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GROWTH PERFORMANCE, NON-SPECIFIC IMMUN AND ANTIOXIDANT RESPONSE OF JUVENILE TILAPIA *OREOCROMIS* SP. FEEDING ON BREWER'S YEAST *SACCHAROMYCES CEREVISIAE* SUPPLEMENTED DIET

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ABSTRACT

A triplicate experiment was conducted to evaluate the effect of the diet suplemented with brewer's yeast on the growth performance and imune response of nila tilapia *Oreocromis* sp. Five isonitrogenous (30% crude protein) and isocaloric diets were used in this experiments. As a control, the diet 1 were formulated without any suplementation of glucan and brewer's yeast; while those diets 2-5 were suplemented with either 0.3% glucan, 3.5% brewer's yeast, 17.5% brewer's yeast, and 35% brewer's yeast respectively. One hundred fish with an initial body weight of 2.4±0.10g were cultured in 2x1x1x1m^3 of floating net cages. The fish were fed on the diets at satiation for 6 weeks. At the end of feeding experiment, 20 fish from each net cage were trasferred in to 35x35x40 cm^3 of aquarium to perform a challenge test with patogen bacteria *Streptococcus agalctiae*. Results showed that fish fed on control, 0.3% glucan and 3.5% brewer's yeast supplemented diets significanly had higer growth rate, feed conversion ratio, and protein retention than others. It was also found that superoxide dismutase (SOD) activity of fish was increased when feeding on the diets suplemented with glucan and brewer's yeast. On the other hand, glutathione peroxidase (GPx) activity decresed and lipid peroxidation increased in the group of fish on the diets suplemented with 17.5% and 35% of brewer's yeast. While, the group of fish in 0.3% glucan and 3.5% brewer's yeast treatment had higer number of total erythrocytes hemoglobin, hematocrite, leukocyte, monocyte as well as phagocytic and lysozyme activities. The fish survival after 12 days challenge test was significantly higer in 0.3% glucan and 3.5% brewer's yeast teratment. Thus feeding on the diets suplemented with glucan and 3.5% berewer's yeast could increase the immune response of tilapia by increasing non-specific response of fish phathogen.

Key word: brewer's yeast, immunostimulan, immun response tilapia

INTRODUCTION

Freshwater aquaculture development in Indonesia, especially tilapia has some problems, such as a high use of imported feed ingredients and outbreak disease. The use of imported raw materials contributed to the depletion of foreign exchange. On the other hand, the disease caused by the bacteria streptococcus agalactiae infection can lead to a reduction survival in tilapia fish farming in South Sumatra up to 60% (Yuasa et al., 2008). For the prevention and control of disease, most farmers usually use probiotics. The results showed that administration of probiotics through feeding on tilapia fish farming can reduce mortality until 20% (Aly et al., 2008), and improve the Feed Conversion Ratio (FCR) from 2.59 into 1.77 (EL-Haroun et al., 2006). Administration probiotics through feeding hardly ever do because mixing probiotics with feed is difficult in mass production. Technical solutions are required, especially to keep the probiotic alive in dry pellets. Probiotics, until now could not be mixed together with other feed ingredients in feed manufacturing process in the factory, because the probiotics are not heat resistant (Gatesoupe, 1999). Thus, it is necessary to find alternatives materials wich can easy to use by farmers.

In order to reduce dependency on imported raw materials, various studies have been conducted to find alternative raw materials. Local raw materials that have been investigated as feed ingredients include palm kernel meal (PKM) (Pamungkas et al., 2011), cocoa-pod husk meal (Jusadi et al., 2013), coconut cake (Zuraida et al., 2013), lamtoro leaf meal (Fitriliyani, 2010). The results show that a variety of local raw materials are only as a carbohydrates source since they are low protein content and high crude fiber.

Brewer's yeast *Sacharomyces cerevisiae* is by-product of the beer industry may potentially be used alternative feed stuff, because it contains high protein (48% dry weight) and immunostimulant, especially β-glucan (Huige, 2006). Approximately 20% of the total dry weight of yeast is cell wall material, yeast cell walls contain 30-60% beta glucan in dry weight (Nerantzis and Tataridis, 2006). According to Cheng et al. (2004), brewer's yeast has a fairly high digestibility (72.4%) in fish Rainbow trout *Oncorhynchus mykiss.*

Brewer's yeast as a source of nutrients and active ingredients has been used since 1990s (Ferreira et al., 2010). Oliva-Tales and Gonsalves (2001) found better feed efficiency and protein utilization when 30% of the fish meal of their control diet (total fish meal protein–based diet) was replaced with brewer's yeast for sea bass, *Dicentrarchus labrax*. Korkmaz and Cakirogullari (2011) Indecated that brewer's yeast can replace up to 20-30% of fish meal in koi carp *Cyprinus carpio* L fingerling diets without andverse effects on suvirval rate, growth performance, and feed utilization efficiency. Brewer's yeast not only uses to replace fish meal but also as a feed supplement. Brewer's yeasts contain various immunostimulating compounds such as b-glucan, nucleic acids, mannan oligosaccharides, and chitin and have been proved to enhance the immune response (Ortuno et al., 2002; Li and Gatlin 2003, 2005). In addition to probable immunostimulant properties of yeast, it also improves fish growth (Lara-Flores et al., 2003; Abdel-Tawwab et al. 2008; Gopalakannan and Arul 2010; Reyes-Becerril et al., 2011) Osman et al., (2010) brewer's yeast is promising as an alternative method to antibiotics for disease prevention in tilapia aquaculture and enhanced growth performance and the optimum level of dietary live brewer's yeast is about 3.0 g/kg diet, when challenge test with Aeromonas hydrophila can reduce mortality up to 80%. However, effect brewer's yeast from by-product beer industry on growth performance, non-specific immun and antioxidant enzyme has not been reported. Therefore, this experiment conducted to evaluate the effect of the diet suplemented with brewer's yeast on the growth performance, non-specific imune and antioxidant enzyme response of tilapia Oreochromis nilaticus.

