North American Journal of Aquaculture 69:400–406, 2007

DOI: 10.1577/A06-080.1

[Article]

Evaluation of the Ability of Partially Autolyzed Yeast and Grobiotic-A to Improve Disease Resistance in Rainbow Trout

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Abstract.—We evaluated the ability of partially autolyzed yeast and Grobiotic-A to improve immune response and disease resistance in rainbow trout *Oncorhynchus mykiss*. Experimental diets were prepared by adding partially autolyzed yeast or Grobiotic-A to a practical trout diet at the manufacturer-recommended level of 2%; the control was the same diet without supplementation. Rainbow trout (initial weight = approximately 14.3 g) were cultured in 145-L fiberglass tanks (50 fish/tank; 3 tanks/diet) in a freshwater flow-through system. Fish were hand-fed the diets to apparent satiation 3 times/d, 6 d/week for 9 weeks. At 3 and 9 weeks postweighing, fish were sampled to determine respiratory burst activity, plasma protein, total immunoglobulin and lysozyme, and tumor necrosis factor-alpha (TNF-α) expression. At the conclusion of the feeding trial (9 weeks), fish remaining after sampling were pooled by diet; one subsample was examined for the ability to respond humorally to infectious hematopoietic necrosis virus (IHNV), and another subsample was challenged by intraperitoneal injection with IHNV. Dietary inclusion of 2% partially autolyzed yeast or Grobiotic-A had no negative impacts on health or growth of rainbow trout. In contrast, although substantial variability was observed for immune response variables, use of 2% partially autolyzed yeast or Grobiotic-A produced striking improvements in survival of rainbow trout after experimental challenge with IHNV.

Disease is consistently responsible for the highest percentage of losses to the trout industry on a national basis (NASS 2005). The large variety of disease agents observed for most commercially cultured aquaculture species limits the application of conventional disease prevention strategies such as vaccines. Also complicating disease management is the limited number of therapeutics approved for use in aquaculture. The limitation of approved drugs and antibiotics, coupled with their inappropriate usage, has rendered a number of these agents largely ineffective. Therefore, new methods are needed to improve immune responses and disease resistance in coldwater carnivorous fish, such as rainbow trout *Oncorhynchus mykiss* and salmon.

Dietary supplements (e.g., prebiotics, probiotics, and immunostimulants) have been used with some success to treat infectious diseases in aquaculture (Sealey 2000; Sealey and Gatlin 2002a; Li and Gatlin 2004). Prebiotics are classified as nondigestible food ingredients that beneficially affect the host by stimulating growth or activity of a limited number of health-promoting bacteria in the intestine while limiting potentially pathogenic bacteria (Gibson and Roberfroid 1995). Effects of probiotics (live microbial feed supplements) on gastrointestinal microbiota have been studied in some fishes, but the primary application of microbial manipulations in aquaculture has been to alter the composition of the aquatic medium (Gatesoupe 1999; Irianto and Austin 2002). Some studies suggest that probiotics function by competing with the often dietary-dependent pathogenic organisms commonly associated with the various conditions, whereas other studies indicate that probiotics interact with and alter immune responses. However, it is often difficult to separate the pathogenic suppression and nutritional effects of probiotic or prebiotic usage (Verschuere et al.

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Received November 1, 2006; accepted February 10, 2007
Published online September 17, 2007

2000). Although mechanisms of immunostimulation by prebiotics, probiotics, and immunostimulants are ill-defined, both have shown promise as disease management tools. Specifically, Li and Gatlin (2003, 2004) have recently demonstrated that partially autolyzed brewer's yeast and a commercial prebiotic mixture (Grobiotic, International Ingredient, St. Louis, Missouri) consisting of partially autolyzed yeast, dairy ingredient components, and dried fermentation products have beneficial effects on growth and disease resistance in hybrids of striped bass *Morone saxatilis* × white bass *M. chrysops*. However, no data were previously available regarding the ability of these commercial products to have similar effects in the coldwater rainbow trout. Therefore, the purpose of this study was to evaluate the ability of partially autolyzed yeast and Grobiotic-A to improve immune response and disease resistance in rainbow trout.

