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Evaluation of the Prebiotic Effect of Chitin Extracted from Shrimp Waste by Bioremediation Method in Commercial Broilers

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ABSTRACT

Shrimp waste was recycled as a source of chitin and the extracted chitin was evaluated for its potential as a prebiotic in broiler birds. Chitin was extracted by the bioremediation method using lactobacillus culture and the protein rich liquid remaining after chitin extraction was recycled to enrich deoiled rice bran (DORB) to increase its crude protein content. The treated DORB was tested as an alternative to fish meal. The extracted chitin was characterized by Fourier Transform Infra Red (FTIR) spectroscopy. Four hundred commercial day-old broiler chicks were divided into five groups of four replicates each with 20 birds per replicate and allotted at random to five experimental diets (T_1 to T_5) formulated for pre-starter (0-14 days), starter (14-28 days) and finisher (28-42 days) phases. The extracted chitin was included at 1 (T_2) and 2% (T_3). The treated deoiled rice bran was included at 3 (T_4) and 6% (T_5) to replace 50 and 100% fish meal of control diet (T_1). The effect of treatments on growth performance of birds, gut pathogen (E. coli and Salmonella) load, RBC, WBC, lymphocyte counts, serum and breast muscle cholesterol and triglyceride content was studied. The overall growth performance of the birds was in the order of $T_3>T_2>T_1>T_4>T_5$. There was a significant reduction ($P<0.01$) in caecal *E.coli* and *Salmonella* count, increase ($P<0.01$) in RBC, WBC and lymphocyte count and decrease in serum cholesterol and muscle triglyceride content of birds fed chitin containing diets. In conclusion, chitin was effective as a prebiotic during the pre -starter and starter phases, more so at 2% than at 1% and the treated DORB was beneficial during finisher phase than during pre-starter and starter phases to replace 50 % of fish meal.

Key words: Shrimp Waste, Chitin, Prebiotic, Broiler, Performance

INTRODUCTION

Scientific evidence suggests that the massive use of antibiotic compounds has led to increased problem of antibiotic resistance (Forgetta *et al.,* 2012) and presence of antibiotics residues in feed and environment (Carvalho and Santos, 2016; Gonzalez Ronquillo and Angeles Hernandez, 2017), compromises human and animal health (Diarra *et al.* 2010). Salmonellosis in humans is another concern of public health importance most frequently associated with the consumption of contaminated fresh and processed poultry products (Lynch *et al.* 2006). According to Foley *et al.* (2011), *Salmonella typhimurium* continues to be among the most common serovars isolated from poultry and a common cause of human salmonellosis. Public health concerns associated with antimicrobial resistance has led to research to find out alternatives to the use of antibiotic feed additives. Shrimp waste is being explored for its potential role as an alternative to antibiotic growth promoters since the chitin and chitosan in shrimp waste have been demonstrated to have pre-biotic effects (Gernat, 2001; Mahata *et al.,* 2008). The shrimp processing industry has been rapidly growing with significant increase in cultured shrimp production in the South-East Asian region. A huge amount of bio-waste consisting of head shells and abdominal shells is produced from the industries because shrimps are normally sold as headless or peeled or both. Shrimp waste constitute 48-56% of the weight of shrimp. The shrimp processing waste generated in India is around 1.25 to 1.50 lakh tonnes per annum. The major components of the waste (DM basis) are protein (35-50%), chitin (15-25%), minerals (10-15%) and carotenoids (Ramyadevi *et al.* 2012). The main commercial process for chitin extraction from shrimp waste is based on demineralization by acid treatment and

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deproteinization by alkali treatment which are corrosive and not environment friendly. Therefore, enzymes, ensiling, bioremediation using probioitics have been tried to extract chitin and chitosan from shrimp waste to overcome the corrosive and environment polluting effects of chemical methods. The remaining liquid portion after chitin extraction is supposed to be rich in protein and ash that can be used to enrich low quality feeds such as rice bran (Prameela *et al.* 2010; Jag Pal *et al.* (2014).

