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FULL LENGTH ARTICLE

**Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*)**



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**Abstract** Twelve practical diets were formulated to contain four levels of *Bacillus licheniformis* ( $0.0$ ,  $0.24 \times 10^6$ ,  $0.48 \times 10^6$  and  $0.96 \times 10^6$  CFU g<sup>-1</sup>), respectively, with three yeast extract levels (0%, 0.5% and 1%), respectively. Each diet was randomly assigned to duplicate groups of 50 Nile tilapia (*Oreochromis niloticus*) ( $5.99 \pm 0.03$  g) in 24 concrete ponds (0.5 m<sup>3</sup> and 1.25 m depth) for 12 weeks. Increasing dietary *B. licheniformis* levels in *O. niloticus* and yeast extract levels significantly ( $P < 0.01$ ) improved growth performance and nutrient utilization. Supplementation of the experimental diets with,  $0.48 \times 10^6$  CFU/g<sup>-1</sup> and 1.0% yeast extract showed the best nutrient utilization compared to other treatments. All probiotic levels significantly ( $P < 0.01$ ) increased chemical composition ( $P < 0.05$ ) compared to the control group, while increasing yeast extract did not significantly alter chemical composition. Hematological indices, total protein and albumin of *O. niloticus* significantly increased while aspartate aminotransferase and alanine aminotransferase significantly ( $P < 0.01$ ) decreased with an increase in *B. licheniformis* level up to  $0.48 \times 10^6$  CFU g<sup>-1</sup>. Increasing levels of yeast extract had no effect on hematological parameters and the diets supplemented with  $0.48 \times 10^6$  CFU g<sup>-1</sup> and 0.5% yeast extract showed the highest hematological values.

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**Introduction**

Probiotics are a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring an improved use of the feed or enhancing its nutritional value, by increasing the host response towards disease, or by improving the quality of its

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environment (Verschuere et al., 2000). Nowadays, probiotics are also becoming an internal part of aquaculture practices to obtain high production. Although considerably low information is available on probiotic application for fish, they offer benefits with regard to improving immune status and fish production (Cerezuela et al., 2011). Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of specific health-promoting bacteria, which can improve host's health (Gibson et al., 2003).

Based on the studies of Mahious and Ollevier (2005) and Gibson et al. (2004) foodstuff that reaches the colon (e. g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids) is a candidate prebiotic (Yousefian and Amiri, 2009). However, most of the studies have focused on non-digestible carbohydrates, mainly oligosaccharides. Synbiotics are nutritional supplements that combine probiotics and prebiotics, enhancing their beneficial effects (Cerezuela et al., 2011).

The use of probiotics and prebiotics has been regarded during recent years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming an integral part of aquaculture practices for improving growth and disease resistance (Rombout et al., 2010). This strategy offers innumerable advantages to overcome the limitation and side effects of antibiotics and other drugs and also leads to high production (Sahu et al., 2008).

In recent years there has been a growing interest in understanding the mechanism of action of probiotics and prebiotics, especially in humans and other mammals. Probiotic activity is mediated by a variety of effects that are dependent on the probiotic itself, the dosage employed, treatment duration and route and frequency of delivery. Some probiotics exert their beneficial effects by elaborating antibacterial molecules such as bacteriocins that directly inhibit other bacteria or viruses and, activity participating in the fight against infections; whereas, others inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules or modulate the inflammatory/immune response (Cerezuela et al., 2011).

On the other hand, the potential mechanism of prebiotics includes a selective increase/decrease in specific intestinal bacteria that modulate local cytokine and antibody production, an increase in the intestinal short chain fatty acid production, an enhanced binding of these fatty acids to G-coupled protein receptors on leucocytes, an interaction with carbohydrate receptors on intestinal epithelial and immune cells, and partial absorption resulting in a local and systemic contact with the immune system (Seifert and Watzl, 2007).

The alternative methods of disease prevention have been used as a means of reducing the presence of opportunistic pathogens and simultaneously stimulating the host immune responses. However, other effects related have been observed, as improved growth performance, feed utilization, digestive enzyme activity, antioxidant enzyme activity, gene expression, disease resistance, larval survival and gut morphology alter the gut microbiota, mediate stress response, improve nutrition, reduce risk of certain cancers (colon, bladder), produce lactase, alleviate symptoms of lactose intolerance and malabsorption (Rombout et al., 2010; Dimitroglou et al., 2011; Yousefian and Amiri, 2009; Ringø et al., 2010).

Synbiotic is defined as a combination of probiotic and prebiotic. It is presumed to impart the beneficial effects of both ingredients. Few data are available regarding the application of synbiotics in aquaculture (Li et al., 2009; Rodriguez-Estrada et al., 2009; Zhang et al., 2010). Synbiotics can help to improve health status, disease resistance, growth performance, feed utilization, carcass composition, gastric morphology, and digestive enzyme activities. As such; many commercial dietary formulations now routinely include probiotics or prebiotics.

Therefore, the aim of the present study is to investigate the effects of supplementation of a probiotic (*Bacillus licheniformis*) and the prebiotic (yeast extract) and their synbiotic interaction on growth performance, chemical composition, hematological and biochemical blood parameters of the Nile tilapia (*Oreochromis niloticus*).

## Materials and methods

### Experimental design and culture technique

A 4 × 3 factorial experiment was designed to study the effect of probiotic (*B. licheniformis*) levels, prebiotic (yeast extract) levels, and their synbiotic interactions on growth performance, feed utilization, proximate chemical analysis of whole fish body, hematological and biochemical blood parameters of the Nile tilapia (*O. niloticus*).

