**UNIVERSIDADE FEDERAL DE SANTA MARIA**
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**PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA**

# POTENCIAL PREBIÓTICO DE DIFERENTES CONCENTRADOS DE FIBRA ALIMENTAR NA DIETA DE JUVENÍS DE JUNDIÁ *(Rhamdia quelen)*

**TESE DE DOUTORADO**

**Fernanda Rodrigues Goulart**

**Santa Maria, RS, Brasil**

**2015**

# POTENCIAL PREBIÓTICO DE DIFERENTES CONCENTRADOS DE FIBRA ALIMENTAR NA DIETA DE JUVENÍS DE JUNDIÁ *(Rhamdia quelen)*

**por**

**Fernanda Rodrigues Goulart**

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Zootecnia, Área de Concentração em Produção Animal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutora em Zootecnia.**

**Orientadora: Profª Drª. Leila Picolli da Silva**

**Santa Maria, RS, Brasil**

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elaborada por
**Fernanda Rodrigues Goulart**

Como requisito parcial para a obtenção do grau de
**Doutora em Zootecnia**

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**João Radünz Neto, Dr.** (UFSM)

Santa Maria, 6 de Fevereiro de 2015

*Dedico*

*Aos meus pais, Birajar (in memorian) e Clarice, com muito carinho, pelo exemplo de força e coragem.*

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*"Nenhum obstáculo é grande demais quando confiamos em Deus"*
*(Aristóteles)*

*"No meio de qualquer dificuldade encontra-se a oportunidade" (Albert Einstein)*

## RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Zootecnia
Universidade Federal de Santa Maria

**POTENCIAL PREBIÓTICO DE DIFERENTES CONCENTRADOS DE FIBRA ALIMENTAR NA DIETA DE JUVENÍS DE JUNDIÁ *(Rhamdia quelen)***

AUTORA: FERNANDA RODRIGUES GOULART
ORIENTADORA: LEILA PICOLLI DA SILVA
Data e Local da Defesa: Santa Maria, 06 de Fevereiro de 2015.

O uso tradicional de antibióticos na aquicultura como promotores de crescimento tem sido limitado em função dos efeitos negativos promovidos por estes medicamentos. Como alternativa ao uso destas drogas, tem se buscado a manipulação da microbiota do trato gastrointestinal dos animais aquáticos através da utilização de oligossacarídeos e de fibras alimentares com potencial prebiótico. Neste sentido, este estudo teve como objetivo aplicar metodologias para obtenção de diferentes Concentrados de Fibras Alimentares (CFAs) = Mucilagem (MG); Pectina (PN) e β-Glicana+Mananas (βG+M) e avaliar o potencial prebiótico destes suplementos na dieta de juvenis de jundiá (*Rhamdia quelen*). A determinação da composição nutricional dos ingredientes revelou que os componentes predominantes em todos os CFAs obtidos foram fibra alimentar total e fibra insolúvel. O CFA que apresentou maior rentabilidade de extração foi a βG+M (19,81%±8,54), seguida da Pectina (14,54%±2,72) e Mucilagem (7,18%±1,54). A composição da Mucilagem e Pectina obtiveram maior diversidade de monossacarídeos, já a βG+M consistiu basicamente de manose (74,5%) e glicose (24,3%). A suplementação dos CFAs na dieta de jundiás foi avaliada durante oito semanas, através de estudo de crescimento, deposição corporal de nutrientes, enzimas digestivas, parâmetros bioquímicos e metabólicos, resposta ao estresse e imunológica e morfometria intestinal. Os jundiás suplementados com os CFAs obtiveram crescimento superior em relação ao grupo controle e similar aos animais suplementados com 5 g $kg^{-1}$ de prebiótico comercial (PC 5). A maioria dos parâmetros somáticos e de composição centesimal de peixe inteiro foram influenciados pela suplementação dos CFAs. A suplementação de Pectina promoveu menor atividade das enzimas digestivas em relação ao grupo controle. Os animais suplementados com os CFAs obtiveram alterações positivas nos parâmetros bioquímicos avaliados. Além disso, os jundiás não mostraram resposta à aplicação do agente estressor, mantendo os níveis de cortisol basal. Os peixes suplementados com os CFAs obtiveram maiores estoques de glicogênio hepático em relação ao grupo controle. Além do mais, a suplementação com os CFAs promoveu aumento na altura de vilos intestinais dos jundiás. Porém, estes valores foram menores em relação aos animais do grupo PC 5. Para espessura do epitélio (EE) esta variável foi maior no grupo Controle comparado aos animais suplementados com β-glicana + Manana.



**Palavras-chave**: Promotor de crescimento. Mucilagem. Pectina. β-glicana+manana. Peixes.

## ABSTRACT

Animal Science Doctoral Thesis
Post-Graduate Program in Animal Science
Federal University of Santa Maria

**POTENTIAL PREBIOTIC OF DIFFERENT DIETARY FIBER CONCENTRATES IN FEED OF JUVENILES JUNDIÁ (*Rhamdia quelen*)**

AUTHOR: Fernanda Rodrigues Goulart
ADVISER: Leila Picolli da Silva
Date and Defense Place: Santa Maria, February 06th, 2015.

The traditional use of antibiotics in aquaculture as growth promoters has been limited due to the negative effects caused by these drugs. As an alternative to the use of these drugs has been sought manipulation of the microbiota of the gastrointestinal tract of aquatic animals through the use of oligosaccharides and dietary fibers with prebiotic potential. Thus, this study aimed to apply different methodologies to obtain Dietary Fiber Concentrates (DFC) = mucilage (MG); pectin (PN) and β-glucan + manan (βG + M) and evaluate the prebiotic potential of these supplements in the diet of juvenile jundiá (*Rhamdia quelen*). The determination of the nutritional composition of the ingredients revealed that the predominant component in all DFCs were dietary fiber and insoluble fiber. The DFC that had higher extraction yield was βG + M (19.81 ± 8.54%), followed by pectin (14.54% ± 2.72), and mucilage (7.18 ± 1.54%). The composition of mucilage and pectin had a greater diversity of monosaccharides, since the βG+M consisted primarily of mannose (74.5%) and glucose (24.3%). The supplementation of DFC in jundiás diet was assessed for eight weeks through study of growth, body nutrient deposition, digestive enzymes, biochemical and metabolic parameters, responses to stress and immune and intestinal morphology. The jundiás supplemented with DFCs achieved higher growth than the control group and similar to animals supplemented with 5 g $kg^{-1}$ commercial prebiotic (CP 5). Most somatic parameters and whole fish proximate composition were influenced by supplementation of DFCs. The supplementation of pectin promoted lower activity of digestive enzymes in relation the control group. The animals supplemented with DFC obtained positive changes in biochemical parameters. Furthermore, jundiás showed no response to application of the stressor, maintaining basal cortisol levels. The fish supplemented with DFCs had higher hepatic glycogen stores in relation the control group. Moreover, supplementation with DFCs increased the height of intestinal villi of jundiá. However, these values were lower for the animals of the group PC 5. For thickness of the epithelium this variable was higher in the control group compared to animals supplemented with β-glucan+Manana.



**Keywords**: Growth promoter. Mucilage. Pectin. β-glucan+mannan. Fish.

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**ESTUDO 1 – "Obtaining of Dietary Fiber Concentrates with prebiotic potential for use in fish nutrition"**



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**ESTUDO 4 – "Effect of Dietary Fiber Concentrates on growth performances, gut morphology and hepatic metabolic intermediates in jundiá (*Rhamdia quelen*)"**

## LISTA DE ILUSTRAÇÕES

**ESTUDO 1 – "Obtaining of Dietary Fiber Concentrates with prebiotic potential for use in fish nutrition"**



**ESTUDO 3 – "Effect of supplementation of Dietary Fiber Concentrates on biochemical parameters, stress response, immune response and skin mucus of jundiá (*Rhamdia quelen*)"**

## LISTA DE ANEXOS

# SUMÁRIO

# 1 INTRODUÇÃO

A produção mundial de pescado tem aumentado a taxa média anual de 3,2% nas últimas cinco décadas, impulsionada pela combinação do crescimento populacional, aumento de renda, urbanização e canais de distribuição mais eficientes (FAO, 2014). Muitas foram as estratégias adotadas para garantir este aumento de produção e sanidade criatória, entre as quais, o emprego de antibióticos como promotores de crescimento nas rações e agentes terapêuticos e profiláticos (REVERTER et al. 2014). No entanto, o uso destas moléculas torna-se cada vez mais restrito devido ao seu potencial para desenvolvimento de bactérias resistentes, comprometimento ao meio ambiente, supressão do sistema imune do animal e risco de bioacumulação no pescado (RINGO et al., 2010). Neste âmbito, uma nova geração de ingredientes ambientalmente corretos, onde incluem-se os prebióticos, vem ganhando destaque como alternativa ao uso dos antibióticos.

Os prebióticos consistem de um grupo complexo de polímeros de polissacarídeos não amiláceos e oligossacarídeos que associados a outros componentes, são resistentes à digestão enzimática no trato gastrointestinal de animais não ruminantes, servindo como substrato para fermentação bacteriana benéfica, agindo positivamente sobre o equilíbrio da microflora intestinal (BACH KNUDSEN, 2001; MONTAGNE et al., 2003; THEUWISSEN; MENSINK, 2008). Os prebióticos são metabolizados principalmente por *Lactobacillus* e *Bifidobacterium*, que inibem a proliferação de agentes patogênicos no trato digestório e aumentam a produção de ácidos graxos de cadeia curta, responsáveis por estimular o crescimento da mucosa intestinal e reduzir a translocação de endotoxinas nos enterócitos, contribuindo com melhorias sobre o desempenho e saúde do hospedeiro (GULLÓN et al., 2011; SONG et al., 2014).

A maioria das formulações comerciais de prebióticos atualmente disponíveis no mercado e de ação comprovada em peixes são compostas de oligossacarídeos de galactose, frutose ou manose (TORRECILLAS et al., 2007; HELLAND et al., 2008; SOLEIMANI et al., 2012; GANGULY et al., 2013; HOSEINIFAR et al., 2013).

No entanto, a ação prebiótica também é intensamente observada em fontes de polímeros mais complexos, como os polissacarídeos não amiláceos insolúveis e solúveis, que compõem parte majoritária da fibra alimentar dos vegetais e resíduos agroindustriais (GOULART et al. 2013). Os polissacarídios não amiláceos apresentam grande diversidade de estrutura química e grau de polimerização, o que se reflete sobre suas características físico químicas e de fermentabilidade. Este fato abre precedentes para o desenvolvimento de novos produtos prebióticos com amplo potencial tecnológico, mas ainda pouco explorados na nutrição de peixes (GULLÓN et al., 2011).

Em paralelo ao crescimento da aquicultura, o Brasil se desenvolve notavelmente no setor agroindustrial, gerando volumes crescentes de resíduos (bagaços, sobras de fermentação, tortas, farelos, etc) com elevado teor de fibras alimentares, os quais exigem estudos de aplicabilidade, a fim de não se tornarem entraves para a sustentabilidade das cadeias produtivas.

Nosso País detêm 50% da produção mundial de suco de laranja (NEVES et al., 2010), o que gera quantidade expressiva de resíduos sólidos (de 100 kg de laranjas utilizadas para produção de suco são gerados 49,2 kg de farelo de polpa cítrica) que normalmente são destinados a suplementação de ruminantes (ALEXANDRINO et al., 2007). No entanto, esta fonte pode deixar de ser resíduo e tornar-se matéria-prima para obtenção de cadeias pécticas de elevado grau de solubilidade e amplamente fermentadas (GULLÓN et al., 2011; CANTERI et al., 2012), sendo excelente alternativa para uma nova geração de prebióticos.

O Brasil também se encontra entre os maiores produtores mundiais de cerveja, gerando grandes quantidades de resíduos sólidos como o bagaço de malte e a levedura (*Saccharomyces cerevisiae*) (SANTOS, 2005). Estes resíduos devem receber atenção especial em relação a sua destinação, pois podem ocasionar sérios problemas ambientais, devido à sua elevada carga poluidora (KLAGENBOECH et al., 2011). Em trabalho realizado por Brochier e Carvalho (2009) foi verificado que a quantidade de resíduos gerados é 32,02% superior a quantidade de cevada utilizada como matéria prima inicial para a produção de cerveja. O resíduo de levedura de cervejaria é constituído de 15 a 30% de parede celular (FLEURI; SATO, 2005; MAGNANI; CASTRO-GÓMEZ, 2008), onde predominam as β-glicanas e as α-mananas, que são amplamente empregadas na nutrição animal como prebióticos ou imunossacarídeos (SCHORER et al., 2009; DIMITROGLOU et al., 2010, SCHWARZ

et al., 2010; ZHAO et al., 2012; SONG et al., 2014). As β-glicanas apresentam habilidades para estimular os mecanismos de defesa do hospedeiro, além de demonstrar efeitos antitumoral, antinflamatório, antimutagênico e proteção contra infecções (MAGNANI; CASTRO-GOMÉZ, 2008). Já as α-mananas são responsáveis por aumentar a imunidade inata e resistência a doenças nos peixes (SONG et al., 2014).

A linhaça (*Linum usitatissimum L*.) é outra fonte rica em nutrientes, composta por aproximadamente, 40% de óleo, 20% de proteína e 30% de fibra alimentar (TARPILA et al., 2005; MORRIS, 2007), apresentando amplo potencial tecnológico ainda pouco explorado. Estudos apontam que a extração de fibras solúveis em meio aquoso dos grãos *in natura* de linhaça, elevam em 30% a eficiência de extração de óleo (SPERONI et al., 2010), além de originar um farelo proteíco parcialmente demucilado e de aplicação factível na nutrição de peixes (GOULART et al., 2013). A fibra remanescente desse processo conhecida como mucilagem é uma excelente candidata a ser empregada na nutrição animal como promotor de crescimento, pois apresenta composição monossacarídica rica em xilose, galactose e arabinose (FEDENIUK; BILIADERIS, 1994; OOMAH et al., 1995; QIAN et al., 2012), compostos que vem sendo amplamente empregados como aditivos na nutrição de peixes, em razão de contribuírem com o crescimento de bifidobactérias em nível intestinal (RINGO et al., 2010).

Para a piscicultura, são escassos os estudos sobre os efeitos biológicos e metabólicos de fibras alimentares, sobretudo abordando o caráter prebiótico destes constituintes alimentares. Este fato leva a uma ampla possibilidade de estudos, onde fontes agroindustriais subutilizadas ou descartadas no meio ambiente poderão ser racionalmente exploradas como matéria prima para obtenção de agentes que auxiliam na melhora do desempenho animal, manutenção da saúde e na segurança alimentar do pescado ofertado ao mercado consumidor.

## 1.1 Objetivos

### 1.1.1 Objetivo geral

Obtenção de Concentrados de Fibra Alimentar de ação prebiótica a partir de fontes agroindustriais e sua aplicação na nutrição de jundiás *(Rhamdia quelen);*

### 1.1.2 Objetivos Específicos

- Aplicar técnicas físicas e/ou químicas para concentração de fibra alimentar a partir de matérias primas agroindustriais;
- Caracterizar química e fisicamente as fontes agroindustriais e os Concentrados de Fibras Alimentares obtidos;
- Adicionar os Concentrados de Fibras Alimentar em rações para jundiás, avaliando seus respectivos efeitos sobre o desempenho produtivo, composição química corporal e enzimas digestivas;
- Avaliar o efeito protetor contra ao estresse agudo sobre parâmetros bioquímicos e imunológicos de jundiás alimentados com dietas suplementadas com diferentes Concentrados de Fibras Alimentares;
- Avaliar o efeito dos Concentrados de Fibras Alimentares sobre parâmetros metabólicos hepáticos e morfometria intestinal de jundiás.

O presente estudo foi desenvolvido em duas fases, sendo que a primeira consistiu na obtenção dos Concentrados de Fibra Alimentar e análises nutricionais e a segunda fase na condução de ensaio biológico. Os resultados estão apresentados na forma de estudos científicos, em que o estudo 1 corresponde a obtenção dos Concentrados de Fibras Alimentares (CFAs) e caracterização da composição química. O efeito da suplementação dos CFAs sobre o desempenho zootécnico e atividade de enzimas digestivas corresponde ao estudo 2. Os efeitos sobre parâmetros bioquímicos, resposta ao estresse e imunológica estão presentes no estudo 3. O estudo 4 é referente aos efeitos sobre morfometria intestinal e parâmetros hepáticos.

# 2 ESTUDO CIENTÍFICO 1

**Obtaining of Dietary Fiber Concentrates with prebiotic potential for use in fish nutrition**

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Keywords: Agroindustrial sources. Pectin. Mucilage. β-glucan+mannan. Growth promoter.

Obtain alternative prebiotics for fish nutrition

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**ABSTRACT:** This study aimed to apply methodologies for concentration of dietary fiber and evaluate their respective nutritional composition and physical chemistry. The Dietary Fiber Concentrates (DFCs) = mucilage, pectin, βglicana + Mannan (βG+M) were obtained from flaxseed, citrus pulp and brewer's yeast (*Saccharomyces cerevisiae*), respectively, through different physical processes chemicals. The nutritional composition determination revealed that the predominant component in all DFCs were dietary fiber and insoluble fiber. The DFC that obtained most extraction yield was βG + M (19.81% ± 8.54), followed by pectin (14.54% ± 2.72) and mucilage (7.18% ± 1.54). The composition of mucilage and pectin have a greater diversity of monosaccharides, already the βG+M consisted primarily of mannose (74.5%) and glucose (24.3%). The pectin showed numerically lower hydration capacity than the other DFCs. For the fat binding ability, all DFCs had similar values. In this study, the DFCs presented nutritional and technological characteristics that indicate potential application of these sources as a prebiotic for supplementation of fish.

## INTRODUCTION

For a long time, dietary fibers represented the inert portion of food because of their low energy content. However, the interest in dietary fibers has been increasing constantly in recent decades, due to their beneficial effects on the microflora of the gastrointestinal tract (Back Knudsen 2001; Wenk 2001; Montagne et al. 2003).

Dietary fibers are formed by non-starch polysaccharides, among which we highlight, cellulose, hemicellulose, pectins, gums, mucilages, β-glucans, among others (Chen et al. 1988; Mudgil and Barak 2013). These components have been receiving a great deal of attention because of their prebiotic properties. This is because they are not digested in the intestine and remain intact when they reach the colon, where metabolized by beneficial bacteria, which alter the colonic microflora, leading to a healthy bacterial microflora capable of inducing important physiological effects on the health and well-being of the host (Catalani et al. 2003).

In the last five decades, the cultivation of aquatic organisms has increased steadily (FAO 2014). Thus, several strategies have been adopted to ensure an increase in production and stock breeding health, including the use of antibiotics as growth promoters in feeds. However, the use of these products has been restricted due to their potential for development of resistant bacteria, hazards to the environment, suppression of the immune system of the animals and risk of bioaccumulation in fish (Ringo et al. 2010). For these reasons, the use of alternative growth promoters in feeds, particularly prebiotics, is currently recommended. Moreover, adding prebiotics in fish nutrition is also important because they are substances rather than living organisms; thus, they are more resistant to processing, as well as extrusion and pelletization (Névoa et al. 2012).

Considering that knowledge of the biological and functional properties of dietary fibers has recently led to the development of methods of obtaining these compounds for use in animal nutrition, this study aimed to apply methodologies to obtain Dietary Fiber Concentrates from agro-industrial sources and determine a nutritional and physicochemical characterization of their components.

## MATERIALS AND METHODS

This study was conducted in the Fish Farming Laboratory, Department of Animal Science of the Santa Maria Federal University, where the nutritional properties of agro-industrial sources were evaluated and methodologies for obtaining different Dietary Concentrate Fiber products were applied.

### RAW MATERIAL

Biomass of yeast (*Saccharomyces cereviseae*) was kindly provided by Santamate Industria e Comercio Ltda, located in Santa Maria-RS. Flaxseed was donated by Giovelli & Cia Ltda (Vegetable Oil Industry). The citrus pulp, comprised of rind or flavedo, albedo, membranes and seeds, was processed in the Fish Farming Laboratory of the Federal University of Santa Maria.