In the present study, chitin was extracted from shrimp waste by the eco-friendly bioremediation method and the extracted chitin was evaluated for its prebiotic effect in broilers. Deoiled rice bran was treated with the remaining liquid portion after extraction of chitin by bioremediation method and used in experimental diets to replace fish meal. The effect of experimental diets on broiler performance and gut pathogen load was studied.

MATERIALS AND METHODS

The shrimp waste (SW), procured from a shrimp processing unit was sun dried for 3 days to about 90% DM, autoclaved for 15 m at 121° C, 15 psi and subjected to grinding in a hammer mill. Chitin was extracted by the bio-remediation method using 5% lactic culture as inoculum, 15% glucose and fermented for 72 h. (Prameela *et al.* 2010). The lactic culture, *Lactococcus lactis* ssp. *lactis* NCDC-134 was procured in lyophilized form in sealed glass ampoule from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal. The culture was propagated in 200 ml sterilized skim milk for inoculation into shrimp waste. The bio-remediation method to extract chitin from shrimp waste was done by taking 15 kg of autoclaved SW in three separate containers of 5 kg each and each container was added with 10 liters of water in which 250 g of lactic acid culture and 750 g glucose was dissolved, fermented for 72 h and later filtered through a muslin cloth. The residue left over was chitin while the filtrate i.e. the remaining liquid fraction was used to treat deoiled rice bran (DORB). Total ash and CP content of SW and

Table 1. Ingredient composition (%) of experimental diets

Ingredients	Pre- starter					Starter					Finisher				
	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5
Maize	57.0	55.0	54.0	57.0	55.0	58.0	58.0	55.85	57.0	54.0	61.9	59.9	57.5	61.5	58.0
Soybean meal	29.15	29.85	29.85	31.85	33.20	28.50	26.80	28.0	31.0	33.0	23.0	23.5	23.85	25.5	28.0
Fish meal	6.0	6.0	6.0	3.0	0	6.0	6.0	6.0	3.0	0	6.0	6.0	6.0	3.0	0
Chitin	0	1.0	2.0	0	0	0	1.0	2.0	0	0	0	1.0	2.0	0	0
De-oiled rice bran	3.0	3.0	3.0	3.0	6.0	3.0	3.0	3.0	3.0	6.0	3.0	3.0	3.0	3.0	6.0
Salt	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Palm oil	2.50	2.80	2.80	2.80	2.80	2.0	2.5	2.5	3.2	3.70	3.5	4.0	5.0	4.3	5.0
Mineral mixture*	1.75	1.75	1.75	1.75	1.75	1.9	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Calcite	0	0	0	0	0.65	0	0	0	0	0.45	0	0	0	0	0.30
DL-methionine	0.15	0.15	0.15	0.15	0.15	0.10	0.15	0.15	0.15	0.15	0.10	0.10	0.15	0.10	0.10
L-lysine	0.20	0.20	0.20	0.20	0.20	0	0.05	0	0.15	0.20	0	0	0	0.10	0.10
Total**	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

*Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35%, and Cu, 100; Mn, 200; Co, 50; I, 100 ppm

fermented residue were estimated to calculate demine-ralization and deproteinization efficiency (Yen *et al.,* 2009). The extracted chitin was characterized using Fourier Transform Infra Red Spectroscopy (FT-IR). A sample of 10 µg was mixed with 100 µg of dried potassium bromide (KBr) and compressed to prepare a salt (10 mm diameter) and characterized by the FT-IR in the range of 500-4000 cm (Kamala *et al.,* 2013). The filtrate was mixed with 15 kg DORB, sundried and used in the experimental diets The ME of ingredients (NRC, 1994) and fish meal (Leeson and Summers, 2008) was predicted from chemical composition to formulate experimental diets. Experimental diets were formulated as per ICAR, 2013 feeding standards (Mandal *et al.,* 2013) for pre-starter (0-14 days), starter (14-28 days) and finisher (28-42 days) phases. The diets during each phase were isonitrogenous and isocaloric and other ingredients like palm oil, calcite, L-lysine and DL-methionine were also used to meet the dietary concentration for energy, protein, Ca, P, lysine and methionine as per Indian Council of Agricultural Research specifications for broiler diets (Mandal *et al.,* 2013). The experimental diets were a basal ration (T_1), into which chitin was included at 1 (T_2) and 2 % (T_3), while the diets T_4 and T_5 were without chitin but contained treated deoiled rice bran at 3 (T_4) and 6 % (T_5) to replace 50 and 100% of fishmeal (Table 1). Four hundred commercial day-old broiler chicks were wing banded and weighed individually on arrival. Each treatment contained four replicates with twenty birds per replicate allotted at random. The experiment was conducted from day old to forty two days.