Nile tilapia, were obtained from the Abbassa hatchery, Abou-Hammad, Sharkia Governorate, Egypt and were acclimated for two weeks at the El-Kanater El-Khayria Fish Research Station, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt. During this period, fish were fed with a commercial diet (30% crude protein) twice a day to be adapted to pelleted feed according to Hassaan et al. (2013). The experiment was conducted in 24 concrete ponds (0.5 m<sup>3</sup> and 1.25 m depth). The ponds were supplied with freshwater from the Darawa irrigation Baranch, Kalubiya, Governorate by a pump machine and a fine net was put in the inlet of each pond. Each pond was stocked with 50 fish with initial weight ranging between 5.69–6.05 g. Two replicates were randomly assigned to each treatment, prior to the start of experiment. During the experiment, fish were hand-fed their respective diets at a level of 3% of body weight, 6 days/week. The daily ratio was divided into three equal amounts and offered three times a day (09:00, 12:00 and 15:00 h). Fish for each pond were weighed biweekly and the amount of daily diet was adjusted accordingly. About one-third of water in each pond was daily renewed by the outlet at the bottom of the pond before feeding. All ponds were provided with continuous aeration to maintain the dissolved oxygen level near saturation and fish were held under natural light.

Water temperature and dissolved oxygen were measured every other day using a YSI model 58 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). Total alkalinity and chloride were monitored twice weekly using the titration; pH was monitored twice weekly using a pH meter (Orion pH meter, Abilene, Texas, USA) (APHA, 1992). The water temperature was 26.17 ± 0.8 °C: dissolved oxygen, 5.6 ± 0.8 mg L<sup>-1</sup>: total

ammonia,  $0.18 \pm 0.12 \text{ mg L}^{-1}$ ; total alkalinity,  $173 \pm 42 \text{ mg L}^{-1}$ ; chlorides,  $570 \pm 151 \text{ mg L}^{-1}$  and pH  $8.52 \pm 0.3$ . Water quality criteria were suitable within the acceptable limits for rearing the Nile tilapia *O. niloticus* fingerlings (El-Greisy and El-Gamal, 2012).

#### Preparation inoculum of probiotics (*B. licheniformis*)

*B. licheniformis* culture was prepared by adding 15 g of dried form (Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams Univ., Egypt) to 100 ml of prepared medium containing ( $\text{g l}^{-1}$ ): (peptone 5.0; beef extract, 3.0) broth and an adjusted pH at 7.0. Incubation was done at 37 °C. After 24 h, 1 ml was inoculated into 100 ml of freshly prepared medium broth that was incubated for a further 48 h at 37 °C. After incubation, the cells were harvested by centrifugation (2000g for 15 min), washed twice with phosphate buffered saline (PBS; pH 7.3; Oxoid) and re-suspended in PBS for the addition to the basal diet. Washed cells were then added dropwise into the basal mixture prior to cold press extruding after Shelby et al. (2006) to produce the probiotic diet with three levels  $0.24 \times 10^6$ ,  $0.48 \times 10^6$  and  $0.96 \times 10^6$  CFU  $\text{g}^{-1}$ . The same volume of PBS (*B. licheniformis*) was added to the basal mixture for the control to maintain an equal volume of PBS.

#### Experimental diets

The basal diet was formulated to contain approximately 30% crude protein and 19.41 KJ/kg diet<sup>1</sup> gross energy (Table 1). *B. licheniformis* was supplemented separately to the basal diet to obtain 0.0,  $0.24 \times 10^6$ ,  $0.48 \times 10^6$  and  $0.96 \times 10^6$  CFU  $\text{g}^{-1}$  after it was pelted. Each level of *B. licheniformis* was supplemented with 0.0, 1 and 1.5% yeast extract (Diamond VXPC®). The ingredients were ground into fine powder through 200  $\mu\text{m}$  mesh. All ingredients were thoroughly mixed with soybean oil and then passed the mixed feed through a laboratory pellet mill (2-mm dye) in the National Institute of Oceanography and Fisheries, Cairo Governorate, Egypt (a California Pellet Mill, San Francisco, CA, USA), and stored at  $-20$  °C until use.

#### Growth and nutrient utilization parameters

Growth performance and feed utilization parameters were measured using the following equations: Weight gain (WG) = final weight (g) – initial weight (g), Specific growth rate (SGR) =  $\frac{\ln W_2 - \ln W_1}{t} \times 100$  where:  $\ln$  = the natural log;  $W_1$  = first fish weight;  $W_2$  = the following fish weight in gram and  $t$  = period in days, Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g), Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g), Protein productive value (PPV)% = (protein gain (g)/protein intake (g)  $\times$  100).

#### Blood sample and hematological and biochemical analysis

At the end of the experiment, blood samples were collected from the caudal vein of all experimental fish treatments and were divided into two groups. The first group was collected with anticoagulant 10% ethylenediaminetetraacetate (EDTA) to measure hematocrit (Ht), hemoglobin (Hb), red blood cells

(RBCs) and white blood cells (WBCs). Ht was determined as described by Reitman and Frankel (1957), hemoglobin (Hb) was determined by the hemoglobin kit which is a standardized procedure of the cyanomet hemoglobin method and the total count of white blood cells (WBCs) was carried out by the indirect method (Martins et al., 2004). The second group of the blood samples was allowed to clot overnight at 4 °C and then centrifuged at 3000 rpm for 10 min. The non-hemolyzed serum was collected and stored at  $-20$  °C until use. Levels of serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined according to the method described by Reitman and Frankel (1957). Total protein (TP) and albumin were determined by the method of (Wotton and Freeman, 1982).

At the termination of the trail, a sample of three fish was randomly sampled from each pond. Fish samples were killed, ground, stored in polyethylene bags and frozen until the chemical analysis. Dry matter, crude protein, lipid and ash contents were determined according to AOAC (1995).

