1109^{NS}
14	34.19±1.15	32.97±1.65	34.15±3.21	33.52±3.26	33.79±4.25	32.71±2.10	32.91±0.56	0.7658^{NS}
21	$59.94±0.99^{A}$	$57.05±0.87^{A}$	$56.74±0.57^{A}$	$60.47±2.25^{A}$	$60.47±2.87^{A}$	$51.24±2.15^{B}$	$57.56±3.10^{A}$	0.0142^{*}
28	$67.47±2.21^{AB}$	$65.98±2.10^{AB}$	$68.15±2.14^{AB}$	$73.01±2.01^{A}$	$70.73±2.54^{A}$	$64.93±3.01^{AB}$	$60.16±0.87^{B}$	0.0434^{*}
35	73.71±1.02	75.92±1.98	74.39±1.06	78.61±1.13	81.86±1.26	75.52±1.59	71.77±1.10	0.7332^{NS}
42	77.33±1.14	85.23±1.87	86.59±0.97	88.00±0.54	84.91±1.58	84.78±3.25	87.45±3.12	0.6767^{NS}

T1, 0.15 kg of corn starch + 1.5 kg of water; T2, T1+ 30 g of exopolysaccharide; T3, 0.15 kg of corn starch + 120 g of exopolysaccharide; T4, 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5, 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6, 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7, T1+ 0.75 kg (glucose:inulin 24:1). ^{A,B,C,D}:Mean values with different superscript within a row differ at $P<0.05$; ^{NS}, No statistically significant difference at $P>0.05$;*, with statistically significant difference at $P<0.05$.

Table 6. Feed conversion rate at different ages of broiler chickens fed with different diets

Bird age (days)	Feed conversion rate (%)							*P*-value
	T1	T2	T3	T4	T5	T6	T7	
7	$1.37±0.05^{C}$	$1.40±0.06^{BC}$	$1.42±0.04^{ABC}$	$1.41±0.01^{ABC}$	$1.37±0.04^{C}$	$1.46±0.05^{A}$	$1.43±0.02^{AB}$	0.0150^{*}
14	1.56±0.01	1.55±0.02	1.56±0.01	1.62±0.03	1.49±0.06	1.58±0.02	1.52±0.01	0.1417^{NS}
21	$1.54±0.01^{BC}$	$1.58±0.01^{B}$	$1.58±0.01^{B}$	$1.50±0.02^{C}$	$1.52±0.01^{BC}$	$1.72±0.02^{A}$	$1.54±0.01^{BC}$	0.0002^{**}
28	$1.82±0.03^{CD}$	$1.92±0.03^{B}$	$1.92±0.01^{B}$	$1.78±0.03^{D}$	$1.75±0.03^{D}$	$2.02±0.03^{A}$	$1.89±0.01^{BC}$	0.0002^{**}
35	2.02±0.02	2.14±0.03	2.15±0.03	2.09±0.03	1.95±0.02	2.07±0.03	2.05±0.02	0.3126^{NS}
42	2.33±0.02	2.13±0.03	2.17±0.03	2.21±0.03	2.20±0.02	2.19±0.01	2.09±0.01	0.4967^{NS}

T1, 0.15 kg of corn starch + 1.5 kg of water; T2, T1+ 30 g of exopolysaccharide; T3, 0.15 kg of corn starch + 120 g of exopolysaccharide; T4, 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5, 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6, 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7, T1+ 0.75 kg (glucose:inulin 24:1). ^{A,B,C,D}:Mean values with different superscript within a row differ at $P<0.05$; ^{NS}, No statistically significant difference at $P>0.05$;*, with statistically significant difference at $P<0.05$;**, with statistically significant difference at $P<0.01$.

