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ENHANCEMENT OF GROWTH, IMMUNITY AND DISEASE RESISTANT TO *YERSINIA RUCKERI* IN *OREOCHROMIS NILOTICUS* FINGERLINGS BY ORAL ADMINISTRATION OF PREBIOTIC (IMMUNOWALL®).

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Abstract

A study was conducted to investigate the effect of Immunowall® commercial prebiotic on *Oreochromis niloticus* fingerlings. Three hundred and sixty fingerlings (8.7 ± 0.4g) were divided into 3 groups (group 1, 2 and 3) of 120 fish each to measure immunity and growth parameters. First group (G1) act as control fed on basal diet. The second (G2) and third (G3) groups were fed on diet supplemented with 1.5 and 3 g kg^{-1} Immunowall® respectively. After 30 days, G3 showed significant improvement in growth and feed utilization parameters followed by G2 compared with G1. At the end of the feeding period (60 days), such parameters did not significantly differ between G2 and G3 except in final body weight. In G3, the heights of the intestinal villi, numbers of goblet cells and numbers of intra-epithelial lymphocytes (IEL) had significant increases compared with control group. The protein and fat contents of the whole body in G3 was higher than G2 and G1. G3 had better effects than G2 on serum total protein and globulin levels. After 15 days of immunological study, G3 was significantly increased in percentage of serum killing and phagocytic activity than G2 and G1. G2 and G3 were higher than control group in lysozyme activity. While no difference between groups in nitric oxide. After 30 days, G3 showed significant increases in percentage of serum killing, phagocytic activity and nitric oxide compared with G1. The relative percentage survival

of fish challenged by *Yersinia ruckeri* was showed enhancement by 60% in G3 and 15.38% in G2 than control.

Keywords: prebiotic, growth, immunity, *Yersinia ruckeri, Oreochromis niloticus.*

INTRODUCTION

The demand for animal protein in the human diet increases daily; therefore, the growth of aquaculture production as an important protein source for human consumption has also increased. Among cichlid fishes, the Nile Tilapia, *Oreochromis niloticus*, is a commercially important fish that is cultured in many tropical and subtropical countries for its excellent growth rate, tolerance to wide ranges of environmental conditions, resistance to various diseases, ease of rearing in captivity and utilization of low trophic levels (Lin *et al.*, 2008). The development of tilapia farming and farming intensification has led to the urgent requirements for a high-quality diet with complementary supplementation.

Several feed supplements have been used to improve growth performance, immunomodulation and disease resistance in various fish. Bacterial diseases are the major problems encountered aquaculture that was combated mainly by antibiotics (Bondad-Reantaso *et al.*, 2005). The uses of antibiotics pose threats such as antibiotic-resistant genes, immunosuppression, destabilization of gastrointestinal (GI) beneficent bacteria and accumulation in the musculature (Romero *et al.*, 2012). Thus, antibiotic uses are strictly regulated worldwide and the attention to the use of alternatives is increased in aquaculture. Prebiotic is a non-digestible food ingredient that beneficially affects the host through improving its intestinal balance (Rurangwa *et al.*, 2009). β-glucan and mannan oligosaccharides (MOS), the commonly used prebiotics for fish, are naturally occurring polysaccharides found in the cell walls of the yeast (*Saccharomyces cerevisiae*), other sources such as brewers and torula yeast, fungi and algae are currently in use (Sang *et al.*, 2010). Numerous studies demonstrated that prebiotic stimulate growth performance,

improve nutrient availability, modulate microbial colonization, improve gut development, modulate innate and acquired immunity responses, enhance the resistance to development of potential pathogen, minimize the side effects associated with vaccines and/ or drug therapy and absorb the mycotoxins found in the nutrients (Torrecillas *et al.*, 2007; Geraylou *et al.*, 2013 and Selim *et al.*, 2013).

Enteric red mouth (ERM) or Yersiniosis is an infectious acute/sub-acute disease of fish caused by gram-negative rod *Yersinia ruckeri*, produce sever mortalities (60-70%) and high economic losses (Frerichs and Roberts, 1989). *Yersinia ruckeri* is an opportunistic pathogen commonly inhabits small intestine, spleen and liver of fish without showing any clinical signs (Busch, 1978). Stress factors such as heat discomfort, oxygen depletion, overcrowding, pollution, bad handling or infectious diseases led to immunosuppression causing outbreak of the disease (Guguianu and Miron, 2002). The severity of the disease varied according to water temperature and age of fish (Busch, 1978). In acute form, controlling of the disease with antibiotics is ineffective; consequently, the dealing with ERM disease depends on use of vaccine and/or immunostimulators (Tebbit *et al.*, 1981).

Based on this, the present study was conducted to evaluate the effects of two-yeast polysaccharide, supplemented to *Oreochromis niloticus* in two levels, on growth performance, feed utilization, gut morphology, body composition, immune response and fish resistance to *Yersinia ruckeri* infection.

MATERIAL AND METHODS

Diet preparation.

