EFFECTS OF PREBIOTICS ON GROWTH AND SURVIVAL OF SILVER CATFISH (*Rhamdia quelen*) JUVENILES

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SUMMARY

Recently, proof of the advantages of employing beneficial microorganisms or organic additives as growth promoters, replacing chemoterapeutic and antimicrobial agents, has been obtained. The aim of this work was to evaluate the effect of two experimental diets, one supplemented by a flavonoid-based metabolic antioxidant (FLAVOXIN®) and the other supplemented by a combination of organic acids and yeast wall (UNIWALL MOS 50®) on growth and survival parameters of Rhamdia quelen *juveniles, in contrast to a control diet. The experiment consisted of three dietary treatments: a control consisting of a commercial diet with 280g of proteins/kg and 120g of lipids/kg, the commercial diet supplemented with FLAVOXIN® (FL) and commercial diet supplemented with UNIWALL MOS® (MOS), both products added on a 2g/kg basis. Nine batches consisting of 42 specimens distributed in outdoor cement tanks of 16m³ were assigned according to a 3×3 Latin square design. The experiment lasted a total of 60 days. Weight gain was determined in all treatments, without statistical differences between them ($p>0.05$). The survival rate was 89% for the control diet and 97 and 95% for FL and MOS, respectively. Both FL and MOS treatments resulted superior and statistically different to the control diet ($p<0.05$). The results show that the use of FLAVOXIN® and UNIWALL MOS 50® at the dose employed for this study has a positive effect on survival of* R. quelen *juveniles, without modifying growth parameters.*

Introduction

In the last decade, growth in worldwide fish consumption and decline in the fishing extraction rate from natural watercourses has stimulated the development of aquatic organism production in captivity, with fish farming as one of the fastest growing productive activities (Luchini and Panné Huidobro, 2008; FAO, 2010).

Rhamdia quelen is a commercially relevant freshwater fish both in Argentina and Brazil, mainly reared under semi-intensive and intensive production systems (Baldisserotto and Radünz Neto, 2004; Luchini and Panné Huidobro, 2008). An increase in its production has been observed in the last couple of years, becoming the most cultivated native species in southern Brazil (Silveira and Müller, 2011).

Intensification of production exposes fish to a great variety of stress factors, such as high-density stock and inadequate water quality, which adversely affect the productive performance (Canario *et al*., 1998; Barton, 2002). Therefore, constant stress may result in a decline in growth rate and alimentary efficiency, favoring also the appearance of diseases (Barcellos *et al*., 2004; Baldwin, 2010), and limiting the economic development of aquaculture (Sado *et al*., 2008).

In order to avoid such inconveniencies, several studies have focused on the use of food supplements as tools to promote the welfare of farmed organisms. Among the most widely employed alternatives are the addition of beneficial microorganisms or probiotics and/or their metabolites (organic acids, bacteriocins), prebiotics (oligosaccharides), organic acidifiers, yeast extracts and other growth stimulants. All of them were aimed at identifying alternatives favoring growth (Apún-Molina *et al*., 2009), health (Gatesoupe, 2007) and the capability to resist diseases, replacing chemotherapeutic and antimicrobial agents (Fritts and Waldroup, 2003), which cause numerous adverse effects and participate in the dissemination of crossed resistance in indigenous microbiota and potentially pathogen microorganisms (Balcázar *et al*., 2006; Salinas *et al*., 2008).

Although probiotics have been widely studied in aquaculture, their application is limited due to high costs, potential environment impact and food-processing difficulties. In contrast, the use of prebiotics is rising due to the fact that it is more practical and allows intestinal microbiota management in aquatic organisms (Yousefian and Amiri, 2009; Ringø *et al*., 2010).