MATERIALS AND METHODS

Diet preparation: The brewer's yeast was obtained from P.T. Multi Bintang Indonesia Tbk, in Jakarta Indonesia. The brewer's yeast used in this study has a protein content of 48% and it was obtained surplus yeast at the end of the second fermentation and maturation in beer production. To reduce moisture content by centrifuged at 8000rpm for 30minutes and super natants were collected. Supernatan was dried in an oven at 70°C until moisture content less than 12%. To get brewer's yeast meal, the dried material was grind. While glucan is a commercial product Ferrrarys ®, with purity of 30%

The study was partitioned into a feeding trial experiment and a digestibility experiment. Five experimental diets were formulated where as a control is without suplemented glucan and brewer's yeast, and than diet suplemented with 0.3% glucan, 3.5%, 17.5% and 35% brewer's yeast. The diets were isonitrogenous (30% crude protein) and isocaloric (Table 1). For measuring the digestibility of the brewerer's yeast, two diets, a reference diet (DR) and a test diet (DT) were formulated according to the procedures set forth in Bureau et al. (1999). The reference diet has similar formulation to the control diet in the feeding trial experiment but included chromic oxide (0.6%) as a digestion indicator. The test diet is formulated in a proportion of 70–30% of the reference diet mix and the test ingredient (brewer's yeast).

Table-1: Ingredients and proximate chemical composition (on dry matter basis) of the experimental diets

	Experimental diets				
	Control	0,3% Glucan	3,5% Brewer's yeast	17,5% Brewer's yeast	35% Brewer's yeast
Ingredients (%)					
Fish meal	25,00	25,00	24,00	20,00	14,00
Soybean meal	31,00	31,00	29,50	20,50	13,00
Polard	15,00	15,00	14,00	13,00	9,00
Tapioca	25,00	25,00	25,00	25,00	25,00

	Experimental diets				
	Control	0,3% Glucan	3,5% Brewer's yeast	17,5% Brewer's yeast	35% Brewer's yeast
Soyben oil	1,50	1,50	1,50	1,50	1,50
Fish oil	1,00	1,00	1,00	1,00	1,00
Promix	1,50	1,50	1,50	1,50	1,50
Brewer's yeast	0	0	3,5	17,50	35,00
Glucan	0	0,30	0	0	0
Total	100,00	100,00	100	100	100
Chemical analysis (%)					
Crude Protein	30,56	30,88	31,43	30,82	31,47
Crude fat	6,74	6,98	7,23	6,81	5,97
NFE	49,08	48,68	48,79	49,71	50,47
Glukan	0,53	1,20	1,21	6,96	9,24
Total Energy (GEkkal/100g)	283,78	282,82	283,61	281,54	281,07
Energy/protein (kkal GE/g protein)	9,38	9,42	9,44	9,37	9,35

NFE (nitrogen-free extract) = 100 - (protein%+lipid%+ ash% + fiber%)
GE (gross energy) was calculated after NRC (1993) as 5.64, 9.44, and 4.11 kcal/g for protein, lipid, and NFE, respectively

Fish culture and feeding regime

Fish culture for growth performance and immun response: Juvenile Nile tilapia with average weight of 2.4±0.10 g, were obtained from hatchery in the Department of Aqua-culture, Bogor Agricultural University, for the feeding trial experiment. Fish were randomly distributed at a rate of 100 fish per net cages (measuring 2x1x1m^3), where were placed in a pond the size of 10x20x1.5 m^3. The fish were fed by hand to apparent visual satiety three times a day (08.00, 12.00 and 16.00 pm), except the day before weighing. The fish were fed on the diets at satiation for 6 weeks. Ten fish from the initial stock population and five fish from each net cages at the end of the trial were collected and frozen for subsequent body composition analysis. The dead fish was daily recorded and removed. Growth performance was determined, and feed utilization was calculated as following: Specific growth rate (SGR) = 100 (Ln W2 - Ln W1)/T, where W1 and W2 are the initial and final weight, respectively, and T is the number of days of the experiment; Feed conversion ratio (FCR) = feed intake/weight gain; Protein retention = proteingain/protein intake.

Disgestibilty test: Two diets, reference diet and test diet, were assigned randomly to six aquariums (measuring 35x35x40 cm^3) each 20 fish with average weight of 2.3±0.1g. The fish were fed by hand to apparent visual satiety three times a day. Feces from each aquarium were collected daily with scoop net. The feces were collected several times a day in an effort to limit leaching into water. After collection, they were dried and then stored frozen for analysis of protein and chromium.

Challenge test: At the end of feeding experiment, 20 fish from each net cage were trasferred in to 35x35x40 cm^3 of aquarium to perform a challenge test with *S. agalctiae.* The fish was challenged with pathogenic *S. agalactiae* using a sublethal dose as described by Reyes-Becerril et al., (2011) where a 0.1 ml dose of 48 h broth from virulent *S.agalactiae* (1x10^7 CFU/mL) was injected interperitonealy (IP) and observed for 12 days to record any abnormal clinical signs and the daily fish mortality. Three replicates were carried out for each treatment.