The control basal diet without any treatment was containing approximately 39.9% crude protein and 10.89% crude lipid was administrated to control group (G1). Two experimental diets contained

1.5 and 3 g kg^{-1} Immunowall® (Pro vet care, animal health product, Brazil, which each kg contain mannan oligosaccharides "MOS" 180 g, β-glucan 300 g and yeast cell wall extracts 520 g) were administrated to group 2, 3(G2 and G3) respectively. Feeding was carried out three times daily at the rate of 5 % of fish body weight.

Fish.

Three hundred and sixty apparently healthy fingerlings of *Oreochromis niloticus* (*O. niloticus*) with an average body weight 8.7 ± 0.4 g were obtained from Abbassa Fish Hatchery, Sharkia Province. Fish were acclimatized to experimental conditions for 15 days in glass aquaria (each, 80x40x30 cm capacity) and feed on basal diet. About 25% of water was changed daily with periodical check to water parameters. *O. niloticus* fingerlings were divided into 3 groups (group 1, 2 and 3) of each 120 fish to measure immunity and growth parameters. Each group was subdivided into two subgroups (subgroup A and B). Each subgroup has 3 aquaria as replicate (20 fish $replicate^{-1}$). Subgroup A in each group served for growth performance study for 60 days after which samples were taken for body composition analysis and intestinal histology. Subgroup B in each group served for immunological study for 30 days where samples were taken at 15 and 30 days. Fish is intraprotenially challenged to examine fish resistance after 30 days.

Growth parameters.

The fish were weighed every two weeks to assess growth performance. The final body weight (FBW), weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) were determined according to De Silva and Anderson (1995) and Yanbo and Zirong (2006). The protein efficacy ratio (PER) was calculated according to Stuart and Hung (1989).

Intestinal histology.

After 8 weeks of feeding, three parts of the intestine, the proximal part (from after the pyloric part of the stomach to before the spiral part of the intestines), the middle part (the spiral part of the intestines) and distal part (from after spiral part of the intestines to 2 cm before anus) were fixed in Bouin's solution. Fixed tissues were processed according to standard histological techniques and tissue sections were stained with Hematoxylin and Eosin (H&E). The heights of intestinal villi in all parts were measured using Image J version 1.36 (National Institutes of Health). An average of 10 villi per section was expressed as the mean villous height for each section. Other intestinal sections were stained by Alcian blue stain. Goblet cells and intra epithelial lymphocytes (IEL) were counted in 10 intestine fold (Samanya and Yamauchi, 2002).

Chemical analysis of fish body composition.

Fifteen fish from each subgroup (A) (5 fish replicate^{-1}) were randomly selected at the end of feeding period; the fish were autoclaved, ground into homogeneous slurry, oven-dried, reground and stored at -20°C until analysis. Carcass samples were analyzed for dry matter (DM) and ash according to AOAC (1995), crude protein (%N x 6.25) by the Kjeldahl method using a Kjeltech auto analyzer (Model 1030, Tecator, Hgans, Sweden) and total lipid according to the method of Bligh and Dyer (1959).

Blood samples collection and nonspecific immune analysis.

Fifteen fish from each subgroup (B) (5 fish replicate^{-1}) were randomly selected on the day 15 and day 30 of the experiment. Blood samples were collected from the caudal vein of fish and were divided into two portions. One part was collected with heparin and used to measure the phagocytic activity; the white blood cells were separated from peripheral blood of the tested fish in the different experimental groups.

Heat-inactivated *Candida albicans* (*C. albicans*) was used to determine the phagocytic activity according to Kumari and Sahoo (2006).

The second portion of the blood sample was allowed to clot and centrifuged at 3000 r.p.m. for 15 min and non-hemolysed serum was collected and stored at -20°C until use. Levels of serum total protein and albumin were measured using spectrophotometry, "Total Protein" and "Albumin" kits (Spectrum, Egyptian Company for Biotechnology, Obour City, Cairo, Egypt). Globulin levels were determined by direct subtracting the values of the albumin from those of the total protein (Coles, 1974). Serum lysozyme, nitric oxide (NO) and serum bactericidal activity were determined (Ellis, 1990; Kajita *et al*., 1990 and Rajaraman *et al*. 1998).

Challenge test.

Fish in subgroup B in each group were intraprotenially (IP) injected after 30 days with 0.1ml of pathogenic *Yersinia ruckeri* (1.5×10^8 cells ml^{-1}) that had been previously isolated from moribund fish and confirmed to be pathogenic. The fish were observed for the presence of disease manifestion. Mortality of fish in each aquarium was observed over 14 days, and mortality was confirmed by reisolating the organism from the kidney and intestinal content of dead fish (Eissa *et al*. 2008). The average of mortality of the triplicate aquarium was used for calculating Relative Percentage Survival (RPS) following Amend (1981).

Statistical analysis.

The mean and standard error were calculated for each variable. The data were analyzed by analysis of variance (ANOVA) to identify the significantly different groups at ($P < 0.05$) using SPSS software statistical program (SPSS for windows ver.15.00, USA).

RESULTS AND DISCUSSION

Growth performance.