The chicks in each replicate were housed in well ventilated individual pens under deep litter system using paddy husk as litter material. All pens were provided with uniform brooding facilities, linear feeders and trough waterers with sufficient feeder and water space per bird.

All diets contained: Meriplex at 10 g/100 kg (providing vitamin-B_1, 8; vitamin -B_6, 16; vitamin E, 80; niacin, 120, folic acid, 8; calcium D pantothenate, 80 mg/& vitamin-B_{12}, 80µg /g), Merivite -AB_2D_3K at 10g/ 100 kg (providing: vitamin A, 500 IU; B_2 52 mg; D_3, 1200 IU and K 10 mg/g) and Cosmodot at 50g/100 kg (3-5, Dinitro-O-Toluamide 25 %W/W)Pre-starter from day old to 14 days, starter from 14 to 28 days and finisher diet from 28 to 42 days of age was offered *ad lib* with free access to fresh water. The body weight of the birds was recorded at weekly intervals upto 6 weeks of age. The feed consumed by each replicate was recorded and the feed efficiency was calculated based on total feed consumed and total weight gain in each replicate. The mortality percent was calculated and the causes there of were ascertained by detailed autopsy. Shrimp waste, treated DORB and the experimental diets were analyzed for proximate composition (AOAC, 2005), calcium and phosphorus (Talapatra *et al.,* 1940). At the end of pre-starter, starter and finisher phases, one bird per replicate of each treatment was slaughtered to collect caecal contents for estimation of the caecal *E.coli* and *Salmonella* counts (Speck, 1984). Blood samples were also collected from slaughtered birds at the end of pre-starter, starter and finisher phases to estimate blood cell (RBC, WBC and lymphocyte) count in whole blood, and glucose (Yong, 1995) and cholesterol (Allian *et al.* 1974) in serum using diagnostic kits. The lipids from breast muscle of slaughtered birds were extracted (Folch *et al.* 1957) to estimate cholesterol and triglyceride content using standard kits (M/s Robonic (India) PVT. LTD) by enzymatic method of Allian *et al.* (1974). The data were subjected to one-way ANOVA (Snedecor and Cochran, 1994).

RESULTS AND DSICUSSION

The shrimp waste contained 26.69 % DM and its other chemical constituents (% DM) were 40.5, 0.85, 28.65, 26.39, 6.78, 3.03, 51.86, 63.76, 54.46, 9.28, 1.81 and 7.50 of CP, EE, CF, TA, Ca, P, Chitin, NDF, ADF, Cellulose, ADL and Silica, respectively. The chemical composition of experimental diets is presented in Table 2. The standard plate count was 2720×10^3 CFU/g in fresh shrimp waste for *E.coli* and *Salmonella* which was reduced to 1648×10^3 CFU/g in sun dried sample and was zero in the autoclaved sample. The treated DORB contained higher CP and TA of 16.7 and 19.9 %