## OBTAINING OF DIETARY FIBER CONCENTRATES (DFC)

### Mucilage

The extraction procedure was based on the methodology described by Goulart et al. (2013). The mucilage was extracted from flaxseed in an aqueous medium at a concentration of 10% w/v, at 60 to 80° C with constant stirring, for 150 minutes. The supernatant was removed and 93% ethanol (final alcohol concentration of 75%) was added in order to precipitate the fiber. The precipitate was collected and dried in an forced air-circulation oven at 60º C for 24 hours, and pulverized in a micro mill.

### Pectin

Pectin was obtained from the citrus pulp, according to the methodology described by Calliari (unpublished data). Before extraction, the orange juice residue was washed with cold water, crushed manually and dried in an oven at 50° C for 24 hours, then ground in a micro mill grinder to obtain the dried citrus pulp. Pectin extracted from citrus pulp in an aqueous medium at a concentration of 8% (w/v) under an average temperature of 100° C for 1 hour. After cooling, the mixture was centrifuged (3500 rpm/10 min) and 93% ethanol was added to the supernatant at a ratio of 1:1 to precipitate the pectin. This mixture was left to stand at 5° C for 24 hours, so that pectin was precipitated. This DFC was dried in an air circulation oven at 55° C for 24-48 hours and ground in a micro mill grinder for 20 seconds.

### β-glucans+mannans

β-glucans+mannans were obtained from brewer's yeast *(Saccharomyces cerevisiae*) according to the methodology described by Matiazi (unpublished data) and Chaud et al. (2007),

with some modifications. Aqueous yeast extract was centrifuged at 3500 rpm and washed three times in distilled water in a 1:1 (w/v) ratio. After that, it was subjected to autolysis at 49° C in a water bath for 8 hours. The extract was recentrifuged at 3500 rpm, and the supernatant was discarded, leaving only the cell wall. This fraction received alkaline treatment with NaOH 1% (1:3 w/v) and heating in a water bath at 75°C with constant agitation for twenty minutes. Subsequently, it was neutralized with 2N HCl, centrifuged at 3500 rpm and washed three times in distilled water in a 1:1 (w/v) ratio. The precipitate was represented by the β-glucans+mannans fraction, which was dried at 40 °C for 24 hours.

## NUTRIENT ANALYSIS

Flaxseed, mucilage, dried citrus pulp, pectin, brewer's yeast (*Saccharomyces cerevisiae)* and β-glucan+mannan were analyzed for chemical composition. The measurements of dry matter (DM - 105 ± 2° C/24 hours), ash (550° C / 6 hours) and crude protein (CP - determination of nitrogen by the micro-Kjeldahl method - N x 6.25) were performed according to the methods described by AOAC (1995). Residual fat present in the ingredients was extracted according to Bligh and Dyer (1959). Total dietary fiber (TDF) and insoluble fiber (IF) contents were determined according to the enzymatic gravimetric method number 991.43, based on AOAC, and soluble fiber (SF) was determined by the difference between them.

### Determination of monosaccharide composition

### Determination of neutral monosaccharides

To determine the monosaccharide composition of the fractions in terms of neutral sugars, samples were hydrolyzed with trifluoroacetic acid 1 M for 5 hours at 100° C. Upon

completion of hydrolysis, the excess acid was removed by evaporation (Biermann 1989). After total acid hydrolysis, the monosaccharides were solubilized in distilled water and reduced by adding approximately 10 mg of sodium borohydride for 16 h at 4° C (Wolfrom and Thompson 1963b). Alditols were submitted to acetylation (Wolfrom and Thompson, 1963a). The extraction of alditol acetate was performed by the addition of chloroform and subsequent elimination of pyridine in successive treatments with 5% copper sulphate and distilled water. After evaporation of the solvent, the alditol acetates were subjected to gas-liquid chromatography (GLC) to determine the composition of neutral monosaccharides.

### Liquid-gas chromatography (GLC)

The resulting alditol acetates were analyzed by GLC using a Trace GC Ultra chromatograph (Thermo Electron Corporation) outfitted with a DB-225 (0.25mm x 30m) capillary column. The temperatures of the injector and flame ionization detector (FID) were 250º C and 300º C, respectively. The oven temperature was set from 100° C to 215° C at a heating rate of 40° C/min. Helium was used as carrier gas at a flow rate of 1.0 ml/min.

### Determination of acidic monosaccharide content (uronic acids)

Uronic acids were measured by the Blumenkrantz and Asboe-Hansen method (1973); galacturonic acid was used as a standard solution, and readings were performed at 520 nm.

## PHYSICOCHEMICAL PROPERTIES

### Hydration Capacity

Hydration capacity of the ingredients was determined according to McConnell et al. (1974). One gram of the sample was weighed in a tared centrifuge tube. 20 mL of distilled water was added, and the mixture was stirred until complete homogenisation. It was left to stand at room temperature for 24 hours. The next day, the material was centrifuged, and the released water was removed. The tube with the sample containing absorbed water was weighed. The hydration capacity of the samples was expressed in grams of water absorbed in one gram of sample.

Fat-binding capacity

Fat-binding capacity was determined according to Abdul-Hamid and Luan (2000). The samples were weighed into the tube, where oil was added. After complete homogenization, the material was centrifuged and the supernatant was removed. The tube with the sediment (sample + absorbed oil) was weighed. Fat-binding capacity was expressed as the number of grams of oil absorbed in one gram of sample.

## RESULTS AND DISCUSSION

### NUTRITIONAL COMPOSITION

All resulting DFCs showed high total dietary fiber (TDF) content (Table 1), which proves that the methodologies for fiber concentrations used in this study were suitable for the proposed objective. For mucilage obtained from flaxseed, Monego (unpublished data) found higher values for TDF (85.07 ± 3.98) and SF (73.21 ± 2.73). These differences in results can be explained by differences in the variety of the raw material employed as well as by the procedure used for obtaining the food products (Kaewmanee et al. 2014).

Although pectin had the lowest TDF content compared with other DFCs, it had a higher SF content (31.47 ± 1.75); this fraction is of great importance to the natural microflora of the colon, as soluble fibers are fermented more quickly and in greater quantity than insoluble ones in the intestine (Puupponen-Pimiä et al. 2002; Catalani et al. 2003). Short-chain fatty acids are produced through fermentation (Saad 2006), they are responsible for various benefits to the host, such as regulation of epithelial proliferation and differentiation of the colonic mucosa; increased blood flow and mucus production; preferred energy source for the colonocytes; decreased pH in the colon; maintenance of the balance of intestinal microflora; beneficial effects on absorption of sodium and water, on lipid and glucose metabolism, on pancreatic secretion and on other hormones (Catalani et al. 2003).

For β-Glucan+mannan, a significant increase was observed in TDF content for *in nature* yeast. Likewise, Chaud et al. (2007) also found approximately 69.7% of TDF in this fraction; however, these authors reported that 60.2% of this fiber is in the soluble form, contrary to the findings of this study. It is suggested that higher levels of insoluble fibers are associated with the shape of the β-glucan present in yeast. According to Magnani and Castro-Gómez (2008), there are two fractions of β-(1-3) glucan on the cell wall of *Saccharomyces cerevisiae* one soluble and one insoluble fraction. The insoluble portion represents the major wall component, while the soluble portion accounts for 15 to 20%. Furthermore, according to Sinha et al. (2011), mannans are highly insoluble polysaccharides in water, and very dense in most cases.

In relation other nutrients, it can be seen that the procedures employed for fiber concentration of the different raw materials caused a reduction in fat content, and this is probably due to the fact that the treatment used for fiber solubilization, combined with the hydrophilic nature of the solvent, has not caused translocation of fat to the resulting extract (Monego unpublished data). Likewise, CP content was reduced in the resulting dietary fiber

fractions. For moisture and ash, the average values found in mucilage and pectin were similar to those found in the literature (Cui and Mazza 1996; Kliemann unpublished data), while the value of ashes for βG+M found by Chaud et al. (2007) was about 40 to 50% lower. Nevertheless, in general, all the DFCs had low ash content, which is a good indication of the purity of the sample (Kliemann unpublished data).

## EXTRACTION YIELD

Figure 1 shows the extraction yields of the different DFCs obtained from agro-industrial sources. The DFC that had the highest extraction was βG+M (β-glucan+mannan), with an average of 19.81 ± 8.54% yield (Figure 1). There are few data in the literature that allow a comparison of the extraction yield of this fraction; the published studies assessed the fractions in isolation, as in the work of Chaud and Sgarbieri (2006) where they obtained 25.13% extraction yield for mannans and 42.92% for glucans, obtained from the cell wall of semi-purified *S. cerevisiae*. For pectin, extraction yield was 14.54 ± 2.72%. In contrast, Calliari (unpublished data) evaluated various methodologies for extraction of pectin from citrus pulp and observed higher yields for extraction in an acid medium a yield of 39.23% for citric acid and 26.70 % for acetic acid. For pectin obtained from passion fruit peel, citric acid was also the best extracting agent, resulting in about 70% yield, while yield was low for nitric and hydrochloric acids: 38% and 26%, respectively (Kliemann unpublished data). Thermal extraction of pectin was not as efficient as extraction in acid medium, which is used in the food industry (Canteri et al. 2012) and has much higher extraction yields. However, it can be considered a cost-effective and environmentally friendly method because it does not generate toxic waste in the environment, and it has low cost.

The extraction yield of flaxseed mucilage averaged 7.18 ± 1.54%, which is consistent with results found in the literature (Monego unpublished data; Qian et al. 2012). Fedeniuk and Biliaderis (1994) tested different methods of flaxseed mucilage extraction and observed lower extraction yield (3.6%) using low temperature water (4°C); however, when using higher temperatures, they had higher yield (9.4%), similar to the finding in the present study. Likewise, Cui et al. (1994) observed higher extraction yield (8%) of flaxseed mucilage using high temperatures between 85-90ºC, pH 6.5-7.0 and water:seed ratio equal to 13. Besides the afore mentioned features, the extraction yield of flaxseed mucilage is variable depending on the culture environment and variety of this grain (Qian et al. 2012).

## MONOSACCHARIDE COMPOSITION

Monosaccharide composition, the nature of binding between monosaccharides, solubility and physicochemical properties are features that directly affect the functional properties of polysaccharides (Tavernari et al. 2008; Bemiller and Huber 2010).

The monomers that comprise the fibers are classified as pentoses: arabinose and xylose; hexoses: glucose, galactose and mannose; 6 deoxihexoses: rhamnose and fucose and uronic, glucuronic and galacturonic acids (Meurer and Hayashi 2003). In addition, knowledge of the polysaccharide composition is extremely important, because the physiological impact will depend on the sugar residues and the nature of bindings between these residues (Sinha et al. 2011).

In the present study, mucilage and pectin were the DFC with the greatest diversity of monosaccharides (Table 2). The monosaccharide composition present in flaxseed mucilage was similar to the findings in the literature (Fedeniuk and Biliaderis 1994; Oomah et al. 1995; Qian et al. 2012). Among the monosaccharides present, xylose, galactose and arabinose were found

in greater quantity. According to Ringo et al. (2010), xylose oligomers (xylooligosaccharides) promote the growth of beneficial bifidobacteria acting as prebiotics. Similarly, galactose molecules (galactooligosaccharides) and arabinose + xylose (arabinoxylo-oligosaccharides) have been widely used as prebiotic source for fish.

In pectin, glucose, arabinose and uronic acid were the neutral monosaccharides found in larger quantities. For commercial pectin, Kliemmann (unpublished data) obtained a similar composition, that is, higher levels of glucose, galactose and rhamnose, but in different concentrations than the findings in the present study. According to BeMiller and Huber (2010), the composition and properties of pectins vary according to their source, the process used during preparation and subsequent treatments. Regarding the functionality of non-starch polysaccharides present in pectin, Canteri et al. (2012) reported that the beneficial effects of the pectin chain can be attributed to its ability to be transformed into short chain fatty acids by the action of pectinolytic enzyme-producing bacteria of the genera Aerobacillus, Lactobacillus, Micrococcus and Enterococcus. Hotchkiss et al. (2003) analyzed the *in vitro* fermentation of oligosaccharides derived from pectin obtained from Valencia oranges, and they concluded that these components exert bifidogenic effects and promote the increase of acetate, propionate and butyrate from such fermentation.

For βG+M, monosaccharides found in larger amounts were mannose (74.5%), followed by glucose (24.3%) and, in lower levels, galactose (1.2%). According to Pinto (unpublished data), certain monosaccharides, as welll as xylose and galactose, are associated with mannoproteins, which may be a probable explanation for the galactose content found in the βG+M fraction. Regarding the beneficial effects of the components present in βG+M, the mannose units (mannanoligosaccharide) are responsible for increasing the performance of fish and feed efficiency, offering protection against pathogens by leveraging the local and systemic immune system, and strengthening gut integrity and functionality (Sinha et al. 2011; Torrecilas

et al. 2014). B-glucan already has shown potential prebiotic because of its immunomodulatory effect when it is recognized by specific cellular receptors, it has the ability to enhance the immune response of the host (Magnani and Castro-Gómez 2008).

PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of dietary fibers have important metabolic and physiological effects on the body of animals, and this is directly related to source of fiber and processing history. Processing can result in important changes that must be taken into account, depending on the final destination of the product and the properties of the product intended for commercialization (Zaragoza et al. 2001).

Figure 2 shows the values found for hydration capacity (HC) and fat-binding capacity (FBC) of the evaluated ingredients. In this study, mucilage showed high HC. Similarly, Monego (unpublished data) also observed high hydration capacity for flaxseed mucilage (17.48 ± 0.23 g water/g sample). In biological conditions, high levels of fiber intake coupled with high water-retention capacity causes greater bolus volume, more satiety, increased viscosity of solutions in the gastrointestinal tract, delayed gastric emptying, among other effects (Brito et al. 2008; Souza et al. 2008; Fabregat et al. 2011). These characteristics are not desirable in fish nutrition.

Although pectin had the highest SF content, this fraction had lower hydration capacity than fresh citrus pulp. It is suggested that pectin had this behavior because it had lower TDF content. Similarly, Macagnan (unpublished data) observed lower HC for orange pulp, even with higher SF levels compared with the other ingredients. This author attributed this result to the low amount of dietary fiber and free pectin in this byproduct.

The βG+M fraction had higher hydration capacity compared with fresh yeast. This behavior may be related to the structure of β-glucan, because unlike cellulose, bindings between

glucose units are variable; they have a branched structure and smaller size. These properties influence their solubility, allowing them to form viscous solutions and acquire greater hydration capacity (Mudgil and Barak, 2013).

Fat-binding capacity (FBC) quantifies how much lipid a fiber is capable of absorbing; it is associated with the fiber's ability to bind to substances in the intestine, as well as bile salts and acids and cholesterol (Souza et al. 2008). Figure 2 shows that there was a small increase in FBC for pectin and mucilage compared with the fresh ingredient, while there was a slight reduction for βG+M. However, FBC was similar for fiber fractions extracted in the present study. Thus, it is suggested that regardless of the origin of the extracted fiber, the behavior of binding to substances in the intestine is similar.

Based on the results found in this study, it can be inferred that all Concentrates of Dietary Fibers obtained from different agro-industrial sources showed nutritional and technological properties that indicate potential application as prebiotics for fish feeds.

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**RESUMO:** O presente estudo teve por objetivo aplicar metodologias para concentração de fibras alimentares e avaliar suas respectivas composição nutricional e físico química. Os Concentrados de Fibras Alimentares (CFAs) = Mucilagem, Pectina e βglicana+manana (βG+M), foram obtidos a partir de grão de linhaça, polpa de cítros e levedura de cervejaria *(Saccharomyces cerevisiae)*, respectivamente, através de diferentes processos físico químicos.

A determinação da composição nutricional revelou que os componentes predominantes em todos os CFAs obtidos foram fibra alimentar total e fibra insolúvel. O CFA que apresentou maior rentabilidade de extração foi a βG+M (19,81%±8,54), seguida da Pectina (14,54%±2,72) e Mucilagem (7,18%±1,54). A composição da Mucilagem e Pectina obtiveram maior diversidade de monossacarídeos, já a βG+M consistiu basicamente de manose (74,5%) e glicose (24,3%). A Pectina apresentou numericamente menor capacidade de hidratação em relação aos demais CFAs. Para capacidade de ligação a gordura, todas os CFA obtiveram valores semelhantes. Neste estudo, os CFAs apresentaram características nutricionais e tecnológicas que indicam potencial de aplicação destas fontes como prebióticos para a suplementação de peixes.

**Palavras-chave**: Fontes agroindustriais. Pectina. Mucilagem. β-glicana+manana. Promotor de crescimento.

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**Table 1.** Nutritional composition (%) of the ingredients *in natura* and Dietary Fiber Concentrates (DFC)

| Components[1] | Ingredients | | | | | |
|---|---|---|---|---|---|---|
| | Flaxseed | Mucilage | Citrus pulp | Pectin | Yeast | βG+M[2] |
| TDF | 53.09±10.11 | 61.73±3.25 | 47.52±2.08 | 32.76±1.05 | 11.88±2.38 | 64.18±0.31 |
| IF | 38.19±8.74 | 39.50±5.44 | 25.37±0.39 | 1.29±0.71 | 1.32±0.15 | 60.98±0.00 |
| SF | 14.90±1.37 | 22.23±2.19 | 22.16±1.69 | 31.47±1.75 | 10.56±2.23 | 3.2±0.31 |
| CP | 16.09±0.11 | 11.29±0.95 | 5.67±0.25 | 5.10±0.15 | 39.44±0.10 | 10.31±0.46 |
| Fat | 31.59±0.57 | 2.67±0.08 | 7.65±0.00 | 0.56±0.11 | 2.24±0.45 | 1.20±0.11 |
| Moisture | 7.76±0.23 | 13.25±0.21 | 11.51±0.01 | 15.39±0.15 | 68.76±0.79 | 10.55±0.00 |
| Ash | 3.41±0.09 | 6.65±0.05 | 3.25±0.13 | 3.62±0.03 | 7.49±0.33 | 7.41±0.15 |

[1]TDF: Total dietary fiber; IF: Insoluble fiber; SF: Soluble fiber; CP: Crude protein. [2]βG+M: βglucan+mannan. Results are expressed as mean ± standard deviation

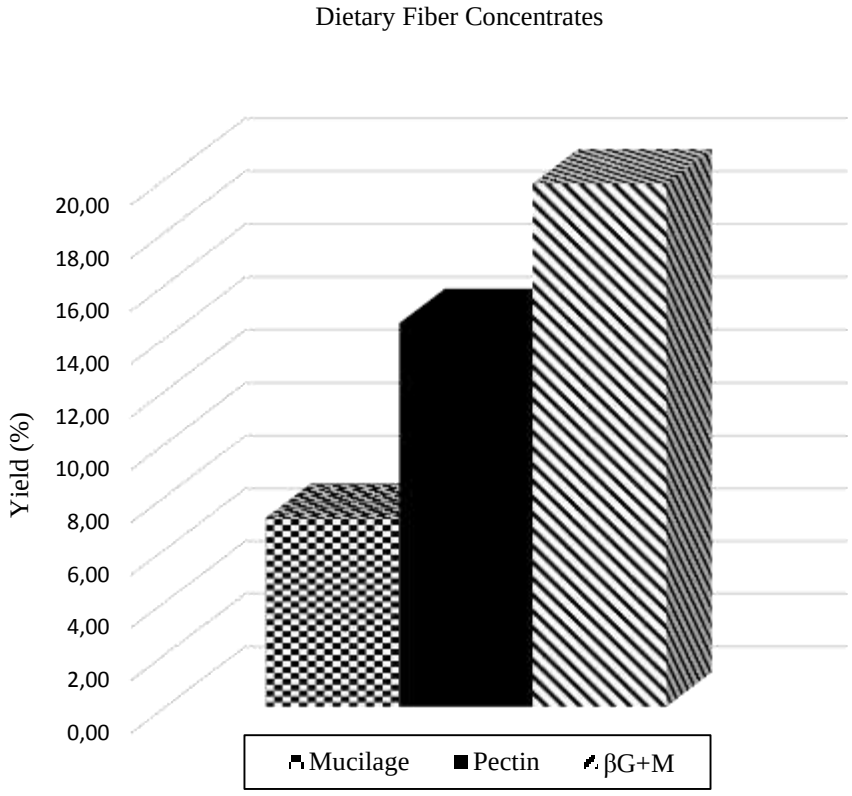


**Figure 1**. Yield obtaining mucilage, pectin and βG+M from agribusiness sources. βG+M: β-glucana+mannan.

**Table 2.** Monosaccharide Composition (%) of Dietary Fiber Concentrates (DFC) obtained from different sources agroindustrial

| | Composition [1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | UA |
| Mucilage | 11 | 3 | 15.5 | 35.4 | 0 | 17.1 | 6.9 | 11.1 |
| Pectin | 2.2 | 0 | 21.6 | 1.2 | 1 | 9.4 | 42.9 | 21.7 |
| βG+M[2] | 0 | 0 | 0 | 0 | 74.5 | 1.2 | 24.3 | 0 |

[1] Rha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose; UA: uronic acid.[2]βglucana+mannan.