Supplementation with Immunowall® significantly improved growth parameters and nutrient utilization in comparison to control group. After

30 days, G3 was significantly higher final body weight (FBW), weight gains (WG) and specific growth rate (SGR) followed by G2. The lowest values were observed in the untreated control group (Fig. 1A). While feed conversion ratio (FCR) was significantly lower in G3 compared to G2 and control (Fig. 1B). After 60 days, G3 showed significant increases in FBW than G2 and G1 while, G2 and G3 were significantly higher than G1 in WG and SGR. The best FCR was obtained with G3 followed by G2 then control group (Fig. 1B). The protein efficacy ratio (PER) in G3 was significantly higher than G2 and G1 (Fig. 1C).

Effects of different prebiotics and their levels on fish growth rate, feed efficiency and digestibility have been investigated in a number of studies (Staykov *et al*., 2007; Salze *et al*., 2008 and Dimitroglou *et al*., 2010). The enhancement of fish growth performance mainly related to presence of MOS which increasing villi height and integrity, modulation intestinal microbiota and adsorption of pathogenic bacteria. As a result, increase digestive tract absorptive efficiency (Dimitroglou *et al*., 2009). Dimitroglou *et al*. (2009) recorded that supplementation of *Oncorhynchus mykiss* diet with MOS at 0.5 to 4.5g kg^{-1} has improve the growth performance. Ta'ati *et al*. (2011 and 2012) observed Immunoster and Immunowall significantly improved growth performance and feed efficiency of juvenile beluga (*Huso huso*). Torrecillas *et al*. (2007) showed that European sea bass fed MOS at two levels of 2 and 4 g kg^{-1} showed a significant increase in body weight and total length. The studies of Staykov *et al*. (2007) demonstrated that 0.2% MOS in rainbow trout diet significantly enhanced body weight and reduced the FCR. In contrary, Dimitroglou *et al*. (2010) reported that diet supplementation with *S. cerevisia* did not improve growth performance of *Sparus aurata* compared to a control diet. The difference could be related to extrinsic factors as temperature, other environmental condition and difference of fish species.