Methods

Experimental approach.—For the control diet, we selected a commercial trout diet (Hardy 2002) formulated to contain 40% crude protein (roughly half from animal sources) and 15% crude lipid and known to meet all known nutrient requirements of rainbow trout (NRC 1993). Two test diets were prepared by adding either partially autolyzed yeast or Grobiotic-A at 2% to the trout diet (Table 1); a like percentage of wheat was excluded from these diets. Both the partially autolyzed brewer's yeast and Grobiotic-A were supplied by International Ingredient. All fish handling and experimental protocols were approved by and conducted in accordance with the guidelines of the University of Idaho's Animal Use and Care Committee.

Feeding trial.—Rainbow trout parr (average weight = 14.3 g) held in 145-L fiberglass tanks were fed one of the three diets (control, autolyzed yeast, or Grobiotic-A) for 9 weeks. Each diet was replicated with three tanks (i.e., 9 tanks total; 50 fish/tank). Fish were fed to apparent satiation (all that they would consume in 20 min) 3 times/d, 6 d/week for 9 weeks. Tanks were supplied with 4–6 L/min of constant-temperature (14.8°C) springwater supplied by gravity to the tanks at the University of Idaho's Hagerman Fish Culture Experiment Station (HFCES). A constant photoperiod was applied (14 h of daylight) using fluorescent lights controlled by a timer. Rainbow trout in the trials were bulk-weighed and counted every 3 weeks, and fish growth rates and feed conversion ratios were calculated. Fish were sampled to determine muscle and whole-body proximate composition at the start of the trial (5 fish), at 3 weeks (3 fish/tank), and at 9 weeks (3 fish/tank). Dry matter and ash analyses of fish and diets were performed according to standard methods (AOAC International 1995). We determined (1) crude protein (N × 6.25) by use of the Dumas method (AOAC International 1995) on a LECO nitrogen analyzer (LECO Corporation, St. Joseph, Michigan; TruSpec N), (2) lipid using a Soxtec HT solvent extractor (Foss Tecator, Höganäs, Sweden; Model HT6), and (3) total energy using an adiabatic bomb calorimeter (Parr Instrument Company, Inc., Moline, Illinois; Model 6300).

Nonspecific immune response evaluations.—To assess the nonspecific immune responses of fish fed the various diets, we randomly sampled 3 fish/tank at 3 and 9 weeks. Fish were anesthetized and blood was collected from the caudal vasculature via a heparinized syringe. Total plasma protein and immunoglobulin were measured using the methods of Siwicki et al. (1994). After blood collection, each fish was killed and the head kidney and spleen were removed. Tissues were pooled by aquarium, and a portion was used to isolate phagocytic cells for functional assays. Respiratory burst activity (Secombes 1990) was determined based on minor modifications of methods described by Sealey and Gatlin (2002b).

TABLE 1.—Ingredients and proximate composition of a rainbow trout control diet (diet 1) containing anchovy meal and experimental diets containing partially autolyzed yeast (diet 2) and Grobiotic-A (diet 3); diets were formulated on an as-fed basis.