Flavonoid prebiotics are natural food additives pres-

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EFECTOS DE PREBIÓTICOS SOBRE EL CRECIMIENTO Y LA SUPERVIVENCIA DE JUVENILES DE *Rhamdia quelen*

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RESUMEN

Recientemente se han obtenido evidencias que demuestran las ventajas de la utilización de microorganismos benéficos o aditivos orgánicos como promotores del crecimiento en reemplazo de agentes quimioterapéuticos y antimicrobianos. El objetivo del presente trabajo fue evaluar el efecto de un alimento suplementado con un reparador metabólico a base de flavonoides (FLAVOXIN®) y otro con una combinación de ácidos orgánicos y pared de levadura (UNIWALL MOS 50®), sobre parámetros de crecimiento y supervivencia en juveniles de Rhamdia quelen*, contrastados con un alimento control. Se utilizaron tres tratamientos alimentarios: control consistente en dieta comercial con 280g de proteínas/kg y 120g de lípidos/kg, dieta comercial suplementada con FLAVOXIN® (FL), y dieta comercial suplementada con UNIWALL MOS® (MOS), ambos productos adicionados a razón de 2g/kg. Nueve lotes de 42 ejemplares distribuidos en estanques de mampostería a cielo abierto de 16m³ fueron asignados de acuerdo a un diseño en cuadrado latino 3×3. La experiencia tuvo una duración total de 60 días, verificándose un incremento de peso en todos los tratamientos alimentarios, aunque sin diferencias estadísticamente significativas entre los mismos (p>0,05). El porcentaje de supervivencia fue de 89% en la dieta control y de 97 y 95% para FL y MOS, respectivamente, resultando ambos tratamientos estadísticamente superiores al control (p<0,05). Los resultados demuestran que el uso de FLAVOXIN® y UNIWALL MOS 50® a la dosis empleada en el presente estudio ejerce un efecto positivo sobre la supervivencia de juveniles de* R. quelen *sin modificar los parámetros de crecimiento.*

EFEITO DE PREBIÓTICOS NO CRESCIMENTO E SOBREVIVÊNCIA DE JUVENIS DE JUNDIÁ, *Rhamdia quelen*

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RESUMO

Em estudos recentes, tem sido provada a vantagem do emprego de microorganismos benéficos ou aditivos orgânicos como promotores de crescimento, substituindo agentes quimioterápicos e antimicrobianos. O objetivo deste trabalho foi avaliar o efeito de duas dietas experimentais, uma suplementada por um reparador metabólico baseada em flavonóides (FLAVOXIN®) e outra suplementada com uma combinação de ácidos orgânicos e parede de levedura (MOS UNIWALL 50®) sobre parâmetros de crescimento e sobrevivência para juvenis de Rhamdia quelen*, em contraste com uma dieta controle. O experimento consistiu em três tratamentos alimentares: controle consistente em dieta comercial contendo 280g de proteínas/kg e 120g de lipídios/kg, dieta comercial suplementada com FLAVOXIN® (FL), e dieta comercial suplementada com UNIWALL MOS® (MOS); ambos os produtos adicionados em base a 2g/kg de ração. Nove lotes de 42 animais foram distribuídos em tanques de cimento de 16m³, atribuídas de acordo com um desenho em quadrado latino 3×3. O experimento teve duração de 60 dias. Ao final do ensaio, foi observado ganho de peso em todos os tratamentos, sem diferença estatística entre eles (p>0,05). A taxa de sobrevivência foi de 89% para a dieta controle e 97 e 95% para FL e MOS, respectivamente. Os tratamentos FL e MOS foram superiores e estatisticamente diferentes ao controle (p<0,05). Os resultados mostram que o uso de FLAVOXIN® e UNIWALL MOS 50® na dose empregada para este estudo tem um efeito positivo na sobrevivência de juvenis de* R. quelen*, sem modificar os parâmetros de crescimento.*

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ent in plants. They have antioxidative, anti-inflammatory and anti-carcinogenic effects (Knekt *et al*., 1997; Nowakowska, 2007; Fariba *et al*., 2009). Several studies on humans have shown that flavonoids can be used therapeutically for their antioxidative actions by reducing free radicals which induces tissue injuries (Barreca *et al*., 2009). Moreover, it is well-known that flavonoids reduce or inhibit the growth of many microorganisms and their toxins (Cushnie and Lamb, 2005; Lee *et al*., 2010).

