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Evaluation of the Ability of GroBiotic®-A to Enhance Growth, Muscle Composition, Immune Responses, and Resistance Against *Aeromonas hydrophila* in Nile tilapia, *Oreochromis niloticus*

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Abstract

This study was conducted to evaluate the potential of graded levels of GroBiotic®-A to improve performance of Nile tilapia, *Oreochromis niloticus*, fed a 29% crude protein (CP) diet. A 29% CP diet was formulated and supplemented with 0, 0.4, 0.8, and 1.2% GroBiotic®-A and compared to performance of fish fed a 33% CP diet. Enhanced weight gain and feed efficiency were generally observed in fish fed the diets supplemented with GroBiotic®-A compared to the 29% CP diet. No significant differences in these responses were observed between fish fed diets supplemented with GroBiotic®-A compared to those fed the 33% CP diet. Supplementation of 0.8 and 1.2% GroBiotic®-A induced significantly lower condition factor and hepatosomatic index compared to fish fed the 29% CP diet, but those values were similar to that of fish fed the 33% CP diet. GroBiotic®-A supplementation and protein reduction had no effect on the viscerosomatic index of fish or moisture, lipid, and protein content of muscle samples. However, muscle ash increased significantly with protein reduction (29% CP diet), but GroBiotic®-A supplementation (0.8 and 1.2%) reduced muscle ash content. Activities of catalase and superoxide dismutase were markedly reduced in fish fed GroBiotic®-A (0.8 and 1.2%) compared to those fed the control diet. GroBiotic®-A supplementation also induced significantly higher neutrophil oxidative radical production compared to fish fed the 29% CP diet, but no significant difference was observed in comparison with the 33% CP diet. After 8 wk of feeding, exposure to *Aeromonas hydrophila* for 3 wk resulted in 40% (0.4, 0.8% GroBiotic®-A) and 27% (1.2% GroBiotic®-A) mortality and reduced signs of disease, while 47% mortality was observed in fish fed the 29% CP diet. Based on the result of this study, it is concluded that 0.8 and 1.2% GroBiotic®-A positively influenced growth performance and feed efficiency of tilapia fed diets containing 29% crude protein to levels comparable to fish fed the 33% CP diet. GroBiotic®-A supplementation also significantly increased neutrophil oxidative radical production as well as resistance to *Ae. hydrophila* infection.

Various dietary supplements including probiotics, prebiotics, and other immunostimulants have recently received heightened attention for use in aquaculture. A rapidly expanding body of literature has been established that many intestinal microbial species may have

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beneficial influences on the performance of fish (reviewed by Irianto and Austin 2002), and diet composition may influence the intestinal microbiota of fishes (Ringø et al. 1998; Ringø and Olsen 1999; Burr et al. 2008a). It is becoming more readily apparent that prebiotics, non-digestible dietary ingredients that can beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of health-promoting bacteria in the intestinal tract, can confer numerous beneficial effects to fish in aquaculture. Responses such as increased weight gain, feed efficiency, and nutrient digestibility as well as enhanced disease resistance have been observed in numerous fish species fed GroBiotic®-A. Li and Gatlin (2004, 2005) evaluated the commercial prebiotic, GroBiotic®-A, a mixture of partially autolyzed brewers yeast, dairy ingredient components, and dried fermentation products, and found it could significantly enhance feed efficiency, selected immune responses, and resistance of juvenile hybrid striped bass to *Streptococcus iniae* and *Mycobacterium marinum*. Subsequent investigations with red drum, *Sciaenops ocellatus*, revealed enhanced growth performance, immunostimulation, and resistance to *Amyloodium ocellatum* infection (Buentello et al. 2009) as well as increased digestibility of crude protein and other dietary constituents (Burr et al. 2008b) with the addition of GroBiotic®-A.