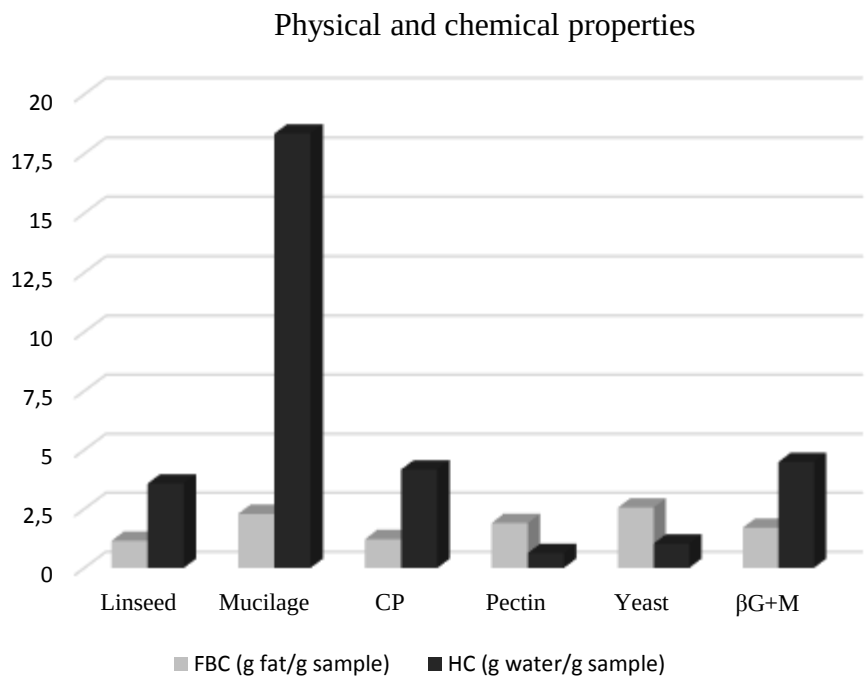


**Figure 2.** Fat binding capacity (FBC) and Hydration capacity (HC) the ingredients *in natura* and different Dietary Fibers Concentrates. CP: citrus pulp; βG+M: βGlucana+Mannan; Yeast: *Saccharomyces cerevisiae.*

# 2 ESTUDO CIENTÍFICO 2

Effects of Dietary Fiber Concentrates on growth performances and digestive enzyme activities of jundiá (*Rhamdia quelen)*

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**Abstract**

This study was conducted to investigate the prebiotic effect of different Dietary Fiber Concentrates (DFC) (Mucilage = MG; Pectin = PN or β-glucan+mannan = βg+M) on growth and somatic parameters, body composition and digestive enzyme activities of jundiá (*Rhamdia quelen*) juveniles. After acclimation, fish (7.16 ± 0.06 g) were allocated into 24 tanks (30 fish per tank) and triplicate groups were fed with Control diet (0 g $kg^{-1}$ of DFC); diet supplemented with 5 g $kg^{-1}$ commercial prebiotic (CP) or diets supplemented with 5 or 10 g $kg^{-1}$ diet of MG; PN or βg+M. At the end of the trial (8 weeks) growth was significantly ($P<0.05$) higher in fish fed diets supplemented with DFCs and did not differ from animals supplemented with CP. The animals that were fed Control diet presented a body protein content higher compared to those supplemented with diets containing pectin or β-glucan+mannan ($P<0.05$). However, fish fed diets added with β-glucan+mannan yielded a higher level of protein deposited in the whole body. The activity of digestive enzymes was lower in the group supplemented with Pectin. Results to indicate that supplementation with DFCs in the diet had positive effects on the performance of jundiá and are prebiotic potential candidate.

## Introduction

In aquaculture, productive success is closely linked to the animals health and well being (Pohlenz & Gatlin 2014). In this line, it has been documented that prebiotics have beneficial effects on the fish immune responses (Sataykov *et al*. 2007; Soleimani *et al*. 2012; Zhang *et al*. 2013) and stress resistance (Soleimani *et al*. 2012; Hoseinifar *et al*. 2013) for stimulating the growth of beneficial bacterial of gastrointestinal tract (Geraylou *et al*. 2012; Akrami *et al*. 2013), increasing the production of short-chain fatty acids (Geraylou *et al*. 2012) and improving the intestinal morphology (Zhou *et al*. 2010), with positive impacts on performance (Li & Gatlin 2005; Xu *et al*. 2009; Zhou *et al*. 2010; Soleimani *et al*. 2012; Talpur *et al*. 2014) and survival (Li & Gatlin 2005; Talpur *et al*. 2014). According Soleimani *et al*. 2012 some of the benefits of including prebiotics on performance and survival can be attributed to increase in the activity of digestive enzymes.

Prebiotics are non-digestible food ingredients that affect positively the host health because they consist of a complex mixture of non-starch polysaccharides and oligosaccharides (Roberfroid *et al.* 2007). Such compounds act as substrate for fermentation of beneficial commensal bacteria and influence the gut microbiota balance positively, which may induce the animal's growth and stimulate the immune system by enhancing the activity of the phagocytic cells and producing lysosomes and antibodies (Bach Knudsen 2001; Montagne *et al*. 2003; Theuwissen & Mensink 2008; Tavechio *et al*. 2009). They also contribute to ensure environmentally suitable and biosafe products delivered to the consumers market.

Most commercial prebiotic formulations currently available in the market and with proven action in fish are made of oligosaccharides of galactose, fructose or

mannose (Torrecillas *et al.* 2007; Helland *et al.* 2008; Soleimani *et al.* 2012; Ganguly *et al*. 2013; Hoseinifar *et al*. 2013). However, prebiotics action is largely observed in sources of more complex polymers, such as insoluble and soluble non-starch polysaccharides that comprise most of the dietary fiber of vegetables and organic wastes (Goulart *et al*. 2013). Such polysaccharides have the most diverse chemical structures and polymerization, with impacts on their physical-chemical and fermentation capabilities, showing a great technological potential still little explored in fish nutrition.

Given the above, this study was conducted to assess the prebiotic effect of Dietary Fiber Concentrates, with diverse chemical constitution, obtained from linseed (mucilage), citrus pulp (pectin) and residues from barley fermentation by S*accharomyces cerevisiae* (β-glucan+mannan), on the productive performance and digestible enzymes of jundiás (*Rhamdia quelen)* juveniles*.*

## Materials and methods

*Preparation of dietary fiber concentrates*

Dietary Fiber Concentrates (DFCs) were obtained from linseed grains, citrus pulp or brewers' yeast and analyzed for monosaccharides composition (Table 1, footnotes). The β-glucans+mannans fraction was obtained from brewer's yeast (*Saccharomyces cerevisiae*) through an autolysis process and NaOH 1% alkaline treatment, according to the method described by Matiazi (2006) and Chaud *et al*. (2007), with some changes. Mucilage was concentrated from whole linseed grains in an aqueous solution at a concentration of 10% w/v, and temperature of 60 to 80º C and constant stirring for 150 minutes, following the experimental method described by

Goulart *et al*. (2013). Pectin was obtained from citrus pulp in an aqueous solution at average temperature of 100ºC, according to the method described by Calliari (2004).

The DFCs neutral monosaccharides were determined by gas-liquid chromatography (GLC) (Biermann 1989; Wolfrom & Thompson 1963a; Wolfrom & Thompson 1963b), and the uronic acids were analyzed by spectrophotometry, following the method described by Blumenkrantz & Asboe-Hansen (1973), having galacturonic acid as standard solution and reading in 520 nm.

*Fish culture and feeding trial*

The study was conducted in the Fish Farming Laboratory of the Department of Animal Science, Santa Maria Federal University (UFSM) - RS, Brazil, after being approved by the UFSM's Ethics Committee on Animal Trials under nº 23081.009051/2014-53. A total of 720 jundiá (*Rhamdia quelen*) juveniles, corresponding to 30 animals per tank, with initial mean weight of 7.16±0.06g, were distributed into 24 fiberglass containers of 125 liters capacity, fitted with individual inlets and outlets, connected to a water recirculating system consisting of two biological filters with gravel, backwash system, and controlled temperature. During the experimental period, the water quality parameters were monitored and maintained as follows: temperature of 23.1±1.29ºC; dissolved oxygen: 5.25±0.65 mg $L^{-1}$; pH: 7.04±0.22 units; total ammonia: 0.28±0.26 mg $L^{-1}$; nitrite: 0.096±0.09 mg $L^{-1}$; alkalinity: 31.6±7.65 mg $L^{-1}$ of $CaCO_3$; and hardness: 32.8±12.04 mg $L^{-1}$ of $CaCO_3$. According to Baldisserotto & Radünz Neto (2004) these parameters are within the optimum range for *Rhamdia quelen* culture.

The fish samples were acclimated in a period of four weeks, when they were fed commercial feed with 360 g $kg^{-1}$ of crude protein (CP), provided three times a day

until apparent satiation. In the experimental period, the animals were fed isocaloric and isoprotein diets for 8 weeks, formulated to meet the requirements of 360 g $kg^{-1}$ of CP and about 13.4 MJ $kg^{-1}$ of Digestible Energy (DE). Eight mixed feeds were formulated for the experiment, containing fish meal, maize starch and cellulose, added with 0 g $kg^{-1}$ of Dietary Fiber Concentrates (DFC) (control diet), 5 g $kg^{-1}$ of commercial prebiotics ( based on mannan-oligosaccharides (Bio-Mos® Alltech, Lexington, Kentucky, USA) (diet CP 5), 5 g $kg^{-1}$ of mucilage (diet MG 5); 5 g $kg^{-1}$ of pectin (diet PN 5); 5 g $kg^{-1}$ of β-glucan+mannan (diet βG+M 5); 10 g $kg^{-1}$ of mucilage (diet MG 10); 10 g $kg^{-1}$ of pectin (diet PN 10) and 10 g $kg^{-1}$ of β-glucan+mannan (diet βG+M 10) (Table 1).

*Data collection and assessed variables*

In the early and late experimental period (eight weeks of treatment) biometrics was performed to collect data of the animals, which had fasted for 18 hours and were anesthetized in diluted Benzocaine (Henrifarma® Produtos Químicos e Farmacêuticos LTDA; Cambuci, SP, Brazil) at the concentration of 100 mg $L^{-1}$, to estimate the following: individual average weight (g); final weight (FW) (g); daily weight gain (DWG) (g/day): (final weight – initial weight)/days; apparent feed conversion (AFC): feed intake/weight gain; specific growth rate (SGR): [(ln (final weight) - ln (initial weight)) /days] x 100, where: In= Neperian logarithm and condition factor (CF): weight/(total length)$^3$ x 100. For the analysis of the somatic parameters, nine animals per treatment were used for determination of the digestive somatic index (DSI) (%): (weight of the digestive tract/weight of the whole fish) x 100; hepatosomatic index (HIS) (%): (weight of the liver/weight of the whole fish) x 100; visceral fat index (VFI) (%): (weight of visceral fat/whole weight) x 100; and intestinal quotient (IQ): length of the digestive tract/total fish length.

For the analysis of proximate body composition, nine animals of each group were used. Crude protein was determined by the micro-Kjeldahl method (method 960.52) using the N x 6.25 factor, and the moisture content and ash content were determined according to AOAC (1995). Fat was extracted and quantified according to the method described by Bligh & Dyer (1959).

The nutrients retention was calculated according to the following equations:

- Protein deposited in the body (g): total deposition of crude protein (TDCP) = [FW * (% FBP/100)] – [IW * (% IBP/100)];
- Fat deposited in the body (g): total fat deposition (TFD) = [FW * (%FBF/100)] – [IW * (%IBF/100)];

Where: FW = final weight; IW = initial weight; IBP = initial body protein; FBP = final body protein; IBF = initial body fat; FBF = final body fat.

*Digestive enzymes assays*

After eight weeks of treatment, three samples of fish of each tank were collected to determine the activity of trypsin and chymotrypsin enzymes. The collected intestine was homogenized in a buffer solution (10mM phosphate/20mM Tris. The samples were then centrifuged, and the supernatants were used in the assays as enzyme source for determination of gut trypsin and chymotrypsin enzymes. To determine the trypsin enzyme activity, TAME (α-ρ-toluenesulphonyl- L-argininemethyl ester hydrochloride; Sigma-Aldrich, St. Louis, MO, USA) was used as substrate. The gut extracts were incubated for two minutes in a 2-ml buffer solution of Tris/$CaCl_2$, pH of 8.1. For determination of chymotrypsin, the substrate used was BTEE (benzoyl-L-tyrosine ethyl ester; Sigma-Aldrich, St. Louis, MO, USA). The extracts were incubated

for two minutes in a 2-ml buffer solution of Tris/CaCl2 (2 ml), pH of 7.8. The trypsin activity was expressed in µmol of hydrolyzed TAME/minute/mg of protein, and the chymotrypsin activity in Mmol of BTEE/minute/mg protein. Readings were taken in a spectrophotometer (Biospectro®, SP220; Curitiba, PR, Brazil) absorbance of 247 and 256 nm respectively, following the methodology described by Hummel (1959). Protein concentration in the supernatant was determined by the Bradford (1976) method using bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) as the standard.

*Statistical analysis*

Data were firstly analyzed for outlier identification. Analyses of variance were performed by F-test, and the treatments were compared through orthogonal contrasts, at 5% probability level.

## Results

*Performance parameters*

All groups supplemented with Dietary Fiber Concentrates (DFCs) showed better results of FW, DWG, AFC and SGR, when compared to the control group ($P<0.05$), except the groups that received pectin, which did not show significant difference for the FW variable ($P>0.05$) (Table 2). CF was higher only in the animals that were fed diets containing β-glucan+mannan ($P<0.05$).

Increased dosages of DFCs addition, from 5 to 10 g $kg^{-1}$, did not result in significant differences in the performance variables ($P>0.05$), which was also observed in the comparison of these treatments added with an equivalent dosage of commercial prebiotics (5 g $kg^{-1}$) ($P>0.05$).

*Somatic parameters*

The control diet showed a higher digestive somatic index (DSI) than treatments with mucilage or pectin ($P$<0.05) (Table 3). The animals fed 5 g $kg^{-1}$ of CP showed higher DSI (3.27±0.59) when compared to the animals supplemented with DFCs ($P$<0.05). Diets with pectin and β-glucan+manann yielded higher values of hepatosomatic index (HSI) ($P$<0.05), however, in the analysis of variance of the other orthogonal contrasts no significant differences were found ($P$>0.05). Concerning the VFI variable (visceral fat index), no significant differences were observed ($P$>0.05). The intestinal quotient was higher in the animals that received the Control diet when compared to those that received diets with pectin or β-glucan+mannan ($P$<0.05).

Moisture, ash, and fat in whole fish values did not indicate significant differences among the animals fed different diets ($P$>0.05) (Table 4). However, the animals that were fed Control diet presented a higher level of crude protein (CP) in the whole fish compared to those supplemented with diets containing pectin or β-glucan+mannan ($P$<0.05). The diets added with β-glucan+mannan yielded a higher level of TDCP and TFD, compared to the Control diet ($P$<0.05). The TDCP and TFD variable was also higher in the animals of the group fed diet CP 5 compared with PN 5 ($P$<0.05).

*Digestive enzymes*

The animals in the Control group showed a greater activity of trypsin and chymotrypsin enzymes when compared to those treated with addition of pectin to the feed ($P$<0.05). The diet containing 10 g $kg^{-1}$ PN yielded a higher activity of trypsin and chymotrypsin enzymes (Table 5) in the fish compared to the diet with 5 g $kg^{-1}$ of PN ($P$<0.05). Fishes that fed the diet containing 5 g $kg^{-1}$ of CP showed a lower enzyme

activity of chymotrypsin in relation to the animals of the group that fed diet with 5 g $kg^{-1}$ of MG ($P$<0.05).

## Discussion

Our results revealed that the addition of Dietary Fiber Concentrates (DFCs) to jundiás diets had significantly positive effects on the zootechnical parameters and equivalent to the commercial prebiotic in the same level of addition (5 g $kg^{-1}$). Such effects are commonly related to improved gut microbiota, which enhances fermentation by beneficial bacteria such as *lactobacilli* and *bifidobacteria* and, consequently, increased production of short-chain fatty acids (SCFA). Among these, butyrate enhances animal performance because it is used as a energy source for colonocytes, resulting in the control of the regulatory mechanisms of apoptosis, reduction of turnover and a higher enterocytes proliferation and differentiation (Hijova & Chmelarova 2007), as well as a better uniformity of the gut membrane, maintenance of the villi sizes, and an increased absorptive capacity of nutrients (Brumano & Gattás 2009). However further analysis of the gut microbiota and production of short-chain fatty acids is warranted to elucidate this.

Similar effects on performance have also been reported for other prebiotics and fish species such as *Sciaenops ocellatus* fed Previda™ (consisted of galactoglucomann) (Zhou *et al*. 2010), tilapia (*Oreochromis niloticus)* and *Channa striata* supplemented with β-glucan (Abu-Elala *et al*. 2013; Talpur *et al*. 2014). In general, our results showed that the addition of 5 g $kg^{-1}$ of DFCs yielded a better animal performance when compared to the 10 g $kg^{-1}$ level. Various studies showed contradictory results regarding the use of prebiotics (Schwarz *et al.* 2010; Dimitroglou *et al.* 2010; Helland *et al*. 2008; Dimitroglou *et al*. 2011; Mansour *et al.* 2011; Zhou *et*

*al.* 2010), which can be associated with under- or overdose, or be attributed to differences in the chemical composition of molecules and in the anatomical and physiological features of fish species. In the present study, among DFCs, the addition of β-glucan+mannan to the diet stands out among the best results, which can be due to their monosaccharide composition, rich in glucose and mannose (Table 1, footnotes), but also to the intrinsic presence of complex B vitamins in the yeast and carried during the process of concentration of this dietary fiber source. In the autolysis process employed for concentration of this dietary fiber, the cell wall breaks up and causes leaking of the cellular content, largely recognized as being rich in complex B vitamins, which can work synergistically with the fiber prebiotic action, optimizing the immunomodulatory responses and the animal performance (Hisano *et al*. 2008). However, more studies are needed to clearly elucidated this topic.

The animals that fed Control diet showed higher intestinal quotient (IQ) and digestive-somatic index (DSI), the latter also being high in the animals supplemented with 5 g $kg^{-1}$ of CP, which may be associated with the morphological adaptation of the fish in response to the diet composition (Abelha *et al*. 2001). This is because such feeds have higher levels of insoluble fiber (Table 1), which increases the water holding capacity and jeopardizes nutrients digestibility, leading to an adaptation of the absorptive area, usually caused by an increase of the intestines length (Fracalossi & Cyrino 2012). The groups that were fed supplementation of pectin or β-glucan-mannan in the feeds showed higher hepatosomatic index (HIS). However, Guerreiro *et al.* (2014) found no differences in the HIS of fish supplemented with prebiotic FOS. Higher HIS may be related to an increase of the glycogen content in the liver (Dimitroglou *et al*. 2010)

Although DFCs have resulted in a lower rate of body protein, with no change in the fats rate, the respective depositions of these nutrients were higher in the animals fed β-glucan+mannan. Similarly, Helland *et al*. (2008) found a lower content of crude protein (CP) in the body composition of Atlantic salmon (*Salmo salar*) supplemented with MOS (mannan-oligosaccharides) or GOS galactooligosaccharides, compared to the control group. Genc *et al*. (2007) also found reduced body proteins in shrimp fed 4.5 g of MOS $kg^{-1}$. In contrast, Mansour *et al.* (2011) and Schwarz *et al*. (2011) did not observe any effect of the MOS supplementation in the proximate body composition of juvenile sturgeons (*Huso huso* Linnaeus, 1754) and Nile tilapias, respectively. Fish body composition can be a useful tool when making a decision on the addition or replacement of ingredients in the diet, once it will directly impact the quality of the product that will be available to consumers (Hisano *et al.* 2007; Fracalossi *et al*. 2012).