#### Statistical analysis

All data were analyzed by SAS (1996). One-way analysis of variance (One-way ANOVA) was used to determine whether significant variation existed between treatments. When overall differences were found, they were tested by Duncan's multiple range test as described by Duncan (1955). Two-way ANOVA was used for analyzing the individual effects of *B. licheniformis* and yeast extract, and the interaction between them. All differences were considered significant at ( $P < 0.05$ ).

## Results and discussion

#### Growth performance

Results in Table 2 indicated that final body weight (BW), body length (BL) weight gain (WG) and specific growth rate (SGR) of *O. niloticus* increased with increasing probiotic (*B. licheniformis*) up to  $0.48 \times 10^6$  CFU  $\text{g}^{-1}$  diet. The diet supplemented with  $0.48 \times 10^6$  CFU  $\text{g}^{-1}$  showed the highest significant ( $P < 0.05$ ) BW, WG and SGR when compared to other fish groups. Such an increase in the growth in aquatic animals that were fed probiotic supplemented diets may be attributed to the improved digestive activity due to enhancing the synthesis of vitamins and enzymatic activities (Ding et al., 2004); consequently, improving digestibility and growth performance. Probiotics have been shown to produce digestive enzymes such as amylase, protease, lipase which may enrich the concentration of intestinal digestive enzymes. In addition, probiotics inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by the competition for nutrients and the alteration of the microbial metabolism (Gatesoupe, 1999). It also improves the nutrition by detoxifying the potentially harmful compounds in feeds by producing vitamins such as biotin and vitamin B<sub>12</sub> (Hoshino et al., 1997), and by stimulating host immunity (Gibson et al., 1997). Soltan and El-Laithy (2008) indicated that supplementation of basal diet with *Bacillus subtilis*, significantly ( $P < 0.001$ ) improved BW, BL, WG and SGR of *O. niloticus*. Similarly, the application of *Enterococcus faecium* as a probiotic was found to enhance the growth performance of Nile tilapia, *O. niloticus* (Wang et al., 2008).

**Table 1** Formulation and chemical proximate analysis of the experimental diets.

Ingredients%	Diet NO. ( <i>B. licheniformis</i> CFU g <sup>-1</sup> /Yeast extract%)												
	D1 0/0	D2 (0/0.05)	D3 (0/1)	D4 (0.24 × 10 <sup>6</sup> /0)	D5 (0.24 × 10 <sup>6</sup> /0.5)	D6 (0.24 × 10 <sup>6</sup> /1)	D7 (0.48 × 10 <sup>6</sup> /0)	D8 (0.48 × 10 <sup>6</sup> /0.5)	D9 (0.48 × 10 <sup>6</sup> /1)	D10 (0.96 × 10 <sup>6</sup> /0)	D11 (0.96 × 10 <sup>6</sup> /0.5)	D12 (0.96 × 10 <sup>6</sup> /1)	
Fish meal	10	10	10	10	10	10	10	10	10	10	10	10	
Soybean meal	46	46	46	46	46	46	46	46	46	46	46	46	
Yellow corn	29.5	29	28.5	29.4	28.9	28.4	29.35	28.85	29	29.3	28.8	28.3	
Wheat bran	10	10	10	10	10	10	10	10	10	10	10	10	
Soybean oil	3	3	3	3	3	3	3	3	3	3	3	3	
Vit. & mineral <sup>a</sup>	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	
<i>B. licheniformis</i> <sup>b</sup>	0	0	0	0.1	0.1	0.1	0.15	0.15	0.15	0.20	0.20	0.20	
Yeast extract	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	
<i>Proximate analysis</i>													
Protein%	30.05	30.70	30.11	30.04	30.68	30.11	30.01	30.65	30.71	30.01	30.62	30.70	
Lipids%	5.70	5.75	5.76	5.65	5.73	5.66	5.63	5.72	5.70	5.70	5.72	5.74	
Ash%	5.13	5.21	5.20	5.15	5.18	5.21	5.13	5.18	5.21	5.13	5.19	5.23	
T. CHO <sup>c</sup> %	59.12	58.34	58.93	59.16	58.49	59.02	59.23	59.06	58.38	59.16	58.47	58.33	
GE (MJ kg <sup>-1</sup> ) <sup>d</sup>	19.49	19.53	19.50	19.47	19.54	19.47	19.46	19.63	19.52	19.48	19.52	19.53	

<sup>a</sup> Vitamin and mineral mix (mg or g/kg diet): MnSO<sub>4</sub>, 40 mg; MgO, 10 mg; K<sub>2</sub>SO<sub>4</sub>, 40 mg; ZnCO<sub>3</sub>, 60 mg; KI, 0.4 mg; CuSO<sub>4</sub>, 12 mg; Ferric citrate, 250 mg; Na<sub>2</sub>SeO<sub>3</sub>, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU;  $\alpha$ -tocopherolacetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg; nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; ascorbic acid, 500 mg.

<sup>b</sup> *B. licheniformis* was prepared to obtain 0.24 × 10<sup>6</sup>, 0.48 × 10<sup>6</sup> and 0.96 × 10<sup>6</sup> CFU g<sup>-1</sup> approximately.

<sup>c</sup> T. CHO total carbohydrate = 100-(crude protein + lipid + ash).

<sup>d</sup> Calculated using gross caloric values of 23.63, 39.52 and 17.15 kJ/g for protein, fat and carbohydrate, respectively according to Brett (1973).

**Table 2** Growth performance of Nile tilapia (*Oreochromis niloticus*) fed the experimental diets with different levels of *Bacillus licheniformis* and yeast extract levels.