Aeromonas hydrophila has been recovered from a wide range of freshwater fish species worldwide (Austin and Adams 1996), and has been described as the dominant infectious agent of "fish-bacterial-septicemia" in freshwater cultured cyprinid fishes, mainly crucian carp, *Carassius carassius*, Wuchang bream, *Megalobrama amblycephala*, and silver carp, *Hypophthalmichthys molitrix*, in China (Qian et al. 1997). Tilapia is another group of fish that are cultured intensively throughout the world and can be negatively affected by *Ae. hydrophila.* Traditionally, antibiotic medications have been used in response to outbreaks of this disease; however, such treatments are expensive and may have limited effectiveness. Prebiotic application was therefore evaluated in this study based on the positive effects of these compounds in protecting fish from various other bacterial pathogens.

The present study was conducted to determine the effects of graded levels of the prebiotic, GroBiotic®-A, in diets with 29% crude protein content on tilapia, *Oreochromis niloticus*, growth performance, immune responses, and resistance to *Ae. hydrophila* infection as compared with fish fed a negative control (29% crude protein) and positive control (33% crude protein) diets.

Materials and Methods

Experimental Design and Diets

Five practical-type diets were formulated to be isocaloric (~4.0 kcal/g diet) but with different crude protein contents (29.2 or 33.3% crude protein) (Table 1). The feed ingredients and proximate composition containing 33% crude protein was according to normal commercial feed formulas in China. The crude protein content of the other four diets was approximately 29% of dry weight. GroBiotic®-A was supplied by International Ingredient (St. Louis, MO, USA). Three incremental levels (0.4, 0.8, and 1.2% of diet) of GroBiotic®-A were added to the unsuplemented 29% CP diet in place of cellulose (Table 1).

All ingredients were finely ground, mixed in a Hobart mixer, and pelleted through a 2.4-mm diameter die in a Hobart meat grinder. The pellets were air-dried at room temperature, broken into small pieces, and stored in a freezer until used.

Fish and Culture Conditions

Tilapia, *O. niloticus*, propagated and reared by the Research Institute for Fisheries (Chongqing, China), were used for the experiment, and maintained in an earthen pond at the Southwest University Fisheries Breeding and Healthy Cultivation Research Centre prior to the feeding trial. Three fish were randomly obtained from this population and analyzed by the Sichuan Agriculture University Fish Disease Diagnostic Laboratory (SAUDDL) using routine bacterial culture and histopathology to confirm the fish

TABLE 1. *Ingredient and proximate composition of experimental diets.*

Constituent	33% Crude protein	29% Crude protein	29% Crude protein with 0.4% GroBiotic®-A (GA1)	29% Crude protein with 0.8% GroBiotic®-A (GA2)	29% Crude protein with 1.2% GroBiotic®-A (GA3)
Fish meal (CP62.5%)	8	8	8	8	8
Soybean meal	22	10	10	10	10
Rapeseed meal	17	17	17	17	17
Cottonseed meal	17.5	17.5	17.5	17.5	17.5
Rice bran meal	10	10	10	10	10
Wheat bran	14	14	14	14	14
Wheat-middlings	3	9	9	9	9
Wheat flour	3.7	9.7	9.7	9.7	9.7
Soybean oil	1	1	1	1	1
$Ca(H_2PO_4)_2$	1.5	1.5	1.5	1.5	1.5
Vitamin premix[1]	0.1	0.1	0.1	0.1	0.1
Mineral premix[2]	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.15	0.15	0.15	0.15	0.15
Vitamin C phosphate[3]	0.05	0.05	0.05	0.05	0.05
Cellulose	1.5	1.5	1.10	0.7	0.30
GroBiotic®-A[4]	—	—	0.40	0.8	1.20
Analyzed proximate composition (% dry matter)					
Total energy(kcal/g)	3.95	4.0	4.0	4.02	4.05
Moisture	9.7	9.4	9.5	9.7	9.4
Crude protein (N × 6.25)	33.3	29.2	29.3	29.4	29.5
Crude fat	4.4	4.6	4.7	4.8	4.9
Nitrogen-free extract	34.2	38.9	39.0	39.0	39.1

[1]Vitamin premix: V_A: 6000 IU; V_D: 2000 IU; V_E: 50.0 μg/kg; V_K: 5.0 μg/kg; V_{B1}: 15.0 μg/kg; V_{B2}: 15.0 μg/kg; V_{B3}: 25.0 μg/kg; V_{B5}: 30.0 μg/kg; V_{B6}: 10.0 μg/kg; V_{B7}: 0.2 μg/kg; V_{B11}: 3.0 μg/kg; V_{B12}: 0.03 μg/kg.