Hematological assays: Five fish per each net cages were anaesthetized with buffered MS222 (30mg/L) and blood was collected with a hypo-dermic syringe from the caudal vessel. The extracted blood was divided into two sets of Eppendorf tubes. In one set, sodium heparinate (500 U/mL) used as an anticoagulant was added and used for hematology (hemoglobin, hematocrit, red and white blood cell counting). The Second set, without anticoagulant, was left to clot at 4^{o}C and centrifuged at 5,000 rpm for

10 min at room temperature. The collected serum was stored at -20°C for further assays. Hematocrit values (Ht) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuging for 5min in a microhematocrit centrifuge. Hemoglobin levels (Hb) were determined colorimetrically by measuring the formation of cyanomethemoglobin after using a commercial kit. Red blood cells (RBCs) were counted under the light microscope using a Neubauer hemocytometer after blood dilution with phosphate-buffered saline.

Total leukocyte count and differential leukocyte count, blood smears were fixed with methanol and stained with Giemsa stain. The slides were washed in tap water and allowed to dry before microscopic examination. Three slides per fish were taken for counting, and 5 fish were used from each net cage at a time. Total leukocytes, differential leukocytes (monocytes, granulocytes, and lymphocytes) were counted under microscope through all fields/ slide to calculate the percentage of differential leukocytes in the blood.

The phagocytic activity of blood macrophage was determined by using $1x10^7$ cells mL^{-1} of *S. aureus* in 0.1 mL of PBS 7.4 were added to 0.1 mL of blood macrophages in a microplate and incubation for 60 min after thorough mixing in humidified atmosphere. After incubation, the plate was mixed gently and 50mL of this suspension was smeared on the glass slide. After air drying, the smears were fixed in ethanol, stained with Giemsa. The phagocytised bacteria were counted. The phagocytic activity was evaluated by estimating the mean percentage of phagocytes containing bacteria, in a random count of 100 phagocytes performed in duplicate.

Lysozyme activity and Protein concentrations: The lysozyme activity of samples (serum) was measured using a method Hanief et al. (2004) based on the ability of lysozyme to lyse the bacterium Micrococcus lysodeikticus. In a 96-well microtray, 100 ml of samples (serum) in four twofold serial dilutions in phosphate buffer (0.05 M, pH 6.2) were mixed with 100 ml of a 0.4 mg ml^{-1} suspension of M. lysodeikticus (Sigma Chemical Co.USA) in phosphate buffer. The microtray was incubated at 22 °C and the O.D. was read at 450 nm at 0, 15, 30 and 60 min. For a positive control, serum was replaced by hen egg white lysozyme (Sigma Chemical Co. USA) (serial dilutions starting at 1.6mgml^{-1}) and for a negative control, buffer replaced serum. A unit of lysozyme activity was defined as the amount of serum causing a decrease in the O.D. reading of 0.001min^{-1}. Protein concentrations were determined by the Bradford assay procedure using bovine serum albumin as the standard (Bradford 1976).

Antioxidant Enzyme assay: Superoxide dismutase (SOD) was examined by method of Misra (1985). The activity was determined from its ability to inhibit the autoxidation of epinephrine. Stimulation of epinephrine autoxidation by traces of heavy metals present as contaminants in the reagents or by the other metals under investigation was prevented by adding 10^{-4} M EDTA in the buffer to chelate these ions. One unit of SOD is defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50 %. The enzyme activity was expressed as U/mg protein.

Glutathione peroxidase (GPx) activity was measured by method of Flohe (1986). The reaction measured the rate of glutathione (GSH) oxidation by tert-butyl hydroperoxide, catalyzed by GPx. GSH was maintained at constant concentration by the addition of exogenous glutathione reductase and nicotinamide adenine dinucleotide phosphate (NAD-PH), which converted the glutathione disulfide (GSSG) to GSH. The rate of GSSG formation was then measured by the change in the absorbance of NADPH at 340 nm and activity expressed as nanomoles of NADPH oxidized/ min/mg protein.

The thiobarbituric acid reacthe substances (TBARS) assay quantifies oxidative stress by measuring the peroxidative damage to lipids that occurs with free radical generation. Free radical damage to lipids results in the production of Malondialdehyde which reacts with thiobarbituric acid (TBA) under conditions of high temperature and acidity generating a chromogen that can be measured spectrophotometrically at 535 nm. The TBARS assay (as follows) was used to quantify the oxidative damage (lipid peroxidation). The concentration of lipid peroxides was expressed as nmols TBARS per mg protein, using tetramethoxypropane as an external standard (Buege and Aust, 1978).

Statistical analysis: In order to determine significant difference, the results were analyzed using one way analysis of variance (ANOVA) and Fisher's method using Minitab 16 for Windows. Differences were considered significant at $P<0.05$.

RESULTS

Digestibility and growth performances: After the six weeks feeding trial, results showed that fish fed on control, 0.3% glucan and 3.5% brewer's yeast supplemented diets significanly had higer growth rate, feed conversion ratio, and protein retention than others (Table 2). The final biomass was increased from 6.7 to 7.1 times on control treatment, 0.3% glucan and 3.5% brewer's yeast. However the treatment of 17.5% and 35% brewer's yeast ware increasing just 5.4 to 5.6 times from the initial biomass and it was concominant with fish growth rate.