Table 7. Bacterial cell numbers in broiler chicken fecal samples collected from new born, 7 and 42 days (d) old chickens fed with different diets

Bacteria	New born (CFU/g dried stool)	Cell number (CFU $\times 10^3$/g dried stool)											
		T1		T2		T3		T4		T5		T6		T7	
		7 d	42 d	7 d	42 d	7 d	42 d	7 d	42 d	7 d	42 d	7 d	42 d	7 d	42 d
E.coli	75±21	8.1±0.6^{D}	830±14^{A}	42±4^{B}	80±1^{CB}	19.8±0.1^{C}	28±1^{D}	17±1^{C}	229 ±18^{B}	15.5±0.7^{C}	35±7.1^{D}	6.6±0.2^{D}	10.5±0.7^{D}	82±1^{A}	27±5^{D}
Coliform	122±6	106±2^{A}	2545±1^{A}	342±20^{C}	250±14^{C}	43.3±2^{B}	56.0±0.3^{C}	45±2^{B}	404±6^{B}	85.5±10^{D}	185±7.1^{B}	15.6±2^{D}	110.5±4^{B}	857±115^{D}	30.5±0.1^{C}
Lactobacilli	107±6	5.5±0.7^{C}	80±3^{C}	250±85^{B}	870±35^{C}	84±3^{C}	550±7^{C}	310±57^{AB}	2550±49^{B}	390±42^{A}	4000±141^{A}	325±49^{AB}	1000±14^{C}	14±3^{C}	275±2^{C}
Vibrio spp.	0	54±1^{A}	75±2^{C}	13±1^{D}	31±1^{B}	2.4±0.2^{C}	30±1^{B}	22±2^{B}	365±9^{C}	54±1^{A}	315±8^{C}	7.0±0.7^{C}	28±2^{B}	17±1^{B}	57±1^{A}
Total bacteria	165±49	2200 ±141^{C}	16850 ±16^{D}	3850 ±495^{BC}	50700 ±11^{A}	4200 ±141^{B}	20250 ±17^{C}	5450 ±1626^{AB}	12950 ±3^{D}	6850 ±212^{A}	35850 ±3^{B}	4200 ±283^{B}	75500 ±8^{D}	2150 ±636^{C}	72500 ±6^{D}

T1, 0.15 kg of corn starch + 1.5 kg of water; T2, T1+ 30 g of exopolysaccharide; T3, 0.15 kg of corn starch + 120 g of exopolysaccharide; T4, 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5, 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6, 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7, T1+ 0.75 kg (glucose:inulin 24:1). ^{A,B,C,D}Mean values with different superscript within a row differ at P<0.05; ^{NS}, No statistically significant difference at P>0.05;*, with statistically significant difference at P<0.05;**, with statistically significant difference at P<0.01.

indicating the beneficial effects of chicken feed supplemented with probiotic or a combination of pre- and probiotics. The results obtained in this study were not significantly different and to obtain clearer results, the number of replicates must be increased. This study emphasized the necessity of having both prebiotic and probiotics in chicken feed to obtain a healthy beneficial effect from the more benign gut commensal bacteria community. The combination of exopolysaccharide from *O. dipterigena* BCC 2073 and *L. acidophilus* BCC 13938 probiotic had positive effects on total body weight, body weight increase, FI, FCR, and growth rate of broiler chickens. Similar results were obtained using a combination of galacto-oligosaccharide and *Bifidobacteria lactis* in chicken diet suggested that using galacto-oligosaccharide and *B. lactis*-based probiotic favored intestinal growth of bifidobacteria in broiler chickens (Jung *et al*. 2008). Feeding broiler chickens with prebiotic (exopolysaccharide) alone did not significantly affect body weight, body weight increase, FI, FCR or growth rate, this might be due to water adsorption and swelling of exopolysaccharide in chicken stomach resulted in lower feed intake. Furthermore, feeding with probiotic (*Lactobacillus*) alone did not also affect body weight, body weight increase, FI, FCR or growth rate due to prebiotic is necessary for their growth promotion. Probiotic, prebiotic and synbiotic supplementation significantly increased body weight and improved feed gain ratio together with decreased the mortality of broiler chickens (El-Banna *et al*. 2010). These results were in agreement with Abdel-Raheem *et al*. (2012) that the final body weight, weight gain, feed conversion efficiency were significantly higher in probiotic and synbiotic supplemented broilers compared with the control and prebiotic groups.