[2]Mineral premix: NaCl: 1.0 μg/kg; $MgSO_4 \cdot 7H_2O$: 15 μg/kg; $NaH_2PO_4 \cdot 2H_2O$: 25 μg/kg; KH_2PO_4: 32 μg/kg; $FeSO_4$: 2.5 μg/kg; Calcium lactate: 3.5 μg/kg; $ZnSO_4 \cdot 7H_2O$: 0.353 μg/kg; $MnSO_4 \cdot 4H_2O$: 0.16 μg/kg; $CuSO_4 \cdot 5H_2O$: 0.031 μg/kg; $CoCl_2 \cdot 6H_2O$: 0.01 μg/kg; KIO_3: 0.003 μg/kg.

[3]Vitamin C phosphate: Vitamin C content is 25%.

[4]International Ingredient (St. Louis, MO, USA).

were pathogen free. Fish were seined from the pond, transported to the culture system, and fed the 29% CP diet to apparent satiation three times per day for a 2-wk conditioning period. Fish were then graded by size and 15 groups of 30 fish averaging 18.0 g each were stocked into individual tanks (200 L), according to a completely randomized design. Water flow rate was provided at approximately 1.5 L/min via a recirculating system which maintained adequate water quality through biological and mechanical filtration. Dissolved oxygen (DO) levels were maintained between 4 and 6 mg/L. Water temperature was controlled by conditioning the ambient air in the building and remained at 25 ± 1 C throughout the trial. A 12 h light : 12 h dark photoperiod was maintained with fluorescent lights controlled by timers.

Fish in randomly assigned triplicate aquaria were fed one of the five experimental diets to apparent satiation three times daily for 8 wk. At each feeding, an excess amount of diet was fed to the fish and uneaten diet was collected 1 h after feeding, dried at 105 C, and reweighed. Leaching of uneaten feed was estimated by placing weighed samples of each diet into a tank without fish for 1 h and then recovered, dried, and reweighed. The average leaching value was used to correct the amount of uneaten feed.

Weight gain and feed efficiency were monitored monthly by collectively weighing each group of fish. Weight gain was expressed

as the increase in total cumulative biomass per tank.

Sample Collection and Analytical Methods

Growth Performance. At the end of the 4- and 8-wk periods, fish in each tank were collectively weighed and sampled for tissue analysis 24 h after the last feeding. The livers and viscera of five fish per tank were weighed for calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI). Dorsal muscles of fish were sampled, sealed in plastic bags, and stored frozen (−18 C) until analysis for muscle nutrient composition.

Crude protein, crude lipid, moisture and ash in diets, and dorsal muscle samples were determined following standard methods (AOAC 1995). Crude protein (N × 6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by the ether-extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Moisture was determined by oven-drying at 105 C until a constant weight was achieved. Ash content was measured after placing the samples in a muffle furnace at 550 C for 24 h.

Immune Response Measurements. At the end of the 8-wk period, three apparently healthy fish (no obvious skin lesions and visceral granulomas) from each tank (nine fish per treatment) were anesthetized with tricaine methanesulfonate (MS-222), and approximately 2 mL of blood was collected from the caudal vasculature using a 1-mL syringe needle with heparin as the anticoagulant. Samples were centrifuged at 700*g* for 30 min at 4 C. After centrifugation, plasma was collected and stored at −20 C for future analysis.

