Influence of probiotic on growth, feed utilization, hematological and biochemical indices of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Twelve practical diets were formulated to contain four levels of *Bacillus licheniformis* (0.0, 0.24×10^6, 0.48×10^6 and 0.96×10^6 CFU g^{-1}), respectively, with three yeast extract levels (0, 0.5% and 1%). Each diet was randomly assigned to duplicate groups of 50 Nile tilapia (*Oreochromis niloticus*) (5.99±0.03 g) 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, 0.48×10^6 CFU/g^{-1} 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 0.48×10^6 CFU g^{-1}. Increasing levels of yeast extract had no effect on hematological parameters and the diets supplemented with 0.48×10^6 CFU g^{-1} and 0.5% yeast extract showed the highest hematological values.

Key words: Nile tilapia, probiotics, prebiotics, synbiotic

Introduction

Probiotcs 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 increase the host response towards disease, or by improving the quality of its 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 probiotics 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 the 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 as 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. Probiotics 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 resonse (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 acids production, an enhanced binding of these fatty acids to G-coupled protein receptors on leycocytes, an interaction with carbohydrates 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 improve 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 and Ringo 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 (*B. Licheniformis*) and the prebiotic (yeast extract) and their synbiotic interaction on growth performance, chemical composition, hematological and biochemical blood parameters of the Nile tilapia (*O. 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 synbiotics interactions on growth performance, feed utilization, proximate chemical analysis of whole fish body, hematological and biochemical blood parameters of the Nile tilapia (*Oreochromis niloticus*).

Nile tilapia, were obtained from Abbassa hatchery, Abou-Hammad, Sharkia Governorate, Egypt and were acclimated for two weeks at El-Kanater El-Khayria Fish Research Station, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt. During this period, fish were fed 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^3 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 ration 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^{-1}: total ammonia, 0.18±0.12 mg L^{-1}: total alkalinity, 173±42mg L^{-1}: chlorides, 570±151mg L^{-1} and pH 8.52±0.3. Water quality criteria were suitable within the acceptable limits for rearing the Nile tilapia *O. niloticus* fingerlings (El-Greirsy and El-Gamal, 2012).

Preparation inoculum of probiotics (*Bacillus licheniformis*)

B. licheniformis culture was prepared by adding 15 g of drid form (Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams Univ., Egypt) to 100 ml of prepared medium containing (gl^{-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 (2000 g 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 0.24×10^6, 0.48×10^6 and 0.96×10^6 CFU 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 19.41KJ/Kg diet[1] gross energy (Table 1). *B. licheniformis* was supplemented separately to the basal diet to obtain 0.0, 0.24×10^6, 0.48×10^6 and 0.96×10^6 CFU 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 µm mesh. All ingredients were thoroughly mixed with soybean oil and then passed the mixed feed through a laboratory pellet mill (2-mm die) 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) = $\frac{InW2 - InW1}{t} x\ 100$ Where: In = 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) ×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), haemoglobin (Hb), red blood cells (RBCs) and white blood cells (WBCs). Ht was determined as described by Reitman and Frankel (1957), haemoglobin (Hb) was determined by the haemoglobin kit which is a standardized procedure of the cyanomet haemoglobin 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-hemolysed serum was collected and stored at -20°C until use. Levels of serum aspartate aminotransferase (AST), 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 the treatments. When overall differences were found, they were tested by Duncan's multiple rang 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

1. 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 0.48×10^6 CFU g^{-1} diet. The diet supplemented with 0.48×10^6 CFU g^{-1} showed the highest significantly ($P<0.05$) BW, WG and SGR when compared to other fish groups. Such increase in the growth in aquatic animals that were fed probiotics 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_{12} (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 *B. 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). Al-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 (0.48×10^6 CUF g^{-1} *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 (Biomin 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 in WG. Also, Ai et al. (2011) indicated that at each dietary FOS level, supplemented by $1.35x10^7$ CFU g^{-1} *B. subtilis* significantly increased SGR and feed efficiency ratio (FER) when compared to the control group for juvenile large yellow croaker, *Larimichthys crocea*.