Table 2. Chemical composition (%) of experimental diets

Parameter	Pre- starter					Starter					Finisher				
	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5
Dry matter	91.4	92.1	91.1	91.3	91.0	90.5	92.4	92.1	91.3	89.3	89.5	90.0	89.8	92.1	91.5
Crude protein	21.8	22.0	22.1	21.8	21.7	21.4	21.5	21.6	21.5	21.4	19.4	19.6	19.6	19.5	19.4
Ether extract	4.7	4.6	4.6	4.1	4.0	3.2	3.3	3.4	3.8	3.6	4.4	4.3	4.4	4.7	4.5
Crude fiber	6.4	7.4	7.1	7.6	8.1	6.4	6.6	6.7	6.8	6.9	6.6	6.8	7.1	6.3	6.9
Total ash	10.3	10.7	10.5	10.2	10.6	9.4	9.1	9.6	9.8	10.1	7.8	8.1	8.2	8.1	8.2
Nitrogen free extract	56.8	55.3	55.7	56.3	55.6	59.6	59.5	58.7	58.1	58.0	61.8	61.2	60.7	61.4	61.0
ME (kcal/kg)*	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.1	3.1	3.1	3.1	3.1
Lysine (%)*	1.20	1.20	1.20	1.20	1.20	1.1	1.1	1.1	1.15	1.15	0.95	0.96	0.96	0.99	0.96
Methionine (%)*	0.50	0.50	0.50	0.50	0.50	0.44	0.50	0.48	0.47	0.45	0.42	0.41	0.41	0.40	0.40
Calcium (%)	1.0	1.0	1.0	1.0	1.0	1.04	1.08	1.08	0.93	0.95	1.07	1.07	1.07	0.92	0.89
Available phosphorus (%)	0.55	0.50	0.50	0.50	0.42	0.57	0.58	0.58	0.50	0.42	0.56	0.56	0.56	0.48	0.43

*Calculated values

than 14.1 and 17.8 % in the untreated DORB, respectively, thus clearly indicating that chitin extraction by the bioremediation method has brought about deproteinization of shrimp waste by the growth of lactic acid bacteria in the culture and demineralization by the lactic acid produced. This has led to an increase in protein and ash content of the filtrate obtained after extraction of chitin from the SW similar to earlier observations (Prameela *et al.,* 2010; Khorrami *et al.,* 2012). The main commercial process for chitin extraction from shrimp waste is based upon demineralization by acid treatment and deproteinization by alkali treatment. In the biological method, chitin is produced by using lactic acid bacteria. It also leads to a protein rich liquid fraction which can be used as human and animal feed. But the use of this methods is still limited to laboratory scale because demineralization and deproteinization are not achieved quite as much efficiently as in the chemical method. Optimization and standardization of the extraction process is necessary to produce high yield with minimum cost (Jag Pal *et al.,* 2014). It has been reported that lactic acid produced by a number of microorganisms such as *Bacillus subtilis, Lactobacillus helveticus, Pseudomonas aeruginosa, Lactobacillus paracasei, Lecanicillium fungicola* and *Penicillium chrysogenum* is easily utilized for demineralization. These microorganisms are responsible for the precipitation of organic salts such as calcium lactate, which is easily removed from media by wash out. Deproteinization is also carried out with the aid of proteolytic activities of some microorganisms. Thus, deproteinization is carried out in the presence of proteases produced by microorganisms meaning that proteolysis causes the breakdown of proteins (Khorrami *et al.,* 2012). The production of chitin and carotenoids from shrimp bio-waste by using natural probiotic has been studied. The fermentation studies were determined by varying inoculum levels from 1 to 10%, glucose concentration from 0 to 15% and incubation time 0 to 72 hours. The maximum decrease of pH and increase of total titratable acidity (TTA) was attained for a incubation period of 72 hours with 5% inoculum and 15% glucose. Deproteinization of 89% and

demineralization of 69% was obtained by fermentation of shrimp bio-waste with natural probiotic. The carotenoid and chitin recovery from shrimp bio-waste was 72.6% and 5.65%, respectively. As the process by using natural probiotic was found to be efficient and economical, it becomes an alternative method for the hazardous chemicals used in extraction of chitin and carotenoids (Prameela *et al.,* 2010). After six days of fermentation of SW with *Lactobacillus plantarum* inoculum at 5%, the deproteinization and demineralization efficiency was 45 and 54%, respectively (Khorrami *et al.,* 2012). Prameela *et al.,* (2010) have reported a deproteination efficiency of 89% and demineralization of 69% when SW was inoculated with 5% natural curd and 15% glucose for 72 h. In the present study, deproteination efficiency of 46.9% and demineralization efficiency of 44.0 % was observed by inoculating with *Lactococcus lactis* culture at 5% along with glucose 15% for 72 hrs. The chitin extracted from shrimp waste was characterized using the Fourier Transform-Infra Red spectroscopy (Fig 1). The peak at 3267, 1629, 1540 and 1317 cm^{-1}, indicated presence of OH group, amide bands I, II and III, respectively. The NH stretching peak of the extracted chitin was at 2924 cm^{-1} which compared well with the NH stretching peak