There were no significant differences between the biomass final weights of the fish fed with suplemented 3.5% berewer's yeast, the control diet and suplemented 0.3% glucan. Suplementation of brewer's yeast (17.5% and 35%) couse slower growth rate compared the other treatments. Reduction daily growth rate of fish that consume diets with suplementation 17.5% and 35% brewer's yeast were suspected by degresing efficiency of feed utilization in the treatment. Protein retention in treatment 17.5% and 35% brewer's yeast were lower than the others treatments. Moreover, impairment of protein retention resulted in higher feed conversion values (Table 2). Survival was not affected by the increased replacement of brewer's yeast. In the digestibility trial, protein of the test diets was found to be 86.92%, respectively and the brewer's yeast was 84.30%.

Table- 2: Growth performance, feed utilization, and survival of Nile tilapia for 6 weeks

Parameter	Experimental diets				
	Control	0,3% Glucan	3,5% brewer's yeast	17,5% brewer's yeast	35% brewer's yeast
Initial weight (g)	232,2±8,30	232,1±5,62	244,0±7,49	252,8±4,92	235,0±11,63
Final weight (g)	1596,1±115,41	1559,6±75,13	1737,9±78,94	1426,6±80,23	1259,2±87,53
SGR (%)	4,7±0,19 ^{a}	4,6±0,15 ^{a}	4,8±0,11 ^{a}	4,3±0,17 ^{b}	4,1±0,29 ^{b}
Feed intake (g)	1544,0±33,74 ^{ab}	1510,6±21,00 ^{b}	1605,4±23,73 ^{a}	1512,7±29,26 ^{b}	1536,2±2,51 ^{b}
FCR	1,1±0,08 ^{b}	1,1±0,05 ^{b}	1,1±0,04 ^{b}	1,3±0,07 ^{a}	1,5±0,16 ^{a}
Protein Retention (%)	41,2±5,66 ^{a}	43,0±5,64 ^{a}	43,6±1,23 ^{a}	34,6±1,95^{bc}	30,4±3,45 ^{c}
Survival (%)	91,7±2,08 ^{a}	93,3±2,08 ^{a}	94,7±1,53 ^{a}	93,0±1,00 ^{a}	92,0±1,73 ^{a}

Data are presented as means ± SD (n = 3).
Different superscript letters within each row represent significant differences (P < 0.05)

Hematology, lysozyme activity and total protein: Diets with suplementation 3.5% brewer's yeast and 0.3% glucan can increase the total erythrocytes, leukocytes, monocytes, hemoglobin, hematocrite and phagocytic activity in fish, contrary diets with suplementation 17.5% and 35% berewer's yeast. Futhermore, suplementation of 0.3% glucan and 3.5% berewer's yeast can increase lysozyme activity. Lysozyme activity at 17.5 % and 35% brewer's yeast were lower than control. In this study, the addition of brewer's yeast did not affect the total amount of protein in the blood serum fish (Table 3).

Table-3: Hematology, lysozyme activity and total protein parameters

Paramater	Experimental diets				
	Control	0,3% glucan	3,5% Brewer's yeast	17,5% Brewer's yeast	35% Brewer's yeast
Total eritrocyte (x10^{6} sel mm^{3})	1,2±0,08^{c}	1,4±0,05^{a}	1,4±0,07^{a}	1,3±0,44^{b}	1,3±0,13^{b}
Total leucocyte (x10^{6} sel mm^{3})	6,8±0,82 ^{b}	8,5±1,38 ^{a}	7,5±1,43 ^{a}	6,3±0,83 ^{c}	5,0±0,23 ^{d}
Hemoglobin(%)	5,1±1,50 ^{b}	6,9±1,15 ^{ab}	7,7±1,60 ^{a}	6,7±0,42 ^{b}	6,8±0,60 ^{b}
Hematocrit (%)	18,2±11,55 ^{c}	20,6±2,00 ^{ab}	23,0±3,60 ^{a}	20,0±1,15 ^{b}	20,4±0,47 ^{b}
Total Monocyte (%)	1,0±1,00 ^{b}	1,3±1,00 ^{a}	1,7±0,58 ^{a}	1,7±1,15 ^{a}	0,3±0,58 ^{c}
Total Neutrophil (%)	8,0±1,73 ^{b}	10,0±2,00 ^{a}	6,3±2,31 ^{b}	5,7±5,03 ^{b}	5,7±2,08 ^{b}
phagocytic activity (%)	20,0±3,79^{b}	38,7±4,89^{a}	36,5±5,47^{a}	20,8±3,75^{b}	24,5±4,30^{b}
Lysozyme activity (unit)	47.1±3.210^{b}	60,2±6,17^{a}	68.7±1.872^{a}	36.9±6.954^{c}	42.8±6.419b^{c}
Total protein serum (mg/g)	3.71±0.26^{a}	3.70±0.34^{a}	3.66±0.21^{a}	3.78±0.27^{a}	3.67±0.35^{a}

Data are presented as means ± SD (n = 3).
Different superscript letters within each row represent significant differences (P < 0.05)

Antioxidant enzyme: Supplemented 0.3% glucan and 3.5% brewer's yeast in diets can increas activity of SOD. At the final treatment, SOD activities were higher on fish that consume diet with high suplementation brewer's yeast. Fish which consume 0.3% glucan and 3.5% brewer's yeast feed had levels of lipid peroxidation was similar with the fish in the control. However, lipid peroxidation increases in diet with the suplementation brewer'yeast 17.5% and 35%.