Diet No ( <i>B. Licheniformis</i> / Yeast extract)	<i>B. Licheniformis</i> cfu g <sup>-1</sup>	Yeast extract (1%)	Growth performance					
			IBW g <sup>a</sup>	FBWg <sup>b</sup>	FBL cm <sup>c</sup>	K <sup>d</sup>	WG g <sup>e</sup>	SGR <sup>f</sup>
Diet 1 (0/0)	0	0	5.96	34.55k	12.30c	1.85ab	28.59j	1.95g
Diet 2 (0/0)	0	0.5	5.98	40.13j	13.40b	1.67b	34.15i	2.12f
Diet 3 (0/0)	0	1	5.98	40.44i	13.25b	1.74ab	34.46h	2.12ef
Diet 4 (0.24 × 10 <sup>6</sup> /0)	0.24 × 10 <sup>6</sup>	0	6.05	40.91h	13.45b	1.67b	34.86g	2.12ef
Diet 5 (0.24 × 10 <sup>6</sup> /0.5)	0.24 × 10 <sup>6</sup>	0.5	6.02	41.15 g	13.45b	1.69ab	35.13f	2.14de
Diet 6 (0.24 × 10 <sup>6</sup> /1)	0.24 × 10 <sup>6</sup>	1	6.01	42.00e	13.40b	1.75b	35.99e	2.16cd
Diet 7 (0.48 × 10 <sup>6</sup> /0)	0.48 × 10 <sup>6</sup>	0	5.98	41.26f	13.05b	1.87a	35.28f	2.15d
Diet 8 (0.48 × 10 <sup>6</sup> /0.5)	0.48 × 10 <sup>6</sup>	0.5	5.97	44.12b	14.45a	1.47d	38.15b	2.22b
Diet 9 (0.48 × 10 <sup>6</sup> /1)	0.48 × 10 <sup>6</sup>	1	6.02	45.84a	14.15a	1.62c	39.82a	2.26a
Diet 10 (0.96 × 10 <sup>6</sup> /0)	0.96 × 10 <sup>6</sup>	0	6.01	40.11j	14.20a	1.40e	34.10i	2.11f
Diet 11 (0.96 × 10 <sup>6</sup> /0.5)	0.96 × 10 <sup>6</sup>	0.5	5.99	42.30d	14.20a	1.49d	36.31d	2.18c
Diet 12 (0.96 × 10 <sup>6</sup> /1)	0.96 × 10 <sup>6</sup>	1	5.98	43.72c	14.15a	1.45d	37.74c	2.22b
<i>One-way ANOVA</i>								
Polled S.E.M*			0.03	0.02	0.17	0.70	0.051	0.0062
<i>F</i> -value ( <i>B. Licheniformis</i> )			1.94	12841.9	24.76	9.00	5838.08	292.23
<i>F</i> -value (Yeast extract)			0.14	13039.9	12.92	3.33	5983.18	321.06
<i>Tow-way ANOVA</i>								
<i>P</i> -value ( <i>B. Licheniformis</i> )			0.182	0.001	0.0001	0.0027	0.0001	0.0001
<i>P</i> -value (Yeast extract)			0.871	0.001	0.0013	0.0742	0.0001	0.0001
<i>P</i> -value (interaction)			0.725	0.001	0.0132	0.0672	0.0001	0.0001

Data are means of triplicate. Means in the same column sharing a same superscript letter are not significantly different ( $P > 0.05$ ).

\* Polled S.E.M standard error of mean.

<sup>a</sup> IBW: Initial body weight.

<sup>b</sup> FBW: Final body weight.

<sup>c</sup> FBL: Final body length.

<sup>d</sup> K: Condition factor.

<sup>e</sup> WG: Weight gain.

<sup>f</sup> SGR: Specific growth rate.

Ai-Dohail et al. (2009) also illustrated that African catfish *Clarias gariepinus* that were fed the *Lactobacillus acidophilus* showed a better growth performance than the control fish group.

As described in Table 2, BW, BL, WG and SGR of *O. niloticus* significantly ( $P < 0.05$ ) increased with increasing prebiotic (yeast extract) levels. Fish that were fed the diet containing 1.0% yeast extract showed the highest BW, BL, WG and SGR when compared to the other two fish groups (control and the diet supplemented with 0.5% yeast extract). In contrary, Jarolowicz et al. (2012) indicated that, the commercial diet supplemented with yeast extract did not have an impact on the final BW of Juvenile European pikeperch, *Sander lucioperca*.

Referring to the effect of synbiotics on growth performance, the highest final BW, WG and SGR were recorded by fish that were fed D9 containing  $0.48 \times 10^6$  CUF g<sup>-1</sup> *B. licheniformis* and 1.0% yeast extract, while the lowest one was shown by fish that were fed the control diet (D1). The study of Mehrabi et al. (2011) showed that after 60 days rainbow trout (*Oncorhynchus mykiss*) fed diets containing different levels of symbiotic (Biomim IMBO) (0.5%, 1.0% and 1.5%) increased in body weight about 50%, 59% and 53%, respectively and improved SGR and FCR in comparison with the control group. Ye et al. (2011) reported that, Japanese flounder fed diet supplemented with fructooligosaccharides (FOS),

mannan oligosaccharides (MOS) and *Bacillus clausii* increased WG. Also, Ai et al. (2011) indicated that at each dietary FOS level, supplemented by  $1.35 \times 10^7$  CFU g<sup>-1</sup> *B. subtilis* significantly increased SGR and feed efficiency ratio (FER) when compared to the control group for juvenile large yellow croaker, *Larimichthys crocea*.