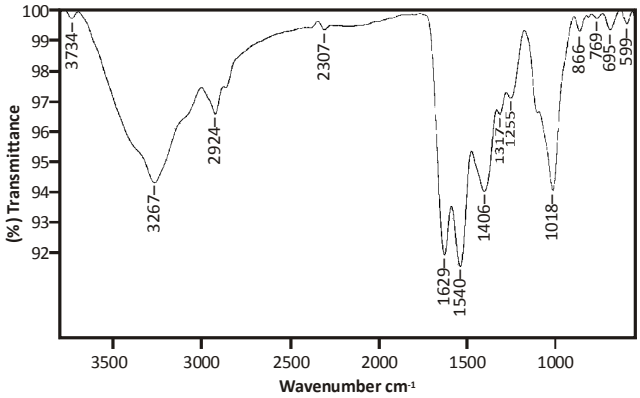

Fig. 1. FTIR spectrum of the extracted chitin

of 3107 cm^{-1} for the standard chitin. These observations are in close agreement with the reported values of 3426 for OH stretching, 3107 for N-H stretching, 1647, 1560 and 1318 for amide I, amide II and amide III bands, respectively in standard chitin (Kamala *et al.,* 2013).

The overall effect of the treatments revealed that the *E.coli* count (log cfu/g) of the cecal content (Table 3) was significantly (P< 0.01) decreased in groups T_2 (5.33) and T_3 (5.31) than in groups T_1 (6.13), T_4 (6.04) or T_5 (6.14). The overall effect of the different phases revealed that the *E.coli* count (log cfu/g) in the cacal content was significantly lower (P<0.01) during the

Table 3. The gut pathogen count (cfu/g) in caecal content of broilers fed experimental diets

	T_1	T_2	T_3	T_4	T_5	SEM	P value	Effect of phase
E. coli								
Pre-starter	6.44 [a]	5.61 [b]	5.54 [b]	6.40 [a]	6.46 [a]	0.08	P<0.01	6.08 [A]
Starter	6.02 [a]	5.40 [b]	5.30 [b]	6.00 [a]	6.02 [a]	0.11	P<0.01	5.75 [B]
Finisher	5.91 [a]	5.00 [b]	5.07 [b]	5.77 [a]	5.93 [a]	0.11	P<0.01	5.54 [C]
Effect of treatment	6.13 [a]	5.33 [b]	5.31 [b]	6.04[a]	6.14 [a]	0.09	P<0.01	P<0.01
								SEM : 0.09
Salmonella								
Pre-starter	4.27[a]	3.86[b]	3.89[b]	3.99[ab]	4.10[ab]	0.10	P<0.01	4.02[B]
Starter	4.43[a]	3.90[b]	3.96[b]	4.32[a]	4.36[a]	0.11	P<0.01	4.19[A]
Finisher	4.05	3.81	3.84	4.13	4.17	0.07		3.90[B]
Effect of treatment	4.25[a]	3.81[b]	3.84[b]	4.13[a]	4.17[a]	0.08	P<0.01	P<0.01
								SEM : 0.09

[a,b] Values in a row not sharing common superscripts differ significantly; [A,B,C] Values in a column not sharing common superscripts differ significantly