Component	Diet 1	Diet 2	Diet 3
Ingredients (%)			
Herring meal[a]	34.35	34.35	34.35
Corn gluten meal	7.20	7.20	7.20
Soybean meal	15.40	15.40	15.40
Grobiotic-A	0	2.0	0
Partially autolyzed yeast	0	0	2.0
Wheat flour[a]	32.14	30.14	30.14
Fish oil[a]	7.89	7.89	7.89
Vitamin premix[b]	0.40	0.40	0.40
Lecithin[a]	2.00	2.00	2.00
Choline chloride[a]	0.50	0.50	0.50
Vitamin C[a]	0.02	0.02	0.02
Trace mineral mix[b]	0.10	0.10	0.10
Analyzed composition[c]			
Crude protein (%)	46.3	42.7	42.1
Lipid (%)	13.5	11.9	11.5
Gross energy (kcal/g)	5,783	5,716	5,703
Ash (%)	3.7	3.4	3.3
Moisture (%)	4.0	7.9	8.8

[a] Sources of ingredients: wheat, choline chloride, and anchovy fish meal were obtained from Nelson & Sons (Murray, Utah); fish oil and vitamin C were from Rangen (Buhl, Idaho).
[b] Same as in Cheng et al. (2003).
[c] Means of two replicate samples per diet on an as-fed basis.

TABLE 2.—Mean growth performance of rainbow trout (3 tanks/diet; 30 fish/tank) fed a control diet or a diet supplemented with 2% Grobiotic-A or partially autolyzed yeast. Fish were sampled at 3 and 9 weeks (weight gain = [group weight-initial group weight initial group weight feed conversion rate [FCR] = g dry feed/g wet weight gain. Statistical differences among diets were examined by ANOVA.

	At 3 weeks		At 9 weeks	
Diet	Weight gain (%)	FCR[a]	Weight gain (%)	FCR
Control	139	0.67 y	532	0.90
Grobiotic-A	136	0.76 z	556	1.00
Yeast	142	0.74 z	542	1.01
P	0.6198	0.0016	0.4186	0.0844
Pooled SE	4.05	0.01	11.69	0.03

[a] Values within this column with a different letter differ significantly ($P \leq 0.05$) based on Duncan's multiple-range test.

To assess immune gene expression, head kidney samples from three additional fish per tank were isolated at 3 and 9 weeks for RNA extraction to examine the inflammatory response markers, lysozyme and tumor necrosis factor-alpha (TNF-α; Overturf et al. 2003) by real-time (RT) polymerase chain reaction (PCR). Isolated tissue was immediately placed into a microtube containing TRIZol (Invitrogen, Carlsbad, California), isolated according to the manufacturer's protocol, and then quantified. The RT-PCR was carried out using a Prism 7900HT sequence detection system (Advanced Biosystems, Inc. [ABI], Foster City, California) and a TaqMan One-Step RT-PCR Master Mix reagents kit (ABI), according to the manufacturer's protocol. The final concentration of each reaction was 1× of Master Mix (contains AmpliTaq Gold enzyme, deoxynucleotide triphosphates including deoxyuracil triphosphate, a passive reference, and buffer components), 0.25 units of MultiScribe reverse transcriptase per microliter, 0.4 units of RNase inhibitor mix per microliter, 100 ng of total RNA, 900 nM of forward and reverse primers for TNF-α and β-actin, 300 nM of forward and reverse primers for lysozyme, 250 nM of probe for TNF-α and β-actin, and 200 nM of probe for lysozyme. For TNF-α and β-actin, primers and probes were designed by the Assay by Design service of ABI. For lysozyme, primers and probe were designed in Primer Express software (ABI). For β-actin (GenBank accession number AF254414), the primer–probe sequence (listed from 5′ to 3′)was CCCTCTTCCAGCCCTCCTT (*βactinF*), AGTTGTAGGTGGTCTCGTGGATA (*βactinR*), and 6FAM-CCGCAAGACTCCATACCGA-NFQ (*βactinMGB*). For lysozyme (AF452171), the primer–probe sequence was TGGGTTTGCCTGTCAAAATG (*Lys131F*), TGTTGATCTGGAAGATGCCATAGT (*Lys227R*), and 6FAM-TCG AGCTACAATACCCAGGCCAC-CA-TAMRA (*Lys7*). For TNF-α (AJ401377), the primer–probe sequence was TGGAGCCTCAGCTG-GAGATATT (*TNFF*), CCGGCAATCTGCTT-CAATGTATT (*TNFR*), and 6FAMCATTGGTCAA-AAGATAC-NFQ (*TNFMGB*). Cycling conditions for TNF-α and β-actin were as follows: 30 min at 48°C, 10 min at 95°C, and 40 cycles of PCR (15 s at 95°C followed by 1 min at 60°C). Cycling conditions for lysozyme were 2 min at 50°C, 30 min at 60°C, 5 min at 95°C, and 40 cycles of PCR (20 s at 95°C followed by 1 min at 62°C). Assays were run in duplicate on RNA samples isolated from individual fish. A serial dilution of six duplicate standards was run with each primer–probe set for quantification. As a cellular messenger RNA (mRNA) control, β-actin levels were determined for each sample and used in the normalization of specific expression data (Kreuzer et al. 1999). The fluorescence output for each PCR cycle was measured and downloaded to a personal computer upon the completion of the entire run. Accumulated data were analyzed using Sequence Detector version 2.1 (ABI). The data for lysozyme and TNF-α are reported as a ratio of absolute mRNA copy number of each specific gene to the absolute copy number of β-actin. Ratios were multiplied by a constant variable for ease of interpretation and are expressed here as means (±SE).