On the other hand, GPx activity decreased when fish consume feed containing brewer's yeast as much as 17.5% and 35% (Table 4).

Table- 4: Enzyme antioxidant paramaters

Experimental diets	SOD (mU/mg protein)	GPx (mU/mg protein)	Lipid peroxidation (mg/g sample)
Control	16,12±1,83 ^{c}	27,85±6,99 ^{a}	0,02±0,002 ^{b}
0,3% glukan	49,28±0,15 ^{b}	28,94 ±0,01 ^{a}	0,02±0,003 ^{b}
3,5% Brewer' yeast	50,74±1,76 ^{b}	25,72±6,43 ^{b}	0,02±0,002^{b}
17,5% brewer's yeast	51,40±1,33 ^{ab}	19,29±3,22 ^{c}	0,03±0,001 ^{a}
35% brewer's yeast	54,79±0,91 ^{a}	22,51±3,22 ^{c}	0,04±0,012 ^{a}

Data are presented as means ± SD (n = 3).
Different superscript letters within each row represent significant differences (P < 0.05)

Challenge test with *Streptococus agalactiae:* The results of challenge test using pathogen *S.agalactiae* showed 96.5%, 89.8%, 71.3%, 67.0% and 61.9% for fish treat with 0.3% glucan, 3.5% berewer's yeast, control, 35% brewer's yeast and 17,5% brewer's yeast, respectively (Figure-1). The fish survival after 12 days challenge test was significantly higer in 0.3% glucan and 3.5% brewer's yeast terat-ment. Thus feeding on the diets suplemented with glucan and 3.5% berewer's yeast could increase the immune response of tilapia by increasing non-specific response of fish phathogen.

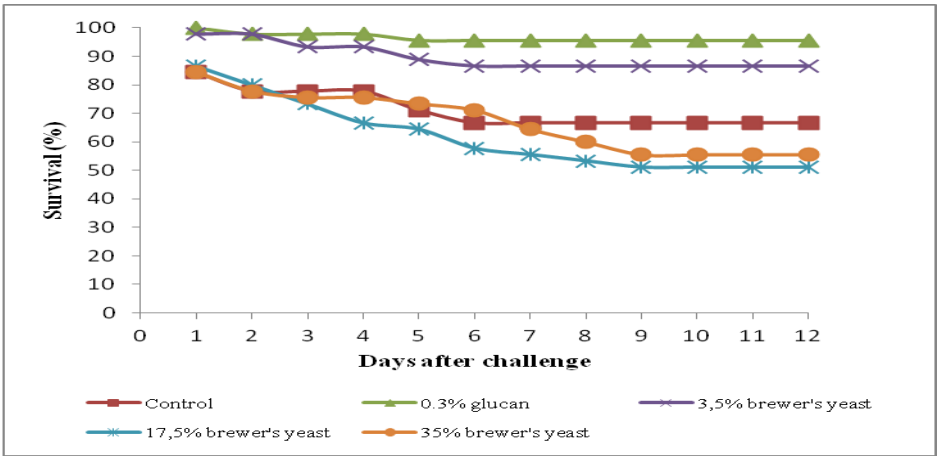

Figure 1 Survival of tilapia after challenge *S.agalactiae*

DISCUSSION

Research on utilization of by-product of beer industry (surplus yeast) is designed not only replace the protein source and but also as a immunostimulant, by replacing the proportional raw material source of protein. The results showed that administration of brewer's yeast by 3.5% in the diet provide the best growth rate. Increased percentage of brewer's yeast (17.5% and 35%) showed a decrease in growth rate. Oliva-Teles and Goncalves (2001) reported that feed conversion of sea bass improved inclusion of up to 30% dietary protein from brewers yeast. There were no significant differences in growth performance with the replacement of 50% of fishmeal protein by brewer's yeast. These trends suggest that the reduced growth rate of tilapia fed successively with increased levels of brewer's yeast could also be, because of low methionine in the high replacement diets. Brewer's yeast is considerably lower in methionine than fish meal and soybean meal. Brewer yeast protein is deficient in sulphur amino acids and several studies showed that supplementation of diets with methionine improved fish growth (Ebrahim and Abou-Seif, 2008). The protein retention in 0.3% glucan and 3,5% brewer's yeast were not significantly different from the protein retention of control diets, but the protein retention 17,5% and 35% brewer's yeast were significantly lower than the other treatments.

In this study, the digestibility of the diets were high (84.3%), but lower than the

feedstuffs it replaced in the diets, fish meal (92.8%) and soybean meal (90.0%) (NRC, 1993). This deference of digestibility value that may cause the low efficiency of feed utilization, and thereby growth rate was low. Another supposition, which cause decline in the growth rate was distrubtion of nutrients absorption. Vechklang t al. (2011) reported that the provision of the by-product yeast from rice wine at high levels can lead damage the intestinal morphology, and that is correlated with nutrients absorption and fish growth rate will be impaired. Intestinal morphological damage allegedly caused the fish undergo oxidative stress conditions, because it has been consumes large quantities of brewer's yeast. Glucans from yeast may enhance the phagocytic activity and increase the production of reactive oxygen species (ROS) metabolites by macrophages (Reyes-Becerril et al., 2008). Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. Zhu et al. (2008) reported that oxidetive stress can inhibit the fish growth. Lipid peroxidation has been used extensively as a marker of oxidative stress (Kelly et al., 1998; Sayeed et al. 2003). Under conditions of oxidetive stress induced by ROS, it will trigger the process of lipid peroxidation (LPO) of cell membrane damage (Sweetman et al., 2010).