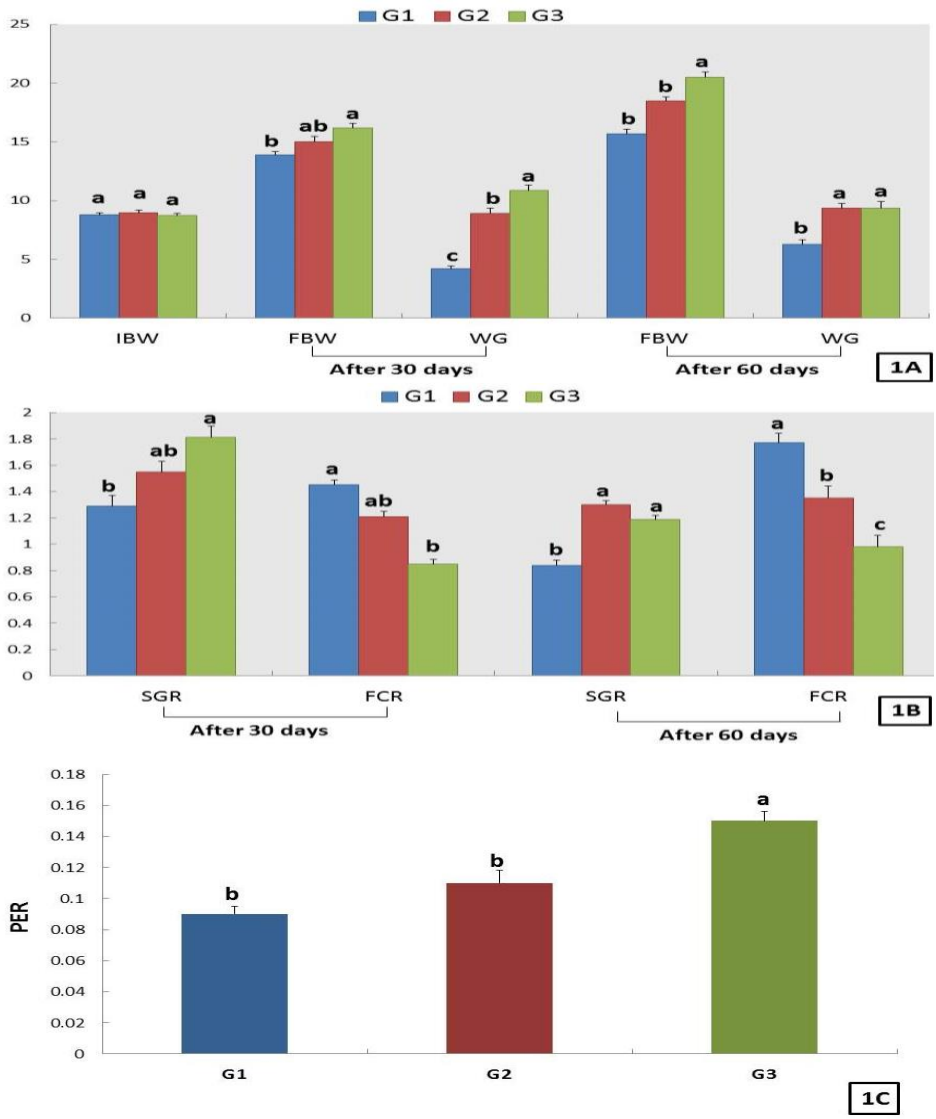

Figure 1. Growth performance of *Oreochromis niloticus* (mean ± SD) fed with two levels of the prebiotic, Immunowall®. (1A) Bars indicate the Initial body weight (IBW), Final body weight (FBW) after 30 and 60 days, Weight gain (WG) after 30 and 60 days. (1B) Bars indicate the specific growth rate (SGR) after 30 and 60 days, feed conversion ratio (FCR) after 30 and 60 days. (1C) Bars indicate the protein efficacy ratio (PER). Bars with different superscripts (a, b and c) are significantly different ($P<0.05$, using One-Way ANOVA).

Intestinal histology.

The light microscopal examination was showed significant increase of intestinal villi height in G3 from anterior till posterior regions of intestine with increase microvilli density and length followed by G2 than the control group (Fig. 2A). The prebiotic-fed fish had higher number of goblet cells than the control. The proximal and middle parts had significantly higher numbers of goblet cells in G3, followed by G2 (Fig. 2B). In the distal part, there were no significant differences between G3 and G2. The G3 showed significant increase in IEL in all parts of the intestine (Fig. 2C). No significant differences were observed in the numbers of IELs between G1 and G2 in the middle parts of intestine, while G2 resulted in significantly higher number of IEL in the proximal and distal parts compared with control. Salze *et al*. (2008) recorded that a 0.2% dietary MOS supplementation to cobia larvae, *Rachycentron canadum* increased intestinal microvilli length. Dimitroglou *et al*. (2010) also reported that MOS supplemented diet increased microvilli densities in both the anterior and posterior intestinal regions of gilthead sea bream (*Sparus aurata*). Conversely, MOS administration at 0.2 or 0.4% to *Dicentrarchus labrax* for 67 days did not affect villi length (Torrecillas *et al*., 2007). Olsen *et al*. (2001) found that Arctic charr *Salvelinus alpinus* L. fed a high concentration of inulin (15% of the diet) lead to destructive effects on microvillous in the hindgut. Little information is available describing the effect of prebiotic on the number of goblet and intraepithelial lymphocyte cells in *O. niloticus*. Zhu *et al*. (2012) demonstrated that 0.1 and 0.2 % yeast polysaccharides supplementation to *Ictalurus punctatus* diet increase intestinal goblet cell number. The intact, healthy mucosal epithelium with increase intestinal height, goblet and intraepithelial lymphocyte cells is improving digestion and absorption and enhancing intestinal defense to resist opportunistic indigenous bacterial infections which cleared the growth enhancement in prebiotic supplemented groups (Staykov *et al*., 2007 and Dimitroglou *et al*., 2009).