Humoral response evaluation.—One-hundred fish from each dietary treatment were transported to the research laboratory at Clear Springs Foods, Inc. (Buhl, Idaho), and placed in 378-L fiberglass aquaria receiving ultraviolet-disinfected, single-pass springwater (mean temperature = 14.5°C). Fish were fed their respective diets ad libitum twice daily. One week after transport, 20 fish were removed from each stock group and were anesthetized in water containing tricaine methanesulfonate at a concentration of 250 mg/L. Each fish was injected intraperitoneally with 10^6 plaque-forming units (PFU) of an infectious hematopoietic necrosis virus (IHNV) cell culture lysate. This virus isolate (039-82) has been previously shown to be significantly less virulent than other IHNV isolates from this area. The virus was isolated, identified, and characterized as previously reported (LaPatra et al. 1994). Each 20-fish group was placed in a separate 378-L fiberglass aquarium on a separate water supply.

At 4 weeks postinjection, 12 fish from each treatment were anesthetized and nonlethally bled. Additionally, five fish from the uninjected stock groups were bled. Blood was obtained by caudal puncture from individual fish after they were anesthetized as previously described. Individual blood samples were

TABLE 3.—Mean muscle and whole-body proximate composition of rainbow trout fed a control diet or a diet containing 2% partially autolyzed yeast or Grobiotic-A for 3 weeks. Statistical differences among diets were examined by ANOVA.

Diet	Muscle (%)			Whole-body (%)				Whole-body energy (kcal/g)
	Moisture	Protein[a]	Ash	Moisture	Protein	Lipid	Ash	
Control	76.95	18.67 y	1.67	74.30	15.72	8.12	1.94	6.552
Grobiotic-A	76.88	19.25 zy	1.67	74.25	16.46	8.16	1.83	6.620
Yeast	76.32	20.04 z	1.68	73.93	15.77	8.41	1.81	6.629
P	0.4697	0.0102	0.9957	0.6446	0.3664	0.7396	0.8359	0.5827
Pooled SE	0.37	0.21	0.10	0.29	0.38	0.29	0.16	54.89

[a] Values within this column with different letters differ significantly ($P \leq 0.05$) based on Duncan's multiple-range test.

stored at 4°C and allowed to clot overnight. The samples were centrifuged for 10 min at 1,600 × gravity, and the sera were tested using a complement neutralization test for determination of anti-IHNV antibody titers (described by LaPatra et al. 1993).