2. Feed intake and feed utilization

Results of Table 3 indicated that, increasing *B. licheniformis* level in *O. niloticus* diets were followed by a significant increase in feed intake (FI) and a significant improvement of FCR, PER and PPV up to the diet supplemented by 0.48×10^6. 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 and 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 (*Morone chrysops×M. saxatilis*) fed commercial diet supplemented with prebiotic Grobiotic®-AE, with 10-20 g kg^{-1} obtained a significantly improved feed efficiency.

Referring to the dietary symbiotic interaction of the experimental diets with, 0.48×10^6 CFU g^{-1} and 1.0% the yeast extract (D9) showed the highest FI, the best FCR, PER and PPV compared to other snybiotic treatments and the control group.These results were parallel to that 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; Wacheç 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 and 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 *Bacillus. Subtilis* 0.96×10^6 CFU g^{-1} significantly improved FCR and PER values when compared to fish group fed the control diet.

3. 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 control group with no effect on the ash content. Bagheri et al. (2008) reported that application of 3.8×10^9 CFU g^{-1} 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 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, the higher body protein content in the rainbow trout (*Oncorhynchus mykiss*) fingerlings implies on this fact that by the application of 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 did not have any significant effect on the lipid content.

4. Hematological indices

Haemoglobin (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 0.48×10^6 CFU g^{-1} *B. licheniformis* then decreased as the diet was supplemented with 0.96×10^6 CFU g^{-1} *B. licheniformis*.

Hematology is an important factor that could be considered for the fish diet quality assessment. Ologhobo, (1992) reported that one of 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 haematological 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 content among the control and fish that were fish groups fed diet enriched with probiotics

As shown in Table (5) Hb, Ht, RBCs and WBCs was not significantly ($P>0.05$) affected by the graded levels of yeast extract used in the study. Fish fed the diet supplemented with synbiotic (0.48×10^6 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 cerevisae* yeast and both of live *Bacillus subtilis* and *S. cerevisae* 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 were significantly ($P<0.05$) different from the control.

5.1. Metabolism enzyems

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

As shown in Table 7, ALT and AST values decreased with increasing the *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 control group. Similarly, Wache¢ 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 *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% g 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 0.96×10^6 and 0.5% yeast extract) recorded the lowest ($P<0.05$) ALT value (82.50 u/L), while the control group showed the highest ($P<0.05$) ALT values, being 89.50 u/L. 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 *Saccharomyces cerevisae* yeast and both of live *Bacillus subtilis* + *S. cerevisae* revealed a significant ($P<0.05$) decrease in ALT and AST when compared to the control group that fed on probiotic-free diet.

5. 2. Total protein and albumin

Table 6 showed that total protein content significantly increased with the first level of probiotic (0.24×10^6) then decreased with increasing the 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 (0.48×10^6 and 0.5% yeast extract) than those that were fed other diets. Meharbi et al. (2012) reported that diet supplemented with synbiotic (Biomin 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* (0.48×10^6 CFU 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.

References

Abd El-Rhman, A. M., Khattab, Y. A. and Shalaby, A. M. (2009): *Micrococcus luteus* and *Pseudomonas species* as probiotics for promoting the growth performance and health of Nile tilapia (*Oreochromis niloticus)*. Fish & Shellfish Immunol., 27:175-180.

Ai, Q., Xu, H., Mai K, Xu W., and Wang, J. (2011): Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture, 317:155-161.

Al-Dohail, M. A., Hashim, R. and Aliyu-Paiko, M. (2009): Anaerobic bacteria isolated from Oscars (*Astronotus ocellatus*), angel animals: a review. Crit. Rev. Food Sci. Nutr., 43:19-60.

AOAC, 1995. Association of Official Analytical Chemists, Official methods of analysis. 16th edition, AOAC, Arlington, VA. 1832pp.