Non-digestible oligosaccharides are another important group of prebiotics which, administered in animal feed, are metabolized by specific intestinal health-promoter bacteria such as *Lactobacillus* and *Bifidobacterium*. Within this group, inulin, fructooligosaccharides (FOS), short-chain fructooligosaccharides (scFOS) and mannan oligosaccharides (MOS) are of special relevance (Yousefian and Amiri, 2009; Ringø *et al*., 2010). MOS, obtained from the cell wall of *Saccharomyces cerevisiae*, present a favorable effect on intestinal health due to improvements in absorption and immune response modulation in productive species (Genç *et al*., 2007; Dimitroglou *et al*., 2009).

Even though several studies on *R. quelen* nutritional requirements in different categories (Meyer and Fracalossi, 2004; Piedras *et al*., 2006; Tronco *et al*., 2007) or food supplements (Montes-Girao and Fracalossi, 2006; Trombetta *et al*., 2006) have been carried out, there are still few evaluations regarding use of nutritional additives (Souza *et al*., 2009). Therefore, the aim of this work was to evaluate the use of two commercial additives, FLAVOXIN® and UNIWALL MOS 50®, in contrast to control food, on growth and survival parameters for *Rhamdia quelen* juveniles.

Materials and Methods

The study was carried out at the Instituto de Ictiología del Nordeste (INICNE), Facultad de Ciencias Veterinarias - Universidad Nacional del Nordeste, Corrientes, Argentina.

Juveniles from crosses of the own broodstock of INIC-NE, Argentina, were used. Three food treatments were evaluated: 1) Control: a commercial diet with 280g proteins/kg and 120g lipids/kg; 2) FL: a commercial diet supplemented with 2g·kg^{-1} of FLAVOXIN® (Vetanco S.A. Laboratory, Buenos Aires, Argentina); and 3) MOS: a commercial diet supplemented with 2g·kg^{-1} of UNIWALL MOS 50® (Vetanco S.A. Laboratory, Buenos Aires, Argentina). Rations were elaborated by grinding the commercial diet and then mixing it with the corresponding additives, adding water (400g·kg^{-1}) to form a soft paste. The paste obtained was passed through a grinder in order to form pellets 4mm in diameter, which were dried with a heater at 40ºC during 48h, and then kept in a refrigerator at -18ºC during the experimental period. The control diet, without additives, was subjected to the same treatment. The trial was conducted in nine 16m^3 cement ponds, assigned according to a 3×3 Latin square design. Forty two silver catfish juveniles (mean weight and standard deviation of 0.11 ±0.015g) were placed in each pond at a density of 2.62 fish/m^{-3}. Fish were fed once a day, at a 5% biomass ratio, during 60 days. The water from the ponds was fertilized with commercial bales of alfalfa (300g/m^3) one week before the beginning of the trial.

Water quality parameters such as temperature (ºC), oxygen (mg·l^{-1}), conductivity (µS·cm^{-1}) and pH were weekly monitored, using YSI® 55 equipment for temperature and dissolved oxygen and HANNA® equipment for conductivity and pH. The obtained mean values and standard deviations for water quality variables were 18.8 ±3.20ºC; 9 ±1.8mg·l^{-1}; 114.8 ±4.9µS·cm^{-1}; and 7.32 ±0.42, for temperature, dissolved oxygen, conductivity and pH, respectively. No significant differences were observed (p>0.05) regarding water quality parameters among the different treatments during the experimental period.

Measured parameters and statistical analysis

At the end of the experiment, final mean weight (MW), absolute growth rate (AGR), specific growth rate (SGR), survival rate (S) and final biomass (FB) were calculated as MW (g)= W_t/N, where W_t: total weight (g) per specimen and N: number of surviving individuals; AGR= 100((W_f-W_i)/T), where W_f : final mean weight (g) and W_i: initial mean weight (g) for given period T; SGR= 100(($\ln W_f$-$\ln W_i$)/T), where $\ln W_f$ and $\ln W_i$ are the natural logarithms of W_f (g) and W_i (g) for a given period T; S(%)= 100(N/N_i), where N_i: initial number of fish; and FB= MW×N.