Whole blood neutrophil oxidative radical production was determined as described by Siwicki et al. (1994). Absorbance was converted to Nitro Blue Tetrazolium (NBT) units based on a standard curve of NBT diformazan/mL blood. Plasma lysozyme (LSZ) activity was determined using a modification of the methods described by Chen et al. (1998). One unit of LSZ activity was defined as the amount of LSZ producing a decrease in absorbance of 0.001/min. Plasma superoxide dismutase (SOD) activity was assayed according to the method of Misra and Fridovich (1972), with enzyme activity expressed as 50% inhibition of epinephrine auto-oxidation/minute/mg protein. Plasma catalase (CAT) activity was assayed according to the method of Aebi (1984). One CAT unit was defined as the enzyme activity necessary to convert 1 μmol of H_2O_2 to $H_2O + O_2$ at 25 C and pH 7 in 1 min.

Challenge Test with Ae. hydrophila. At the end of the feeding trial, a challenge test was performed on each experimental group with *Ae. hydrophila*. The BYK-038 strain of *Ae. hydrophila*, which had originally been isolated in Shanghai, China, was used for the test. This strain was chosen because in our preliminary experiments, it proved to be a very virulent strain, and its LC_{50} to tilapia was 2.61×10^5 CFU/mL (or 0.2 mL injected per fish). Bacteria were inoculated into 10 mL of liquid tryptic soy broth (TSB, Sigma, Shanghai, China) medium and were grown overnight at 28 C. Cultures were centrifuged at 1000*g* for 10 min. Supernatant was removed and the pelleted bacteria were washed twice in sterile phosphate buffered saline (PBS) solution. The concentration of bacteria was adjusted to 5×10^5 CFU/mL by the optical density of suspension. Then, 0.1 mL of suspended bacteria was injected into the peritoneal cavity of each fish. Mortality was recorded for 21 d following infection.

Statistical Analysis

Results are expressed as mean ± SD. All data were subjected to one-way ANOVA. When significant differences occurred, the group means were further compared with Duncan's multiple range tests. All statistical analyses were performed using SPSS 11.5 (SPSS, Chicago, IL, USA).

Results

Growth Performance

After the 4- and 8-wk feeding period, significantly enhanced weight gain was observed in fish fed diets GA1, GA2, and GA3 compared to those fed the 29% CP diet ($P < 0.05$), and no significant differences were observed among GA2 and GA3 groups and the 33% CP diet ($P > 0.05$) (Table 2). Feed efficiency was significantly affected by the supplement of GroBiotic®-A (0.4, 0.8, and 1.2%) at 4 and 8 wk of feeding compared to the 29% CP diet ($P < 0.05$), and no significant difference was observed between the GA3 and the 33% CP diet at 4 and 8 wk of feeding ($P > 0.05$).

HSI, VSI, and Condition Factor

The HSI of fish in GA2 and GA3 was significantly lower than that of fish fed the 29% CP diet ($P < 0.05$), and no significant difference was observed among fish fed the GroBiotic®-A-supplemented diets and the 33% CP diet ($P > 0.05$) (Table 3). No differences in VSI were observed among fish fed any of the five experimental diets ($P > 0.05$).

After the 8-wk feeding period, fish fed the diets GA2 and GA3 had a significantly higher condition factor compared to those fed the 29% CP diet, while no significant differences were noted between those fed GA2 and GA3 and those fed the 33% CP diet ($P > 0.05$).

TABLE 3. *Condition factor, hepatosomatic index, and viscerosomatic index of tilapia fed the experimental diets.*[1]

Diets	Condition factor (g/mm^3)	Hepatosomatic index (HSI) (%)	Viscerosomatic index (VSI) (%)
33% Crude protein	2.87 ± 0.06^a	1.63 ± 0.16^a	9.19 ± 0.43^a
29% Crude protein	2.36 ± 0.08^b	1.90 ± 0.11^b	9.36 ± 0.10^a
GA1	2.50 ± 0.14^b	1.75 ± 0.04^{ab}	8.84 ± 0.22^a
GA2	2.75 ± 0.09^a	1.70 ± 0.04^a	8.90 ± 0.44^a
GA3	2.81 ± 0.09^a	1.69 ± 0.03^a	9.13 ± 0.41^a

GA1 = 29% crude protein with 0.4% GroBiotic®-A; GA2 = 29% crude protein with 0.8% GroBiotic®-A; GA3 = 29% crude protein with 1.2% GroBiotic®-A.