APHA, 1992. Standard Methods for the Examination of Water and Waste Waters, 18th ed. American Public Health Association, Washington, DC, 1268 pp.

Bagheri, T., Hedayati, S. A., Yavari, V., Alizade, M. and Farzanfar, A. (2008): Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turk. J. Fish. Aquat. Sci., 8:43-48.

Bairagi, A., Sarkar Ghosh, K., Sen, S. K. and Ray, A. K. (2004): Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. Aquac. Res., 35:436-446.

Boyd, C. E. (1990): Water quality in ponds for aquaculture. Birmingham, AL: Birmingham Publishing

Brett, J. R. (1973): Energy expenditure of Sockeye salmon *Oncorhynchus nerka*, during sustained performance. Journal of the Fisheries Research Board of Canada, 30:1799-1809.

Carver, J. D. (1994): Dietary nucleotides: cellular immune, intestinal and hepatic system effects. J. Nutr., 124:144-148.

Cerezuela, C., Meseguer, J. and Esteban, A. (2011): Current knowledge in synbiotic use for fish aquaculture: A review. Aquac. Res Development S1:008. doi:10.4172/2155.9546.S1-008.

Dimitroglou, A., Merrifield, D. L., Carnevali, O., Pichietti, S. and Avella, M. (2011): Microbial manipulations to improve fish health and production–A Mediterranean perspective. Fish Shellfish Immunol., 30:1-16.

Ding, X., Li, Z. Q., Chen,Y. Q., Lin, H. Z., Yang, Y. Y. and Yang, K. (2004): Effects of probiotics on growth and activities of digestive enzymes of *Pennaus vannamei*. J. Fish. Sci., China, 1(6):580-584.

Duncan, M.B. (1955): Multiple ranges and multiple F-tests. Biometrics,11:1-42.

El-Greirsy, Z.A., El-Gamal, A.E., 2012. Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17a-methyltestosterone with respect to the degree of sex stability after one year of treatment. Egypt. J. Aquat. Res. 38, 59–66.

Ferguson, R. M., Merrifield, D. L., Harper, G. M., Rawling, M. D., Mustafa, S., Picchietti, S., Balcázar, J. L. and Davies, S. J . (2010): The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing Nile tilapia (*Oreochromis niloticus*). J. Appl. Microbiol., 109:851-862.

Firouzbakhsh, F., Mehrabi, Z., Heydari, M., Khalesi, M. K. and Tajick, M. A. (2012): Protective effects of a synbiotic against experimental *Saprolegnia parasitica* infection in rainbow trout (*Oncorhynchus mykiss*) Aquaculture Research, doi:10.1111/j.1365-2109.2012.03261.x

Gatesoupe, F. J. 1999: The use of probiotics in aquaculture. Aquaculture, 180:147-165.

Gibson, G. R., Saavendra, J. M., MacFarlane, S. and MacFarlane, G. T. (1997): Probiotics and intestinal infections. In: Probiotics 2, Application and Practical Aspects (ed. by R. Fuller), pp.10-32, Chapman & Hall, London.

Gibson, G. R., Rastall, R. A. and Fuller, R. (2003): The health benefits of probiotics and prebiotics. In Fuller R, Perdigigon G. (Eds), gut Flora, Nutrition, immunity and Health. Blackwell Publishing Ltd., Oxford, UK, pp, 55-76.

Gibson, G. R., Probert H. M. Loo, V., Rastall, R. A. and Roberfroid, M. B. (2004): Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. Nutr. Res. Rev., 17:259-275.

Hassaan, M.S., Soltan, M.A., Agouz, H.M., Badr, A.M. (2013): Influences of calcium/phosphorus ratio on supplemental microbial phytase efficiency for Nile tilapia (*Oreochromis niloticus*) Egyptian J. of Aquatic Research, 39, 205–213.