The results from the *R. quelen* feeding trial were analyzed through a three-way analysis of variance (ANOVA) without interaction, according to a Latin square design. This design was chosen to eliminate the effect of environmental variables associated to the localization of the ponds (light and wind incidence). Comparisons of treatment means were later carried out through the Tukey test (p<0.05). Data were analyzed using Statistica software for Windows, version 6.0.

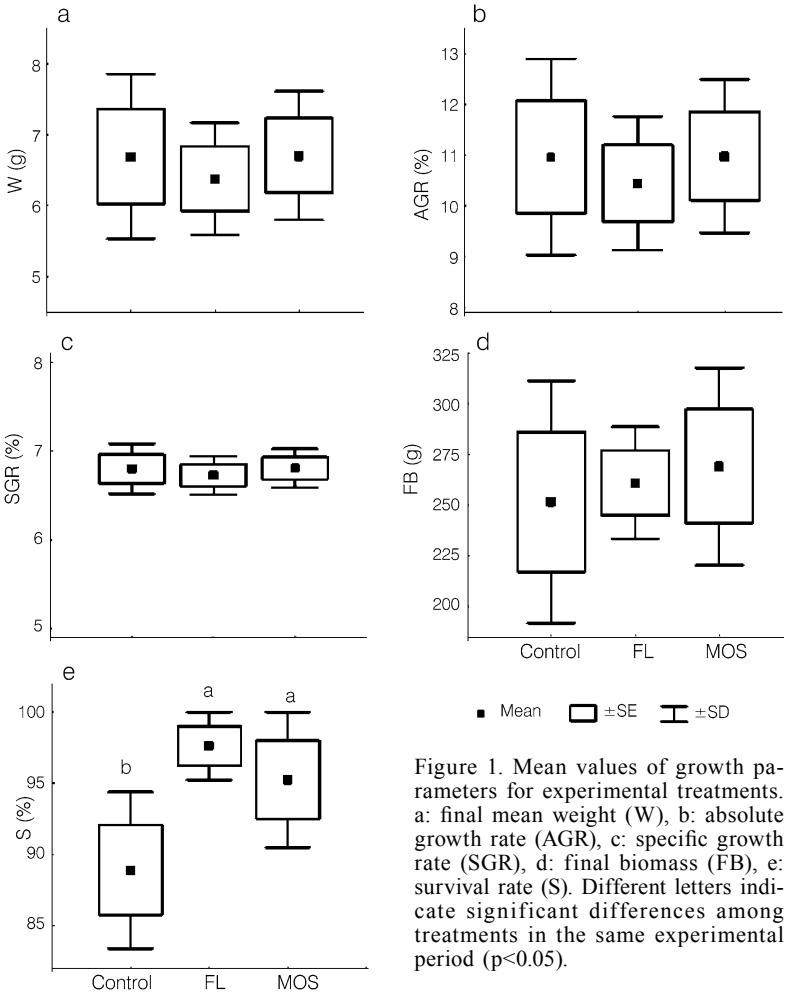

Figure 1. Mean values of growth parameters for experimental treatments. a: final mean weight (W), b: absolute growth rate (AGR), c: specific growth rate (SGR), d: final biomass (FB), e: survival rate (S). Different letters indicate significant differences among treatments in the same experimental period (p<0.05).

Results and Discussion

At the end of the experiment, all the feeding groups presented a similar weight (p>0.05), reaching values of 6.74, 6.37 and 6.72g in the control, FL and MOS groups, respectively (Figure 1a). No significant differences were found among the groups for the rest of the analyzed growth variables (AGR, SGR and FB; p>0.05; Figures 1b-d). The same analysis, carried out on survival values, allowed the detection of significant differences among treatments, with 97% and 95% as estimated averages for the FL and MOS groups, respectively, which did not differ between them (p>0.05) but showed a statistically significant higher than that of the control group (89%; p<0.05; Figure 1e).