#### Feed intake and feed utilization

Results of Table 3 indicated that, increasing *B. licheniformis* level in *O. niloticus* diets was followed by a significant increase in feed intake (FI) and a significant improvement in FCR, PER and PPV up to the diet supplemented by  $0.48 \times 10^6$ . In practical terms, this means that the use of probiotics can decrease the amount of feed necessary for animal growth which could result in a reduction in the production cost. Several studies on probiotics have been published in recent years which suggested that, probiotics provide nutritional benefits in diets for tilapia fingerling (Khattab et al., 2004; Ferguson et al., 2010).

Increasing yeast extract levels (from 0%, 0.5% or 1.0% yeast extract) significantly ( $P < 0.01$ ) increased FI, and significantly improved FCR, PER and PPV (Table 3) which was subsequently followed by an increase in the growth performance. Generally, the high demand for nucleotides occurs during periods of rapid growth (Carver, 1994). Li and Gatlin (2005) indicated that sub-adult hybrid striped sea bass

**Table 3** Feed utilization of Nile tilapia (*Oreochromis niloticus*) fed the experimental diets with different levels of *Bacillus licheniformis* and yeast extract levels.

Diet No ( <i>B. Licheniformis</i> / Yeast extract)	<i>B. Licheniformis</i> cfu g <sup>-1</sup>	Yeast extract (1%)	Feed utilization				
			Fig/fish <sup>a</sup>	FCR <sup>b</sup>	PER <sup>c</sup>	PPV% <sup>d</sup>	FR% <sup>e</sup>
Diet 1 (0/0)	0	0	52.95d	1.85a	1.830e	23.51g	33.32e
Diet 2 (0/0)	0	0.5	54.64cd	1.60bc	2.12cd	26.38ef	38.53d
Diet 3 (0/0)	0	1	55.34bc	1.61bc	2.12cd	27.27de	38.63d
Diet 4 (0.24 × 10 <sup>6</sup> /0)	0.24 × 10 <sup>6</sup>	0	56.45ab	1.62b	2.10d	25.66f	38.79d
Diet 5 (0.24 × 10 <sup>6</sup> /0.5)	0.24 × 10 <sup>6</sup>	0.5	56.79a	1.62b	2.10d	28.49bc	43.27b
Diet 6 (0.24 × 10 <sup>6</sup> /1)	0.24 × 10 <sup>6</sup>	1	58.04a	1.61b	2.10d	26.87e	40.59c
Diet 7 (0.48 × 10 <sup>6</sup> /0)	0.48 × 10 <sup>6</sup>	0	56.81ab	1.61b	2.11cd	26.60e	41.63c
Diet 8 (0.48 × 10 <sup>6</sup> /0.5)	0.48 × 10 <sup>6</sup>	0.5	55.75bc	1.47e	2.32a	29.34b	43.69b
Diet 9 (0.48 × 10 <sup>6</sup> /1)	0.48 × 10 <sup>6</sup>	1	56.75ab	1.43e	2.38a	31.57a	49.22a
Diet 10 (0.96 × 10 <sup>6</sup> /0)	0.96 × 10 <sup>6</sup>	0	54.57de	1.60bc	2.12cd	28.85bc	43.31b
Diet 11 (0.96 × 10 <sup>6</sup> /0.5)	0.96 × 10 <sup>6</sup>	0.5	56.60ab	1.56cd	2.18Bc	27.97cd	40.59c
Diet 12 (0.96 × 10 <sup>6</sup> /1)	0.96 × 10 <sup>6</sup>	1	58.05a	1.54d	2.21b	29.34b	44.07b
<i>One-way ANOVA</i>							
Polled S.E.M <sup>*</sup>			0.55	0.014	0.020	0.44	0.29
<i>F</i> -value ( <i>B. Licheniformis</i> )			14.46	87.44	76.59	93.29	182.20
<i>F</i> -value (Yeast extract)			11.39	89.12	71.09	89.06	79.53
<i>Tow-way ANOVA</i>							
<i>P</i> -value ( <i>B. Licheniformis</i> )			0.004	0.0001	0.0001	0.0001	0.0001
<i>P</i> -value (Yeast extract)			0.0021	0.0001	0.0001	0.0001	0.0001
<i>P</i> -value (interaction)			0.1025	0.0355	0.0495	0.0001	0.0001

Data are means of triplicate. Means in the same column sharing a same superscript letter are not significantly different ( $P > 0.05$ ).

\* Polled S.E.M standard error of mean.

<sup>a</sup> FI: feed intake g/fish.

<sup>b</sup> FCR: feed conversion ratio.

<sup>c</sup> PER: protein efficiency ratio.

<sup>d</sup> PPV: protein productive value.

<sup>e</sup> FR: fat retention.

(*Morone chrysops* × *M. saxatilis*) fed commercial diet supplemented with prebiotic Grobiotic®-AE, with 10–20 g kg<sup>-1</sup> obtained a significantly improved feed efficiency.

Referring to the dietary symbiotic interaction of the experimental diets with, 0.48 × 10<sup>6</sup> CFU g<sup>-1</sup> and 1.0% the yeast extract (D9) showed the highest FI, the best FCR, PER and PPV compared to other synbiotic treatments and the control group. These results were parallel to those obtained for other growth parameters (BW, BL, WG and SGR) obtained in the present study.

Gastrointestinal bacteria take part in the decomposition of nutrients, provide the microorganisms with physiologically active materials, such as enzymes, amino acids, and vitamins (Bairagi et al., 2004; Wacheć et al., 2006; Wang, 2007; Wang and Xu, 2006), and thus facilitate feed utilization and digestion. This may account for the enhanced FCR, PER and PPV by dietary *B. licheniformis* supplementation in the present study and previous studies (Bairagi et al., 2004; Bagheri et al., 2008). Mehrabi et al. (2011) came to similar results. They found that the addition of synbiotic to the feed of rainbow trout, *Oncorhynchus mykiss* fingerlings produced a better significant ( $P < 0.05$ ) FCR values than the control. Ye et al. (2011) reported that, Japanese flounder fed diet supplemented with FOS, MOS and *Bacillus clausii* improved FCR than other diets. Also, Ai et al. (2011) showed that juvenile large yellow croaker, *Larimichthys crocea* fed the diet supplemented with FOS and *B. subtilis* 0.96 × 10<sup>6</sup> CFU g<sup>-1</sup> significantly improved FCR and PER values when compared to the fish group fed with the control diet.