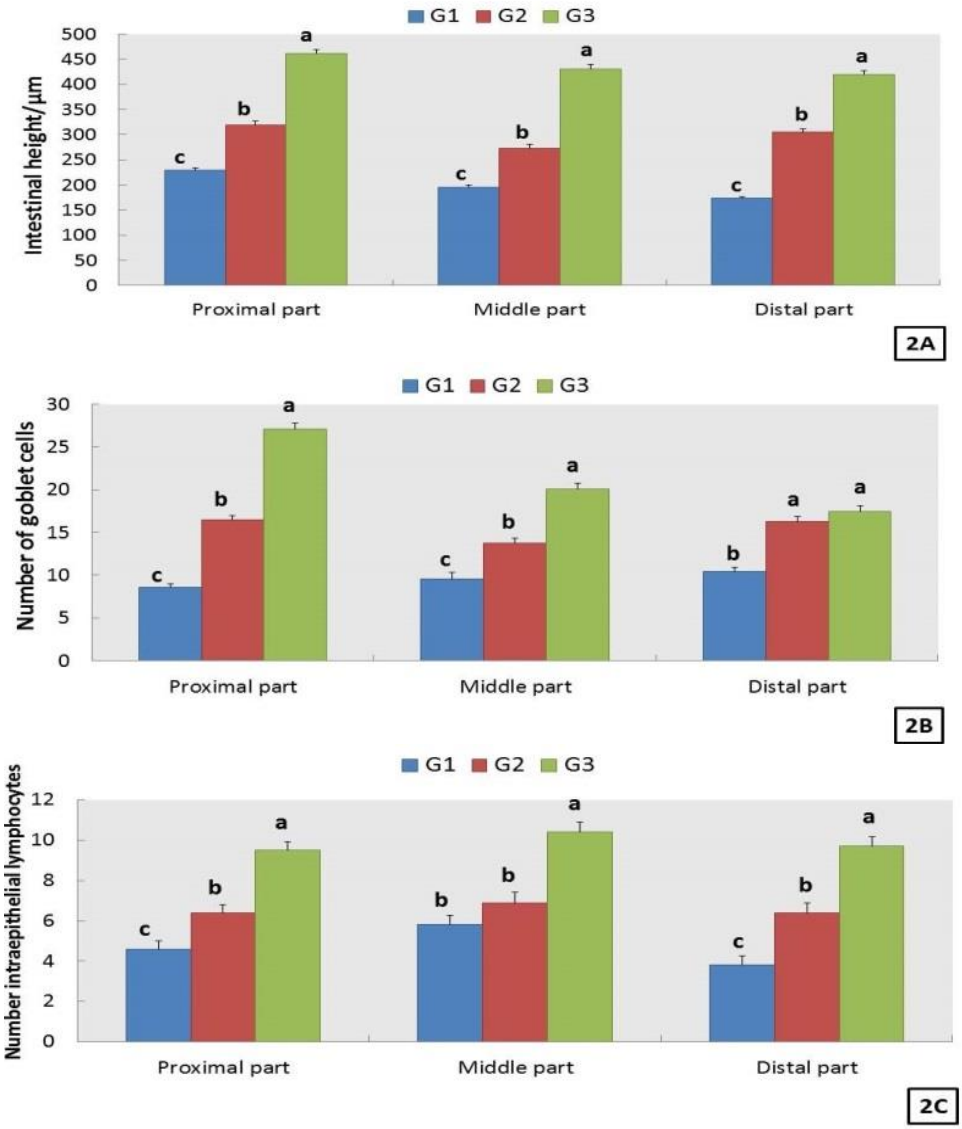

Figure 2. Intestinal histology of *Oreochromis niloticus* fed with 2 levels of the prebiotic, Immunowall®, for 2 months. (2A) Bars indicate the villus height (mean ± SD) in the proximal, middle and distal parts of the intestine. (2B) Bars indicate the number of goblet cells (mean ± SD) in the proximal, middle and distal parts of the intestine. (2C) Bars indicate the number of IELs (mean ± SD) in the proximal, middle and distal parts of the intestine. Bars with different superscripts (a, b and c) are significantly different ($P<0.05$, using One-Way ANOVA).

Body composition analysis.

Crude protein and lipid percentages showed higher significant values in G3 than G2 and G1. However, Whole-body moisture showed higher significant values in G1. Ash contents were not significantly affected by dietary supplementation with Immunowall® (Fig. 3A and B). Similar result has been observed in rainbow trout and hybrid tilapia which fed on 4.5 g kg^{-1} MOS supplemented diet (Genc *et al*., 2007; Yilmaz *et al*. 2007 and Ebrahimi *et al*., 2011) reported that *Cyprinus carpio* fed a diet containing 2.5 g kg^{-1} prebiotic Immunogen® showed highest protein content ($p < 0.05$). While, Immunoster and Immunowall prebiotic dietary administration to *Huso huso* significantly decreased carcass moisture and non-significantly increased carcass crude lipid (Ta'ati *et al.*, 2011). Grisdale-Helland *et al.* (2008) has been proved that supplementing the diet with 10 g kg^{-1} MOS resulted in a decrease in the protein concentration in the body of the salmon.