Disease resistance evaluation.—To examine disease resistance, 20 fish from each dietary treatment group were challenged with IHNV at two different time points. Twenty mock-infected control fish from each treatment were also included at each time point. Fish were anesthetized and injected intraperitoneally with 10^6 PFU of IHNV strain 220-90, which has been classified as highly virulent (LaPatra et al. 1994). Fish were monitored for mortality and fed their respective diets for 18 d. At least 20% of each day's mortalities were examined for virus presence and titer via plaque assay procedures described by LaPatra et al. (1989).

Statistical analyses.—The MIXED procedure in the Statistical Analysis System version 7.00 (SAS Institute 1990) was used to conduct analysis of variance (ANOVA) for a mixed effects model (Ott 1977) in which treatment was the fixed effect and tank within treatment was a random effect. Binomial data were arcsine transformed prior to analysis. Differences among treatment means were determined via Tukey's procedure for pairwise comparisons. Treatment effects in all statistical analyses were evaluated at a significance level of 0.05.

Results

Growth Performance

Throughout the growth trial, all of the fish appeared to be in good health and readily accepted the experimental diets (Table 1). There were no mortalities observed during the growth trials. At 3 weeks, fish fed the experimental diets containing partially autolyzed yeast and Grobiotic-A at 2% had feed conversion ratios that were significantly higher ($P = 0.0016$) than those of control fish (Table 2). At 9 weeks, there were no significant differences in weight gain or final feed conversion ratio between the dietary treatments.

Body Composition

Fish fed the experimental diets containing partially autolyzed yeast at 2% had significantly higher muscle protein than control fish ($P = 0.0102$; Table 3). Final (9-week) proximate composition analysis of muscle displayed the same trend as the 3-week data ($P = 0.0237$; Table 4). Fish fed the experimental diets containing partially autolyzed yeast and Grobiotic-A at 2% had significantly higher whole-body energy stores than those fed the control diet ($P = 0.0144$; Table 4).

Nonspecific Immune Responses

Individual immune responses within treatments were highly variable (Table 5). At 3 and 9 weeks, neither lysozyme nor TNF-α mRNA expression differed

TABLE 4.—Mean muscle and whole-body proximate composition of rainbow trout fed a control diet or a diet containing 2% partially autolyzed yeast or Grobiotic-A for 9 weeks. Statistical differences among diets were examined by ANOVA.

Diet	Muscle (%)					Whole-body (%)				Whole-body energy[a] (kcal/g)
	Moisture	Protein[a]	Lipid	Ash	Energy	Moisture[a]	Protein	Lipid	Ash	
Control	75.37	20.38 y	4.98	1.47	6,435	69.54 zy	17.19	11.96	1.62	7,035 y
Grobiotic-A	76.43	20.90 zy	3.86	1.55	6,710	70.68 z	16.60	9.67	1.68	7,269 z
Yeast	75.59	21.79 z	4.44	1.57	6,601	68.96 y	17.49	12.14	1.71	7,220 z
P	0.1866	0.0237	0.2035	0.2495	0.4225	0.0308	0.2327	0.0895	0.8839	0.0144
Pooled SE	0.41	0.34	0.43	0.04	0.19	0.41	0.08	23.33		

[a] Values within this column with different letters differ significantly ($P \leq 0.05$) based on Duncan's multiple-range test.

TABLE 5.—Immune gene expression and nonspecific immune responses of rainbow trout fed a control diet or a diet containing 2% partially autolyzed yeast or Grobiotic-A for 3 and 9 weeks. Lysozyme or TNF-α messenger RNA (mRNA) expression, as determined by real-time PCR and normalized to β-actin (a housekeeping gene), is expressed as a ratio. Respiratory burst response was determined by nitroblue tetrazolium reduction and is expressed as optical density (OD) at 620 nm.