#### Proximate analysis

Proximate analysis of *O. niloticus* which was affected by probiotic (*B. licheniformis*) and prebiotic (yeast extract) is presented in Table 4. With respect to the effect of *B. licheniformis* supplemented to the experimental diets, it is shown that all probiotic levels significantly ( $P < 0.05$ ) increased dry matter, lipid and protein content when compared to the control group, while ash content was not significantly affected. Soltan and El-laithy, 2008 indicated that, *O. niloticus* fed diet supplemented with *B. subtilis* recorded a high level of dry matter and lipid content than the control group with no effect on the ash content. Bagheri et al. (2008) reported that application of 3.8 × 10<sup>9</sup> CFU g<sup>-1</sup> of *Bacillus spp.* in diet of rainbow trout fry made a significant increase in fish body protein content when compared to the control group.

Results of proximate analysis (Table 4) showed that, increasing yeast extract from 0 to 1.0% did not significantly alter crude protein, dry matter, lipid or ash content. The interaction between probiotic and the prebiotic (Table 4) showed no clear trend in the proximate analyses of whole fish. Ye et al. (2011) in Japanese flounder showed an increase in the body protein content in fish fed with a FOS, MOS and/or *B. clausii*-containing diet when compared to the control, body lipid content demonstrated an opposite trend to body protein content. Mehrabi et al. (2011) indicated that, higher body protein content in the rainbow trout (*Oncorhynchus mykiss*) fingerlings implies on this fact that by the application of

**Table 4** Body composition of whole body fish of Nile tilapia (*Oreochromis niloticus*) fed the experimental diets with different levels of *Bacillus licheniformis* and yeast extract levels.

Diet No ( <i>B. Licheniformis</i> / Yeast extract)	<i>B. Licheniformis</i> cfu g <sup>-1</sup>	Yeast extract (1%)	Chemical analysis of whole fish%			
			Dry matter	Lipid	Protein	Ash
Diet 1 (0/0)	0	0	24.16d	14.40f	53.10c	14.30ab
Diet 2 (0/0)	0	0.5	23.88d	14.80f	53.30c	14.50ab
Diet 3 (0/0)	0	1	24.36d	14.60f	53.35c	14.75ab
Diet 4 (0.24 × 10 <sup>6</sup> /0)	0.24 × 10 <sup>6</sup>	0	23.70e	15.05cd	53.10c	14.75a
Diet 5 (0.24 × 10 <sup>6</sup> /0.5)	0.24 × 10 <sup>6</sup>	0.5	26.20a	15.25bc	53.50ab	14.50ab
Diet 6 (0.24 × 10 <sup>6</sup> /1)	0.24 × 10 <sup>6</sup>	1	24.58d	15.25bc	53.65ab	14.55ab
Diet 7 (0.48 × 10 <sup>6</sup> /0)	0.48 × 10 <sup>6</sup>	0	24.65c	15.80 a	53.55ab	14.35ab
Diet 8 (0.48 × 10 <sup>6</sup> /0.5)	0.48 × 10 <sup>6</sup>	0.5	24.46d	15.20bc	54.10ab	14.55ab
Diet 9 (0.48 × 10 <sup>6</sup> /1)	0.48 × 10 <sup>6</sup>	1	25.55b	15.85a	53.65a	14.70a
Diet 10 (0.96 × 10 <sup>6</sup> /0)	0.96 × 10 <sup>6</sup>	0	25.67a	15.75a	53.50ab	14.80a
Diet 11 (0.96 × 10 <sup>6</sup> /0.5)	0.96 × 10 <sup>6</sup>	0.5	24.29d	15.55ab	54.25a	14.15b
Diet 12 (0.96 × 10 <sup>6</sup> /1)	0.96 × 10 <sup>6</sup>	1	24.88c	15.50ab	54.15ab	14.45ab
<i>One-way ANOVA</i>						
Polled S.E.M*			0.21	0.15	0.26	0.15
<i>F</i> -value			9.47	32.47	4.85	0.39
<i>F</i> -value			1.99	0.47	3.90	1.56
<i>Tow-way ANOVA</i>						
<i>P</i> -Value ( <i>B. Licheniformis</i> )			0.0022	0.0001	0.0218	0.7633
<i>P</i> -value (Yeast extract)			0.1836	0.6342	0.0526	0.2535
<i>P</i> -value (interaction)			0.0001	0.0529	0.8104	0.0987

Data are means of triplicate. Means in the same column sharing a same superscript letter are not significantly different ( $P > 0.05$ ).

\* Polled S.E.M standard error of mean.

**Table 5** Hematological parameters of Nile tilapia (*Oreochromis niloticus*) fed the experimental diets with different levels of *Bacillus licheniformis* and yeas extract levels.