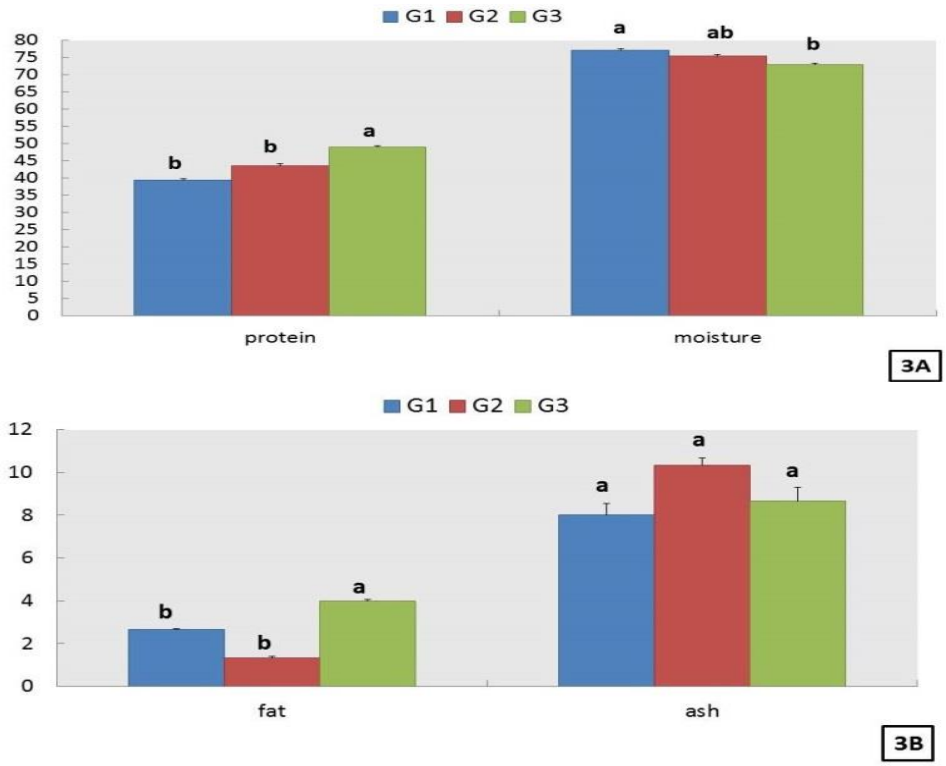

Figure 3. Body composition of *Oreochromis niloticus* fed with 2 levels of the prebiotic, Immunowall®, for 2 months. Bars indicate crude protein %, moisture % (3A), crude lipid %, Ash content (3B). Groups with different superscripts (a, b, c) are significantly different ($P<0.05$, using One-Way ANOVA).

Nonspecific immune response.

Total serum protein, albumin and globulin were significantly increased in G3 compare to G2 and G1 (Fig. 4). There are significant differences in the percentage of serum killing, phagocytic activity, lysozyme activity and nitric oxide of G3 with respect to the values obtained in the control groups at all time of experimental period (30 days) (Fig. 5). After 15 days, G3 showed significantly higher percentage of serum killing and phagocytic activity than G2 and G1. Lysozyme activity had significantly increased in G3 and G2 than control while the result showed no significant difference between groups in nitric oxide (mmol $^{-1}$). At the end of experiment (30 days), G3 showed significant increases in percentage of serum killing, phagocytic activity and nitric oxide followed by G2 in compare with G1. G3 and G2 were significantly higher in lysozyme activity than control group (Fig. 5A, B, C and D). Glucans was given their effect through interaction with membrane receptors on macrophages, lectins, scavenger receptors, neutrophils, natural killer (NK) cells and various lymphocyte cells (Brown and Gordon 2003). Based on this receptors interaction, β -glucan may induce activation of leukocytes, phagocytic activity, lysozyme and complement activity and production of cytokines (interleukins "IL-1, IL-6, IL-8, IL-12", tumor necrosis factor α "TNF-α") and inflammatory mediators (nitric oxide, NO, and hydrogen peroxide, H_2O_2) (Brown *et al*., 2003 and Misra *et al*. 2006) and influenced fish resistance against infection with significant reduced mortality rate (Misra *et al*., 2006). Ta'ati *et al*., (2012) reported that Immunoglobulin M concentration and lysozyme activity in *Huso huso* fed Immunowall at 3% were higher than the control group. The dietary effect of ß-1, 3-glucan on immune responses in healthy and aflatoxin-induced immunocompromised rohu (*Labeo rohita* Hamilton) for 7 days was significantly raised non-specific immunity as measured by serum bactericidal activity, phagocytic activity and antibody titers (Sahoo and Mukherjee, 2002). Feeding tilapia β-glucan for 4 week and then

switching to the basal diet for 2 week caused a significant increase in the respiratory burst of polymorphonuclear lymphocytes compared to catfish fed the control diet or the β-glucan diet continuously, but other immune parameters were unaffected (Welker *et al*., 2007). *Oreochromis niloticus* fed on *Saccharomyces* and vaccinated intraperitoneally with *Aeromonas hydrophila* bacterin had a lower mortality percent and higher relative level of protection than fish received only either the bacterin or yeast cells when challenged by immersion in bacterial suspension with $3x10^7$ virulent life cells / ml at 7, 14 and 21 days post vaccination (Zaki, 2004). Siwicki *et al*. (2004) observed that increase the number of specific antibody secreting cells and specific Ig levels in serum of *Oncorhynchus mykiss* fed on pellets containing beta-1.3/1.6- glucan (Macrogard) at a dose of 0.5 g/100 g of pellets (0.5%) per day then were immunized by immersion of the anti-*Yersinia ruckeri* vaccine. Lin *et al*. (2011) reported that *Cyprinus carpio* koi fed on basal diet containing 0.5% glucan reached the maximum level on respiratory burst, phagocytic activities and lysozme activity at the 21st day, and subsequently decreased at the end of