Hoshino, T., Ishizaki, K., Sakamoto, T., Kumeta H., Yumoto I., Matsuyama, H. and Ohgiya, S. (1997): Isolation of a *Pseudomonas* species from fish intestine that produces a protease active at low temperature. Letters in Applied Microbiology, 25:70-72.

Jarolowicz, S., Zakes, Z., Siwicki, Z., Kowalska, A., Hopoko, M, Glabski, E., Demsk- Zakes, K. and Partyka, P. (2012): Effect of brewer yeast extract on growth performance and health of juvenile pikeperch, *Sander Lucioperca* (L.) Aquacuture Nutrition, doi: 10.1111/j.1365-2095.2011.00915.x

Khattab, Y. A. E, Shalaby, A. M. E., Sharaf Saffa, M., El-Marakby, H. and RizlAlla, E. H. (2004): The physiological changes and growth performance of the Nile Tilapia *Oreochromis niloticus* after feeding with Biogen® as growth promoter. Egypt. J. Aquat. Biol. And Fish., 8(2): 145-158.

Kumar, V., Makkar, H. P. S. and Becker, K. (2011): Nutritional, physiological haematological responses in rainbow trout (*Oncorhynchus mykiss*) juvenile fed detoxified *jatropha curcas* kernel meal. Aquaculture Nutrition, 17:415-467.

Li, J. Q., Tan, B. P. and Mai, K. S. (2009): Dietary probiotic *Bacillus spp*. and isomalto-oligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (*Litopenaeus vannamei*). Aquaculture 291: 35–40.

Mahious, A. S. and Ollevier, F. (2005): Probiotics and prebiotics in Aquaculture. 1st regional Workshop on Techniques for Enrichment of Live Food for use in Larviculture, 2005, AAARC, Urmia, Iran. P. 67.

Martins, M. L., Pilarsky, F., Onaka, E. M., Nomura, D. T., Fenerick, J., Ribeiro, K., Myiazaki, D. M. Y., Castro, M .P. and Malheiros, E. B. (2004): Hematology and inflammatory response in *Oreochromis niloticus* subjected to single stimuli and consecutive stress of capture. Bulletin of the Institute of Fisheries, 30:71-80.

Marzouk, M. S. Moustafa, M. M. and Mohamed, N. M. (2008): The Influence of some probiotics on the growth performance and intestinal microbial flora of *Oreochromis niloticus*. Proceedings of 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, pp. 1059-1071.

Mehrabi, Z., Firouzbakhsh, F. and Jafarpour, A. (2011): Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. Journal of Animal Physiology and Animal Nutrition, 96: 474-481.

Ologhobo, A. D. (1992): Nutritive values of some tropical (West African) legumes for poultry. Journal Applied Animal Research, 2:93-104.

Palikova, M., Navratil, S., Krejcf, R., Sterba, F., Tichy, F. andKubala, L. (2004): Outcomes of repeated exposure of carp (*Cyprinus carpio* L) to Cyanobacteria extract. ACTA Veterinaria Brno., 73:259-265.

Ringø, E., Olsen, R. E., Gifstad, T. Ø., Dalmo, R.A., Amlund, H., Hemre, G.-I. and Bakke, A. M., (2010): Prebiotics in aquaculture: a review. Aquacult. Nutr., 16:117-136.

Reitman, S., and Frankel, S. (1957): Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. Journal of Clinical Pathology, 28:56-59.

Rodriguez-Estrada U., Satoh S., Haga Y., Fushimi H. and Sweetman J. (2009). Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydrobutyric acid on growth performance and immune response of rainbow trout *(Oncorhynchus mykiss)*. Aquaculture Science, 57: 609–617.

Rombout, J. H., Abelli, L. Picchietti, S., Scapigliati, G. Kiron, V. (2010): Teleost intestinal immunology. Fish Shellfish immunol., 31:616-626.

Sahu, M. K., Swamakumar, N. S., Sivakumar, K., Thangaradjou, T. and Kannan, L. (2008): Probiotics in aquaculture: importance and future perspectives. Ind J. Microbiol., 48:299-308.