Diet No ( <i>B. Licheniformis</i> / Yeast extract)	<i>B. Licheniformis</i> cfu g <sup>-1</sup>	Yeast extract (1%)	Hematological parameters			
			Hb(g/dl) <sup>a</sup>	Hct% <sup>b</sup>	RBC <sup>c</sup>	WBC(×10 <sup>3</sup> mm <sup>-3</sup> ) <sup>d</sup>
Diet 1 (0/0)	0	0	10.21e	15.05de	1.80h	36.29e
Diet 2 (0/0)	0	0.5	10.51d	14.10g	1.83g	37.05c
Diet 3 (0/0)	0	1	10.83c	14.70ef	1.85fg	37.15c
Diet 4 (0.24 × 10 <sup>6</sup> /0)	0.24 × 10 <sup>6</sup>	0	10.49d	15.25d	1.87de	36.90d
Diet 5 (0.24 × 10 <sup>6</sup> /0.5)	0.24 × 10 <sup>6</sup>	0.5	10.97bc	14.35fg	1.85ef	37.12c
Diet 6 (0.24 × 10 <sup>6</sup> /1)	0.24 × 10 <sup>6</sup>	1	10.88bc	14.70ef	1.85fg	37.60b
Diet 7 (0.48 × 10 <sup>6</sup> /0)	0.48 × 10 <sup>6</sup>	0	10.95bc	15.15d	1.88cd	37.20bc
Diet 8 (0.48 × 10 <sup>6</sup> /0.5)	0.48 × 10 <sup>6</sup>	0.5	11.28a	17.12a	1.97a	38.36a
Diet 9 (0.48 × 10 <sup>6</sup> /1)	0.48 × 10 <sup>6</sup>	1	11.07ab	16.15c	1.91bc	38.15a
Diet 10 (0.96 × 10 <sup>6</sup> /0)	0.96 × 10 <sup>6</sup>	0	10.97bc	16.75b	1.90bc	37.38bc
Diet 11 (0.96 × 10 <sup>6</sup> /0.5)	0.96 × 10 <sup>6</sup>	0.5	10.91bc	17.10a	1.84fg	36.94c
Diet 12 (0.96 × 10 <sup>6</sup> /1)	0.96 × 10 <sup>6</sup>	1	11.09ab	16.41bc	1.92b	37.16bc
<i>One-way ANOVA</i>						
Polled S.E.M*			0.0070	0.11	0.0094	0.13
<i>F</i> -value ( <i>B. Licheniformis</i> )			40.28	234.67	57.43	34.91
<i>F</i> -value (Yeast extract)			22.96	23.30	4.52	20.14
<i>Tow-way ANOVA</i>						
<i>P</i> -value ( <i>B. Licheniformis</i> )			0.0001	0.0001	0.0001	0.0001
<i>P</i> -value (Yeast extract)			0.0001	0.0001	0.0369	0.0002
<i>P</i> -value (interaction)			0.068	0.0001	0.001	0.0016

Data are means of triplicate. Means in the same column sharing a same superscript letter are not significantly different ( $P > 0.05$ ).

\* Polled S.E.M standard error of mean.

<sup>a</sup> Hb: hemoglobin.

<sup>b</sup> Hct: hematocrit.

<sup>c</sup> RBC: red blood.

<sup>d</sup> WBCs: white blood cell.

synbiotics, the ingested food was converted more effectively into the structural protein and subsequently resulted in more muscle, which is a desirable aspect in fish farming. However, the application of synbiotic in trout fingerlings diet has insignificant effect on the lipid content.

#### Hematological indices

Hemoglobin (Hb), hematocrit (Ht), red blood cells (RBCs) and white blood cells (WBCs) of *O. niloticus* significantly increased with each increase in *B. licheniformis* level, up to  $0.48 \times 10^6$  - CFU  $g^{-1}$  *B. licheniformis* and then decreased as the diet was supplemented with  $0.96 \times 10^6$  CFU  $g^{-1}$  *B. licheniformis*.

Hematology is an important factor that could be considered for the fish diet quality assessment. Ologhobo (1992) reported that the most common blood variables consistently influenced by diet are the hematocrit (Ht) and hemoglobin (Hb) levels. Probiotics and prebiotics have been used alone and together in various animals including the synbiotic, in tilapia (Abd El-Rhman et al., 2009), which reported positive effects on hematological parameters. On the other hand, *O. niloticus* fed diet supplemented with *B. subtilis* (Soltan and El-Laithy, 2008) or supplemented with *Pediococcus acidilactici* (Ferguson et al. (2010) showed some variation (but not significant) in Hb and Ht contents among the control and fish that were fish groups fed diet enriched with probiotics.

As shown in Table 5 Hb, Ht, RBCs and WBCs were not significantly ( $P > 0.05$ ) affected by the graded levels of yeast

extract used in the study. Fish fed the diet supplemented with synbiotic ( $0.48 \times 10^6$  CFU  $g^{-1}$  and 0.5% yeast extract) showed the highest values of Hb, RBCs and WBCs. Marzouk et al. (2008) reported that both fish groups fed the diet supplemented with dead *Saccharomyces cerevisiae* yeast and both of live *B. subtilis* and *S. cerevisiae* showed significant ( $P < 0.05$ ) increase in the Ht level when compared to fish fed the control diet. Also, Firouzbakhsh et al. (2012) reported that, Hb concentration, in rainbow trout (*Oncorhynchus mykiss*) fed different levels of synbiotic was significantly ( $P < 0.05$ ) different from the control.

#### Metabolism enzymes

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes are important liver enzymes. They are indicators for liver health and function through controlling the transferring amino group function of alpha-amino acids to alpha-keto acids. Large amounts of ALT and AST are released into animal blood, mostly during liver cell damage (Kumar et al., 2011).

As shown in Table 6, ALT and AST values decreased with increasing *B. licheniformis* level. Soltan and El-Laithy (2008) found that, ALT and AST levels significantly decreased when Nile tilapia fed diets supplemented with probiotics were compared to the control group. Similarly, Wacheć et al. (2006) observed a decrease in the activity of AST, ALT and lactate dehydrogenase in *O. niloticus* after being fed with diet containing *Pseudomonas* spp. and a mixture of *Micrococcus luteus* and

**Table 6** Biochemical blood parameters of Nile tilapia (*Oreochromis niloticus*) fed the experimental diets with different levels of *Bacillus licheniformis* and yeas extract levels.