In the current study, the trypsin and chymotrypsin digestive enzymes showed higher activity in the animals fed the Control diet compared to those that were fed pectin. Peptic substances are included in the group that has the greatest importance in the water holding process, which determines the increased viscosity of the food (Brito *et al*. 2008) and prevents the access of the enzymes to the substrates, resulting in decreased digestive enzyme activity and lower zootechnical performance (Meurer & Hayashi 2003). In contrast to our results, inulin supplementation in the diet of *Cyprinus carpio* did not cause significant effects on the activity of digestive enzymes (Eshaghzadeh *et al*. 2014). However, digestive enzyme activities were significantly elevated with increasing levels of dietary FOS (fructooligosaccharides) in diets of Caspian roach (*Rutilus rutilus*) (Soleimani *et al.* 2012). The animals that were supplemented with 5 g $kg^{-1}$ of CP in the diet showed lower enzyme activity compared to those that were fed 5 g $kg^{-1}$ of MG, which can be due to the gut microbiota ability to

ferment prebiotics with distinct chemical compositions. However, as the topic has not been clearly elucidated, further studies are needed.

The results indicated that supplementation of diets of jundiá with 5 g $kg^{-1}$ of DFCs yielded improved performances of the animals and effects similar to commercial prebiotics. Thus, suggestion is to supplement diets with 5 g $kg^{-1}$ of mucilage or β-glucan+mannans to induce growth of jundiás juveniles. Further studies should be conducted in order to demonstrate the benefits of dietary fibers on other fish species, and to study the potential of its combined use to optimize the beneficial effects on animals performance.

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**Table 1**. Dietary formulations and proximate composition of the experimental diets (g kg$^{-1}$)

| Ingredients (%) | Treatments[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | CP 5 | MG 5 | PN 5 | βg+M 5 | MG 10 | PN 10 | βg+M 10 |
| Fish meal[2] | 660 | 660 | 660 | 660 | 660 | 660 | 660 | 660 |
| Maize starch | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 |
| Cellulose | 60 | 55 | 55 | 55 | 55 | 50 | 50 | 50 |
| Mucilage[3] | - | - | 5 | - | - | 10 | - | - |
| Pectin[4] | - | - | - | 5 | - | - | 10 | - |
| βg+M[5] | - | - | - | - | 5 | - | - | 10 |
| CPre[6] | - | 5 | - | - | - | - | - | - |
| Melbond | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Oil | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| VMP[7] | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| NaCl[8] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Inert[9] | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| BHT[10] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| **Calculated composition**[11] | | | | | | | | |
| CP | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 |
| DE (MJ kg$^{-1}$)[12] | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 |
| Ether extract | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 |
| Ash | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 |
| TDF[13] | 256.2 | 258.3 | 317.8 | 219.5 | 238.6 | 322.9 | 249.5 | 228.9 |
| IF[14] | 201.7 | 200.7 | 159.6 | 183.2 | 196.2 | 168.9 | 183.6 | 100.6 |
| SF[15] | 54.4 | 57.7 | 158.2 | 36.4 | 42.4 | 154.0 | 65.9 | 128.2 |

[1] Control: addition of 0 g kg$^{-1}$ of DFC; CP 5: addition of 5 g kg$^{-1}$ of commercial prebiotic; MG 5: addition of 5 g kg$^{-1}$ of mucilage; PN 5: addition of 5 g kg$^{-1}$ of pectin; βG+M 5: addition of 5 g kg$^{-1}$ of β-glucan+mannan; MG 10: addition of 10 g kg$^{-1}$ of mucilage; PN 10: addition of 10 g kg$^{-1}$ de pectin; βg+M 10: addition of 10 g kg$^{-1}$ of β-glucan+mannan;
[2] Residue filleting tilapia (COPISCES®, Toledo-PR)
[3] Composition of mucilage (g kg$^{-1}$): Rhamnose-Rha: 110; Fucose-Fuc: 30; Arabinose-Ara: 155; Xylose-Xyl: 354; Galactose-Gal: 171; Glucose-Glc: 69; Uronic acid (UA): 111.
[4]Composition of Pectin (g kg$^{-1}$): Rha: 110; Ara: 216; Xyl: 12; Man: 10; Ga: 94; Glc: 429; UA: 217.
[5] Composition of βg+M (g kg$^{-1}$): Man: 745; Gal: 12; Glc: 243.
[6]CPre: commercial prebiotic (Bio-MOS, Alltech®, Lexington, Kentucky, USA)
[7] Vitamin and mineral mixture (Mig Plus®; Casca, PR, Brasil): Folic acid: 300 mg, Pantothenic acid: 3000 mg, Glutamic acid: 1 mg, Cobalt: 60 mg, Copper: 1000 mg, Choline: 102120 mg, Iron: 5000 mg, Biotin: 60 mcg, Iodine: 45 mg, Manganese: 8000 mg, Magnesium: 5%, Selenium 60 mg, Vit. A: 1000 IU, Vit. B1: 1500 mg, Vit. B2: 1500 mg, Vit. B6: 1500 mg, Vit. B12: 2000 mcg, Vit. C: 15000 mg, Vit. D: 240 IU, Vit. E: 10000 mg, Vit. K: 400 mg, Zinc: 14000 mg, Inositol 10000 mg, Niacin 9000 mg, antioxidant: 792 mg.
[8]sodium chloride;
[9]sand;
[10]Butylated hydroxytoluene (BHT)
[11]Calculated from the ingredients analyses
[12]Digestible energy = [(CP * 23.61 MJ kg$^{-1}$ * 0.9) + Fat * 39.82 MJ kg$^{-1}$ * 0.85) + CSDN * 17.21 MJ kg$^{-1}$ *0.50)] (Jobling 1983).
[13]Values analyzed in the Fisheries Laboratory of UFSM and expressed on dry matter basis; TDF: total dietary fiber; IF: insoluble fiber; SF: soluble fiber.

**Table 2.** Growth parameters of jundiá (*Rhamdia quelen)* supplemented with different Dietary Fiber Concentrates (DFC)

| Treatments | FW (g) | DWG (g) | AFC | SGR (%) | CF |
|---|---|---|---|---|---|
| Control | 47.43±12.1 | 0.72±0.05 | 1.34±0.06 | 3.25±0.13 | 1.02±0.1 |
| CP 5 | 56.25±17.3 | 0.82±0.04 | 1.22±0.03 | 3.44±0.04 | 1.03±0.1 |
| MG 5 | 53.42±14.7 | 0.81±0.01 | 1.25±0.03 | 3.41±0.03 | 1.03±0.1 |
| PN 5 | 52.36±16.6 | 0.81±0.01 | 1.24±0.03 | 3.38±0.08 | 0.99±0.2 |
| βg+M 5 | 58.06±19.1 | 0.85±0.05 | 1.19±0.05 | 3.48±0.11 | 1.06±0.1 |
| MG 10 | 49.34±13.1 | 0.78±0.05 | 1.23±0.06 | 3.39±0.10 | 1.03±0.1 |
| PN 10 | 48.88±12.2 | 0.78±0.05 | 1.24±0.08 | 3.34±0.07 | 1.04±0.1 |
| βg+M 10 | 55.82±17.8 | 0.84±0.03 | 1.19±0.04 | 3.47±0.04 | 1.05±0.1 |
| | | | Orthogonal contrasts | | |
| Control x treatments | * | * | * | * | ns |
| Control x MG | * | * | * | * | ns |
| Control x PN | ns | * | * | * | ns |
| Control x βg+M | * | * | * | * | * |
| MG 5 x MG 10 | ns | ns | ns | ns | ns |
| PN 5 x PN 10 | ns | ns | ns | ns | ns |
| βg+M 5 x βg+M 10 | ns | ns | ns | ns | ns |
| CP 5 x DFCs | ns | ns | ns | ns | ns |
| CP 5 x MG 5 | ns | ns | ns | ns | ns |
| CP 5 x PN 5 | ns | ns | ns | ns | ns |
| CP 5 x βg+M 5 | ns | ns | ns | ns | ns |

Values presented as means ± standard deviation (±SD). ns = not significant ($P>0.05$); *$P<0.05$.
FW: final weight; DWG: daily weight gain; AFC: apparent feed conversion; SGR: specific growth rate; CF: condition factor.

**Table 3.** Somatic parameters of jundiá (*Rhamdia quelen)* supplemented with different Dietary Fiber Concentrates (DFC)

| Treatments | DSI[1] | HSI[2] | VFI[3] | IQ[4] |
|---|---|---|---|---|
| Control | 3.33±0.56 | 1.30±0.11 | 2.14±0.90 | 1.29±0.20 |
| CP 5 | 3.27±0.59 | 1.37±0.12 | 2.38±1.00 | 1.11±0.11 |
| MG 5 | 2.82±0.23 | 1.34±0.20 | 2.31±1.03 | 1.07±0.31 |
| PN 5 | 2.96±0.35 | 1.41±0.11 | 2.19±0.77 | 1.03±0.20 |
| βg+M 5 | 2.97±0.27 | 1.42±0.17 | 2.34±0.64 | 1.03±0.13 |
| MG 10 | 3.07±0.52 | 1.31±0.13 | 1.62±0.94 | 1.24±0.14 |
| PN 10 | 2.89±0.46 | 1.43±0.09 | 2.54±0.92 | 1.10±0.25 |
| βg+M 10 | 3.11±0.14 | 1.57±0.28 | 3.19±1.12 | 1.02±0.18 |
| Orthogonal contrasts | | | | |
| Control x treatments | * | ns | ns | * |
| Control x MG | * | ns | ns | ns |
| Control x PN | * | * | ns | * |
| Control x βg+M | ns | * | ns | * |
| MG 5 x MG 10 | ns | ns | ns | ns |
| PN 5 x PN 10 | ns | ns | ns | ns |
| βg+M 5 x βg+M 10 | ns | ns | ns | ns |
| CP 5 x DFCs | * | ns | ns | ns |
| CP 5 x MG 5 | * | ns | ns | ns |
| CP 5 x PN 5 | ns | ns | ns | ns |
| CP 5 x βg+M 5 | ns | ns | ns | ns |

Values presented as means ± standard deviation (±SD). ns = not significant ($P>0.05$); *$P<0.05$.
[1] Digestive somatic index: (digestive tract (g)/ body weight (g))*100.
[2] Hepatosomatic index: (liver weight (g)/ body weight (g)) *100.
[3] Visceral fat index: (visceral fat (g)/ body weight (g)) *100.
[5] Intestinal quotient: length of the digestive tract/total fish length.

**Table 4.** Proximate body composition of whole fish (g $kg^{-1}$) and nutrients deposition of jundiá (*Rhamdia quelen)* supplemented with different Dietary Fiber Concentrates (DFC)

| Treatments | Moisture | Crude protein | Fat | Ash | TDCP[1] | TFD[2] |
|---|---|---|---|---|---|---|
| Control | 749±1.6 | 155±0.6 | 72±1.1 | 30±0.6 | 72±0.3 | 35±0.5 |
| CP 5 | 752±1.4 | 148±0.7 | 69±0.5 | 27±0.6 | 77±0.4 | 37±0.3 |
| MG 5 | 749±1.6 | 158±1.9 | 73±1.0 | 31±0.9 | 79±1.1 | 38±0.6 |
| PN 5 | 748±2.1 | 144±1.5 | 70±1.3 | 32±0.4 | 69±0.8 | 36±0.8 |
| βg+M 5 | 744±2.3 | 146±0.8 | 78±2.0 | 31±0.4 | 77±0.5 | 44±1.2 |
| MG 10 | 745±1.4 | 149±1.8 | 73±1.2 | 29±0.8 | 72±1.0 | 37±0.7 |
| PN 10 | 738±2.1 | 150±1.0 | 85±2.8 | 33±1.0 | 72±0.5 | 44±1.6 |
| βg+M 10 | 737±0.9 | 145±0.6 | 76±1.0 | 34±1.1 | 76±0.4 | 42±0.6 |
| | | | Orthogonal contrasts | | | |
| Control x treatments | ns | * | ns | ns | ns | ns |
| Control x MG | ns | ns | ns | ns | ns | ns |
| Control x PN | ns | * | ns | ns | ns | ns |
| Control x βg+M | ns | * | ns | ns | * | * |
| MG 5 x MG 10 | ns | ns | ns | ns | ns | ns |
| PN 5 x PN 10 | ns | ns | ns | ns | ns | ns |
| βg+M 5 x βg+M 10 | ns | ns | ns | ns | ns | ns |
| CP 5 x DFCs | ns | ns | ns | ns | ns | ns |
| CP 5 x MG 5 | ns | ns | ns | ns | ns | ns |
| CP 5 x PN 5 | ns | ns | ns | ns | * | ns |
| CP 5 x βg+M 5 | ns | ns | ns | ns | ns | ns |

Values presented as means ± standard deviation (±SD). ns = not significant ($P$>0.05); *$P$<0.05.
[1] Total deposition of crude protein.
[2] Total fat deposition.

**Table 5.** Digestive enzymes activity of jundiá (*Rhamdia quelen)* supplemented with different Dietary Fiber Concentrates (DFC)

| Treatments[1] | Trypsin (µmol/tame/min/mg prot) | Chymotrypsin (mmol/btee/min/mg prot) |
|---|---|---|
| Control | 13.22±4.0 | 12.22±3.3 |
| CP 5 | 8.68±0.4 | 8.03±1.1 |
| MG 5 | 12.05±4.6 | 11.12±3.0 |
| PN 5 | 7.22±1.3 | 7.06±1.0 |
| βg+M 5 | 10.32±4.4 | 9.27±3.2 |
| MG 10 | 11.93±5.2 | 11.32±4.4 |
| PN 10 | 11.90±4.1 | 11.15±2.2 |
| βg+M 10 | 11.61±2.6 | 10.74±1.9 |
| | Orthogonal contrasts | |
| Control x treatments | ns | ns |
| Control x MG | ns | ns |
| Control x PN | * | * |
| Control x βg+M | ns | ns |
| MG 5 x MG 10 | ns | ns |
| PN 5 x PN 10 | * | * |
| βg+M 5 x βg+M 10 | ns | ns |
| CP 5 x DFCs | ns | ns |
| CP 5 x MG 5 | ns | * |
| CP 5 x PN 5 | ns | ns |
| CP 5 x βg+M 5 | ns | ns |

Values presented as means ± standard deviation (±SD). ns = not significant ($P>0.05$); *$P<0.05$.

# 3 ESTUDO CIENTÍFICO 3

Effect of supplementation of Dietary Fiber Concentrates on biochemical parameters, stress response, immune response and skin mucus of jundiá (*Rhamdia quelen*)

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Abstract

The aim of this study was to assess the effects of different Dietary Fiber Concentrates (DFC: Mucilage = MG; Pectin = PN or β-glucan+mannan = βg+M), on biochemical parameters, stress response, immune response and skin mucus of jundiá (*Rhamdia quelen).* The fish (7.16 ± 0.06g) were fed with Control diet (0 g $kg^{-1}$ of DFC); diet supplemented with 5 g $kg^{-1}$ of commercial prebiotic (CP 5) or diets supplemented with 5 or 10 g $kg^{-1}$ of MG; PN or βg+M. After 8 weeks of the feeding trials, biochemical parameters (cholesterol, glucose, albumin and total protein), cortisol, immunoglobulin IgM and mucoproteins of skin mucus were assessed. Results demonstrated that at the end of the trial supplementation with PN increased cholesterol levels ($P<0.05$). After application of the stressor, most fish, except those in the PN and 10 g $kg^{-1}$ MG groups, showed significant increases ($P<0.05$) in cholesterol, glucose and albumin levels. The jundiás showed no difference in cortisol levels after application of the stressor ($P>0.05$). IgM levels were significantly high in fish supplemented with DFC ($P<0.05$). However, the concentration of mucoproteins in skin mucus was not influenced in the different treatments ($P>0.05$). The results showed that supplementation with DFC promoted beneficial effects on the metabolism of jundiá and immune response.

## Introduction

The jundiá (*Rhamdia quelen*) is a species of fish endemic to South America with high productive potential in temperate or subtropical climate [1], because it is well adapted to intensive farming systems. However, intensive farming of this species can be commercially successful if supported by control of stress on fish, because stress leads to infections by bacteria and protozoa, causing decrease in livestock breeding and performance [2]. For a long time, the inclusion of sub-therapeutic doses of antibiotics in feeds was used as a strategy to control these pathogens. However, bacterial strains with increased resistance to antibiotics [3], the presence of antibiotic residues in meat for human consumption and contamination of farming systems have restricted the use of this sanitary practice [4].

Currently, there is growing demand for healthy aquaculture [5], preferably where the use of specific food additives or dietary raw materials positively influence animal performance and animal welfare, while promoting food security and avoiding negative impacts to the farming environment. In this context, a new generation of environment friendly products has emerged, and prebiotics play an effective role in promoting and maintaining the growth and health of animals.

Prebiotics are defined as "*nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health*" [6]. Prebiotics that are being studied as promoters of fish growth include inulin, fructooligosaccharides (FOS), short-chain fructooligosaccharides, mannanooligosaccharides (MOS), galactooligosaccharides

(GOS), xylooligosaccharides (XOS), arabinoxylooligosaccharides (AXOS), isomalto-oligosaccharides (IMO) and GroBiotic®-A [4]. Currently, dietary fibers, composed mostly of polysaccharides, have also demonstrated prebiotic action associated with their levels of inclusion and source of origin. Results have shown that prebiotic supplementation not only contributes positively to intestinal microbiota [7-8], production of short chain fatty acids [7], intestinal morphology [9] and survival [10-11], but may also positively affect animal performance [10,12, 9,13,11]. The beneficial effects of fibers are attributed largely to the products generated from the fermentation by commensal bacterial (lactic acid and short chain fatty acids) [14], which are readily absorbed in the large intestine, thus serving as a source of energy for the host [15].

According to Maita [16] nutritional strategies, such as adjusting the levels of specific nutrients in the diet, manipulation of nutritional status through diets and administration of immunostimulant ingredients, can be used to maintain fish health. Thus, the aim of this study was to evaluate the effects of high-fiber food products (mucilage, pectin and β-glucan + mannan) on biochemical responses and stress response, interference with the immune system and skin mucus of jundiá (*Rhamdia quelen*).

## Material and methods

*Preparation of Dietary Fiber Concentrates (DFC)*

The β-glucan+mannan fraction was produced from brewer's yeast (*Saccharomyces cerevisiae*) by autolysis and alkali treatment (1% NaOH) according to the methodology described by Matiazi [17] and Chaud et al. [18], with some modifications.

Mucilage was obtained from the grain of linseed in an aqueous medium at a 10% w/v concentration, at a temperature of 60 to 80°C with constant stirring, for 150 minutes, based on the methodology described by Goulart et al. [19]. Pectin was produced from citrus pulp in an aqueous medium under average temperature of 100°C, according to the methodology described by Calliari [20].

*Experimental diet*

A basal diet was formulated for *Rhamdia quelen* (Table 1), this basal served as the control diet. The experimental diets were produced by supplementation of the basal formulation with 5 g $kg^{-1}$ of commercial prebiotic ( based on mannanoligosaccharides: Bio-Mos® Alltech, Lexington, Kentucky, USA) (diet CP 5), 5 g $kg^{-1}$ of mucilage (diet MG 5); 5 g $kg^{-1}$ of pectin (diet PN 5); 5 g $kg^{-1}$ of β-glucan+mannan (diet βG+M 5); 10 g $kg^{-1}$ of mucilage (diet MG 10); 10 g $kg^{-1}$ of pectin (diet PN 10) and 10 g $kg^{-1}$ of β-glucan+mannan (diet βG+M 10) (Table 1). The experimental diets were formulated to contain approximately 360 g kg-1 of CP and about 13.4 MJ kg-1 of Digestible Energy (DE). To prepare the diets, the dry ingredients were mixed manually until complete homogenization; soybean oil and water were added shortly afterwards. The diets were pelleted, oven dried at 50° C for a period of 24 hours, crushed, placed in plastic bags and stored in a freezer ( -18° C) until the fish were fed.