In this study, treatment diets with high level of brewer's yeast (17.5% and 35%) produce higher lipid peroxidation values compared with others. The extent of LPO is determined by the balance between the production of ROS and the removal and scavenging of those ROS by antioxidants. The typical reaction during oxidative stress is peroxidative damage to unsaturated fatty acids, the elevation of the LPO level in the liver indicated that oxidative damage was occurring (Kelly et al., 1998). This oxidative stress in this study was occuring because overload brewer's yeast, because at higher concentrations of glucan could be attributed to the continuous overproduction of superoxide anions and free radicals generated from the respiratory burst activity of the phagocytic haemocytes (Reyes-Becerril et al. 2008; Sajeevan et al., 2009). These defense systems include antioxidant enzymes (e.g., SOD and Catalase) and numerous low-molecular-weight, nonenzymatic antioxidants (e.g., Glutations peroksidase). The SOD-Catalase system pro-vides the first defense against oxygen toxicity. Superoxide dismutase catalyzes the trans-formation of to O_2 and H_2O_2, and catalase contributes to convert H_2O_2 to water and oxygen. Oxidative stress occurs when there is an imbalance in the generation and removal of radical species within an organism. The majorities of these radicals involves oxygen and are referred to as reactive oxygen species (ROS) and the potential to damage tissues and cellular components such as membranes, protein (Kelly et al., 1998; Sweetman et al., 2010). Under oxidative stress induced by ROS, the lipid peroxidation process can lead to cell membrane damage (Sweetman et al. 2010). ROS could be effectively scavenged by the antioxidant defence systems including super-oxide dismutase (SOD), catalase and gluta-thione peroxidase (GPx) (Atencio et al. 2009). Some fish diseases, such as muscular dystro-phy, hemolysis, and jaundice, are thought to be induced by oxidative damage to tissue (Nakano et al., 1985; Han et al, 2011; Torlt 2011). Increa-sing the antioxidant system capacity in farmed fish is important because the disease states, resulting from oxidative stress (Santacroce et al., 2012).

Many studies have been reported that administration of brewer's yeast can affect to the immune system, depending on the dose and rearing conditions (Abdel-Tawab et al, 2008). In our study, feeding doses of brewer's yeast (3.5%) induced the best protection when administered for six weeks. The non-specific immune parameters variables were significantly affected by brewer's yeast levels. Fish fed on 0. 3% glucan and 3.5% yeast-supplemented diets exhibited higher values than the others. Similar results were obtained by Zhu et al., (2012) who reported that the immunology variables of catfish were significantly influenced by yeast supplements. El Boshy et al., (2010) reported that the use of yeast in diets for Nile tilapia enhanced their non-specific immune parameters such as lysozyme activity, phagocytic activity; respiratory burst activity and bactericidal activity resulting in improvement of fish resistance to A. hydrophila infection.

However, we have found that when administered high dose (17.5% and 35%), brewer's yeast had negative effects on the immune system. Unlike many chemotherapeutics, immunostimulants do not show a linear dose effect relationship (Bliznakov and Adler, 1972; Bricknell and Dalmo, 2005). In fact they often show a distinct maximum at a certain

intermediate concentration and even a complete absence of effect or an adverse toxic effect at higher concentrations. It presumes that higher concentrations of glucan caused excessive degranulation of both granular and semigranular haemocytes resulting in an exhaustion of immune system (Couso et al., 2003). Robertsen et al., (1990) found sometimes higher mortalities among Atlantic salmon (Salmo salar L.) that had been injected intraperitoneally with high doses of glucan than in the control. These authors suggested that the phagocytic cells become overloaded with glucan particles, decreasing their capacity to phagocytose bacteria.

It could be concluded that use of brewer's yeast on high level dose (17% and 35%) may suppress the immunity in fish and could be attributed to the continuous overproduction of superoxide anions and free radicals generated from the respiratory burst activity of the phagocytic haemocytes, causing non-specific injury. Based on these results, the use of a 3.5% for Nile tilapia diet was recommended to stimulate their production performance and enhance their resistance against *S. agalactiae* infection

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REFERENCES

Abdel-Tawwab, M., A. M. Abdel-Rahman and N.E. Ismael, Evaluation of commercial live bakers' yeast, Saccharomyces cerevisiae as a growth and immunity promoter for Fry Nile tilapia, (*Oreochromis niloticus* (L.) challenged in situ with Aeromonas hydrophila. *Aquaculture* 280 (1):185-189 (2008).

Aly, S. M., M. F. Mohamed and G. John, Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). *Aquaculture Research* **39** (6): 647-656 (2008).

Atencio, L., I. Moreno, Á. Jos, A. I. Prieto, R. Moyano, A. Blanco and A. M. Cameán, Effects of dietary selenium on the oxidative stress and pathological changes in tilapia (*Oreochromis niloticus*) exposed to a microcystin producing cyanobacterial water bloom. *Toxicon* **53** (2): 269-282 (1999).

Bliznakov, E. G. and A. D. Adler, Non-linear response of the reticuloendothelial system upon stimulation. *Pathol. Microbiol.* **38**: 393–410 (1972).

Bradford, M. M., A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254 (1976).