Vibrio spp. were not found in faeces of new born chicks (Table 8). Faecal samples of 7 day-old chicks contained an increase in numbers of *E. coli* compared to new borns, with those in feed groups T4, T5 and T6 having lower numbers compared to the groups. The highest total *Coliform* counts were found in T7 feed group. *Lactobacilli* spp. counts in T4, T5 and T6 feed groups were higher than the other feed groups. Feed groupT1 had the highest total *Vibrio* spp. counts and the highest total bacteria count was in T5 feed group. On day 42, T4, T5 and T6 feed groups contained higher fecal *Lactobacilli* spp. counts compared to the other groups, while T1 feed group had the highest total *E. coli* and *Coliform* counts, T4 feed group had the highest total *Vibrio* spp. counts and highest total bacteria counts were found in T6 feed group (Table 7). Sequencing of 16S rDNA amplicons from faecal DNA separated by DGGE revealed that on day 7, *Streptococcus* spp. were present in feed groups T3, T4, T5 and T6; *Escherichia* spp. in all groups; and *Lactobacilli* spp. in T2, T3 and T6 (Table 8). On day 42, *Lactobacilli* spp. were found in feed groups T2, T3, T4, T6 and T7; *Firmicutes* spp. in T5; *Clostridium* spp. in T4 and T7; and *Bacillus sporothermodurans* and *B. licheniformis* in T6 (Table 9). The microbial community in faecal samples of the broiler chickens given a diet supplemented with *O.*

Table 8. Microorganisms in faeces of 7 days old broiler chickens fed with different diets detected from sequences of 16S rDNA separated by denaturing gradient gel-electrophoresis (DGGE)

Diet	Microorganism
T1	1. Uncultured bacterium (JX183824.1, 91%), 2. *Escherichia coli* (CAJT01000035.1, 93%), 3. Uncultured Enterobacteriales bacterium (EU434894.1, 93%), 4. Uncultured Enterobacteriales bacterium (HM074352.1, 80%), 5. Uncultured *Streptococcus* sp. (JN173175.1, 91%), 6.-, 7.-
T2	1. *Lactobacillus johnsonii* (NZ_AFQJ01000004.1, 88%), 2. Uncultured bacterium (HQ701230.1, 73%), 3. *E. Coli* (KC138796.1, 87%), 4. *Staphylococcus* sp. (FR719724.1, 72%), 5. *E. coli* (GU646192.1, 97%), 6. Uncultured bacterium (AB698035.1, 94%), 7. Uncultured bacterium (FJ658570.1, 82%), 8. *L. reuteri* (JN183871.1, 69%), 9. Uncultured bacterium (JX839289.1, 83%)
T3	1. *L. acidophilus* (1, NZ_ACHN01000039.1, 81%), 2. *Streptococcus* sp. (JN998528.1, 80%), 3. Uncultured bacterium (FJ682070.1, 92%), 4. Uncultured bacterium (JQ695610.1, 90%), 5. *Streptomyces* sp. (GU130103.1, 89%), 6. Uncultured bacterium (GU600141.1, 80%), 7. *E. coli* (KC138804.1, 89%), 8. Uncultured *Escherichia* sp. (DQ856917.1, 83%), 9. *L. gasseri* (AB289138.1, 83%)
T4	1. Uncultured bacterium (GQ137993.1, 85%), 2. Uncultured bacterium (HQ716677.1, 87%), 3. *Streptococcus* sp. (AB371967.1, 78%), 4. *Streptococcus* sp. (AF084834.1, 75%), 5. Uncultured *Escherichia* sp. (EF674507.1, 72%), 6. *Streptococcus* sp. (JQ937302.1, 74%), 7. Uncultured *Escherichia* sp. (DQ856917.1, 96%), 8. Uncultured bacterium (JQ284446.1, 71%), 9. *Enterococcus* sp. (FJ513907.1, 86%), 10. Uncultured bacterium (EU791270.1, 86%)
T5	1. Uncultured bacterium (EU457527.1, 83%), 2. *E. faecalis* (FJ821314.1, 78%), 3. Uncultured *Streptococcus* sp. (JN173175.1, 92%), 4. Uncultured *Escherichia* sp. (DQ856861.1, 85%), 5. *Arthrobacter* sp. (FJ626801.1, 70%), 6. *E. fergusonii* (HQ407221.1, 96%), 7. Uncultured bacterium (JQ410865.1, 94%), 8. Uncultured bacterium (JX457166.1, 92%)
T6	1. *L. johnsonii* (EU381128.1, 96%), 2. *Streptococcus* sp. (JQ316647.1, 95%), 3. *E. coli* (GU646183.1, 96%), 4. Uncultured bacterium (JX457256.1, 88%), 5. -
T7	1. *Serratia marcescens* (JQ068796.1, 70%), 2. *S. gallolyticus* (JQ912076.1, 94%), 3. *E. coli* (JQ993881.1, 97%), 4. *E. coli* (JQ975905.1, 82%), 5. *Staphylococcus* sp. (FR719724.1, 75%), 6. *Serratia* sp. (DQ297665.1, 95%), 7. -