*Fish farming and feeding trial*

The experiment was conducted at the Fish Farming Laboratory, Department of Animal Science, Santa Maria Federal University , after approval by the Ethics Committee on Animal Experimentation of UFSM under nº23081.009051 / 2014-53. The experiment was divided into two phases. In the first phase, the jundiás (initial weight of 7.16 ± 0.06) were randomly distributed into 24 tanks (125L) at a density of 30 fish per tank (three tanks per treatment). During the experimental period the water quality parameters were monitored and maintained as follows: temperature of 23.1±1.29º C; dissolved oxygen: 5.25±0.65 mg $L^{-1}$; pH: 7.04±0.22 units; total ammonia: 0.28±0.26 mg $L^{-1}$; nitrite: 0.096±0.09 mg $L^{-1}$; alkalinity: 31.6±7.65 mg $L^{-1}$ of $CaCO_3$; and hardness: 32.8±12.04 mg $L^{-1}$ of $CaCO_3$. According to Baldisserotto & Radünz Neto [21] these parameters are within the optimum range for *Rhamdia quelen* culture. During the first experimental period, which lasted 8 weeks, the fish were fed three times a day to apparent satiation. Daily siphoning was held for removal of waste debris.

*Sampling*

At the end of the experimental period, three fish per tank were sampled for plasma collection through previously heparinized syringes. After centrifugation (1000g, 10 min), the obtained plasma was stored in Eppendorf tubes and refrigerated at -20 ° C until further analysis.

Blood tests

The levels of cholesterol, glucose, albumin and total circulating proteins were determined from plasma by using Doles® commercial kits.

*Acute stress challenge and blood tests*

In the second phase, after five days of completion of the first phase, the jundiás used for experimental feeding were subjected to an acute stressor; they were chased with dip nets for 60 seconds, according to Barcellos et al. [22] . To evaluate the response of cortisol and biochemical parameters to the treatments, 3 fish per tank were sampled for blood collection 1 hour after application of the stressor [22]. Through previously heparinized syringes, blood was collected of nine fish per treatment. After centrifugation (1000g, 10 min) the obtained plasma was stored in Eppendorf tubes and refrigerated at -20° C until further analysis. Cortisol concentration was determined in duplicate using a commercially available assay kit (EIAgen™ Cortisol Test; BioChem Immuno Systems). Likewise, levels of cholesterol, triglycerides, glucose, total circulating proteins and albumin were determined by using DOLES® commercial colorimetric kits.

Immunological analysis

The concentration of immunoglobulin M (IgM) was determined by Bioclin® colorimetric kit. The principle of this kit is to form an insoluble complex between the IgM and the specific antiserum, producing turbidity whose intensity increases absorbance, which is proportional to the concentration of IgM in the sample.

Analysis of mucus

After being kept 18 h without feeding, 9 fish per treatment were anaesthetized with Benzocaine (100 mg $L^{-1}$) and their skin mucus was collected by carefully scraping to avoid contamination with blood. The mucus was homogenized with potassium phosphate buffer (20 mM, pH = 7.5) and centrifuged at 1000 g for 10 min. Mucoprotein concentrations of the resulting supernatants were determined by a Bioclin® commercial kit. This method is based on precipitating proteins in perchloric acid solution, which results in a glycoprotein fraction known as seromucoids or mucoproteins. They are precipitated from the filtrate with phosphotungstic acid and then dissolved, and their tyrosine content is determined.

*Statistical analyses*

Data were subjected to analysis of outlier detection. Later, variance analysis by the F-test and the comparison between treatments by orthogonal contrasts ($P<0.05$) were performed (Table 2). Student's t-test was used to compare plasma biochemical results before and after application of stress.

## Results

Higher levels of plasma cholesterol were observed in fish supplemented with PN compared with the control group before application of stress ($P<0.05$) (Figure 1a). The stressor caused a significant increase in cholesterol levels ($P<0.05$), except in fish

supplemented with pectin and 10 g $kg^{-1}$ of MG. After stress, there were no significant differences in cholesterol levels between treatments.

For glucose (Figure 1b), there were no significant differences between treatments ($P$>0.05) before and after application of stress. However, after application of stress, glucose levels increased significantly ($P$<0.05) for all fish except for those supplemented with 5 g $kg^{-1}$ of CP.

Albumin levels (Table 3) were higher in the βg+M 10 group compared with the βg+M 5 group ($P$<0.05) before and after application of stress. Furthermore, stress caused a significant increase of albumin, regardless of supplementation with DFC.

The total protein (TP) levels differed significantly between treatments only after stress ($P$>0.05), with higher levels in fish supplemented with 5 g $kg^{-1}$ of MG compared with the CP 5 group ($P$<0.05). Only fish fed with the treatments MG 5, βg+M 5 and MG 10 had significant elevation of PT after stress.

The induction of acute stress caused no significant differences in cortisol levels ($P$>0.05) (Figure 2).

Supplementation with DFCs led to a significant increase in IgM levels compared with the control group ($P$<0.05) (Figure 3). Mucilage showed the best prebiotic effect at its lowest level of supplementation ($P$<0.05). The lowest level of pectin (5 g $kg^{-1}$) caused a decrease in IgM production compared to supplementation of 10 g $kg^{-1}$ and compared to supplementation with 5 g $kg^{-1}$ of PC ($P$<0.05).

The levels of mucoprotein in the skin was not altered ($P$>0.05) by the treatments (Figure 4).

## Discussion

The effectiveness of prebiotics in animal nutrition has been evidenced by the growth of luminal populations, anatomical characteristics of the gastrointestinal tract, as well as improved immune system, performance and fish health [4]. However, there are still few studies assessing their effect on biochemical parameters in native fish.

Levels of blood components such as total protein, glucose and cholesterol are directly associated with resistance to bacterial infections and stress [23], and they help monitor the physiological condition of fish [24, 16, 25]. The results revealed that jundiás supplemented with Pectin showed high plasma cholesterol levels before application of stress. It is suggested that elevated cholesterol levels may be attributed to increased production of SCFA (short chain fatty acids), mainly acetate, which is the main product of pectin fermentation at the intestinal level [26] and the primary substrate for cholesterol synthesis [15]. Cholesterol is a precursor of several important hormones that protect fish against stress. Thus, this metabolite is considered to play an important role in immune defenses [27]. As an example, fish from the species *Seriola quinqueradiata* with higher cholesterol levels (274 mg / 100 ml) had higher survival rates when compared to fish with lower levels of total cholesterol (238mg / 100ml), which were more susceptible to infestation by *Lactococcus garvieae* (238mg/100ml) [23].

After the stressor was applied, the levels of total cholesterol of most fish supplemented with DFCs increased between 37 and 51%. This was not observed for fish fed diets with pectin and 10 g $kg^{-1}$ of MG. The groups that did not show high cholesterol

increase seem to have had better metabolic control against the stressor; however, this topic should be explored further.

The results showed that the DFC did not affect blood glucose levels. Similarly, Hoseinifar et al. [28] found no significant differences in glucose levels of beluga sturgeons *(Huso huso)* after they were fed inactive brewer's yeast *Saccharomyces cerevisiae*. In the present study, there was a rise in blood glucose levels after stress in all the treatments. Likewise, Jeney et al. [29] observed a significant increase in glucose levels after stress in rainbow trout (*Oncorhyncus mykiss*) previously supplemented with β-glucan. The glucose increase after application of stress is caused by elevation of cortisol and epinephrine, which accelerate glycogenolysis and hepatic gluconeogenesis, resulting in hyperglycemia to meet energy demands during stress, allowing the animals to react against the stressor [29].

Higher levels of total proteins, albumin and globulin are associated with more efficient innate immune response [16, 8, 30]. The differences in the levels of albumin and total protein among the animals supplemented with different sources of DFC reflect differences in prebiotic potential of the study sources. Similarly to the results of the present study, juvenile *Labeo rohita* supplemented with mannanooligosaccharides (MOS) and yeast extract had higher albumin and total protein levels [31]. However, Akrami et al. [8] found no significant effect for these parameters on sturgeons *(Huso huso; Linnaeus, 1754*) fed diets containing *inactive Saccharomyces cerevisiae* and MOS.

Glucocorticoids are secreted in response to stress; they are known for being responsible for increasing the susceptibility of fish to diseases [29]. For jundiás, basel cortisol levels range between 23 and 28 ng $mL^{-1}$ [32, 33, 34b, 35]. However, after

application of acute stress, levels rise within a range between 130,35 and 176,78 ng $mL^{-1}$ [33, 34b,35], which are higher than those found in the present study. Under experimental conditions, fish are continually subjected to small levels of stress caused by daily feeding and tank cleaning activities, sampling for biometric measurements and verification of disease status. This sequence of stressors can lead to accumulation of response or habituation of cortisol levels [36], which remain low even after application of acute stress. Unlike the findings of this study, Jeney et al. [29] observed a direct relationship between β-glucan supplementation and plasma cortisol levels after stress in rainbow trout *(Oncorhynchus mykiss)*.

Immunoglobulin M (IgM) is the primary antibody of humoral immune response and its concentration is indicative of physiological status and health of fish [11]. Our results showed that supplementation with DFC increased IgM levels after stress, which is indicative of an increase in immune function and activation of the animals' ability to defend against pathogens [11].

The skin and the mucus are non-specific defense mechanisms which work as a natural barrier against pathogens [16]. Fish mucus plays an important role in mechanical and physiological protection against unfavorable environmental conditions and pathogenic infections. In addition, it is essential for preventing colonization by bacteria, fungi and other aquatic parasites [37]. Our results showed no differences in the concentration of glycoproteins in the mucus. Contrary to the findings of the present study, Hoseinifar et al. [38] revealed that supplementation with xylooligosaccharides increased the antibacterial activity and the protein levels of fish mucus in *Rutilus frisii kutum*.

Overall, the results of this study showed that supplementation of DFC promoted beneficial effects on biochemical parameters of jundiás and positive effects on stress resistance and immune system. This preliminary study encourages further research on the use of DFC (mucilage, pectin and β-glucan + mannans) in other fish species that are relevant in fish farming.

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**Table 1**. Dietary formulations and proximate composition of the experimental diets (g $kg^{-1}$)

| INGREDIENTS (%) | Treatments[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | CP 5 | MG 5 | PN 5 | βg+M 5 | MG 10 | PN 10 | βg+M 10 |
| Fish meal[2] | 660 | 660 | 660 | 660 | 660 | 660 | 660 | 660 |
| Maize starch | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 |
| Cellulose | 60 | 55 | 55 | 55 | 55 | 50 | 50 | 50 |
| Mucilage[3] | - | - | 5 | - | - | 10 | - | - |
| Pectin[4] | - | - | - | 5 | - | - | 10 | - |
| βg+M[5] | - | - | - | - | 5 | - | - | 10 |
| CPre[6] | - | 5 | - | - | - | - | - | - |
| Melbond | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Oil | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| VMP[7] | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| NaCl[8] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Inert[9] | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| BHT[10] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| **Calculated composition**[11] | | | | | | | | |
| CP | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 |
| DE (MJ $kg^{-1}$)[12] | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 |
| Ether extract | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 |
| Ash | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 |
| TDF[13] | 256.2 | 258.3 | 317.8 | 219.5 | 238.6 | 322.9 | 249.5 | 228.9 |
| IF[13] | 201.7 | 200.7 | 159.6 | 183.2 | 196.2 | 168.9 | 183.6 | 100.6 |
| SF[13] | 54.4 | 57.7 | 158.2 | 36.4 | 42.4 | 154.0 | 65.9 | 128.2 |

[1] Control: addition of 0 g $kg^{-1}$ of DFC; CP 5: addition of 5 g $kg^{-1}$ of commercial prebiotic; MG 5: addition of 5 g $kg^{-1}$ of mucilage; PN 5: addition of 5 g $kg^{-1}$ of pectin; βG+M 5: addition of 5 g $kg^{-1}$ of β-glucan+mannan; MG 10: addition of 10 g $kg^{-1}$ of mucilage; PN 10: addition of 10 g $kg^{-1}$ de pectin; βg+M 10: addition of 10 g $kg^{-1}$ of β-glucan+mannan;

[2] Residue filleting tilapia (COPISCES®, Toledo-PR)

[3] Composition of mucilage (g $kg^{-1}$): Rhamnose-Rha: 110; Fucose-Fuc: 30; Arabinose-Ara: 155; Xylose-Xyl: 354; Galactose-Gal: 171; Glucose-Glc: 69; Uronic acid (UA): 111.

[4] Composition of Pectin (g $kg^{-1}$): Rha: 110; Ara: 216; Xyl: 12; Man: 10; Ga: 94; Glc: 429; UA: 217.

[5] Composition of βg+M (g $kg^{-1}$): Man: 745; Gal: 12; Glc: 243.

[6] CPre: commercial prebiotic (Bio-MOS, Alltech®, Lexington, Kentucky, USA)

[7] Vitamin and mineral mixture (Mig Plus®; Casca, PR, Brasil): Folic acid: 300 mg, Pantothenic acid: 3000 mg, Glutamic acid: 1 mg, Cobalt: 60 mg, Copper: 1000 mg, Choline: 102120 mg, Iron: 5000 mg, Biotin: 60 mcg, Iodine: 45 mg, Manganese: 8000 mg, Magnesium: 5%, Selenium 60 mg, Vit. A: 1000 IU, Vit. B1: 1500 mg, Vit. B2: 1500 mg, Vit. B6: 1500 mg, Vit. B12: 2000 mcg, Vit. C: 15000 mg, Vit. D: 240 IU, Vit. E: 10000 mg, Vit. K: 400 mg, Zinc: 14000 mg, Inositol 10000 mg, Niacin 9000 mg, antioxidant: 792 mg.

[8] sodium chloride;

[9] sand;

[10] Butylated hydroxytoluene (BHT)

[11] Calculated from the ingredients analyses

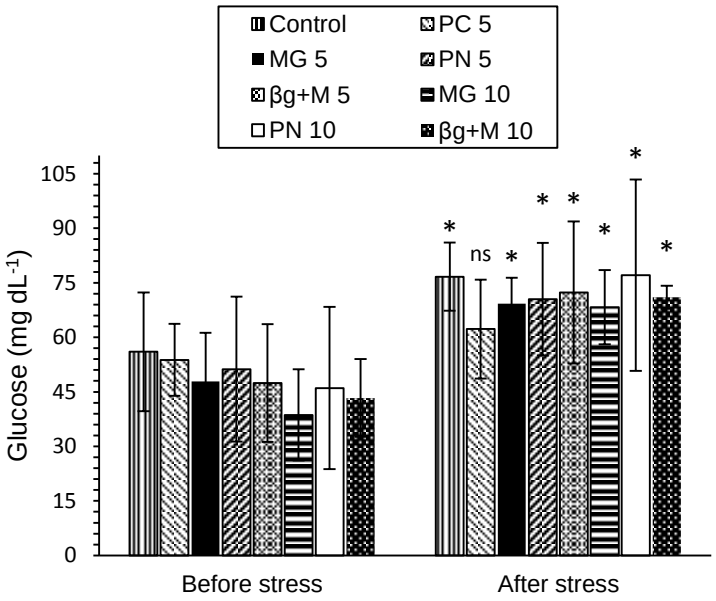
[12] Digestible energy = [(CP * 23.61 MJ $kg^{-1}$ * 0.9) + Fat * 39.82 MJ $kg^{-1}$ * 0.85) + CSDN * 17.21 MJ $kg^{-1}$ *0.50)] (Jobling 1983).

[13] Values analyzed in the Fisheries Laboratory of UFSM and expressed on dry matter basis; TDF: total dietary fiber; IF: insoluble fiber; SF: soluble fiber.

**Table 2.** Description of orthogonal contrasts

| Contrasts | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | CP 5 | MG 5 | PN 5 | βg+M 5 | MG 10 | PN 10 | βg+M 10 |
| Control x treatments | -6 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| Control x MG | -2 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Control x PN | -2 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| Control x βg+M | -2 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| MG 5 x MG 10 | 0 | 0 | -1 | 0 | 0 | 1 | 0 | 0 |
| PN 5 x PN 10 | 0 | 0 | 0 | -1 | 0 | 0 | 1 | 0 |
| βg+M 5 x βg+M 10 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 1 |
| CP 5 x DFCs | 0 | -3 | 1 | 1 | 1 | 0 | 0 | 0 |
| CP 5 x MG 5 | 0 | -1 | 1 | 0 | 0 | 0 | 0 | 0 |
| CP 5 x PN 5 | 0 | -1 | 0 | 1 | 0 | 0 | 0 | 0 |
| CP 5 x βg+M 5 | 0 | -1 | 0 | 0 | 1 | 0 | 0 | 0 |

**A**




**B**

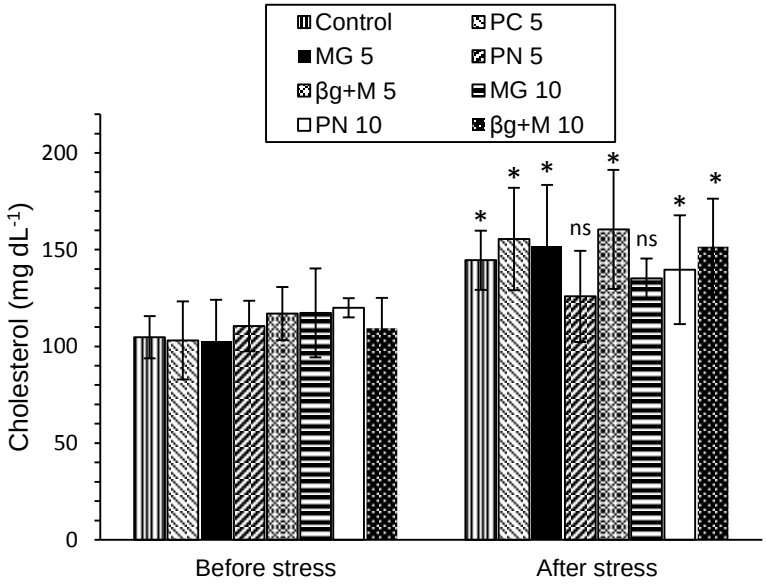



**Figure 1**. Plasma levels of cholesterol (A) and glucose (B), before and after application of an acute stressor in jundiás (*Rhamdia quelen*) fed different Dietary Fibers Concentrates. Mean ± standard deviation. ns = not significant. *Significant difference between groups of the same treatment at different times (Student's t-test, $P$ <0.05).

**Table 3.** Plasma levels of albumin and total protein before and after treatment of acute stressor in jundiá (*Rhamdia quelen*) fed different Dietary Fibers Concentrates (DFC)

| | Before stress | | After stress | |
|---|---|---|---|---|
| Treataments | Albumin[1] | TP[2] | Albumin[1] | TP[2] |
| Control | 0.37±0.08 | 3.59±0.53 | 0.59±0.10* | 3.96±0.09 |
| CP 5 | 0.38±0.03 | 3.58±0.39 | 0.58±0.02* | 3.87±0.18 |
| MG 5 | 0.38±0.05 | 3.47±0.15 | 0.63±0.12* | 4.27±0.33* |
| PN 5 | 0.40±0.07 | 3.59±0.29 | 0.60±0.05* | 3.71±0.33 |
| βg+M 5 | 0.35±0.04 | 3.47±0.34 | 0.56±0.05* | 4.07±0.09* |
| MG 10 | 0.36±0.04 | 3.41±0.48 | 0.59±0.07* | 4.05±0.43* |
| PN 10 | 0.45±0.04 | 3.89±0.52 | 0.58±0.04* | 4.06±0.13 |
| βg+M 10 | 0.41±0.04 | 3.75±0.38 | 0.64±0.09* | 3.92±0.42 |
| | | | | |
| Control x treatments | ns | ns | ns | ns |
| Control x MG | ns | ns | ns | ns |
| Control x PN | ns | ns | ns | ns |
| Control x βg+M | ns | ns | ns | ns |
| MG 5 x MG 10 | ns | ns | ns | ns |
| PN 5 x PN 10 | ns | ns | ns | ns |
| βg+M 5 x βg+M 10 | * | ns | * | ns |
| CP 5 x DFCs | ns | ns | ns | ns |
| CP 5 x MG 5 | ns | ns | ns | * |
| CP 5 x PN 5 | ns | ns | ns | ns |
| CP 5 x βg+M 5 | ns | ns | ns | ns |

[1]g $dL^{-1}$. [2]Total protein: g $dL^{-1}$.