Bricknell, I. and R. A. Dalmo, The use of immunostimulants in fish larval aquaculture. *Fish and Shellfish Immunology* **19**(5): 457-472 (2005).

Buege J A. and S. D. Aust, *Microsomal lipid peroxidation. In: Fleischer S, Packer L (eds) Methods in enzymology*. New York: Academic Press (1987).

Buege, J. A. and S. D. Aust, Microsomal lipid peroxidation. *Methods in Enzymology* **52**: 302-310 (1978).

Bureau, D. P., A. M. Harris and C. Y. Cho, Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). *Aquaculture* **180** (3–4): 345-358 (1999).

Cheng, Z. J., R. W. Hardy and N. J. Huige, Apparent digestibility coefficients of nutrients in brewer's and rendered animal by-products for rainbow trout (*Oncorhynchus mykiss* (Walbaum)). *Aquaculture Research* **35** (1): 1-9 (2004).

Couso, N., R. Castro, B. Magariños, A. Obach and J. Lamas, Effect of oral administration of glucans on the resistance of gilthead seabream to pasteurellosis. *Aquaculture* **219** (1–4): 99-109 (2003).

Dalmo, R. A., Ontogeny of the fish immune system. *Fish & Shellfish Immunology* **19** (5): 395-396 (2005).

Ebrahim, M. and R. Abou-Seif, Fish meal replacement by yeast protein supplemented with biogenic L-Carnitine as source of Methionine plus Lysine mixture in feed for Nile tilapia. Paper read at 8th International Symposium on Tilapia in Aquaculture (2008).

El-Boshy, M.E., M.Ahmed, F.M. Abdel Hamid and H. A. Gadalla, Immuno-modulatory effect of dietary Saccharomyces cerevisiae, β-glucan and laminaran in mercuric chloride treated Nile tilapia (*Oreochromis niloticus*) and experimentally infected with Aeromonas hydrophila. *Fish & Shellfish Immunology* **28** (5): 802-808 (2010).

El-Haroun, E. R., A. M. A. S. Goda and M. A. Kabir Chowdhury, Effect of dietary probiotic Biogen® supplementation as a growth

promoter on growth performance and feed utilization of Nile tilapia (*Oreo-chromis niloticus* L.). *Aquaculture Research* **37** (14): 1473-1480 (2006).

Ferreira, I., O. Pinho, E. Vieira and J.Tavarela, Brewer's Saccharomyces yeast biomass: characteristics and potential applications. *Trends in Food Science & Technology* **21** (2): 77-84 (2010).

Fitriliyani,I., Evaluation of the nutritional value of Leucaena leucophala leaf meal hydrolyzed by sheep rumen liquor enzyme extract on the growth performance of Nile tilapia (*Oreochromis niloticus*). *Jurnal Akuakultur Indonesia* **9** (1): 30-37 (2010).

Flohe, L., Glutathione peroxidase. In *Oxygen Radicals in Biology and Medicine*,Springer Pp. 663-668 (1988).

Gatesoupe, F., The use of probiotics in aquaculture. *Aquaculture* **180** (1):147-165 (1999).

Gopalakannan, A. and V.Arul, Enhancement of the innate immune system and disease resistant activity in Cyprinus carpio by oral administration of β-glucan and whole cell yeast. *Aquaculture Research* **41**(6): 884-892 (2010).

Han, D., S. Xie, M. Liu, X. Xiao, H. Liu, X. Zhu and Y.Yang, The effects of dietary selenium on growth performances, oxidetive stress and tissue selenium concentration of gibel carp (Carassius auratus gibelio). *Aquaculture Nutrition* **17**(3): e741 -e749 (2011).

Hanif, A., V. Bakopoulos and G. Dimitriadis, Maternal transfer of humoral specific and non-specific immune parameters to sea bream (Sparus aurata) larvae. *Fish and Shellfish Immunology* **17**(5):411-435(2004).

Huige, N.J., Brewery by-products and effluents. *Food Science And Technology-NEW York-Marcel Dekker* **157**: 655 (2006).

Jusadi, D., J. Ekasari and A. Kurniansyah, The effectiveness of sheep rumen liquor enzyme addition on the reduction of cocoapod husk meal fiber content and its digestibility in tilapia diet. *Jurnal Akuakultur Indonesia* **12** (1):43-51 (2013).

Kelly, K., C. M. Havrilla, T. C. Brady, K. H. Abramo and E. D. Levin, Oxidative stress in toxicology: established mammalian and emerging piscine model systems. *Environmental Health Perspectives* **106**(7): 375 (1998).

Korkmaz, A.S. and G. C. Cakirogullari, Effects of partial replacement of fish meal by dried baker's yeast (*Saccharomyces cerevisiae*) on growth performance, feed utilization and digestibility in Koi carp (*Cyprinus Carpio* L., 1758) fingerlings. *Journal of Animal and Veterinary Advances* **10** (3): 346-351 (2011).

Lara-Flores, M., M. A. Olvera-Novoa, B.Z. E. Guzmán-Méndez and W. López-Madrid, Use of the bacteria Streptococcus faecium and Lactobacillus acidophilus, and the yeast Saccharomyces cerevisiae as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **216**(1): 193-201 (2003).

Li, P., and D. M. Gatlin, Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture* **219** (1): 681-692 (2003).