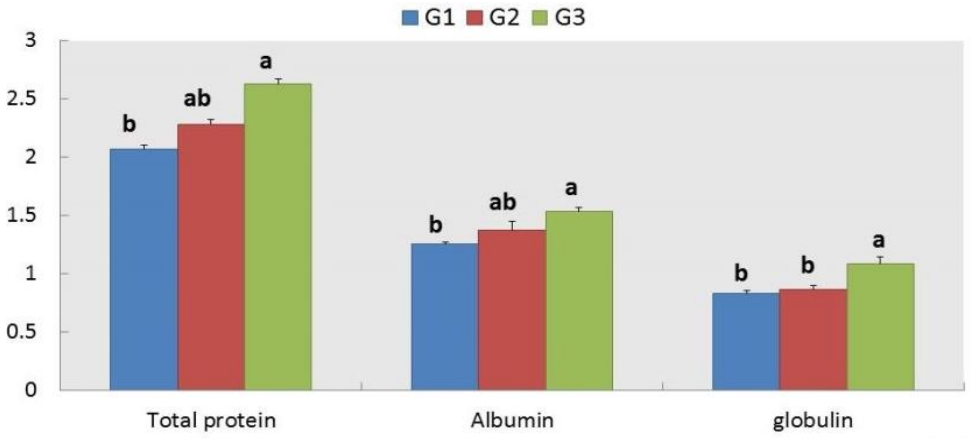

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Figure 4. Total protein, albumin and globulin of *Oreochromis niloticus* fed with 2 levels of the prebiotic, Immunowall®, for 1 months. Groups with different superscripts (a, b, c) are significantly different (P<0.05, using One-Way ANOVA).

the 56 day of feeding trial. Similar result obtained by Misra *et al*. (2006) who found the immunostimulatory effect of glucan on *Labeo rohita* fingerlings peaked at day 42 and decreased at the end of the 56 day of feeding trial. Also, Ortuno *et al*. (2002) and Rodriguez *et al*. (2003) recorded that yeast supplemented diets improved nonspecific immunity of sea bream after a 4-week feeding experiment.

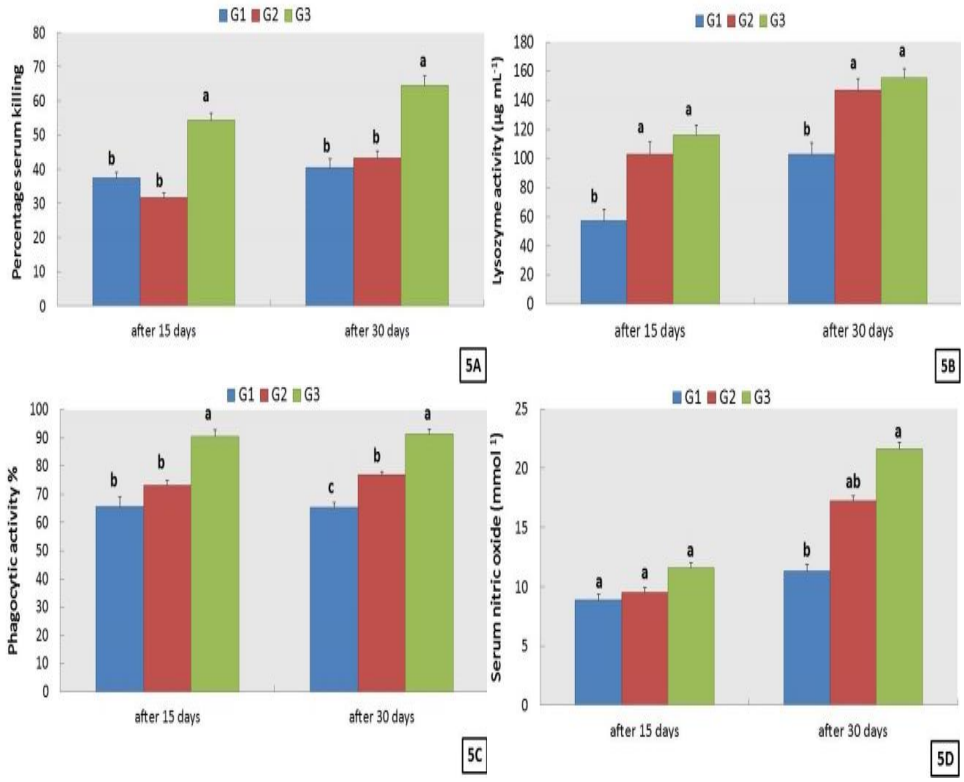

Figure 5. Nonspecific immune response of *Oreochromis niloticus* fed with 2 levels of the prebiotic, Immunowall®. (5A) Bars indicate serum killing percentage (%) after 15 and 30 days. (5B) Bars indicate serum lysozyme activity (µg ml^{-1}) after 15 and 30 days. (5C) Bars indicate phagocytic activity (%) after 15 and 30 days. (5D) Bars indicate serum nitric oxide (mmol $^{-1}$) after 15 and 30 days. Bars with different superscripts (a, b and c) are significantly different ($P<0.05$, using One-Way ANOVA).

Challenge study.

The challenge test showed that Immunowall® administration enhanced the protection against *Yersinia ruckeri* infection. Moribund fish characterized by hemorrhage in different part of body, around mouth with congestion in internal organs. Mortality and relative percentage survival (RPS) of *O. niloticus* fed on Immunowall® supplemented diet and the control diet after challenge is represented in Fig. (6). The mortality percentage was significantly high (65±5%) in the control group and was significantly low (26±4%) in G3. The relative percentage survival was high in G3 (60%) followed by G2 (15.38%) in compare to control (0/%). Siwicki *et al*. (2004) observed that beta-1.3/1.6 – glucan (Macrogard) at a dose of 0.5 g/100 g of pellets (0.5%) per day increased the effectiveness of anti-*Yersinia ruckeri* vaccine in fish. The best disease resistance in red tilapia (*Oreochromis niloticus* x *O. mossambicus*) was found in the group injected with inactivated *Streptococcus iniae* vaccine plus β-(1, 3/1,6)-glucan with the relative percent survival (RPS) of 95.12% (Suanyuk and Itsaro, 2011).