Mean ± standard deviation. ns = not significant. *Significant difference between groups of the same treatment at different times (Student's t-test, $P$ <0.05).

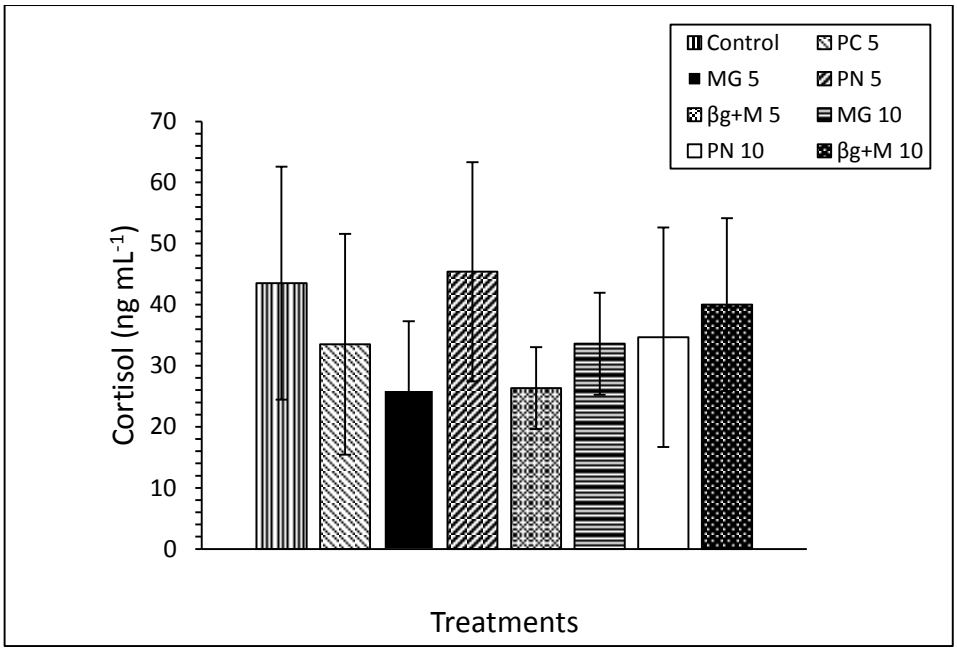



**Figure 2.** Levels of plasma cortisol of jundiás (*Rhamdia quelen*) fed different Dietary Fibers Concentrates, after application of an acute stressor. Mean ± standard deviation.

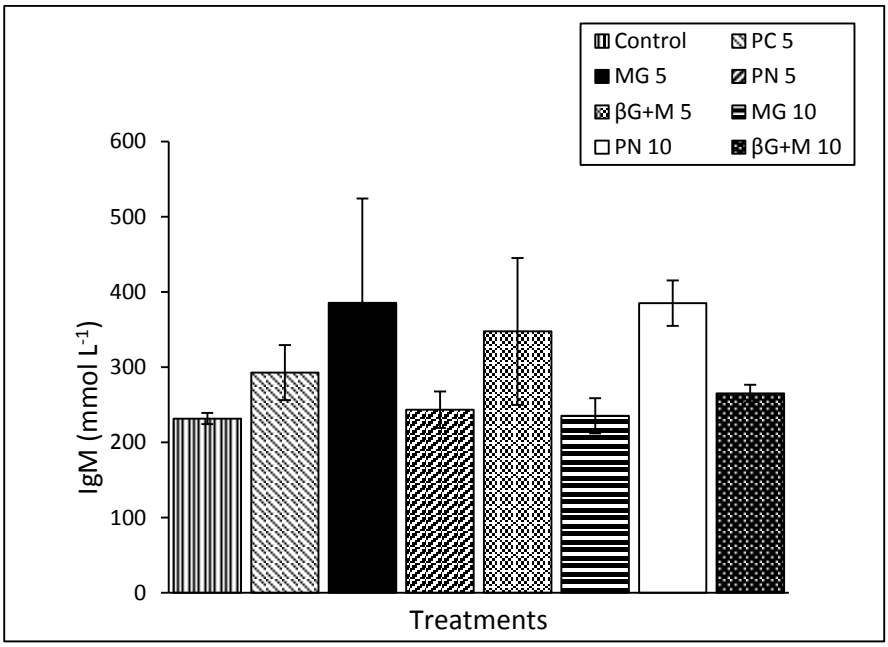



**Figure 3**. Levels of IgM of jundiás (*Rhamdia quelen)* fed different Dietary Fibers Concentrates, after application of an acute stressor. Mean ± standard deviation.

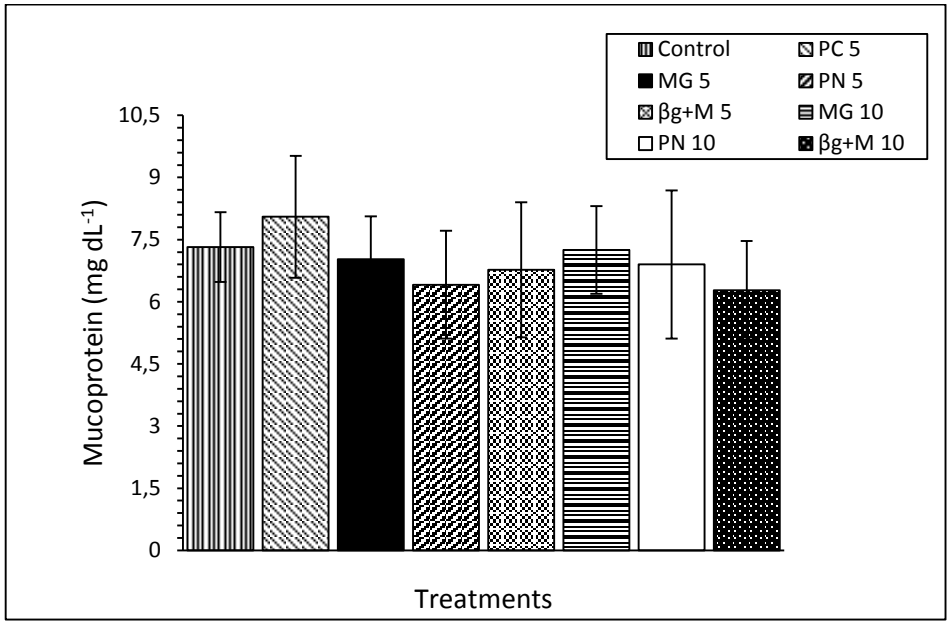



**Figure 4.** Levels of mucoproteins skin jundiá (*Rhamdia quelen*) fed different Dietary Fibers Concentrates, after application of an acute stressor. Mean ± standard deviation.

# 5 ESTUDO CIENTIFICO 4

Effect of Dietary Fiber Concentrates on growth performances, gut morphology and hepatic metabolic intermediates in jundiá (*Rhamdia quelen)*

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**Abstract**

A study was conducted to investigate the effect of Dietary Fiber Concentrates (DFC: Mucilage = MG; Pectin = PN or β-glucan+mannan = βg+M) on growth performances, gut morphology and hepatic metabolic intermediates in jundiá (*Rhamdia quelen)*. The fish were fed with Control diet (0 g $kg^{-1}$ of DFC); diet supplemented with 5 g $kg^{-1}$ commercial prebiotic (CP 5) or diets supplemented with 5 or 10 g $kg^{-1}$ diet of MG; PN or βg+M. At the end of the trial (8 weeks) growth was significantly ($P<0.05$) higher in fish fed diets supplemented with DFCs and did not differ from animals supplemented with CP. Fish fed with DFCs supplemented diets obtained significantly greater intestinal villus height. However, the animals in CP 5 group showed higher values for this variable compared to the other treatments. Regarding the thickness of the epithelium (TE) bowel, it was greater in the Control group compared to animals supplemented with βg+M. Likewise, treatment with CP 5 showed higher values of TE compared to the DFCs. No effect of treatment on the amounts of protein and hepatic glucose were observed. However, the fish supplemented with DFCs, had higher glycogen storage compared to the control group. Similarly, animals supplemented with 10 g $kg^{-1}$ of PN showed greater liver glycogen value higher when compared to fish supplemented with 5 g $kg^{-1}$ of PN. These results indicate that DFCs can be considered as a beneficial dietary supplement for improving growth performance, gut morphology and hepatic metabolic intermediates of jundiá.

**Keywords**: Prebiotics. Fiber dietary. *Rhamdia quelen*. Mucilage. Pectin. β-glucan + mannan.

## Introduction

Much of the success in intensive fish farming is in the control of infestation by bacteria and protozoa, which are responsible for the decline in zootechnical and reproductive performances. In order to diminish these effects, the widespread use of antibiotics and other therapeutic chemical treatments led to those drugs resistance problems in aquaculture. In order to reduce the use of those drugs there has been an increase on the search for alternative nutraceuticals products such as prebiotics and probiotics, which contribute to the host health and ensure the maximum performance (Hoseinifar et al. 2011; Zhu et al. 2012).

Prebiotics are defined as non-digestible oligosaccharides that beneficially affect the host by stimulating growth and/or activity of a limited number of bacteria in the intestine (Gibson 2004). Examples of prebiotics with proven actions to increase growth, improve immune response, modulating the gut microflora, increase resistance against stress and diseases, are mainly composed of oligosaccharides of galactose, fructose or mannose (Torrecillas et al. 2007; Helland et al. 2008; Soleimani et al. 2012; Ganguly et al. 2013; Hoseinifar et al. 2013). However, the prebiotic action has also been intensely observed in sources of more complex polymers, such as non-starch polysaccharides, insoluble and soluble, which compose the major portion of the dietary fiber and vegetable organic residues. These polysaccharides present great diversity of chemical structure and polymerization degree, which is reflected on their physicochemical and fermentability characteristics, demonstrating wide technological potential yet unexplored in fish nutrition.

The available information on the prebiotic effect of non-starch polysaccharides to fish, especially for native species, are scarce when compared to the existing knowledge about their beneficial potential to humans and terrestrial species (Ringo et al. 2010). Therefore, this study aimed to evaluate the prebiotic effect of different Dietary Fiber Concentrates (DFCs) obtained from agribusiness sources (flaxseed, citrus pulp and brewer's yeast), on growth, intestinal morphology and hepatic parameters of juvenile jundiás (*Rhamdia quelen).*

## Material and methods

*Preparation of diets*

Obtaining β-glucan + mannan from brewer's yeast was done according to the methodology described by Matiazi (2006) and Chaud et al. (2007), with some modifications. The flaxseed mucilage was obtained from whole grain flaxseed in aqueous medium, having the experimental methodology described by Goulart et al. (2013) as basis. Pectin was isolated from citrus pulp in aqueous media, according to the methodology described by Calliari (2004).

In the experimental period, the animals were fed isocaloric and isoprotein diets for 8 weeks, formulated to meet the requirements of 360 g $kg^{-1}$ of CP and about 13.4 MJ $kg^{-1}$ of Digestible Energy (DE). Eight mixed feeds were formulated for the experiment, containing fish meal, maize starch and cellulose, added with 0 g $kg^{-1}$ of Dietary Fiber Concentrates (DFC) (control diet), 5 g $kg^{-1}$ of commercial prebiotics ( based on mannan-oligosaccharides (Bio-Mos® Alltech, Lexington, Kentucky, USA) (diet CP 5), 5 g $kg^{-1}$ of mucilage (diet MG 5); 5 g $kg^{-1}$ of pectin (diet PN 5); 5 g $kg^{-1}$ of β-glucan+mannan (diet

βG+M 5); 10 g $kg^{-1}$ of mucilage (diet MG 10); 10 g $kg^{-1}$ of pectin (diet PN 10) and 10 g $kg^{-1}$ of β-glucan+mannan (diet βG+M 10) (Table 1). In order to prepare the diets, the dry ingredients were mixed manually until complete homogenization, then oil and, finally, water were added. The diets were pelleted, dried at the temperature of 50°C for a period of 24 hours; then, they were crushed, placed in plastic bags and stored in a freezer (-18°C) until the animal feeding.

*Fish culture and feeding regime*

The trials were conducted at the Fish Farming Laboratory, Department Animal Science, Santa Maria Federal University, after the approval by the Ethics Committee in Animal Experimentation of the UFSM under nº 23081.009051/2014-53. Jundiá (initial weight of 7.16 ± 0.06) were randomly distributed into 24 tanks (125L) at a density of 30 fish per tank (three tanks per treatment). During the experimental period, the water quality parameters were monitored and maintained as follows: temperature of 23.1±1.29ºC; dissolved oxygen: 5.25±0.65 mg $L^{-1}$; pH: 7.04±0.22 units; total ammonia: 0.28±0.26 mg $L^{-1}$; nitrite: 0.096±0.09 mg $L^{-1}$; alkalinity: 31.6±7.65 mg $L^{-1}$ of $CaCO_3$; and hardness: 32.8±12.04 mg $L^{-1}$ of $CaCO_3$. According to Baldisserotto and Silva (2004), these parameters are within the optimum range for *Rhamdia quelen* culture. During the rearing trial (8 weeks), the fish were hand-fed with experimental diets to apparent satiation three times a day, at 9:00 a.m., 01:30 p.m. and 05:00 p.m. Daily siphoning were performed for removal of waste debris.

*Growth performance and survival*

All fish were weighed at the beginning and at the end of 8 week-feeding trial for estimation of growth. Growth performance and survival rate of jundiás were calculated using the following formula: Weight gain (WG) (g) = final weight - initial weight and relative weight gain (RWG) (%) = [(final weight - initial weight) / initial weight] x 100, feed efficiency index (FEI) (%) = (weight gain / total feed intake) x 100. In addition, survival rate was calculated at the end of the experiment: survival = (Nf/N0) x 100; where $N_0$ is the initial number of fish and $N_f$ is the final number of fish.

*Sample collection*

After 8 weeks of feeding, 3 fish (starved for 18h) were sampled from each tank for histological analysis and liver analysis.

For histological analyzes of the gut, samples from the intestine intermediate portion were collected. The samples were fixed in 10% formalin, cleaved, dehydrated in alcohol, starting from 70% to absolute alcohol, cleared in xylene (xylene) and embedded in molten paraffin. After solidification, the blocks were cut in six micrometers on a rotary microtome, and the cuts were wedged to the histological slides. For staining, the HE (hematoxylin-eosin) technique was used. Nine replicates of each treatment were used in order to observe the histological structure of the intestine. In each repetition, the villus height was estimated in six villi and the thickness of the villi (epithelium) muscular layer using ImageJ software.

In the liver, the glucose concentration was determined by the method of Park and Johnson (1949). This method consisted of incubating a sample aliquot in a medium containing potassium ferricyanide and sodium carbonate, for 15 minutes in a water bath. Afterwards, ferricammonium sulphate and acid solution with sodium lauryl sulfated were added. The reading was performed in an absorbance spectrophotometer at 660nm. Liver glycogen was extracted hot as determined by the methodology of Bidinotto et al. (1998). Liver Protein was determined by the method of Bradford (1976) using bovine serum albumin as standard.

*Statistical analysis*

Means and standard deviations (SD) were calculated for each parameter measured. Results were expressed as mean ± SD. Data were first analyzed for outlier identification. Analyses of variance were performed by F-test, and the treatments were compared through orthogonal contrasts, at 5% probability level.

## Results

*Growth performances*

Jundiás fed with diets supplemented with DFCs had higher FW, WG, RWG and FEI compared to animals fed with the control diet ($P<0.05$) (Table 2). For these variables, no differences were observed for animals fed with 0.5% DFCs related to the animals supplemented with 5 g $kg^{-1}$ of CP. The survival rate was 100% in all treatments.

*Intestinal histology*

Villus height (VH) and epithelial thickness (ET) of jundiás intestine were significantly affected by the diets ($P<0.05$) (Table 3). Fish fed with diets supplemented with DFCs, except for those supplemented with 5 g $kg^{-1}$ of CP, obtained higher villus height ($P<0.05$) when compared to Control. The intestinal epithelial thickness was significantly higher in the Control group compared to animals supplemented with β-glicana+Manana. Likewise, animals treated with CP 5 showed higher ET when compared to the ones supplemented with PN 5 or with βg+M 5 ($P<0.05$).

*Hepatic metabolic intermediates*

No influence of the treatments were observed on the values of protein and hepatic glucose ($P>0.05$) (Table 4). However, the fish supplemented with Mucilage and Pectin had higher glycogen levels compared to the jundiás on the Control group ($P>0.05$). Likewise, the animals in group PN 10 showed higher liver glycogen values than the PN 5 group ($P>0.05$).

## Discussion

The effect of prebiotics on growth parameters in several species of fish have been intensively studied, but the data regarding the effect of non-starch polysaccharides as ecofriendly promoter is still limited. Thus, this study was the first to investigate the effect of DFC as a prebiotic to jundiás.

The use of non-starch polysaccharides concentrated promoted similar effects to commercial prebiotic (Bio Mos®, Alltech), with significant improvement in the performance

of juvenile jundiá. Those effects may be associated with the modulation of beneficial intestinal microflora and consequently increase digestibility and nutrient absorption. These results are equivalent to those reported for other prebiotics proven action. Soleimani et al. (2012) and Hoseinifar et al. (2013) found high growth in fish of the *Rutilus rutilus* specie supplemented with fructooligosaccharide (FOS) and galactooligosaccharide (GOS), respectively. Yellow catfish (*Pelteobagrus gulvidraco*) supplemented with mannanoligosaccharide have also presented better performance compared to animals receiving the basal diet (Wu et al. 2014). In contrast to these positive effects, *Sciaenops ocellatus* supplemented with Bio-MOS® (mannanoligosaccharide) did not differ from the control. However, this same species when supplemented with FOS and Previda™ (galactoglucomannan), demostrated better performance compared to MOS and to the Control (Zhou et al. 2010). Likewise, Sado et al. (2008) related that the adition of MOS (Active MOS®) in the diet of Nile tilapia (*Oreochromis niloticus*), it did not made improvements on the species performance. These contradictory effects are associated with anatomical and physiological traits between different species of fish and the inability of the intestinal microbiota in fermented prebiotics with different compositions.

Supplementation with DFCs promoted improvements in the heights of intestinal villi, with little interference on the epithelium thickness. The observed increase in fish VH is very desirable because alterations in intestinal morphology, as shorter villi and deeper crypts, are associated with increased susceptibility to diseases caused by intestinal pathogens (Brumano and Gattás 2009; Ferreira 2012). The greater the height of the intestinal villi the better digestion and nutrient absorption, being reflected in positive

effects on animal performance, which may explain the increase in weight gain in the groups supplemented with DFCs in this study. Similar to our results, Zhou et al. (2010) also observed improvements in microvilli height of red drum *(Sciaenops ocellatus)* supplemented with prebiotics. In contrast, the administration of prebiotics did not promote improvements in gut morphology of the species *Rutilus frisii kutum* (Hoseinifar et al. 2014).

Metabolic parameters in order to elucidate the nutritional quality of different ingredients can be used. Our results showed that hepatic parameters, except glycogen levels, were not affected by the inclusion of DFCs. For glycogen, higher levels were found in jundiás supplemented with Mucilage and Pectin. The not hydrolyzed carbohydrates in the small intestine are fermented by colonic bacteria, producing short-chain fatty acids rapidly absorbed by enterocytes (Hyjova and Chmelarova 2007). In those SCFA, propionate and butyrate are captured by the liver and converted into glucose through gluconeogenesis (Champe and Harvey, 1997), and stored in a rapidly deployable form, in this case as glycogen, which explains the higher glycogen levels observed in fish supplemented with DFCs in our study.