SAS (1996): Statistical Analysis system, Procedure Guide version 6.12 Ed. SAS Institute Inc., Cary, NC, USA.

Seifert, S. and Watzl, B. (2007): Inulin and oligofructose: Review of Experimental data on immune modulation. J. Nutr., 137:2563-2567.

Shelby, R., Lim, C., Yildirm-Aksoy, M. and Delaney, M. (2006): Effects of probiotic diet supplements on disease resistance and immune response of young Nile Tilapia (*Oreochromis niloticus*). J Appl Aquac 18, 22–34.

Soltan, M. A. and El-Laithy, S. M. M. (2008): Effect of probiotics and some spices as feed additives on the performance and behaviour of Nile tilapia, *Oreochromis niloticus*. Egypt. J. Aquat. Biol. & Fish., 12(2):63-80.

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. (2000): Probiotic bacteria as biological control agents in aquaculture. Microbial Mol Biol Rev 64:655-671.

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

Wang, Y. B. (2007): Effect of probiotics on growth performance and digestive enzyme activity of the shrimp Penaeus vannamei. Aquaculture, 269:259-264.

Wang, Y. B. and Xu, Z. R. (2006): Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol., 127:283-292.

Wang, Y. B., Li, J. R. and Lin, J. (2008): Probiotics in aquaculture: challenges and outlook. Aquaculture, 281:1-4.

Wotton, I.D. and Freeman, H., (1982): Micro analysis in Medical Biochemistry. Churchill, New York, USA.

Ye, J. D., Wang, K., Li, F. D. and Sun, Y. Z. (2011): Single or combined effects of fructo-and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder *Paralichthys olivaceus*. Aquac. Nutr., 17:902-911.

Yousefian, M. and Amiri, M. S. (2009): A review of the use of prebiotic in aquaculture for fish and shrimp. Afr. J. Biotechnol., 8:7313-7318.

Zhang, Q., Ma, H.M., Mai, K.S., Zhang, W.B. Liufu, Z.Q. and Xu, W. (2010). Interaction of dietary *Bacillus subtilis* and fructooligosaccharide on the growth performance, non-sp Food and Agriculture Organization of the United Nations ecific immunity of sea cucumber (*Apostichopus japonicas)*. Fish & Shellfish Immunol., 29: 204–211.

Table 1: Formulation and chemical proximate analysis of the experimental diets

Ingredients %	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
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[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L. acidophulus[2]	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
Proximate analysis												
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
NFE[3]%	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^{-1})[4]	19.49	19.53	19.50	19.47	19.54	19.47	19.46	19.63	19.52	19.48	19.52	19.53

[1]Vitamin and mineral mix (mg or g / Kg diet): MnSO4, 40 mg; MgO, 10 mg; K2SO4, 40 mg; ZnCO3, 60 mg; KI, 0.4 mg; CuSO4, 12 mg; Ferric citrate, 250 mg; Na2SeO3, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU; α-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.

[2]*Lactobacillus acidophilus* was prepared to obtain (1.0×10^{10} CFU g^{-1} approximately.

[3]NFE (Nitrogen free extract)=100-(crude protein + lipid + ash).

[4]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: Effect of probiotic, prebiotic levels and synbiotics on growth performance of *O. niloticus*.