	Lysozyme mRNA at week		TNF-α mRNA at week		Respiratory burst OD at week		Plasma protein (mg/mL) at week		Plasma immunoglobulin (mg/mL) at week	
Diet	3	9	3	9	3	9	3	9	3	9
Control	1.67	5.50	0.06	0.12	0.502	0.473	49.51	47.26	32.61	28.14
Grobiotic-A	1.32	7.82	0.07	0.08	0.650	0.599	58.74	51.21	36.18	40.13
Yeast	0.82	4.89	0.03	0.20	0.526	0.492	50.89	46.83	33.53	43.72
P	0.8045	0.6923	0.1269	0.5925	0.3634	0.1349	0.2331	0.1425	0.9232	0.4582
Pooled SE	1.37	2.39	0.01	0.08	0.18	0.10	9.97	6.47	10.35	13.27

among the diets. Similarly, respiratory burst response, plasma protein, and total antibody level displayed no significant effects of dietary treatment at either time point ($P > 0.05$).

Humoral Response Evaluation

At 30 d postimmunization, considerable variation in humoral response was observed among individual fish (Table 6). Titers of 20 or greater were seen in 9 of 12 fish fed the partially autolyzed yeast supplemented diet, 7 of 12 fish fed the Grobiotic-A supplemented diet, and 6 of 12 fish fed the control diet.

Disease Resistance Evaluation

In contrast to the nonspecific immune response evaluations, substantial effects of dietary treatment were observed after experimental challenge with IHNV (Table 7). Average percent survival was nearly three times greater for fish fed the partially autolyzed yeast (61.5%) and Grobiotic-A (63.5%) supplemented diets than for control fish (22%).

TABLE 6.—Infectious hematopoietic necrosis virus neutralizing antibody titers in rainbow trout fed a control diet or a diet containing 2% partially autolyzed yeast or Grobiotic-A for 9 weeks. Values are for 12 replicate fish and all fish had negligible titers before immunization.

Fish number	Control	Grobiotic-A	Yeast
1	20	40	40
2	20	<20	40
3	<20	<20	80
4	<20	<20	<20
5	<20	<20	20
6	<20	20	<20
7	≥160	40	<20
8	≥160	<20	20
9	80	40	40
10	≥160	80	80
11	<20	≥160	80
12	<20	40	80

Discussion

Numerous studies have suggested that for the efficient enhancement of immune responses, pulsed feeding regimes with immunostimulants are preferable to long-term feeding (Siwicki et al. 1994; Jeney and Anderson 1993). Our assessment of feeding duration effects on nonspecific immune responses of rainbow trout indicated no significant differences between partially autolyzed yeast and Grobiotic-A diets and, importantly, no detrimental effects of continuous feeding for 9 weeks. The lack of correlation between immune responses and partially autolyzed yeast or Grobiotic-A probably indicates that the number of fish sampled was not sufficient to detect immune response changes because of the high variability within the assays. Alternatively, the choice of housekeeping gene for normalization in the RT-PCR analysis may not have been optimal. Thus, our methods may have reduced our ability to detect significant dietary-induced immune response changes.

In contrast, 9 weeks of dietary supplementation with 2% partially autolyzed yeast or Grobiotic-A provided striking benefits to survival in rainbow trout that were experimentally challenged with IHNV. Previously, Li and Gatlin (2004) demonstrated that dietary brewer's yeast and Grobiotic-AE supplementation significantly enhanced hybrid striped bass survival after bath immersion exposure to the bacterial pathogen *Streptococcus iniae*. Our study furthers their work by demonstrating Grobiotic-A's efficacy against viral as well as bacterial pathogens. Additional research is needed to fully address the mechanisms by which autolyzed yeast or Grobiotic-A increases IHNV disease resistance in rainbow trout.

The growth rate of fish fed the control diet was comparable to growth rates attained in previous HFCES studies that used identical experimental conditions, similar diets, and similar fish sizes (Cheng et al. 2003; Cheng and Hardy 2004). These observa-

TABLE 7.—Survival of rainbow trout that were fed a control diet or a diet containing 2% partially autolyzed yeast or Grobiotic-A for 9 weeks and that were challenged with an intraperitoneal injection of infectious hematopoietic necrosis virus.