In conclusion, this study showed that administration of DFCs (mucilage, pectin or β-glucan + mannan) exerted positive effects on growth parameters and intestinal morphology of jundiás. Furthermore, mucilage and pectin promoted glycogen reserves increase. However, more research should be done with these concentrates of dietary fiber, in order to confirm its prebiotics effects in other fish species.

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**Table 1**. Dietary formulations and proximate composition of the experimental diets (g $kg^{-1}$)

| Ingredients (%) | Treatments[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | CP 5 | MG 5 | PN 5 | βg+M 5 | MG 10 | PN 10 | βg+M 10 |
| Fish meal[2] | 660 | 660 | 660 | 660 | 660 | 660 | 660 | 660 |
| Maize starch | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 | 149.8 |
| Cellulose | 60 | 55 | 55 | 55 | 55 | 50 | 50 | 50 |
| Mucilage[3] | - | - | 5 | - | - | 10 | - | - |
| Pectin[4] | - | - | - | 5 | - | - | 10 | - |
| βg+M[5] | - | - | - | - | 5 | - | - | 10 |
| CPre[6] | - | 5 | - | - | - | - | - | - |
| Melbond | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Oil | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| VMP[7] | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| NaCl[8] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Inert[9] | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| BHT[10] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| **Calculated composition**[11] | | | | | | | | |
| CP | 360 | 360 | 360 | 360 | 360 | 360 | 360 | 360 |
| DE (MJ $kg^{-1}$)[12] | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 | 13.49 |
| Ether extract | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 | 107.5 |
| Ash | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 | 154.1 |
| TDF[13] | 256.2 | 258.3 | 317.8 | 219.5 | 238.6 | 322.9 | 249.5 | 228.9 |
| IF[13] | 201.7 | 200.7 | 159.6 | 183.2 | 196.2 | 168.9 | 183.6 | 100.6 |
| SF[13] | 54.4 | 57.7 | 158.2 | 36.4 | 42.4 | 154.0 | 65.9 | 128.2 |

[1] Control: addition of 0 g $kg^{-1}$ of DFC; CP 5: addition of 5 g $kg^{-1}$ of commercial prebiotic; MG 5: addition of 5 g $kg^{-1}$ of mucilage; PN 5: addition of 5 g $kg^{-1}$ of pectin; βG+M 5: addition of 5 g $kg^{-1}$ of β-glucan+mannan; MG 10: addition of 10 g $kg^{-1}$ of mucilage; PN 10: addition of 10 g $kg^{-1}$ de pectin; βg+M 10: addition of 10 g $kg^{-1}$ of β-glucan+mannan;
[2] Residue filleting tilapia (COPISCES®, Toledo-PR)
[3] Composition of mucilage (g $kg^{-1}$): Rhamnose-Rha: 110; Fucose-Fuc: 30; Arabinose-Ara: 155; Xylose-Xyl: 354; Galactose-Gal: 171; Glucose-Glc: 69; Uronic acid (UA): 111.
[4] Composition of Pectin (g $kg^{-1}$): Rha: 110; Ara: 216; Xyl: 12; Man: 10; Ga: 94; Glc: 429; UA: 217.
[5] Composition of βg+M (g $kg^{-1}$): Man: 745; Gal: 12; Glc: 243.
[6] CPre: commercial prebiotic (Bio-MOS, Alltech®, Lexington, Kentucky, USA)
[7] Vitamin and mineral mixture (Mig Plus®; Casca, PR, Brasil): Folic acid: 300 mg, Pantothenic acid: 3000 mg, Glutamic acid: 1 mg, Cobalt: 60 mg, Copper: 1000 mg, Choline: 102120 mg, Iron: 5000 mg, Biotin: 60 mcg, Iodine: 45 mg, Manganese: 8000 mg, Magnesium: 5%, Selenium 60 mg, Vit. A: 1000 IU, Vit. B1: 1500 mg, Vit. B2: 1500 mg, Vit. B6: 1500 mg, Vit. B12: 2000 mcg, Vit. C: 15000 mg, Vit. D: 240 IU, Vit. E: 10000 mg, Vit. K: 400 mg, Zinc: 14000 mg, Inositol 10000 mg, Niacin 9000 mg, antioxidant: 792 mg.
[8] sodium chloride;
[9] sand;
[10] Butylated hydroxytoluene (BHT)
[11] Calculated from the ingredients analyses
[12] Digestible energy = [(CP * 23.61 MJ $kg^{-1}$ * 0.9) + Fat * 39.82 MJ $kg^{-1}$ * 0.85) + CSDN * 17.21 MJ $kg^{-1}$ *0.50)] (Jobling 1983).
[13] Values analyzed in the Fisheries Laboratory of UFSM and expressed on dry matter basis; TDF: total dietary fiber; IF: insoluble fiber; SF: soluble fiber.

**Table 2.** Growth parameters of jundiá (*Rhamdia quelen)* supplemented with different Dietary Fiber Concentrates (DFCs)

| Treatments[1] | IW (g) | WG (g) | RWG (%) | IFE (%) | Survival (%) |
|---|---|---|---|---|---|
| Control | 7.15±0.1 | 42.97±3.2 | 601.72±51.4 | 74.65±3.4 | 100 |
| CP 5 | 7.12±0.1 | 49.14±2.0 | 690.64±22.5 | 82.32±2.2 | 100 |
| MG 5 | 7.20±0.1 | 48.45±0.6 | 672.80±12.1 | 80.17±1.8 | 100 |
| PN 5 | 7.18±0.1 | 47.27±2.0 | 658.98±37.0 | 80.15±1.9 | 100 |
| βg+M 5 | 7.22±0.1 | 50.85±2.9 | 705.37±52.7 | 84.41±3.6 | 100 |
| MG 10 | 6.96±0.2 | 48.13±2.9 | 665.37±47.2 | 83.71±1.1 | 100 |
| PN 10 | 7.23±0.1 | 46.40±3.0 | 641.61±34.8 | 80.39±4.9 | 100 |
| βg+M 10 | 7.22±0.1 | 50.72±2.0 | 702.38±22.6 | 84.27±3.1 | 100 |
| | | | Orthogonal contrasts | | |
| Control x treatments | ns | * | * | * | ns |
| Control x MG | ns | * | * | * | ns |
| Control x PN | ns | * | ns | * | ns |
| Control x βg+M | ns | * | * | * | ns |
| MG 5 x MG 10 | ns | ns | ns | ns | ns |
| PN 5 x PN 10 | ns | ns | ns | ns | ns |
| βg+M 5 x βg+M 10 | ns | ns | ns | ns | ns |
| CP 5 x DFCs | ns | ns | ns | ns | ns |
| CP 5 x MG 5 | ns | ns | ns | ns | ns |
| CP 5 x PN 5 | ns | ns | ns | ns | ns |
| CP 5 x βg+M 5 | ns | ns | ns | ns | ns |

Values presented as means ± standard deviation (±SD). ns = not significant ($P>0.05$); *$P<0.05$.
IW: initial weight; WG: weight gain; RWG: relative weight gain; IFE: index of food efficiency.

**Table 3.** Intestinal histology of jundiás *(Rhamdia quelen)* supplemented with different Dietary Fiber Concentrates (DFCs)

| Treatments[1] | Villus height (µm) | Thickness epithelium (µm) |
|---|---|---|
| Control | 50.7±13.5 | 23.44±3.1 |
| CP 5 | 92.8±9.6 | 27.26±5.3 |
| MG 5 | 76.5±20.9 | 24.32±4.8 |
| PN 5 | 91.6±41.8 | 23.22±5.4 |
| βg+M 5 | 65.8±21.7 | 19.39±3.4 |
| MG 10 | 76.6±22.5 | 25.05±5.4 |
| PN 10 | 85.5±34.9 | 23.11±3.8 |
| βg+M 10 | 76.9±20.6 | 18.63±4.8 |
| | Orthogonal contrasts | |
| Control x treatments | * | ns |
| Control x MG | * | ns |
| Control x PN | * | ns |
| Control x βg+M | ns | * |
| MG 5 x MG 10 | ns | ns |
| PN 5 x PN 10 | ns | ns |
| βg+M 5 x βg+M 10 | ns | ns |
| CP 5 x DFCs | * | * |
| CP 5 x MG 5 | * | ns |
| CP 5 x PN 5 | ns | * |
| CP 5 x βg+M 5 | * | * |

Values presented as means ± standard deviation (±SD). ns = not significant ($P>0.05$); *$P<0.05$.

**Table 4.** Liver parameters of jundiás (*Rhamdia quelen)* supplemented with different Dietary Fiber Concentrates (DFCs)

| Treatments[1] | Protein[1] | Glucose[2] | Glycogen[3] |
|---|---|---|---|
| Control | 11.62±1.7 | 195.43±45.3 | 6.17±1.1 |
| CP 5 | 11.99±2.3 | 200.05±24.9 | 7.28±1.3 |
| MG 5 | 11.45±2.5 | 195.14±23.2 | 7.37±1.1 |
| PN 5 | 11.62±1.1 | 197.07±22.9 | 7.05±1.1 |
| βg+M 5 | 10.21±1.7 | 186.30±15.5 | 6.75±1.0 |
| MG 10 | 10.52±1.5 | 226.19±74.2 | 7.07±0.6 |
| PN 10 | 10.32±3.4 | 168.95±47.0 | 8.51±1.0 |
| βg+M 10 | 10.21±1.7 | 186.30±15.5 | 6.75±1.0 |
| | Orthogonal contrasts | | |
| Control x treatments | ns | ns | * |
| Control x MG | ns | ns | * |
| Control x PN | ns | ns | * |
| Control x βg+M | ns | ns | ns |
| MG 5 x MG 10 | ns | ns | ns |
| PN 5 x PN 10 | ns | ns | * |
| βg+M 5 x βg+M 10 | ns | ns | ns |
| CP 5 x DFCs | ns | ns | ns |
| CP 5 x MG 5 | ns | ns | ns |
| CP 5 x PN 5 | ns | ns | ns |
| CP 5 x βg+M 5 | ns | ns | ns |

Values presented as means ± standard deviation (±SD). ns = not significant ($P>0.05$); *$P<0.05$.
[1]mg $g^{-1}$;
[2]µmol de glicose $g^{-1}$;
[3]µmol de glicose $g^{-1}$.

# 6 DISCUSSÃO GERAL

Nas últimas décadas, o tradicional uso de antibióticos na aquicultura como promotores de crescimento tem sido limitado em função dos seus efeitos negativos sobre o hospedeiro, ambiente e alimentos. Como alternativa ao uso destes medicamentos, tem se buscado a manipulação da microbiota do trato gastrointestinal dos animais aquáticos através da utilização de oligossacarídeos e de fibras alimentares com potencial prebiótico. Estes suplementos fortalecem a microbiota benéfica do trato digestório, reduzindo a susceptibilidade à doenças do organismo hospedeiro e refletindo-se positivamente sobre o crescimento dos peixes (RINGO et al., 2010). Neste contexto, esta tese foi desenvolvida com o objetivo principal de avaliar o efeito prebiótico de diferentes Concentrados de Fibras Alimentares (CFAs), obtidos a apartir de fontes agroindustriais com amplo potencial tecnológico ainda pouco explorado.

No ensaio biológico (Estudos 2 e 4) verificou-se aumento do desempenho dos jundiás quando suplementados com os CFAs em relação ao grupo Controle. Além disso, os animais que receberam 5 g $kg^{-1}$ dos diferentes CFAs obtiveram resultados semelhantes à adição de 5 g $kg^{-1}$ de prebiótico comercial e prebióticos já consolidados, como frutoligossacarídeos, galactoligossacaríeos e mananoligossacarídeos (SOLEIMANI et al., 2012; HOSEINIFAR et al., 2013; WU et al., 2014). Estes efeitos positivos estão atrelados a composição monossacarídica presente nestes Concentrados de Fibras, com grande diversidade de estrutura química (Estudo 1) e grau de polimerização, o que se reflete sobre suas características físico químicas e de fermentabilidade. Dentre os monossacarídeos presentes na mucilagem de linhaça, oligômeros de xilose, galactose e arabinose+xilose foram encontrados em maiores quantidades e são responsáveis por promover o crescimento de bifidobactérias benéficas que contribuem para o aumento do crescimento do animal (RINGO et al., 2010). No concentrado βG+M os monossacarídeos encontrados em maior quantidade foram manose (74,5%) e glicose (24,3%) (Estudo 1).

As unidades de manose são responsáveis por aumentar o desempenho de peixes e eficiência alimentar, protege contra patógenos através da potencialização do sistema imune local e sistêmico, além de promover reforço sobre a funcionalidade e integridade intestinal (SINHA et al., 2011; TORRECILAS et al., 2014). Já a β-glicana é considerada um modificador da resposta biológica devido ao seu potencial imunomodulador, pois ao ser reconhecida por receptores celulares específicos tem habilidade de realçar a resposta imune do hospedeiro (MAGNANI; CASTRO-GÓMEZ, 2008).

A melhora do desempenho encontrada nos animais suplementados com os CFAs também pode ser reflexo das melhorias ocorridas sobre a morfometria intestinal (Estudo 4). A suplementação com os CFAs promoveram aumento na altura de vilosidade intestinal (AV). Este acréscimo é bastante desejável, pois alterações na morfologia intestinal, como vilos mais curtos e criptas mais profundas, estão associados à maior susceptibilidade de doenças provocadas por patógenos intestinais (BRUMANO; GATTÁS, 2009; FERREIRA, 2012). Quanto maior a altura das vilosidades intestinais melhor será a digestão e absorção de nutrientes, se refletindo em efeitos positivos sobre desempenho zootécnico, como ocorreu no presente estudo.

Os animais suplementados com Pectina não apresentaram diferenças no peso final em relação ao grupo controle e além disso, obtiveram menor atividade das enzimas tripsina e quimiotripsina (Estudo 2) em comparação aos animais alimentados com a dieta controle. Provavelmente, isto se deve pelo fato das substâncias pécticas estarem incluídas no grupo que tem maior importância no processo de retenção de água, que determina o aumento na viscosidade do alimento (BRITO et al., 2008) e dificulta o acesso das enzimas aos substratos, se refletindo em diminuição da atividade enzimática digestiva e menor desempenho zootécnico (MEURER; HAYASHI, 2003). O que confirma os resultados encontrados, pois dentre os CFAs a Pectina foi a que obteve resultados menos satisfatórios.

Além dos benefícios observados dos CFAs sobre o crescimento dos peixes, deve se ressaltar os efeitos benéficos promovidos sobre o metabolismo e sistema imunológico dos jundiás. Embora a Pectina tenha apresentado menor efeito sobre o desempenho animal dentre os CFAs, esta fibra promoveu aumento nos níveis de colesterol dos jundiás antes da aplicação do estresse, fazendo com que estes níveis não tivessem um aporte

tão elevado após aplicação do agente estressor. O que foi atribuído à melhor controle metabólico frente ao agente estressor, no entanto, este tópico merece maiores esclarecimentos. O colesterol é precursor de vários hormônios importantes que protegem os peixes contra o estresse, dessa forma tem sido indicado que este metabólito desempenha papel importante sobre as defesas do sistema imune (DENG et al., 2013).

Conforme nossos resultados, todos os CFAs adicionados à dieta promoveram acréscimo nos níveis de IgM após o estresse, o que indica aumento na função imune. A imunoglobulina IgM é considerada como principal anticorpo da resposta imune humoral nos peixes e sua concentração apresenta significante importância fisiológica e patológica para estes animais (TALPUR et al., 2014). Talpur et al. (2014) descreve que o aumento de imunoglobulina após desafio em peixes, sugere que sua capacidade de defesa contra patógenos foi ativada.

Além dos benefícios citados dos CFAs sobre o sistema imune de jundiás, estes suplementos promoveram maior aporte de glicogênio hepático (Estudo 4). Confirmando estes resultados, os animais suplementados com Pectina e β-glicana+manana apresentaram maiores valores de relação hepatossomática (Estudo 2), o qual se encontra diretamente relacionado ao aumento de glicogênio no fígado. Os carboidratos que não sofrem hidrólise em nível intestinal são fermentados por bactérias no cólon, obtendo-se como principais produtos desta fermentação os ácidos graxos de cadeia curta (AGCC), os quais são rapidamente absorvidos pelo cécum e colón (HYJOVA; CHMELAROVA, 2007). Destes AGCC, o propionato e butirato, podem ser captados pelo fígado, o qual é capaz de converte-los em glicose através da gliconeogênese, e armazenar este suprimento de glicose em uma forma rapidamente mobilizável, neste caso como glicogênio (CHAMPE; HARVEY, 1997), assim como observado no presente estudo. Sugere-se que o aumento dos níveis de glicogênio através do propionato e butirato é de grande importância para a nutrição de peixes, porque reduz o catabolismo de proteínas para produção de energia através da gliconeogênese, promovendo um efeito poupador de proteína. Este fato pode ter permitido maior deposição de proteína corporal nos jundiás suplementados com a βG+M, onde os peixes passam a utilizar a proteína dietética para crescimento e formação de tecidos e não como fonte de energia

(MORO, 2013), a qual seria suprida pela produção de ácidos graxos de cadeia curta advindos dos CFAs. O aumento na deposição de proteína no peixe é extremamente desejável, pois seu aporte possibilita a competição com outras fontes proteicas amplamente utilizadas na nutrição humana como a carne bovina, suína e aves (ARBELÁEZ-ROJAS et al., 2002).

# 7 CONSIDERAÇÕES FINAIS

Com base nos resultados obtidos neste trabalho, pode-se concluir que:

- A análise nutricional realizada sobre os ingredientes *in natura* e seus respectivos Concentrados de Fibras Alimentares (CFAs) revelou que estes obtiveram concentração no conteúdo de fibra alimentar total, sendo apontados como ingredientes com amplo potencial prebiótico para serem adicionados na dieta de jundiás;
- Apesar das metodologias de concentração de fibras não proporcionarem rendimentos de extração tão elevados, são consideradas tecnicamente viáveis e de baixo risco de contaminação ambiental;
- A suplementação dos jundiás com os CFAs proporcionou melhorias no desempenho e resultados semelhantes ao prebiótico comercial avaliado no presente estudo;
- Não houve influência dos CFAs sobre os valores de cortisol plasmáticos dos jundiás após a aplicação do agente estressor agudo;
- Os CFAs adicionados as dietas promoveram efeitos benéficos sobre parâmetros metabólicos e resposta imune dos jundiás frente ao agente estressor;
- O efeito dos CFAs sobre parâmetros hepáticos e morfometria intestinal foram considerados satisfatórios.
- A elevação da dose dos CFAs sobre os parâmetros de desempenho zootécnico não promoveu efeito significativo, dessa forma, a adição de 5 g $kg^{-1}$ dos CFAs é a mais indicada para nutrição de jundiás

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# ANEXOS

## ANEXO 1- Normas de publicação da Revista Anais da Academia Brasileira de Ciências


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### INSTRUÇÕES AOS AUTORES



**Objetivo e política editorial**

A revista **ANAIS DA ACADEMIA BRASILEIRA DE CIÊNCIAS** encoraja fortemente as submissões online. Uma vez o artigo preparado de acordo com as instruções abaixo, visite o site de submissão online (http://aabc.abc.org.br).

As instruções devem ser lidas cuidadosamente e seguidas integralmente. Desta forma, a avaliação e publicação de seu artigo poderão ser feitas com mais eficiência e rapidez. Os editores reservam-se o direito de devolver artigos que não estejam de acordo com estas instruções. Os artigos devem ser escritos em inglês claro e conciso.

### OBJETIVO E POLÍTICA EDITORIAL

Todos os artigos submetidos devem conter pesquisa original e ainda não publicada ou submetida para publicação. O primeiro critério para aceitação é a qualidade científica. O uso excessivo de abreviaturas ou jargões deve ser evitado, e os artigos devem ser compreensíveis para uma audiência tão vasta quanto possível. Atenção especial deve ser dada ao Abstract, Introdução e Discussão, que devem nitidamente chamar a atenção para a novidade e importância dos dados relatados. A não observância desta recomendação poderá resultar em demora na publicação ou na recusa do artigo.

Os textos podem ser publicados como uma revisão, um artigo ou como uma breve comunicação. A revista é trimestral, sendo publicada nos meses de março, junho, setembro e dezembro.