Diet No ( <i>B. Licheniformis</i> / yeast extract)	<i>B. Licheniformis</i> CFU $g^{-1}$	Yeast extract (%)	Biochemical blood parameters			
			ALT( $\mu$ /l) <sup>a</sup>	AST( $\mu$ /l) <sup>b</sup>	TP(g/dl) <sup>c</sup>	AL(g/dl) <sup>d</sup>
Diet 1 (0/0)	0	0	89.50a	17.85a	2.95d	1.250d
Diet 2 (0/0)	0	0.5	86.50b	17.25ab	3.45c	1.450c
Diet 3 (0/0)	0	1	86.00bc	17.06b	3.65abc	1.45c
Diet 4 ( $0.24 \times 10^6/0$ )	$0.24 \times 10^6$	0	85.50bc	16.25cd	3.75ab	1.55bc
Diet 5 ( $0.24 \times 10^6/0.5$ )	$0.24 \times 10^6$	0.5	83.50e	16.05cd	3.70abc	1.63ab
Diet 6 ( $0.24 \times 10^6/1$ )	$0.24 \times 10^6$	1	84.50 d	16.15cd	3.80a	1.74a
Diet 7 ( $0.48 \times 10^6/0$ )	$0.48 \times 10^6$	0	86.50b	16.55bc	3.45c	1.45c
Diet 8 ( $0.48 \times 10^6/0.5$ )	$0.48 \times 10^6$	0.5	82.50f	15.00e	3.80a	1.75a
Diet 9 ( $0.48 \times 10^6/1$ )	$0.48 \times 10^6$	1	84.50d	16.06cd	3.60abc	1.25d
Diet 10 ( $0.96 \times 10^6/0$ )	$0.96 \times 10^6$	0	85.50c	15.90cd	3.50bc	1.25d
Diet 11 ( $0.96 \times 10^6/0.5$ )	$0.96 \times 10^6$	0.5	84.50d	15.65de	3.55abc	1.11d
Diet 12 ( $0.96 \times 10^6/1$ )	$0.96 \times 10^6$	1	84.00de	15.85cd	3.55abc	1.160d
<i>One-way ANOVA</i>						
Polled S.E.M*			0.19	0.24	0.081	0.047
<i>F-value</i>			153.27	28.29	12.80	52.58
<i>F-value</i>			184.80	7.32	10.37	5.99
<i>Tow-way ANOVA</i>						
<i>P-value (B. Licheniformis)</i>			0.0001	0.0001	0.0007	0.6830
<i>P-value (Yeast extract)</i>			0.0001	0.0095	0.0029	0.2121
<i>P-value (interaction)</i>			0.0001	0.1095	0.0115	0.5245

Data are means of triplicate. Means in the same column sharing a same superscript letter are not significantly different ( $P > 0.05$ ).

\* Polled S.E.M standard error of mean.

<sup>a</sup> ALT: alanine aminotransferase.

<sup>b</sup> AST: aspartate aminotransferase.

<sup>c</sup> TP: total protein.

<sup>d</sup> AL: albumin.



*Pseudomonas* spp. Similar results were also observed in *Cyprinus carpio* fed the extract of Cyanobacteria (Palikova et al., 2004).

Control fish group showed the highest ( $P < 0.05$ ) ALT value, while fish fed 0.5% yeast extract achieved the lowest ( $P < 0.05$ ) ALT and AST values indicating the positive effects of probiotic and yeast extract in enhancing and protecting liver cells.

Fish fed diet D8 (supplemented with synbiotic  $0.96 \times 10^6$  and 0.5% yeast extract) recorded the lowest ( $P < 0.05$ ) ALT value (82.50  $\mu\text{L}$ ), while the control group showed the highest ( $P < 0.05$ ) ALT values, being 89.50  $\mu\text{L}$  respectively. Recently, Jarolowicz et al. (2012) reported that juvenile pikeperch, *Sander lucioperca* that received yeast extract in their diets exhibited a significantly lower AST and ALT activity in comparison to the control group ( $P < 0.05$ ).

Results of the present study also showed that, all levels of synbiotics significantly decreased the serum levels of ALT and AST. Marzouk et al. (2008) found that, fish groups fed on diets supplemented with dead *S. cerevisiae* yeast and both of live *B. subtilis* + *S. cerevisiae* revealed a significant ( $P < 0.05$ ) decrease in ALT and AST when compared to the control group that fed on probiotic-free diet.

#### Total protein and albumin

Table 6 showed that total protein content significantly increased with the first level of probiotic ( $0.24 \times 10^6$ ) then decreased with increasing probiotic level. The same trend was also observed for albumin. Increasing yeast extract levels 0.5 and 1.0% increased total protein (TP) and albumin (AL) and the differences between values are significant ( $P < 0.05$ ). As described in Table 6, TP and ALT recorded the highest values for fish that were fed the diet supplemented with synbiotic ( $0.48 \times 10^6$  and 0.5% yeast extract) than those that were fed other diets. Mehrabi et al. (2011) reported that diet supplemented with synbiotic (Biomim IMBO) increased the serum protein, albumin and globulin level of rainbow trout.

#### Conclusions

The results of the present study clearly indicated that the supplementation of *B. licheniformis* ( $0.48 \times 10^6$  CFU  $\text{g}^{-1}$ ) not only enhanced the growth performance and feed utilization of Nile tilapia, but also hematological and biochemical blood parameters. Moreover, the supplementation of yeast extract had significant beneficial effects and there were significant interactions between dietary *B. licheniformis* and yeast extract.

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