[1]Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$ based on Duncan's multiple range tests.

Moisture, Crude Protein, Crude Lipid, and Ash of Dorsal Muscle Samples

In terms of muscle composition, no significant differences among treatments were detected for moisture, crude protein, and lipid content ($P > 0.05$), but muscle ash content was affected ($P < 0.05$) (Table 4). The ash content of muscle from fish in GA2 and GA3 was lowest, but similar to fish fed the 33% CP diet ($P > 0.05$).

Immune Response Assays

Immune response was measured by SOD, LSZ and CAT activity, and NBT test (Table 5). CAT activity in plasma of fish fed all GroBiotic®-A-supplemented diets was not significantly different from that of fish fed 29% CP diet ($P > 0.05$), but was significantly lower

TABLE 2. *Weight gain (g gain/fish) and feed conversion ratio (g feed /g gain) of tilapia fed diets containing various amounts of dried GroBiotic®-A for 8 wk.*[1]

	0–4 wk		0–8 wk	
Diets	Weight gain (g gain/fish)	Feed conversion ratio (g feed/g gain)	Weight gain (g gain/fish)	Feed efficiency (g gain/g feed)
33% Crude protein	49.07 ± 2.27^a	1.36 ± 0.02^a	102.09 ± 3.10^a	1.27 ± 0.03^a
29% Crude protein	35.76 ± 1.75^c	1.76 ± 0.10^c	81.73 ± 2.82^c	1.54 ± 0.05^d
GA1	42.98 ± 3.06^b	1.59 ± 0.03^b	91.58 ± 3.74^b	1.46 ± 0.03^c
GA2	47.25 ± 0.80^a	1.41 ± 0.02^a	97.87 ± 1.34^a	1.34 ± 0.00^b
GA3	47.49 ± 1.22^a	1.36 ± 0.03^a	98.13 ± 0.95^a	1.28 ± 0.2^a

[1]Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$ based on Duncan's multiple range tests.

TABLE 4. *Moisture, crude protein, crude lipid, and ash (% fresh weight) of dorsal muscle samples from tilapia fed experimental diets.*[1]

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
33% Crude protein	76.94 ± 0.46[a]	18.34 ± 0.59[a]	2.77 ± 0.11[a]	1.84 ± 0.12[a]
29% Crude protein	77.00 ± 0.58[a]	18.09 ± 0.76[a]	2.73 ± 0.06[a]	2.07 ± 0.16[bc]
GA1	77.35 ± 0.39[a]	17.90 ± 0.18[a]	2.65 ± 0.06[a]	2.22 ± 0.13[c]
GA2	77.60 ± 0.75[a]	18.57 ± 0.12[a]	2.82 ± 0.15[a]	1.67 ± 0.19[a]
GA3	77.76 ± 0.86[a]	18.36 ± 0.38[a]	2.78 ± 0.31[a]	1.66 ± 0.19[a]

GA1 = 29% crude protein with 0.4% GroBiotic®-A; GA2 = 29% crude protein with 0.8% GroBiotic®-A; GA3 = 29% crude protein with 1.2% GroBiotic®-A.

[1]Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$ based on Duncan's multiple range tests.

than that of the 33% CP diet except for the GA1 group ($P < 0.05$). LSZ activity in plasma of fish fed all the GroBiotic®-A-supplemented diets was not significantly different compared to the 29% CP diet ($P > 0.05$), while GA2 and GA3 groups resulted in significantly higher LSZ than fish fed the 33% CP diet ($P < 0.05$). Blood neutrophil oxidative radical production was similar for fish fed all GroBiotic®-A-supplemented diets and 33% CP diet ($P > 0.05$), but significantly higher than those fed the 29% CP diet ($P < 0.05$). SOD activity in plasma of fish fed all GroBiotic®-A-supplemented diets was not significantly different compared to fish fed 29% CP diet ($P > 0.05$), but was significantly lower than that of fish fed the 33% CP diet ($P < 0.05$).