	Challenge 1			Challenge 2			Combined		
Diet	Challenged *N*	Mortality *N*	Survival (%)	Challenged *N*	Mortality *N*	Survival (%)	Challenged *N*	Mortality *N*	Average survival[a] (%)
Control	20	17	15	21	15	29	41	32	22 y
Grobiotic-A	19	9	53	20	6	70	39	15	61.5 z
Yeast	18	5	72	20	9	55	38	14	63.5 z

[a] Values in this column with different letters differ significantly ($P \leq 0.05$) based on Duncan's multiple-range test.

tions indicate that 2% partially autolyzed yeast or Grobiotic-A has no negative impacts on growth of rainbow trout. Of note, the differences in feed conversion ratio observed at 3 weeks reflect the increased consumption of the partially autolyzed yeast or Grobiotic-A diets. Importantly, the negative effects of supplementation on feed conversion efficiency at 3 weeks were not observed at 9 weeks. Literature reports (Spurlock 1997; Bosworth et al. 1998; Guttridge et al. 2000; Overturf et al. 2003; Acharyya et al. 2004) and studies conducted at HFCES in animals fed in pairs (Johansen et al. 2006) have demonstrated tradeoffs between growth efficiency and elevated immune responses. Thus, the transient increase in feed intake may simply represent a short-term compensatory effort of fish to overcome the initial metabolic costs of an acute immune response to the dietary supplements and would probably not be discernible in a production setting.

Acknowledgments

We thank the staff at HFCES and Clear Springs Foods for their contributions. Funding for the study was provided, in part, by the University of Idaho and the Trout Grains Project of the Small Grains and Potato Germplasm Research Unit (U.S. Department of Agriculture, Agricultural Research Service), Aberdeen, Idaho. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation by the U.S. Department of Agriculture or the University of Idaho.

References

Acharyya, S., K. J. Ladner, L. L. Nelson, J. Damrauer, P. J. Reiser, S. Swoap, and D. C. Guttridge. 2004. Cancer cachexia is regulated by selective targeting of skeletal muscle gene products. Journal of Clinical Investigation 114:370–378.

AOAC International. 1995. Official methods of analysis, 15th edition. AOAC International, Arlington, Virginia.

Bosworth, B. G., W. R. Wolters, D. J. Wise, and M. H. Li. 1998. Growth, feed conversion, fillet proximate composition and resistance to *Edwardsiella ictaluri* of channel catfish, *Ictalurus punctatus* (Rafinesque), blue catfish, *Ictalurus furcatus* (Lesueur), and their reciprocal F1 hybrids fed 25% and 45% protein diets. Aquaculture Research 29:251–257.

Cheng, J., and R. W. Hardy. 2004. Effects of microbial phytase supplementation in corn distiller's dried grain with solubles on nutrient digestibility and growth performance of rainbow trout, *Oncorhynchus mykiss*. Journal of Applied Aquaculture 15(3):83–100.

Cheng, J., R. W. Hardy, and M. Blair. 2003. Effects of supplementing methionine hydroxyl analogue in soybean meal and distiller's dried grain-based diets on the performance and nutrient retention of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research 34:1303–1310.

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

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125:1401–1412.

Guttridge, D. C., M. W. Mayo, L. V. Madrid, C. Y. Wang, and A. S. Baldwin, Jr. 2000. NF-κB-induced loss of *MyoD* messenger RNA: possible role in muscle decay and cachexia. Science (Washington, D.C.) 289:2363–2366.

Hardy, R. W. 2002. Rainbow trout, *Oncorhynchus mykiss*. Pages 184–202 *in* C. D. Webster and C. Lim, editors. Nutrient requirements and feeding of aquaculture fish. CAB International Publishers, New York.