## TIPOS DE TRABALHOS

**Revisões.** Revisões são publicadas somente a convite. Entretanto, uma revisão pode ser submetida na forma de breve carta ao Editor a qualquer tempo. A carta deve informar os tópicos e autores da revisão proposta e declarar a razão do interesse particular do assunto para a área.

**Artigos.** Sempre que possível, os artigos devem ser subdivididos nas seguintes partes: 1. Página de rosto; 2. Abstract (escrito em página separada, 200 palavras ou menos, sem abreviações); 3. Introdução; 4. Materiais e Métodos; 5. Resultados; 6. Discussão; 7. Agradecimentos quando necessário; 8. Resumo e palavras-chave (em português - os autores estrangeiros receberão assistência); 9. Referências. Artigos de algumas áreas, como Ciências Matemáticas, devem observar seu formato usual. Em certos casos pode ser aconselhável omitir a parte (4) e reunir as partes (5) e (6). Onde se aplicar, a parte de Materiais e Métodos deve indicar o Comitê de Ética que avaliou os procedimentos para estudos em humanos ou as normas seguidas para a manutenção e os tratamentos experimentais em animais.

Breves comunicações

Breves comunicações devem ser enviadas em espaço duplo. Depois da aprovação não serão permitidas alterações no artigo, a fim de que somente correções de erros tipográficos sejam feitos nas provas.
Os autores devem enviar seus artigos somente em versão eletrônica.

**Preparação de originais**

## PREPARO DOS ARTIGOS

Os artigos devem ser preparados em espaço duplo. Depois de aceitos nenhuma modificação será realizada, para que nas provas haja somente correção de erros tipográficos.

Tamanho dos artigos. Embora os artigos possam ter o tamanho necessário para a apresentação concisa e discussão dos dados, artigos sucintos e cuidadosamente preparados têm preferência tanto em termos de impacto quando na sua facilidade de leitura.

**Tabelas e ilustrações.** Somente ilustrações de alta qualidade serão aceitas. Todas as ilustrações serão consideradas como figuras, inclusive desenhos, gráficos, mapas,

fotografias e tabelas com mais de 12 colunas ou mais de 24 linhas (máximo de figuras gratuitas: cinco figuras). A localização provável das figuras no artigo deve ser indicada.

**Figuras digitalizadas.** As figuras devem ser enviadas de acordo com as seguintes especificações: 1. Desenhos e ilustrações devem ser em formato .PS/.EPS ou .CDR (Postscript ou Corel Draw) e nunca inseridas no texto; 2. Imagens ou figuras em meio tom devem ser no formato .TIF e nunca inseridas no texto; 3. Cada figura deve ser enviada em arquivo separado; 4. Em princípio, as figuras devem ser submetidas no tamanho em que devem aparecer na revista, i.e., largura de 8 cm (uma coluna) ou 12,6 cm (duas colunas) e com altura máxima para cada figura menor ou igual a 22 cm. As legendas das figuras devem ser enviadas em espaço duplo e em folha separada. Cada dimensão linear das menores letras e símbolos não deve ser menor que 2 mm depois da redução. Somente figuras em preto e branco serão aceitas. 5. Artigos de Matemática, Física ou Química podem ser digitados em Tex, AMS-Tex ou Latex; 6. Artigos sem fórmulas matemáticas podem ser enviados em .RTF ou em WORD para Windows.

**Página de rosto.** A página de rosto deve conter os seguintes itens: 1. Título do artigo (o título deve ser curto, específico e informativo); 2. Nome (s) completo (s) do (s) autor (es); 3. Endereço profissional de cada autor; 4. Palavras-chave (4 a 6 palavras, em ordem alfabética); 5. Título abreviado (até 50 letras); 6. Seção da Academia na qual se enquadra o artigo; 7. Indicação do nome, endereço, números de fax, telefone e endereço eletrônico do autor a quem deve ser endereçada toda correspondência e prova do artigo.

**Agradecimentos.** Devem ser inseridos no final do texto. Agradecimentos pessoais devem preceder os agradecimentos a instituições ou agências. Notas de rodapé devem ser evitadas; quando necessário, devem ser numeradas. Agradecimentos a auxílios ou bolsas, assim como agradecimentos à colaboração de colegas, bem como menção à origem de um artigo (e.g. teses) devem ser indicados nesta seção.

**Abreviaturas.** As abreviaturas devem ser definidas em sua primeira ocorrência no texto, exceto no caso de abreviaturas padrão e oficial. Unidades e seus símbolos devem estar de acordo com os aprovados pela ABNT ou pelo Bureau International des Poids et Mesures (SI).

**Referências.** Os autores são responsáveis pela exatidão das referências. Artigos publicados e aceitos para publicação (no prelo) podem ser incluídos. Comunicações pessoais devem ser autorizadas por escrito pelas pessoas envolvidas. Referências a teses, abstracts de reuniões, simpósios (não publicados em revistas indexadas) e artigos em preparo ou submetidos mas ainda não aceitos, podem ser citados no texto

como (Smith et al. unpublished data) e não devem ser incluídos na lista de referências.

As referências devem ser citadas no texto como, por exemplo, (Smith 2004), (Smith and Wesson 2005) ou, para três ou mais autores, (Smith et al. 2006). Dois ou mais artigos do mesmo autor no mesmo ano devem ser distinguidos por letras, e.g. (Smith 2004a), (Smith 2004b) etc. Artigos com três ou mais autores com o mesmo primeiro autor e ano de publicação também devem ser distinguidos por letras.

As referências devem ser listadas em ordem alfabética do primeiro autor sempre na ordem do sobrenome XY no qual X e Y são as iniciais. Se houver mais de 10 autores, use o primeiro seguido de et al. As referências devem ter o nome do artigo. Os nomes das revistas devem ser abreviados. Para as abreviações corretas, consultar a listagem de base de dados na qual a revista é indexada ou consulte a World List of Scientific Periodicals. A abreviatura para os Anais da Academia Brasileira de Ciências é An Acad Bras Cienc. Os seguintes exemplos são considerados como guia geral para as referências.

*Artigos*

ALBE-FESSARD D, CONDES-LARA M, SANDERSON P AND LEVANTE A. 1984a. Tentative explanation of the special role played by the áreas of paleospinothalamic projection in patients with deafferentation pain syndromes. Adv Pain Res Ther 6: 167-182.

ALBE-FESSARD D, SANDERSON P, CONDES-LARA M, DELANDSHEER E, GIUFFRIDA R AND CESARO P. 1984b. Utilisation de la depression envahissante de Leão pour l'étude de relations entre structures centrales. An Acad Bras Cienc 56: 371-383.

KNOWLES RG AND MONCADA S. 1994. Nitric oxide synthases in mammals. Biochem J 298: 249-258.

PINTO ID AND SANGUINETTI YT. 1984. Mesozoic Ostracode Genus Theriosynoecum Branson, 1936 and validity of related Genera. An Acad Bras Cienc 56: 207-215.

*Livros e Capítulos de Livros*

DAVIES M. 1947. An outline of the development of Science, Athinker's Library, n. 120. London: Watts, 214 p.

PREHN RT. 1964. Role of immunity in biology of cancer. In: NATIONAL CANCER CONFERENCE, 5, Philadelphia Proceedings …., Philadelphia: J.B. Lippincott, p. 97-104.

UYTENBOGAARDT W AND BURKE EAJ. 1971. Tables for microscopic identification of minerals, 2nd ed., Amsterdam: Elsevier, 430 p.

WOODY RW. 1974. Studies of theoretical circular dichroism of Polipeptides: contributions of B-turns. In: BLOUTS ER ET AL. (Eds), Peptides, polypeptides and proteins, New York: J Wiley & Sons, New York, USA, p. 338-350.

*Outras Publicações*

INTERNATIONAL KIMBERLITE CONFERENCE, 5, 1991. Araxá, Brazil. Proceedings ... Rio de Janeiro: CPRM, 1994, 495 p.

SIATYCKI J. 1985. Dynamics of Classical Fields. University of Calgary, Department of Mathematics and Statistics, 55 p. Preprint n. 600.

## ANEXO 2- Normas de publicação da Revista Aquaculture Nutrition

**Page Charges:** Original research articles exceeding 8 pages when in proof will be subject to a page charge of GBP 100 per additional page. The first 8 pages will be published free of charge. An average 8-page article will have approximately 6200 words in manuscript, with approximately 5 figures or tables and 50 references. An invoice will be sent to authors for these charges upon print publication of their article. Invited and review articles are excluded from this rule. Download Page Charge Form.

**Preparation of the Manuscript:** All sections of the manuscript should be double-spaced and with 30mm margins. Articles are accepted for publication only at the discretion of the Editor(s). Authors will receive prompt acknowledgement of receipt of their paper and a decision will be reached within 3 months of receipt. A manuscript should consist of the following sections:

**Title page:** This should include: the full title of the paper; the full names of all the authors; the name(s) and address(es) of the institution(s) at which the work was carried out (the present addresses of the authors, if different from the above, should appear in a footnote); the name, address, and telephone and fax numbers of the author to whom all correspondence and proofs should be sent; a suggested running title of not more than fifty characters, including spaces; and six key words to aid indexing.

**Main text:** Generally, all papers should be divided into the following sections and appear in the order: (1) Abstract or Summary, not exceeding 150-200 words, (2) Introduction, (3) Materials and Methods, (4) Results, (5) Discussion, (6) Acknowledgements, (7) References, (8) Figure legends, (9) Tables, (10) Figures. The Results and Discussion sections may be combined and may contain subheadings. The Materials and Methods section should be sufficiently detailed to enable the experiments to be reproduced. Trade names should be capitalized and the manufacturer's name and address given. All pages must be numbered consecutively from the title page, and include the acknowledgements, references and figure legends, which should be submitted on separate sheets following the main text. The preferred position of tables and figures in the text should be indicated in the left-hand margin.

**Units and spellings:** Système International (SI) units should be used. The salinity of sea water should be given as g $L^{-1}$. Use the form g $mL^{-1}$ not g/mL. Avoid the use of g per 100g, for example in food composition, use g $kg^{-1}$. If other units are used, these should be defined on first appearance in terms of SI units, e.g. mmHg. Spelling should conform to that used in the Concise Oxford Dictionary published by Oxford University Press. Abbreviations of chemical and other names should be defined when first mentioned in the text unless they are commonly used and internationally known and accepted.

**Scientific names and statistics:** Complete scientific names should be given when organisms are first mentioned in the text and in tables, figures and key words. The generic name may subsequently be abbreviated to the initial, e.g. Gadus morhua L., otherwise G. morhua. Carry out and describe all appropriate statistical analyses.

**References (Harvard style)**: References should be cited in the text by author and date, e.g. Lie & Hemre (1990). Joint authors should be referred to by et al. if there are more than two, e.g. Hemre et al. (1990). More than one paper from the same author(s) in the same year must be identified by the letters a, b, c, etc., placed after the year of publication. Listings of references in the text should be chronological. At the end of the paper, references should be listed alphabetically according to the first named author. The full titles of papers, chapters and books should be given, with the first and last page numbers; journal titles should be abbreviated according to World List of Scientific Periodicals.

Lie, O., Lied, E. & Lambertsen, G. (1988) Feed optimization in Atlantic cod (Gadus morhua): fat versus protein content in the feed. Aquaculture, 69, 333-341.
Lall, S.P. (1989) The minerals. In: Fish Nutrition (Halver, J.E. ed.), 2nd edn, Vol. 1, pp. 219-257. Academic Press Inc., San Diego, CA, USA.

Work that has not been accepted for publication and personal communications should not appear in the reference list, but may be referred to in the text (e.g. A. Author, unpubl. observ.; A.N. Other, pers. comm.). It is the authors' responsibility to obtain permission from colleagues to include their work as a personal communication. A letter of permission should accompany the manuscript.

**References in Articles:** We recommend the use of a tool such as EndNote (http://www.endnote.com/) or Reference Manager (http://www.refman.com/) for reference management and formatting. EndNote reference styles can be searched for here: http://www.endnote.com/support/enstyles.asp. Reference Manager reference styles can be searched for here: http://www.refman.com/support/rmstyles.asp

**Illustrations and tables:** These should be referred to in the text as figures using Arabic numbers, e.g. Fig. 1, Fig. 2, etc., in order of appearance. Three copies of each figure should be submitted and each figure should be marked on the back with its appropriate number, together with the name(s) of the author(s) and the title of the paper. Where there is doubt as to the orientation of an illustration the top should be marked with an arrow. Photographs and photomicrographs should be un mounted glossy prints and should not be retouched. Labelling should be clearly indicated on an overlay or photocopy. Colour illustrations are acceptable when found necessary by the Editor; however, the author may be asked to contribute towards the cost of printing. Line drawings should be on separate sheets of white paper in black indelible ink (dot matrix illustrations are not permitted); lettering should be on an overlay or photocopy and should be no less than 4 mm high for a 50% reduction. Please note,

each figure should have a separate legend; these should be grouped on a separate page at the end of the manuscript. All symbols and abbreviations should be clearly explained. Tables should be self-explanatory and include only essential data. Each table must be type written on a separate sheet and should be numbered consecutively with Arabic numerals, e.g. Table 1, and given a short caption. No vertical rules should be used. Units should appear in parentheses in the column headings and not in the body of the table. All abbreviations should be defined in a footnote. All tables and figures that are reproduced from a previously published source must be accompanied by a letter of permission from the Publisher or copyright owner.

**Colour figures:** It is the policy of Aquaculture Nutrition for authors to pay the full cost for the reproduction in print of their colour artwork. Therefore, please note that if there is colour artwork in your manuscript when it is accepted for publication, Wiley-Blackwell requires you to complete and return a colour work agreement form before your paper can be published. This form can be downloaded as a PDF* here. If you are unable to access the internet, or are unable to download the form, please contact the Production Editor at anu@wiley.com**.** Once completed, please post or courier all pages of your completed form to OPI at the address below. Please note that electronic or faxed copies cannot be accepted. Any article received by Wiley-Blackwell with colour work will not be published until the form has been returned.

**Acknowledgements:** These should be brief and must include references to sources of financial and logistical support.

## Anexo 3 - Normas de publicação da Revista Fish and Shellfish Imunology

Fish and Shellfish Immunology rapidly publishes high-quality, peer-refereed contributions in the expanding fields of fish and shellfish immunology. It presents studies on the basic mechanisms of both the specific and non-specific defense systems, the cells, tissues, and humoral factors involved, their dependence on environmental and intrinsic factors, response to pathogens, response to vaccination, and applied studies on the development of specific vaccines for use in the aquaculture industry.

### INTRODUCTION

*Types of Papers*

*Review articles*
*Short Communications*: Where the subject matter does not warrant a full paper, a Short. Communication is more likely to be acceptable for publication. Communications should be restricted in length to no more than the equivalent of two printed pages of Fish and Shellfish Immunology, that total length to include references and normally no more than one illustration and one short table. No abstract will be required, nor should a communication necessarily have subheadings or be subdivided as are full papers, but an introductory sentence or sentences must make its purport clear. In other respects submitted manuscripts should comply with the instructions given above. A Short Communication may be concerned with any subject within the scope of Fish and Shellfish Immunology but should be confined to a single point or issue of progress, and to such as an unusual occurrence, an interesting observation or a topical and timely finding.

*Short Sequence Reports*: Where the reporting of a novel fish or shellfish sequence with limited functional analysis does not warrant a full paper, a Short Sequence Report is more likely to be acceptable for publication. Communications should be restricted in length as for Short Communications (see above). No abstract or subheadings are required but an introductory sentence or sentences must make it clear why the sequence is novel and the approach used to obtain the gene. In other respects the submitted manuscripts should comply with the instructions given above, and be within the subject scope of the journal. Accepted reports will undergo rapid publication.

***Language (usage and editing services):*** Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

## PREPARATION

### *NEW SUBMISSIONS*

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process. As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or layout that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

***References:*** There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

***Formatting requirements:*** There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions. If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes. Divide the article into clearly defined sections. Please ensure your paper has consecutive line numbering - this is an essential peer review requirement.

*Figures and tables embedded in text:* Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file.

***Revised Submissions:*** *Use of word processing software*

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

***Revised Submissions:*** *Use of word processing software*
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

***Highlights:*** Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples. Immediately after the abstract, provide 5-10 keywords, avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible.

***Abbreviations:*** Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

***Acknowledgements:*** Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

***Nomenclature and units:*** Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUB: Biochemical Nomenclature and Related Documents: http://www.chem.qmw.ac.uk/iubmb/ for further information.

***Database linking:*** Elsevier encourages authors to connect articles with external databases, giving their readers oneclick access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

***Footnotes:*** Footnotes should be used sparingly. Number them consecutively throughout the article. Many Word processors build footnotes into the text, and this feature may be

used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

*Table footnotes*
Indicate each footnote in a table with a superscript lowercase letter.

***Artwork***
*Electronic artwork*
*General points*
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
• Please note that individual figure files larger than 10 MB must be provided in separate source files.
A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions.
**You are urged to visit this site; some excerpts from the detailed information are given here.**
*Formats*
Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below): EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi
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**Please do not:**
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
• Submit graphics that are disproportionately large for the content.
*Color artwork*
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the**

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Please note: Because of technical complications that can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.
*Figure captions*
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

***Tables:*** Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

***References:*** *Citation in text*
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.
*Web references*
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.
*References in a special issue*
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.
*Reference management software*
This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to word processing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.
*Reference formatting*
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume

number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

*Reference style*

*Text:* Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.

*List:* Number the references (numbers in square brackets) in the list in the order in which they appear in the text.

*Examples:*

Reference to a journal publication:

[1] Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun 2010;163:51–9.

Reference to a book:

[2] Strunk Jr W, White EB. The elements of style. 4th ed. New York: Longman; 2000.

Reference to a chapter in an edited book:

[3] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith
RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

Note shortened form for last page number. e.g., 51–9, and that for more than 6 authors the first 6 should be listed followed by 'et al.' For further details you are referred to 'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (J Am Med Assoc 1997;277:927–34) (see also http://www.nlm.nih.gov/bsd/uniform_requirements.html).

## ANEXO 4 – Normas para publicação da Revista Fish and Phisiology Biochemistry

*Fish Physiology and Biochemistry* is an international journal publishing original research papers in all aspects of the physiology and biochemistry of fishes. Papers dealing with experimental work in the following areas will be given preference, but other topics will be given consideration: Biochemistry of organisms, organs, tissues and cells. Structure of organs, tissues, cells and organelles related to their function. Nutritional, osmotic, ionic, respiratory and excretory homeostasis. Nerve and muscle physiology. Endocrinology. Reproductive physiology. Energetics. Biochemical and physiological effects of toxicants. Molecular biology and biotechnology. Categories of articles include full papers, brief communications, rapid communications, unsolicited and invited reviews and editorial comments and announcements.

**Title Page**
The title page should include: The name(s) of the author(s), A concise and informative title, The affiliation(s) and address (es) of the author(s), The e-mail address, telephone and fax numbers of the corresponding author

**Abstract**
Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

**Keywords**
Please provide 4 to 6 keywords which can be used for indexing purposes.

**Text Formatting**
Manuscripts should be submitted in Word. Use a normal, plain font (e.g., 10-point Times Roman) for text. Use italics for emphasis. Use the automatic page numbering function to number the pages. Do not use field functions. Use tab stops or other commands for indents, not the space bar. Use the table function, not spreadsheets, to make tables. Use the equation editor or MathType for equations. Save your file in docx format (Word 2007 or higher) or doc format (older Word versions). Manuscripts with mathematical content can also be submitted in LaTeX. LaTeX macro package (zip, 182 kB).

**Headings**
Please use no more than three levels of displayed headings.

**Abbreviations**
Abbreviations should be defined at first mention and used consistently thereafter.

**Footnotes**
Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference

citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols. Always use footnotes instead of endnotes.

**Acknowledgments**

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

Citation

Cite references in the text by name and year in parentheses. Some examples: Negotiation research spans many disciplines (Thompson 1990). This result was later contradicted by Becker and Seligman (1996).This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

**Reference list :** The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list. Reference list entries should be alphabetized by the last names of the first author of each work.

**Journal article**: Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8.

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted: Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI: Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Book: South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter: Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document: Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation: Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

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