Table 4. Growth performance of broilers

Phase	Parameter	T_1	T_2	T_3	T_4	T_5	SEM	P value
Pre-starter	Body weight gain (kg)	8.49 [b]	8.62 [ab]	9.00 [a]	7.52 [c]	6.05 [d]	0.12	P<0.01
	Feed intake (kg)	12.50 [a]	12.46 [a]	12.56 [a]	11.72 [b]	10.75 [c]	0.14	P<0.01
	Feed/ kg gain	1.47 [bc]	1.44 [c]	1.39 [c]	1.55 [b]	1.77 [a]	0.02	P<0.01
Starter	Body weight gain (kg)	13.26 [b]	14.31 [a]	15.02 [a]	10.61 [c]	7.36 [d]	0.28	P<0.01
	Feed intake (kg)	19.98 [a]	20.01 [a]	20.14 [a]	20.15 [a]	14.26 [b]	0.21	P<0.01
	Feed/ kg gain	1.50 [b]	1.39 [b]	1.34 [b]	1.91 [a]	1.94 [a]	0.04	P<0.01
Finisher	Body weight gain (kg)	11.29	11.98	11.83	12.00	11.23	0.46	
	Feed intake (kg)	24.95 [a]	25.00 [a]	24.96 [a]	24.97 [a]	20.65 [b]	0.12	P<0.01
	Feed/ kg gain	2.21 [a]	2.09 [a]	2.08 [a]	2.11 [a]	1.84 [b]	0.14	P<0.01
Overall	Body weight gain (kg)	33.05 [b]	34.92 [a]	35.85 [a]	30.13 [b]	24.64 [c]	0.36	P<0.01
	Feed intake (kg)	57.44 [a]	57.48 [a]	57.67 [a]	56.85 [a]	45.67 [b]	0.39	P<0.01
	Feed/kg gain	1.73 [a]	1.64 [a]	1.61 [a]	1.89 [b]	1.85 [b]	0.03	P<0.01

[a,b,c,d] Values in a row not sharing common superscripts differ significantly

finisher phase (5.54) followed by starter phase (5.75) and pre-starter phase (6.08). *Salmonella* count (log cfu/ g) was significantly (P< 0.01) reduced in birds of group T_2 or T_3 than in other treatments and the values were 4.25, 3.81, 3.84, 4.13 and 4.17 in groups T_1 to T_5, respectively. It was significantly (P<0.01) lower during pre-starter and finisher phases than during starter phase and the values were 4.02, 4.19, and 3.90 during pre-starter, starter and finisher phases, respectively. The observations of present study reinforce the claims of previous authors (Chen *et al.,* 2002, Vishu Kumar *et al.,* 2005 and Li *et al.,* 2006) that feeding of low level of SW containing chitin and extracted chitin-chitosan was effective as pre-biotic in reducing the gut pathogens, particularly *E.coli* and *Salmonella.* Wang *et al., (*2003) suggested that the chitin oligosaccharides might affect the colonization of *Salmonella* strains by blocking bacterial attachment to the gut mucosa, thereby reducing the prevalence of *Salmonella*. The use of shrimp waste meal as a protein source in poultry diets may have a beneficial effect in that it can potentially alter the microbial ecology of the intestine. Acidic digestive fluid in the proventriculus and gizzard may degrade the shrimp shell to release chitin, and some

Table 5. Effect of dietary treatments on blood cells, serum metabolites and muscle cholosterol and triglyceride content

Parameter	T_1	T_2	T_3	T_4	T_5	SEm	P-value
RBC count (10^6/µl)	2.90[b]±0.09	3.30[a]±0.10	3.21[a]±0.11	2.97[b]±0.10	2.94[b]±0.09	0.09	P<0.01
WBC count (10^3/µl)	29.20[c]±0.18	30.3[a]±0.17	30.2[a]±0.14	29.7[b]±0.16	29.8[b]±0.14	0.15	P<0.01
Lymphocyte (%)	60.78[b]±0.25	63.66[a]±1.16	63.74[a]±1.20	60.84[b]±1.31	60.99[b]±1.09	1.01	P<0.01
Blood glucose (mg%)	380.0[b]±3.92	392.4[a]±3.02	388.9[ab]±3.53	375.0[b]±5.07	382.1[ab]±3.09	3.01	P<0.01
Serum cholesterol (mg%)**	124.9[a]±5.4	103.9[b]±1.8	103.7[b]±1.9	121.2[a]±1.7	120.1[a]±2.3	1.8	P<0.01
Muscle Cholesterol (mg/dl)	58.9±1.18	55.8±1.73	55.6±1.28	55.9±1.01	57.9±1.62	1.28	
Muscle Triglycerides (mg/dl)**	147.0[a]±4.16	123.9[b]±4.55	121.2[b]±4.89	134.3[ab]±10.25	130.7 [ab]±3.68	4.25	P<0.05