Irianto, A., and B. Austin. 2002. Probiotics in aquaculture. Journal of Fish Diseases 25:633–642.

Jeney, G., and D. P. Anderson. 1993. Glucan injection or bath exposure alone or in combination with a bacterin enhances the non-specific defense mechanisms in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 116:315–329.

Johansen, K. A., W. M. Sealey, and K. Overturf. 2006. The effects of chronic immune stimulation on muscle growth in rainbow trout. Comparative Biochemistry and Physiology 144B:520–531.

Kreuzer, K. A., U. Lass, O. Landt, A. Nitsche, J. Laser, H. Ellerbrok, G. Pauli, D. Huhn, and C. A. Schmidt. 1999. Highly sensitive and specific fluorescence reverse transcription–PCR assay for the pseudogene-free detection of beta-actin transcripts as quantitative reference. Clinical Chemistry 45:297–300.

LaPatra, S. E., K. A. Lauda, and G. R. Jones. 1994. Antigen

variants of infectious hematopoietic necrosis virus and implications for vaccine development. Diseases of Aquatic Organisms 20:119–126.

LaPatra, S. E., K. A. Roberti, J. S. Rohovec, and J. L. Fryer. 1989. Fluorescent antibody test for the rapid diagnosis of infectious hematopoietic necrosis. Journal of Aquatic Animal Health 1:29–35.

LaPatra, S. E., T. Turner, K. A. Lauda, G. R. Jones, and S. Walker. 1993. Characterization of the humoral response of rainbow trout to infectious hematopoietic necrosis virus. Journal of Aquatic Animal Health 5:165–171.

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

Li, P., and D. M. Gatlin III. 2004. Dietary brewers yeast and the prebiotic Grobiotic™AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. Aquaculture 231:445–456.

NASS (National Agricultural Statistics Service). 2005. Trout production, February 25, 2005. U.S. Department of Agriculture, National Agricultural Statistics Service. Available: usda.mannlib.cornell.edu/reports/nassr/other/ztp-bb/. (October 2006).

NRC (National Research Council). 1993. Nutrient requirements of fish. National Academy Press, Washington, D.C.

Ott, L. 1977. An introduction to statistical methods and data analysis. Duxbery Press, North Scituate, Massachusetts.

Overturf, K., M. T. Casten, S. L. LaPatra, C. Rexroad III, and R. W. Hardy. 2003. Comparison of growth performance, immunological response and genetic diversity of five strains of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 217:93–106.

SAS Institute. 1990. SAS user's guide: statistics, version 5 edition. SAS Institute, Cary, North Carolina.

Sealey, W. M. 2000. Probiotics and immunostimulants. Pages 676–680 *in* R. R. Stickney, editor. Encyclopedia of aquaculture. Wiley, New York.

Sealey, W. M., and D. M. Gatlin III. 2002a. Nutritional strategies affecting health of marine fish. Pages 103–112 *in* C. E. Lim and C. D. Webster, editors. Nutrition and fish health. Haworth Press, Binghamton, New York.

Sealey, W. M., and D. M. Gatlin III. 2002b. In vitro manipulations of vitamin C and vitamin E concentration alter intracellular superoxide production of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) head kidney cells. Fish and Shellfish Immunology 12:131–140.

Secombes, C. J. 1990. Isolation of salmonid macrophages and analysis of their killing activity. Pages 137–154 *in* J. S. Stolen, T. C. Fletcher, D. P. Anderson, B. S. Roberson, and W. B. Van Muiswinkel, editors. Techniques of fish immunology. SOS Publications, Fair Haven, New Jersey.

Siwicki, A. K., D. P. Anderson, and G. L. Rumsey. 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Veterinary Immunology and Immunopathology 41:125–139.

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: an overview of cytokine function. Journal of Animal Science 75:1773–1783.

Verschuere, L., G. Rombaut, P. Sorgeloos, and W. Verstraete. 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews 64:655–671.