Challenge Test with A. hydrophila

After 8 wk of feeding, fish were challenged with *Ae. hydrophila* and cumulative mortality was recorded for 21 d (Fig. 1). Mortality of fish fed all GroBiotic®-A-supplemented diets was significantly decreased compared to 29% CP diet ($P < 0.05$). Mortality of fish in GA3 group also was significantly lower than that of fish fed the 33% CP diet ($P < 0.05$).

Discussion

The results of the present study showed that increased weight gain and feed efficiency were generally observed in tilapia fed diets supplemented with GroBiotic®-A at each sampling time (4 and 8 wk). Fish fed the diet with higher protein content (33% CP diet) and lower protein diets supplemented with GroBiotic®-A (0.4, 0.8, 1.2%) had consistently and significantly better growth performance throughout the feeding trial compared to fish fed the 29% CP diet. Fish fed the 29% CP diet did appear to exhibit substandard performance which may have been related to other factors besides simply the reduction in crude protein level such as a disproportionate reduction in essential amino acid or digestible protein levels. Thus, the suboptimal performance of fish fed this diet likely accentuated the positive responses to supplementation of GroBiotic®-A.

TABLE 5. *Neutrophil oxidative production (NBT test), lysozyme, catalase, and superoxide dismutase in plasma of tilapia fed experimental diets.*[1]

Diets	Catalase (U/mL)	Lysozyme (U/mL)	NBT test (mg/mL)	Superoxide dismutase (U/mL)
33% Crude protein	0.16 ± 0.01[a]	54.01 ± 2.13[a]	6.19 ± 0.43[a]	228.46 ± 12.39[a]
29% Crude protein	0.10 ± 0.02[b]	59.62 ± 2.42[ab]	4.93 ± 0.15[b]	169.29 ± 3.49[b]
GA1	0.13 ± 0.01[ab]	59.14 ± 2.91[ab]	6.24 ± 0.35[a]	189.63 ± 15.68[b]
GA2	0.10 ± 0.02[b]	66.09 ± 5.42[b]	7.09 ± 0.30[a]	172.33 ± 7.84[b]
GA3	0.10 ± 0.02[b]	65.11 ± 5.68[b]	6.75 ± 0.38[a]	172.45 ± 8.29[b]

[1]Values in a column that do not have the same superscript are significantly different at $P \leq 0.05$ based on Duncan's multiple range tests.

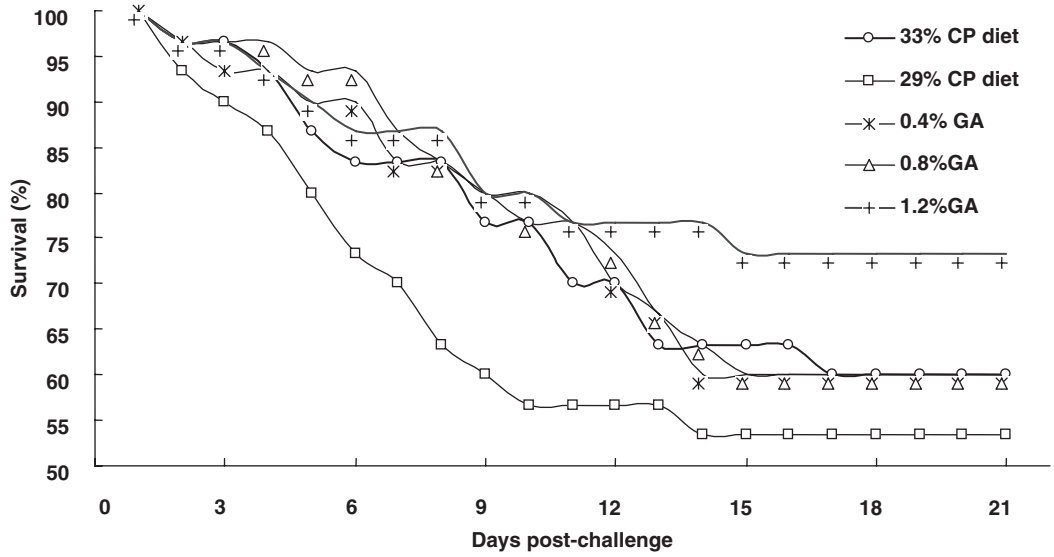

FIGURE 1. *Percent cumulative survival rate of tilapia fed incremental levels of GroBiotic® −A (0.4, 0.8, and 1.2%) for 3 wk with* Aeromonas hydrophila *injection ($P < 0.05$). Symbols represent means of three replicate tanks per treatment.*