[a,b,c] values in a row not sharing common superscripts differ significantly

chitin may simply dissolve. The degradation products of chitin can inhibit the growth of harmful bacteria (Chen *et al.,* 2002). Data pertaining to growth performance of broilers during different phases and overall growth performance is presented in Table 4. During the pre-starter and starter phases, the body weight gain and feed intake of birds in T_2 and T_3 was significantly($P<0.01$) higher than in other treatments and the lowest ($P<0.01$) body weight gain and feed intake was observed in T_5 followed by T_4. A superior ($P<0.01$) feed conversion ratio was observed in groups T_1, T_2 and T_3 than in groups T_4 and T_5. Contrary to the pre-starter and starter phases, the body weight gain of birds during finisher phase was comparable among treatments. The growth performance of the birds in present study revealed that supplementation of chitin as prebiotic was beneficial in promoting better growth rate. Similar to the results of present study, earlier studies of Shi *et al.,* (2005), Khambualai *et al.,* (2008 and 2009), Huang *et al.,* (2005), (Ramachandran Nair *et al.,* 1987) and Zhou *et al.,* (2009) also reported beneficial effects of supplementing chito-oligosaccharides at low levels in promoting higher ($P<0.01$) growth rate and feed efficiency in broilers. However, Okoye *et al.,* (2005), Khempaka *et al.,* (2006 and 2011) and Razdan *et al.,* (1996 and 1997) have reported inferior ($P<0.01$) performance of broilers by feeding higher ($P<0.01$) levels of shrimp waste or chitin. Enhanced ileal digestibility of nutrient due to reduced number of pathogenic bacteria i.e. *Esche-richia coli, Salmonella typhimurium* (Choi *et al.,* 1994; Wang *et al.,* 2003) was reported. Increased immune function (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003), better absorption of nutrients (Huang *et al.,* 2005), increased feed intake and nutrient digestibility (Li *et al.,* 2007; Khambualai *et al.,* 2008, 2009) were attributed for better performance of broilers by supplementing chito oligosaccharides at low levels and a similar trend was observed in the present study.

The inclusion of treated DORB to replace 50 and 100% of fish meal in diets T_4 and T_5 resulted in poor performance of birds during pre-starter and starter phases. However, during the finisher phase, the BWG and feed intake of birds in groups T_4 and T_5 was higher than during earlier phases and this could be attributed to the maturity and/ or age of the birds. Okoye *et al.,* (2005) observed that as the birds age the gastrointestinal tract matures and nutrient absorption efficiency increases. The RBC, WBC and lymphocyte count (Table 5) was higher ($P<0.01$) in birds of groups T_2 and T_3 than those in other treatment groups whereas the blood glucose was higher ($P<0.01$) in group T_2 as compared to birds of groups T_1 and T_4. Serum concentration of cholesterol (mg/dl) was decreased ($P<0.01$) in birds of groups T_2 and T_3 as compared to those of groups T_1, T_4 or T_5. The breast muscle cholesterol (mg/dl) was not significantly different among the treatments. However, the triglyceride (mg/dl) content of muscle in birds of groups T_2 and T_3 was lower ($P<0.01$) than those in group T_1 and comparable with those of groups T_4 and T_5. Similar to the observations of present study, several earlier reports (Jameela *et al.,* 1994; Kanuachi *et al.,* 1995; Razdan and Patterson 1996; Razdan *et al.,* 1997; Li *et al.,* 2007) have indicated the beneficial effect of chito-oligosaccharides in reducing blood cholesterol and triglyceride levels.

CONCLUSIONS

It was concluded that bioremediation method using lactobacillus culture was effective to extract chitin from shrimp waste. The extracted chitin was effective as a prebiotic during the pre -starter and starter phases of broilers, more so at 2% than at 1% and the treated DORB can be better included during finisher phase than during pre-starter and starter phases to replace 50 % of fish meal.

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