Items	Body weight (g)		Body length (cm)		WG (g/fish)	SGR (%)
	Initial BW	Final BW	Initial BL	Final BL		
Effect of probiotic (CFU/g)						
0.00 (Pro1)	5.97	38.37 d	4.65	12.98 c	32.40 c	2.06 d
0.42×10^7 (Pro2)	6.03	41.35 c	5.05	13.43 b	35.32 b	2.14 c
0.84×10^7 (Pro3)	5.99	43.74 a	4.90	13.88 a	37.75 a	2.21 a
1.35×10^7 (Pro 4)	5.99	42.04 b	5.05	13.18 a	36.05 b	2.17 b
Polled SE	*0.02*	*0.02*	*0.10*	*0.11*	0.03	0.01
Effect of prebiotic (g/Kg diet)						
0 (pre 1)	6.00	39.21 c	5.06	13.25	33.21 c	2.08 c
5 (pre 2	5.99	41.93 b	4.88	13.87	35.94 b	2.16 b
10 (pre 3)	5.99	43.00 a	4.80	13.94	37.00 a	2.19 a
Polled SE	*0.01*	*0.02*	*0.09*	*0.09*	*0.03*	*0.01*
Interactions (probiotic × prebiotic (synbiotic)						
D1 (Pro1×pre 1)	5.96	34.55 k	5.00	12.30 c	28.59 j	1.95 g
D2 (Pro1×pre 2)	5.98	40.13 j	4.45	13.40 b	34.15 i	2.12 f
D3 (Pro1×pre 3)	5.98	40.44 i	4.50	13.25 b	34.46 h	2.12 ef
D4 (Pro2×pre 1)	6.05	40.91 h	5.15	13.45 b	34.86 g	2.12 ef
D5 (Pro2×pre 2)	6.02	41.15 g	5.10	13.45 b	35.13 f	2.14 de
D6 (Pro2×pre 3)	6.01	42.00 e	4.90	13.40 b	35.99 e	2.16 cd
D7 (Pro3×pre 1)	5.98	41.26 f	4.95	13.05 b	35.28 f	2.15 d
D8 (Pro3×pre 2)	5.97	44.12 b	4.75	14.45 a	38.15 b	2.22 b
D9 (Pro 3×pre 3)	6.02	45.84 a	5.00	14.15 a	39.82 a	2.26 a
D10 (Pro4×pre 1)	6.01	40.11 j	5.15	14.20 a	34.10 i	2.11 f
D11 (Pro4×pre 2)	5.99	42.30 d	5.20	14.20 a	36.31 d	2.18 c
D12 (Pro4×pre 3)	5.98	43.72 c	4.80	14.15 a	37.74 c	2.22 b
Pooled SE	*0.03*	*0.02*	*0.17*	*0.18*	*0.05*	*0.01*

Table 3: Effect of probiotic, prebiotic and synbiotic levels on feed intake and feed conversion ratio of *O. niloticus.*

Items	FI (g/fish)	FCR	PER	PPV
Effect of probiotic ($CFUg^{-1}$)				
0.00 (Pro1)	54.31 b	1.69 a	2.02 d	25.72 c
0.42×10^7 (Pro2)	57.09 a	1.62 b	2.10 c	27.00 b
0.84×10^7 (Pro3)	56.44 a	1.50 d	2.27 a	29.17 a
1.35×10^7 (Pro 4)	56.40 a	1.57 c	2.17 b	28.72 b
Polled SE	*0.32*	*0.01*	*0.01*	*0.26*
Effect of prebiotic (g/Kg diet)				
0 (pre 1)	55.19 b	1.67 a	2.04 b	26.15 c
5 (pre 2	55.94 b	1.56 b	2.18 a	28.04 b
10 (pre 3)	57.04 a	1.55 b	2.20 a	28.76 a
Polled SE	*0.28*	*0.01*	*0.01*	*0.22*
Interactions (probiotic × prebiotic (synbiotic)				
D1 (Pro1×pre 1)	52.95 c	1.85 a	1.83 e	23.51 f
D2 (Pro1×pre 2)	54.64 b	1.60 bc	2.12 cd	26.38 d
D3 (Pro1×pre 3)	55.34 b	1.61 bc	2.12 cd	27.27 c
D4 (Pro2×pre 1)	56.45 a	1.62 b	2.10 cd	25.66 e
D5 (Pro2×pre 2)	56.79 a	1.62 b	2.10 cd	28.49 b
D6 (Pro2×pre 3)	58.04 a	1.61 b	2.10 cd	26.87 d
D7 (Pro3×pre 1)	56.81 a	1.61 b	2.11 cd	26.60 d
D8 (Pro3×pre 2)	55.75 b	1.47 e	2.32 a	29.34 b
D9 (Pro 3×pre 3)	56.75 a	1.43 e	2.38 a	31.57 a
D10 (Pro4×pre 1)	54.57 b	1.60b c	2.12 cd	28.85 b
D11 (Pro4×pre 2)	56.60 a	1.56c d	2.19 b	27.97 c
D12 (Pro4×pre 3)	58.05a	1.54 d	2.21 b	29.34 b
Pooled SE	*0.55*	*0.014*	*0.02*	*0.44*