The lower protein diets supplemented with GroBiotic®-A at 0.8 and 1.2% had nearly the same weight gain and feed efficiency compared to the positive control diet with higher protein content. Li and Gatlin (2004) supplemented GroBiotic®-A at either 1 or 2% of dry weight in experimental diets, and reported that after a 7-wk period trial, significantly enhanced weight gain and feed efficiency were observed in juvenile fish compared to those fed the basal diet. Increased resistance to *St. iniae* also was observed with GroBiotic®-A supplementation in that study. In a separate study with advanced hybrid striped bass juveniles, Li and Gatlin (2005) also found that supplementation of GroBiotic®-A significantly enhanced weight gain and feed efficiency as well as enhanced resistance to *My. marinum*. Subsequent investigations in which GroBiotic®-A was fed to red drum at 1% of diet resulted in enhanced growth performance, immunostimulation, and resistance to *Am. ocellatum* infection (Buentello et al. 2009) as well as increased digestibility of crude protein and other dietary constituents (Burr et al. 2008b) with the addition of GroBiotic®-A to practical diets.

Brewers yeast has been recognized to have potential as a potential replacement for fish meal (Rumsey et al. 1991; Oliva-Teles and Goncalves 2001). According to Rumsey et al. (1992) and Cabib et al. (1982), yeast cells provide about 7.7% crude glucan and 1% chitin. It is known that glucan is capable of enhancing innate immune responses, including respiratory burst of head kidney macrophages, serum complement activity, and serum LSZ (Engstad et al. 1992; Jørgensen et al. 1993) when administered by injection. However, the increase of serum LSZ was not observed in fish orally administered glucan or chitin. GroBiotic-A and brewers yeast apparently influenced immune response in very similar ways (Li and Gatlin 2004), which agrees with the results of the present study. Serum LSZ, CAT, and SOD activities of tilapia in the present study had no significant differences among the experimental groups and 29% CP diet (basal diet), except that NBT test was activated for the GroBiotic-A supplement.

Following challenge with *Ae. hydrophila*, all fish fed diets with GroBiotic®-A supplementation showed a significantly reduced mortality compared to those fed the 29% CP diet,

but the best survival rate was observed in fish fed the diet with 1.2% GroBiotic®-A. Survival rates of infected fish usually increase after treatment with various immunostimulants (Anderson 1992; Sakai 1999). Feeding common carp with chitosan and levamisole reduced mortality after challenge with *Ae. hydrophila* (Gopalakkanan and Arul 2006). Li and Gatlin (2004, 2005) demonstrated statistically improved survival of hybrid striped bass fed a diet supplemented with GroBiotic®-A when exposed to *St. iniae* and *Mycobacterium* sp. via immersion. This dairy/yeast prebiotic also has been reported to improve survival of rainbow trout after experimental exposure to infectious hematopoietic necrosis virus (IHNV) (Sealey et al. 2007). Similarly, golden shiners fed this dairy/yeast prebiotic experienced reduced mortality when experimentally exposed to columnaris, *Flavobacterium columnare*, which is a major disease problem in golden shiner aquaculture (Sink et al. 2007; Sink and Lochmann 2008). Theoretically, the fermentation products of oligosaccharides, nucleotides, and other immunostimulants from the yeast fractions of GroBiotic®-A all could have a positive effect on the survival of tilapia exposed to *Ae. hydrophila*. This also could be attributed to the synergistic effects of the active components.

Based on the results of this study, GroBiotic®-A was able to enhance growth performance of tilapia fed diets with reduced protein content to levels comparable to that of fish fed the 33% CP diet. Increased protection against *Ae. hydrophila* also was observed in fish fed the GroBiotic®-A-supplemented diets.

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