DNA sequences were aligned using NCBI DNA sequence database (accession number and % identity are in bracket, respectively). T1 , 0.15 kg of corn starch + 1.5 kg of water; T2 , T1+ 30 g of exopolysaccharide; T3 , 0.15 kg of corn starch + 120 g of exopolysaccharide; T4 , 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5 , 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6 , 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7 , T1+ 0.75 kg (glucose:inulin 24:1). Number refers to band number on DGGE (data not shown).

dipterigena BCC 2073 exopolysaccharide as prebiotic resulted in reduced numbers of pathogenic bacteria (*E. coli, Staphylococcus* sp., *Streptococcus* sp., *Enterococcus* sp.) and increased numbers of beneficial bacteria (*Lactobacilli* and *Bacilli*). The numbers of pathogenic bacteria in faecal samples were inversely correlated with total body weight, body weight increase, FI, FCR and growth rate. The addition to broiler chicken feeds of both pre- and probiotics favored the growth of probiotics (*Lactobaclli*) in the GI tract. The results obtained in this study showed the effects of prebiotic of exopolysaccharide used in which it promoted the growth of intestinal probiotics. The combination of exopolysaccharide from *O. dipterigena* BCC 2073 and probiotic (*L. acidophilus* BCC 13938) showed the effects of pre- and probiotic in which the reduction of gut pathogenic bacteria were revealed and increased number of intestinal commensal bacteria. Similar phenomena have reported in other animals, suplementation of dietary inulin in broiler chicken does not affect intestinal bacterial communities but their metabolic activity changes (Rehman *et al*. 2008). Studies of the effects of fructo-oligosaccharide supplemented diet on non-Bifidobacteria in piglet gut microflora showed an increase in *Bifidobacterium* spp. and a decrease in *Bacteroides* spp. (Shen *et al*. 2010). Arabonoxylan-oligosacchardies stimulate the growth of lactic acid bacteria and *Clostridium* spp. in juvenile Siberian sturgeon (*Acipenser baerii*) intestines (Geraylou *et al*. 2012). Supplementation of short-chain fructo-oligosaccharides influences gastrointestinal microbiota composition and immunity characteristics of Pacific white shrimp (*Litopenaeus vannamei*) but does not improve weight gain, feed conversion ratio or survival (Li *et al*. 2007). The supplementation of probiotic, prebiotic or synbiotic improved weight gain and feed conversion rate of broiler chickens, also increased the caecal populations of lactobacilli/bifidobacteria, decreased caecal *E. coli* and increased the caecal volatile fatty acids (Mookiah *et al*. 2014).