Flavonoids, as well as MOS, are commonly used as additives in feed rations for fish and crustaceans (Genç *et al*., 2007; Yilmaz *et al*., 2007; Zhou *et al*., 2010) in order to improve growth, sanitary conditions or pathogen resistance (Ardó *et al*., 2008; Citarasu, 2010), but results have been inconsistent (Peterson *et al*., 2010).

In agreement with the results from the present study, Peterson *et al*. (2010) did not observe improvement regarding weight gain in juvenile channel catfish (*Ictalurus punctatus*) by adding Bio-MOS® in similar percentages to those used in this study, but did observe a higher survival rate as compared to a control diet. Dimitroglou *et al*. (2010) evaluated the addition of two concentrations of MOS (2 and 4g·kg^{-1}) for *Sparus aurata* juveniles, observing that the final mean weight, the specific growth rate, the conversion index and the protein efficiency percentage were not affected by the use of this supplement as compared to a control diet. Moreover, Sado *et al*. (2008) reported that Nile tilapia juveniles (*Oreochromis niloticus*) presented a decrease in daily food consumption and growth rate, which was proportional

to the addition of MOS to the diet from 2 to 10g·kg^{-1}.

Shin *et al*. (2010) found higher weights in olive flounders, *Paralichthys olivaceus*, by feeding them with quercetin flavonoids at 5g·kg^{-1}, reporting also a hypocholesterolemic, antioxidative effect and a rise in the lysozyme activity as compared to the control group, suggesting that this flavonoid improves the immune response against external stress. Moreover, several researchers (Staykov *et al*., 2007; Yilmaz *et al*., 2007; Dimitroglou *et al*., 2009) obtained improvements in the growth response of rainbow trout (*Oncorhynchus mykiss*) by feeding them with mannan-MOS in the diet at different levels of supplementation (0.5 to 4.5g·kg^{-1}). In addition, Mazlum *et al*. (2011) found greater growth in *Astacus leptodactylus* juveniles fed with MOS (3g·kg^{-1}), while the survival rate was not affected. According to Peterson *et al*. (2010) there is a significant variation regarding the effects of derivative mannan oligosacharides in diets on growth efficiency, which probably depends upon the species, feeding duration and level of inclusion in the food, as well as type of yeast used.

In the present study, *R. quelen* juveniles fed with a ration supplemented with FLAVOXIN® and UNIWALL MOS 50® (2g·kg^{-1}) showed no improvement in any of the growth parameters as compared with a control diet. These results would indicate that the dose employed had no influence over growth in the animal category used. Besides, low-density breeding conditions would not entail the inconveniences habitually observed in intensive or over-intensive farming, where a beneficial effect of the additives on fish growth could be expected.

Diet and nutritional conditions are fundamental factors for fish farming exploitation due to the fact that they determine the physiological conditions of the animals, with a direct influence on energetic conditions, and an indirect one through influence on stress tolerance (Blazer, 1992). Thus, an adequate diet may favor growth, alimentary efficacy, immunologic condition and physiology of the fish, through immune system improvement and enhancement of use of nutrients (Staykov *et al*., 2007).

Conclusion

The results from this work show that the use of FLAVOXIN® and UNIWALL MOS 50® at a 2g·kg^{-1} ratio does not modify growth parameters, while it determines improvements in survival rate. These results need to be studied in depth in order to further adjust the use of these additives in diets for *R. quelen* juveniles.

REFERENCES

Apún-Molina JP, Santamaría-Miranda A, Luna-González A. Martínez-Díaz SF, Rojas-Contreras M (2009) Effect of potential probiotic bacteria on growth and survival of tilapia *Oreochromis niloticus* L., cultured in the laboratory under high density and suboptimum temperature. *Aquacult. Res. 40*: 887-894.