+ Means with the same letter in each column are not significantly different.

Table 4: Effect of probiotic, prebiotic and synbiotic levels on proximate chemical analysis of *O. niloticus*.

Items	Dry matter	Lipid	protein	Ash
Effect of probiotic ($CFUg^{-1}$)				
0.00 (Pro1)	24.13 b	14.60 b	53.25 b	14.52
0.42×10^7 (Pro2)	24.83 a	15.18 a	53.42 a	14.60
0.84×10^7 (Pro3)	24.89 a	15.62 a	53.77 a	14.53
1.35×10^7 (Pro 4)	24.95 a	15.60 a	53.97 a	14.47
Polled SE	*0.04*	*0.08*	*0.15*	*0.09*
Effect of prebiotic (g/Kg diet)				
0 (pre 1)	24.54	15.25	53.31	14.55
5 (pre 2	24.71	15.20	53.79	14.43
10 (pre 3)	24.84	15.30	53.70	14.61
Polled SE	*0.107*	*0.073*	*0.13*	*0.076*
Interactions (probiotic×prebiotic (synbiotic)				
D1 (Pro1×pre 1)	24.16 c	14.40f	53.10 b	14.30 b
D2 (Pro1×pre 2)	23.88 d	14.80 c	53.30 a	14.50 ab
D3 (Pro1×pre 3)	24.36 c	14.60 c	53.35 a	14.75 a
D4 (Pro2×pre 1)	23.70 e	15.05 c	53.10 b	14.75 a
D5 (Pro2×pre 2)	26.20 a	15.25 b	53.50 a	14.50 ab
D6 (Pro2×pre 3)	24.58 c	15.25 b	53.65 a	14.55 ab
D7 (Pro3×pre 1)	24.65 c	15.80 a	53.55 a	14.35 ab
D8 (Pro3×pre 2)	24.46 c	15.20 b	54.10 a	14.55 ab
D9 (Pro 3×pre 3)	25.55 ab	15.85 a	53.65 a	14.70 a
D10 (Pro4×pre 1)	25.67 a	15.75 a	53.50 a	14.80 a
D11 (Pro4×pre 2)	24.29 c	15.55 a	54.25 b	14.15 b
D12 (Pro4×pre 3)	24.89 b	15.50 a	54.15 b	14.45 ab
Pooled SE	*0.21*	*0.15*	*0.26*	*0.15*

Table 5: Effect of probiotic, prebiotic and synbiotic on hemoglobin, hematocrit, blood cells of *O. niloticus*.