Li, P. and D.M. Gatlin, Evaluation of the prebiotic GroBiotic®-A and brewers yeast as dietary supplements for sub-adult hybrid striped bass (*M. chrysops* × *M. saxatilis*) challenged in situ with Mycobacterium marinum. *Aquaculture* ***248***(1-4): 197–205 (2005)

Misra, H.P., *Adrenochrome assay In: Green wald RA (ed) Handbook of methods for oxygen radical research*. Boca Raton: CRC Press (1985).

Nakano, T., T. Kanmuri, M. Sato and M. Takeuchi, Effect of astaxanthin rich red yeast (*Phaffia rhodozyma*) on oxidative stress in rainbow trout. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1426** (1): 119-125 (1985)

Nerantzis, E. and P. Tataridis, Integrated enology-utilisation of winery by-products into high added value products. *J. Sci. Tech* **1**: 79-89 (2006).

NRC (National Research Council). Nutrient requirements of fish. Committee on Animal Nutrition. Board on Agriculture. National Research Council. National Academy Press. Washington DC., USA. Pp. 114 (1993).

Oliva-Teles,A. and P.Gonçalves, Partial replacement of fishmeal by brewers yeast (Saccaromyces cerevisae) in diets for seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture* **202** (3): 269-278 (2001).

Ortuño, J., A. Cuesta, A. Rodríguez, M. A. Esteban and J. Meseguer, Oral administration of yeast, Saccharomyces cerevisiae, enhances the cellular innate immune

response of gilthead seabream (*Sparus aurata L.*). *Veterinary Immunology and Immunopathology* **85** (1): 41-50 (2002).

Osman, H., T. Ibrahim, W. Soliman and M. M. Monier, Influence of dietary commercial Baker's yeast, Saccharomyces cerevisae on growth performance, survival and immunostimulation of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *Nature and Science* **8**: 96-103 (2010).

Pamungkas W, D. Jusadi and N.B.P. Utomo, The effectivity test of sheep rumen liquor enzyme added to palm kernel meal on its decrease of crude fiber and apparent digestibility coefficient for catfish pangasius hypophthalmus diet. Indonesia Aquaculture Journal **2**(1): 149-156 (2011)

Reyes-Becerril, M., D. Tovar-Ramírez, F. Ascencio-Valle, R. Civera-Cerecedo, V. Gracia-López and V. Barbosa-Solomieu, Effects of dietary live yeast Debaryomyces hansenii on the immune and antioxidant system in juvenile leopard grouper *Mycteroperca rosacea* exposed to stress. *Aquaculture* **280** (1):39-44 (2008).

Robertsen, B., G. RΘRSTAD, R. Engstad and J.Raa, Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar L.*, by a glucan from Saccharomyces cerevisiae cell walls. *Journal of Fish Diseases* **13** (5):391-400 (1990).

Sajeevan, T., R. Philip and I. B. Singh, Dose/ frequency: a critical factor in the administration of glucan as immune-stimulant to Indian white shrimp (*Fennero-penaeus indicus)*. *Aquaculture* **287**(3): 248-252 (2009).

Santacroce, M. P., E. Merra, G. Centoducati, V. Zacchino and E. Casalino, Effects of dietary yeast Saccaromyces cerevisiae on the antioxidant system in the liver of juvenile sea bass Dicentrarchus labrax. *Fish physiology and biochemistry* **38**(5): 1497-1505 (2012).

Sayeed, I., S.Parvez, S. Pandey, B. Bin-Hafeez, R. Haque and S.Raisuddin. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicology and Environmental safety* **56** (2): 295-301 9 2003).

Sweetman, J. W., S. Torrecillas, A. Dimitroglou, S. Rider, S. J. Davies and M. S. Izquierdo, Enhancing the natural defences and barrier protection of aquaculture species. *Aquaculture Research* **41** (3):345-355 (2010).

Tort, L., Stress and immune modulation in fish. *Developmental and Comparative Immunology* **35** (12): 1366-1375 (2011).

Vechklang, K., S. Boonanuntanasarn, S. Ponchunchoovong, N. Pirarat and C. Wanapu, The potential for rice wine residual as an alternative protein source in a practical diet for Nile tilapia (*Oreochromis niloticus*) at the juvenile stage. *Aquaculture Nutrition* **17** (6):685-694 (2011).

Yuasa, K., T. Kamaishi, K. Hatai, M. Bahnnan and P.Borisutpeth, Two cases of streptococcal infections of cultured tilapia in Asia. *Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila* (2008).

Zerai, D. B., K. M. Fitzsimmons, R. J. Collier and G.C. Duff, Evaluation of brewer's waste as partial replacement of fish meal protein in Nile tilapia, *Oreochromis niloticus* diets. *Journal of the World Aquaculture Society* **39** (4): 556-564 (2008).

Zhu, H., H. Liu, J. Yan, R. Wang and L. Liu, Effect of yeast polysaccharide on some hematologic parameter and gut morphology in channel catfish (*Ictalurus punctatus*). *Fish physiology and biochemistry* **38**(5): 1441-1447 (2012).

Zhu, X., L. Zhu, Y. Lang and Y. Chen, Oxidative stress and growth inhibition in the freshwater fish Carassius auratus induced by chronic exposure to sublethal fullerene aggregates. *Environmental Toxicology and Chemistry* **27**(9): 1979-1985 (2008).

Zuraida, D. Jusadi and N. B. P. Utomo, The Evaluation of Utilization of Sheep Rumen Liquor Enzyme for Hydrolisis of Crude Fiber Content in Coconut Meal Basic Material Fish Diet. Jurnal Akuakultur Rawa Indonesia **1**(2): 117-126 (2013)