Ardó L, Yin G, Xu P, Váradi L, Szigeti G, Jeney Z, Jeney G (2008) Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture 275*: 26-33.

Balcázar JL, De Blas I, Zarzuela-Ruiz I, Cunningham D, Vendrell D, Múzquiz JL (2006) The role of probiotics in aquaculture (Review). *Vet. Microbiol. 114*: 173-186.

Baldisserotto B, Radünz Neto J (2004) *Criação de Jundiá*. Santa Maria. Brasil. 232 pp.

Baldwin L (2010) The effects of stocking density on fish welfare. *Plymouth Stud. Scient. 4*: 372-383.

Barcellos LJG, Kreutz LC, Quevedo RM, Fioreze I, Cericato L, Benck Soso A, Fagundes M, Conrad J, Krammer Baldissera R, Bruschi A, Ritter F (2004) Nursery rearing of jundiá, *Rhamdia quelen* (Quoy & Gaimard) in cages: cage type, stocking density and stress response to confinement. *Aquaculture 232*: 383-394.

Barreca D, Lagana G, Tellone E, Ficarra S, Leuzzi U, Galtieri A, Bellocco E (2009) Influences of flavonoids on erythrocyte membrane and metabolic implication through anionic exchange modulation. *J Membr. Biol. 230*: 163-171.

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr. Compar. Biol. 42*: 517-525.

Blazer VS (1992) Nutrition and disease resistance in fish. *Annu. Rev. Fish Dis. 2*: 309-323.

Canario A, Condeça J, Power DM, Ingleton PM (1998) The effect of stocking density on growth in the gilthead sea-bream, *Sparus aurata* (L.). *Aquacult. Res. 29*: 177-181.

Citarasu T (2010) Herbal biomedicines: a new opportunity for aquaculture industry. *Aquacult. Int. 18*: 403-414.

Cushnie TPT, Lamb AJ (2005) Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents 26*: 343-356.

Dimitroglou A, Merrifield DL, Moate R, Davies SJ, Spring P, Sweetman J, Bradley G (2009) Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Anim. Sci. 87*: 3226-3234.

Dimitroglou A, Merrifield DL, Spring P, Sweetman J, Moate R, Davies SJ (2010) Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture 300*: 182-188.

FAO (2010) *El Estado Mundial de la Pesca y la Acuicultura* (SOFIA). Departamento de Pesca y Acuicultura. FAO. Roma, Italia. 219 pp.

Fariba S, Gholamreza DN, Mansour M (2009) Major flavonoids with antioxidant activity from L. *Food Chem. 112*: 885-888.

Fritts CA, Waldroup PW (2003) Evaluation of Bio-Mos mannan oligosaccharide as a replacement for growth promoting antibiotics in diets of turkeys. *Int. J. Poultry Sci. 2*: 19-22.

Gatesoupe FJ (2007) Live yeasts in the gut: Natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture 267*: 20-30.

Genç MA, Aktas M, Genç E, Yilmaz E (2007) Effects of dietary mannan oligosaccharide on growth, body composition and hepatopancreas histology of *Penaeus semisulcatus* (de Haan 1844). *Aquacult. Nutr. 13*: 156-161.

Knekt P, Jarvinen R, Seppanen R, Helibvaara M, Teppo L, Pukkala E, Aromaa A (1997) Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am. J. Epidemiol. 146*: 223-230.

Lee K-A, Moon SH, Kim K-T, Mendonca AF, Paik H-D (2010) Antimicrobial effects of various flavonoids on *Escherichia coli* O157:H7 cell growth and lipopolysaccharide production. *Food Sci. Biotechnol. 19*: 257-261.

Luchini L, Panné Huidobro S (2008) *Perspectivas en Acuicultura: Nivel Mundial, Regional y Local*. Dirección de Acuicultura -Subsecretaría de Pesca y Acuicultura- SAGPyA. Argentina. 99 pp.