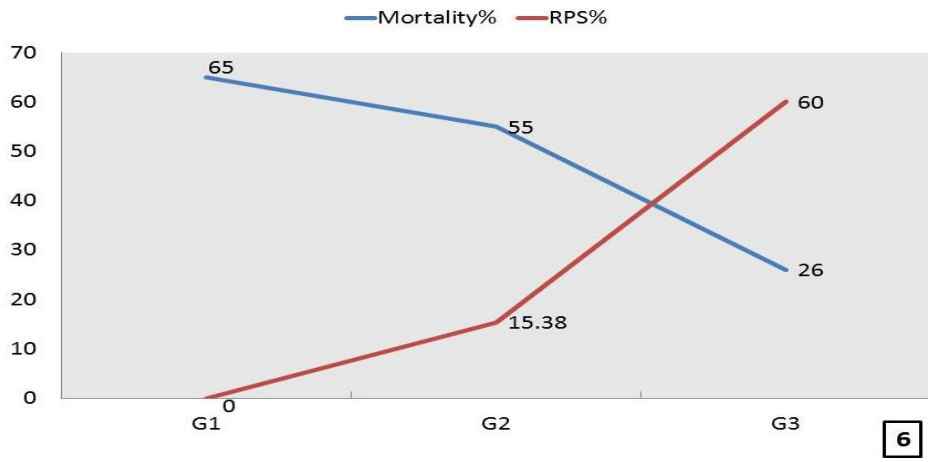

Figure 6. Mortality (%) and Relative Percentage Survival (RPS) (%) of challenged *Oreochromis niloticus* fed Immunowall® supplemented diet and the control diet for 30 days.

CONCLUSION

In conclusion, the addition of Immunowall® to dry food at a dose of 0.5 and 1 g kg^{-1} improved the growth performance of *O. niloticus* fingerlings, increased the intestinal villous heights, goblet cell numbers and intra-epithelial lymphocytes (IEL) numbers. The prebiotic increased the protein and lipid content of the carcass in a dose- dependent manner. This prebiotic supplement also ameliorated the immunological status and the fish resistance to *Yersinia ruckeri* infection, thereby acting as a robust immunostimulant.

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تحسين النمو و المناعة والمقاومة لليرسينيا روكاري في إصبعيات البلطي النيلي بتناول البريبيوتك (امينووال) من خلال الفم.

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الملخـــص العربـــى

أجريت هذه الدراسة لاستبيان مدي تأثير البريبيوتيك التجاري "اميونووال" علي اصبعيات (متوسط الوزن الاولي= ٨.٧± ٠.٤ جم) البلطي النيلي. لقد تم تقسيم الاصبعيات الي ثلاث مجموعات (مجموعة ١، ٣,٢) كلا منها ١٢٠ سمكة لدراسة التأثيرعلي قياسات النمو و المناعة. المجموعة الاولي هي المجموعة الضابطة و التي تتغذي علي وجبة الاساسية بدون اضافات. المجموعة الثانية و الثالثة التي تتغذي علي عليقة مضاف اليها الاميونووال بمقدار ١.٥ و ٣ جم لكل كجم عليقة علي التوالي. كل مجموعة مقسمة لمجموعتين فرعيتين (مجموعة فرعية أ، ب). كل مجموعة فرعية لها ثلاث أحواض بمثابة تكرار (٢٠ سمكة للتكرار الواحد). المجموعة الفرعية (أ) في كل مجموعة استخدمت لدراسة أداء النمو (٨ أسابيع) وعند نهاية ٨ أسابيع تم أخذ عينات لتحليل تكوين الجسم والأنسجة المعوية. المجموعة الفرعية (ب) في كل مجموعة استخدمت للدراسات المناعية (٤ أسابيع) حيث تم أخذ العينات في ٢ أسابيع و ٤ أسابيع، وفي نهاية من ٤ أسابيع تم الحقن البريتونى لدراسة مقاومة الأسماك لليرسينيا روكاري. ولقد اظهرت نتيجة ٨ أسابيع تغذية ارتفاع معنوي في أداء النمو لاصبعيات البلطي في مجموعة ٣ مقارنة بالمجموعة الضابطة. وأظهرت نتيجة الدراسة المناعية بعد ٤ أسابيع تغذية زيادة معنوية في النشاط القاتل للبكتيريا، البلعمة، النشاط الليزوزيم وأكسيد النيتريك في المجموعة ٣ عن المجموعة الضابطة مع تحسين لنسبة الاعاشة النسبية تصل الي ٦٠٪. و بذلك نستنتج أن اميونووال محسن قوي لنمو و مناعة أسماك البلطي النيلي مع زيادة مقاومة العدوى باليرسينيا روكاري.