Here, we report the potential of supplementing broiler chicken diet with a combination of fungal

Table 9. Microorganisms in faeces of 42 days old broiler chickens fed with different diets detected from sequences of 16S rDNA separated by denaturing gradient gel-electrophoresis

Diet	Microorganism
T1	1. Uncultured *Lactobacillaceae* bacterium (FJ975946.1, 73%), 2. Uncultured bacterium (GQ179294.1, 79%), 3. -, 4. -, 5. -, 6. -
T2	1. Uncultured *Lactobacillus* sp. (JQ960617.2, 87%), 2. Uncultured bacterium (JQ336532.1, 87%), 3. -, 4. -, 5. -, 6. -, 7. -, 8. -, 9. -, 10 -
T3	1. Uncultured *Lactobacillus* sp. (GQ280119.1, 76%), 2. Uncultured *Lactobacillus* sp. (JQ962805.2, 75%), 3. Uncultured rumen bacterium (GU303313.1, 80%), 4. *Streptomyces* sp. (FJ626802.1, 81%), 5. Uncultured bacterium (JF633402.1, 72%), 6. Uncultured *Lactobacillus* sp. (JQ962830.2, 75%), 7. Uncultured bacterium (FJ163666.1, 73%), 8. -
T4	1. Uncultured bacterium (GU619794.1, 75%), 2. Uncultured bacterium (AY702848.1, 77%), 3. *Lactobacillus acidophilus* (GU413899.1, 84%), 4. Uncultured rumen bacterium (EU382039.1, 80%), 5. Uncultured rumen bacterium (GU303227.1, 77%), 6. Uncultured bacterium (FJ369165.1, 76%), 7. Uncultured *Escherichia* sp. (DQ856911.1, 78%), 8. *Clostridium paraputrificum* (NR_026135.1, 73%)
T5	1. Uncultured Firmicutes bacterium (FJ968489.1, 87%), 2. Uncultured rumen bacterium (GU303475.1, 75%), 3. Uncultured bacterium (EU452347.1, 81%), 4. Uncultured bacterium (DQ807981.1, 91%), 5. Uncultured bacterium (EU886393.1, 74%), 6. Uncultured bacterium (DQ904829.1, 74%), 7. Uncultured bacterium (GU060386.1, 86%), 8. -, 9. -, 10.-
T6	1. Uncultured bacterium (JF549232.1, 82%), 2. Uncultured bacterium (EU475583.1, 82%), 3. *Bacillus sporothermodurans* (FJ973536.1, 71%), 4. *B. licheniformis* (GU723480.1, 72%), 5. Uncultured bacterium (GQ175416.1, 91%), 6. *L. acidophilus* (JX255677.1, 77%), 7. *L. oris* (AB596981.1, 73%), 8. -, 9. -
T7	1. Uncultured bacterium (FJ682680.1, 91%), 2. Uncultured *Lactobacillus* sp. (JQ962353.2, 75%), 3. Uncultured *Lactobacillus* sp. (EU381137.1, 76%), 4. *L. crispatus* (AM117143.1, 84%), 5. *L. reuteri* (JX963641.1, 93%), 6. Uncultured sp. (DQ856758.1, 79%)

DNA sequences were aligned using NCBI DNA sequence database (accession number and % identity are in bracket, respectively). T1 , 0.15 kg of corn starch + 1.5 kg of water; T2 , T1+ 30 g of exopolysaccharide; T3 , 0.15 kg of corn starch + 120 g of exopolysaccharide; T4 , 1.5 kg of water + 150 g of *L. acidophilus* BCC 13938; T5 , 0.75 kg of water +150 g of *L. acidophilus* BCC 13938 + 30 g of exopolysaccharide; T6 , 150 g of *L. acidophilus* BCC 13938 + 120 g of exopolysaccharide; T7 , T1+ 0.75 kg (glucose:inulin 24:1). Number refers to band number on DGGE (data not shown).

exopolysaccharide from *O. dipterigena* BCC 2073 as prebiotic and *Lactobacillus acidophilus* BCC 13938 as probiotic in generating a less pathogenic intestinal commensal bacterial community, which will impact on the health of the host animal. This study hopefully may lead to the adoption of fungal exopolysaccharide as a prebiotic supplement (together with appropriate probiotics) in animal feeds and thereby reduce the over-use of antibiotic in the industrial livestock production.

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