Mazlum Y, Yılmaz E, Genc MA, Guner O (2011) A preliminary study on the use of mannan oligosaccharides (MOS) in freshwater crayfish, *Astacus leptodactylus* Eschscholtz, 1823 juvenile diets. *Aquacult. Int. 19*: 111-119.

Meyer G, Fracalossi DM (2004) Protein requirement of jundia fingerlings, *Rhamdia quelen*, at two dietary energy concentrations. *Aquaculture 240*: 331-343.

Montes-Girao PJ, Fracalossi DM (2006) Dietary lysine requirement as basis to estimate the essential dietary amino acid profile for jundiá, *Rhamdia quelen*. *J. World Aquacult. Soc. 37*: 388-396.

Nowakowska Z (2007) A review of anti-infective and anti-inflammatory chalcones. *Eur. J. Med. Chem. 42*: 125-137.

Peterson BC, Bramble TC, Manning BB (2010) Effects of Bio-Mos® on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. *J. World Aquacult. Soc. 41*: 149-155.

Piedras SRN, Pouey JLF, Moraes PRR, Rodrigues FV (2006) Resposta de alevinos de jundiá (*Rhamdia* sp.) alimentados com diferentes níveis de

proteína bruta e energia digestível. *Rev. Brás. Agrociênc. 12*: 217-220.

Ringø E, Olsen RE, Gifstad TØ, Dalmo RA, Amlund H, Hemre GI, Bakke AM (2010) Prebiotics in aquaculture: a review. *Aquacult. Nutr. 16*: 117-136.

Sado RY, De Almeida Bicudo AJ, Possebon Cyrino JE (2008) Feeding dietary mannan oligosaccharides to juvenile Nile tilapia, *Oreochromis niloticus*, has no effect on hematological parameters and showed decreased feed consumption. *J. World Aquacult. Soc. 39*: 821-826.

Salinas I, Abelli L, Bertoni F, Picchietti S, Roque A, Furones D, Cuesta A, Meseguer J, Esteban MÁ (2008) Monospecies and multispecies probiotic formulations produce different systemic and local immunostimulatory effects in the gilthead seabream (*Sparus aurata* L.). *Fish Shellfish Immunol. 25*: 114-123.

Shin HS, Yoo JH, Min TS, Lee KY, Choi CY (2010) The effects of quercetin on physiological characteristics and oxidative stress resistance in olive flounder, *Paralichthys olivaceus*. *Asian-Austr. J. Anim. Sci. 23*: 588-597.

Silveira F, Müller F (2011) *Síntese informativa da atividade de piscicultura de água doce 2011*. Centro de Desenvolvimento em Aqüicultura e Pesca (Cedap). Epagri. Florianópolis, Brasil. 8 pp.

Souza DM, Martins GB, Piedras SRN, Pouey JLOF, Robaldo RB, Leite FPL (2009) Potencial probiótico de *Bacillus cereus* variedade Toyoi e *Saccharomyces boulardii* no cultivo de *Rhamdia quelen*. In *2ª Conf Latinoam. Sobre Cultivo de Peces Nativos*. Chascomús, Argentina. p. 58.

Staykov Y, Spring P, Denev S, Sweetman J (2007) Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Int. 15*: 153-161.

Trombetta CG, Radünz Neto J, Lazzari R (2006) Suplementação vitamínica no desenvolvimento de larvas e jundiá (*Rhamdia quelen*). *Ciênc. Agrotecnol. 30*: 1224-1229.

Tronco AP, Radünz Neto J, Medeiros TS, Lima RL (2007) Alimentação de larvas de jundiá (*Rhamdia quelen*) com dietas semipurificadas e fontes lipídicas. *Bol. Instit. Pesca 33*: 9-17.

Yilmaz E, Genc MA, Genc E (2007) Effects of dietary mannan oligosaccharides on growth, body composition, and intestine and liver histology of rainbow trout, *Oncorhynchus mykiss*. *Isr. J. Aquacult-Bamid 59*: 182-188.

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

Zhou QC, Buentello JA, Gatlin III DM (2010) Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). *Aquaculture 309*: 253-257.