Items	Hb (g/dl)	Ht (%)	RBC (10^6)/cmm	WBC(10^3)
Effect of probiotic ($CFUg^{-1}$)				
0.00 (Pro1)	10.51 c	14.62 c	1.82 d	36.83 c
0.42×10^7 (Pro2)	10.78 b	14.77 c	1.85 c	37.21 b
0.84×10^7 (Pro3)	11.10 a	15.33 b	1.92 a	37.90 a
1.35×10^7 (Pro 4)	10.99 a	16.75 a	1.88 b	37.16 b
Polled SE	*0.04*	*0.06*	*0.01*	*0.08*
Effect of prebiotic (g/Kg diet)				
0 (pre 1)	10.65	15.55	1.86	36.94
5 (pre 2	10.92	15.06	1.87	37.36
10 (pre 3)	10.97	15.49	1.88	37.51
Polled SE	*0.04*	*0.06*	*0.01*	*0.07*
Interactions (probiotic×prebiotic (synbiotic)				
D1 (Pro1×pre 1)	10.21 b	15.05 c	1.80 d	36.29 c
D2 (Pro1×pre 2)	10.51 b	14.10 d	1.83 c	37.05 b
D3 (Pro1×pre 3)	10.83 ab	14.70 d	1.85 c	37.15 b
D4 (Pro2×pre 1)	10.49 b	15.25 c	1.87 c	36.90 c
D5 (Pro2×pre 2)	10.97 a	14.35 d	1.85 c	37.12 b
D6 (Pro2×pre 3)	10.88 a	14.70 d	1.85 c	37.60 b
D7 (Pro3×pre 1)	10.95 a	15.15 c	1.88 c	37.20 b
D8 (Pro3×pre 2)	11.28 a	14.70 d	1.97 a	38.36 a
D9 (Pro 3×pre 3)	11.07 a	16.15 b	1.91 b	38.15 a
D10 (Pro4×pre 1)	10.97 a	16.75 b	1.90 b	37.38 b
D11 (Pro4×pre 2)	10.91 a	17.10 a	1.84 c	36.94 c
D12 (Pro4×pre 3)	11.09 a	16.41 b	1.92 b	37.16 b
Pooled SE	*0.01*	*0.11*	*0.01*	*0.13*

Table 6: Effect of probiotic, prebiotic and synbiotic levels on ALT and AST of *O. niloticus*.

Items	ALT (µ/L)	AST (µ/L)	Total protein g/dl	Albumin g/dl
Effect of probiotic (CFU/g)				
0.00 (Pro1)	87.33 a	17.39 a	3.35 c	1.38 c
0.42×10^7 (Pro2)	84.50 b	16.15 b	3.75 a	1.64 a
0.84×10^7 (Pro3)	84.50 b	15.87 b	3.62 b	1.48 b
1.35×10^7 (Pro 4)	84.67 b	15.80 b	3.53 b	1.47 b
Polled SE	*0.11*	*0.14*	*0.047*	*0.027*
Effect of prebiotic (g/Kg diet)				
0 (pre 1)	86.75 a	16.64 a	3.41b	1.38b
5 (pre 2	84.25 c	15.99 b	3.63a	1.48a
10 (pre 3)	84.75 b	16.28 ab	3.65a	1.40b
Polled SE	*0.097*	*0.12*	*0.041*	*0.023*
Interactions (probiotic×prebiotic (synbiotic)				
D1 (Pro1×pre 1)	89.50 a	17.85 a	2.95 b	1.25 d
D2 (Pro1×pre 2)	86.50 b	17.25 ab	3.45 ab	1.45 b
D3 (Pro1 ×pre 3)	86.00 b	17.06 b	3.65 a	1.45 b
D4 (Pro2×pre 1)	85.50 b	16.25 c	3.75 a	1.55 b
D5 (Pro2×pre 2)	83.50 e	16.05 c	3.70 a	1.63 ab
D6 (Pro2×pre 3)	84.50 c	16.15 c	3.80 a	1.74 a
D7 (Pro3×pre 1)	86.50 b	16.55 c	3.45 ab	1.45 c
D8 (Pro3×pre 2)	82.50 f	15.00 d	3.80 a	1.75 a
D9 (Pro 3×pre 3)	84.50 d	16.06 c	3.60 a	1.25 d
D10 (Pro4×pre 1)	85.50 c	15.90 c	3.50 ab	1.25 d
D11 (Pro4×pre 2)	84.50 d	15.65 c	3.55 ab	1.11 d
D12 (Pro4×pre 3)	84.00 d	15.85 c	3.55 ab	1.16 d
Pooled SE	*0.19*	*0.24*	*0.08*	*0.05*

+ Means with the same letter in each column are not significantly different.