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CONCENTRADOS DE FIBRA ALIMENTAR COMO AGENTE PREBIÓTICO EM DIETAS DE JUNDIÁ (*Rhamdia quelen*)

DISSERTAÇÃO DE MESTRADO

Taida Juliana Adorian

Santa Maria, RS, Brasil
2015

CONCENTRADOS DE FIBRA ALIMENTAR COMO AGENTE PREBIÓTICO EM DIETAS DE JUNDIÁ (*Rhamdia quelen*)

Taida Juliana Adorian

Dissertação apresentada ao Curso de Mestrado 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
Mestre em Zootecnia.

Orientadora: Profª Drª Leila Picolli da Silva

Santa Maria, RS, Brasil
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CONCENTRADOS DE FIBRA ALIMENTAR COMO AGENTE PREBIÓTICO EM DIETAS DE JUNDIÁ (*Rhamdia quelen*)

elaborada por
Taida Juliana Adorian

Como requisito parcial para a obtenção do grau de
Mestre em Zootecnia

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Nilton Garcia Marengoni, Dr. (UNIOESTE)

Santa Maria, 10 de Fevereiro de 2015.

Aos meus **pais**,
Pela compreensão e incentivo.

Aos meus sobrinhos **Julia e Gustavo**,
Pelo carinho incondicional.

As minhas irmãs **Tarcéli e Tâmina**,
Pelo apoio de sempre.

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“Viver é isto:
Ficar se **equilibrando**, o tempo todo,
Entre **escolhas e consequências**.”
Jean-Paul Sartre

A tarefa não é tanto ver aquilo que ninguém viu, mas **pensar o que ninguém ainda pensou** sobre aquilo que todo mundo vê.”
Arthur Schopenhauer

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
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CONCENTRADOS DE FIBRA ALIMENTAR COMO AGENTE PREBIÓTICO EM DIETAS DE JUNDIÁ (*Rhamdia quelen*)
AUTORA: Taida Juliana Adorian
ORIENTADORA: Leila Picolli da Silva
Data e Local da Defesa: Santa Maria, 10 de Fevereiro de 2015.

Este trabalho teve como objetivo avaliar o efeito da inclusão de fibra alimentar concentrada como agente prebiótico em dietas sob as respostas metabólicas, imunológicas, parâmetros de desempenho, deposição de nutrientes e produção de enzimas digestivas de juvenis de jundiá. Foram avaliados concentrados de fibra alimentar preparados a partir da polpa cítrica, biomassa de levedura de cervejaria e grão de linhaça e incluídos em dietas mistas, além de uma dieta contendo o prebiótico comercial a base de mananoligossacarídeos Actigen® e uma dieta controle sem adição de agente prebiótico. Durante 50 dias, 600 juvenis de jundiá com peso médio inicial de 3,54±0,53g foram mantidos em um sistema de recirculação de água dotado de dois filtros biológicos, caixa de decantação, reservatório de água, aquecimento e 20 tanques com capacidade de 230 litros. Distribuiu-se ao acaso 30 peixes por unidade experimental, os quais receberam as dietas experimentais, três vezes ao dia (8:00, 13:00 e 17:00 horas) até a saciedade aparente. Ao final do experimento os animais foram submetidos a biometria onde coletou-se sangue, fígado, muco e intestino dos peixes, além de dados de peso e comprimento e uma amostra de peixes. O delineamento experimental o inteiramente casualizado, com cinco tratamentos e quatro repetições, os dados foram submetidos a análise de variância e as médias comparadas pelo teste de Tukey ($P<0,05$). Os níveis de colesterol, proteína total, globulina e mucoproteína, foram superiores nos animais alimentados com autolisado de levedura e fibra de linhaça na dieta. Observou-se maior quantidade de glicogênio hepático nos peixes alimentados com dieta controle e Actigen®, o conteúdo de proteína hepática foi superior ($P<0,05$) para os animais que receberam dieta contendo fibra de linhaça. Os peixes alimentados com dietas contendo autolisado de levedura e linhaça fibra apresentaram desempenho superior ($P<0,05$) aos demais tratamentos testados, bem como maiores valores de proteína bruta e gordura corporal depositada. Animais alimentados com dietas contendo polpa cítrica mostraram menor desempenho e deposição de nutrientes. O rendimento de carcaça, índices digestivos e produção de enzimas digestivas não foram afetados pelos tratamentos testados. O autolisado de levedura e a fibra de linhaça proporcionam efeito prebiótico quando adicionados a dietas para juvenis de jundiá, uma vez que beneficiam o sistema imune e proporcionam maior desempenho e deposição de nutrientes pelos animais.

Palavras-chave: Fibra solúvel. Fibra insolúvel. Promotor de crescimento. Peixes. Imunoestimulante.

ABSTRACT

Animal Science Master Dissertation
Post-Graduate Program in Animal Science
Federal University of Santa Maria

CONCENTRATES OF DIETARY FIBER AS AGENT PREBIOTIC IN DIETS OF SILVER CATFISH (*Rhamdia quelen*)

AUTHOR: Taida Juliana Adorian
ADVISER: Leila Picolli da Silva
Date and Defense Place: Santa Maria, February 10th, 2015.

This study aimed to evaluate the effect of inclusion of concentrated dietary fiber as prebiotic agent in diets on metabolic, immune responses, performance parameters, deposition of nutrients and production of digestive enzymes of juvenile silver catfish. Concentrates were prepared from dietary fiber citrus pulp, biomass of yeast brewery and grain included in linseed and mixed diets followed a diet containing the prebiotic commercial mananoligossacarids base Actigen® and control treatment without added prebiotic agent. For 50 days, 600 juvenile silver catfish with average initial weight of 3.54±0.53 g were kept in a water recirculation system with two biological filters, settling box, heating and 20 tanks with a capacity of 230 liters. Were randomly assigned to 30 fish per experimental unit, which were fed the experimental diets, three times a day (8:00, 13:00 and 17:00) to apparent satiation. At the end of the experiment the animals were subjected to biometrics were collected blood, liver, mucous, intestine and data length and weight, beyond a sample of fish. The experimental design was a randomized, with five treatments and four replications, the data were subjected to analysis of variance and means were compared by Tukey test ($P<0.05$). Cholesterol levels, total protein, globulin and mucoprotein were higher in animals fed yeast autolysate and linseed fiber in the diet. A higher amount of liver glycogen in fish fed with control diet and Actigen®, the liver protein content was higher ($P<0.05$) in a diet containing linseed fiber. Fish fed diets containing yeast autolysate and linseed fiber were superior ($P<0.05$) to the other treatments tested, as well as higher crude protein values and deposited body fat. Animals fed diets containing citrus pulp showed lower performance and nutrient deposition. The yield of body, digestive indices and production of digestive enzymes were not affected by the tested treatments. The yeast autolysate and linseed fibers provide a prebiotic effect when added to diets for juvenile silver catfish, since they benefit the immune system and provide improved performance and deposition of nutrients by the animal.

Keywords: Soluble fiber. Insoluble fiber. Growth promoter. Fish. Immunostimulant.

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AFC: apparent feed conversion

AGCC: ácidos graxos de cadeia curta

AL: autolisado de levedura

CF: condition factor

CFAs: concentrados de fibra alimentar

CH: capacidade de hidratação

CLG: capacidade de ligação a gordura

CP: citrus pulp

CY: corporal yeld

DSI: digestive somatic index

FBF: final body fat

FBP: final body protein

FI: fibra insolúvel

FL: fibra de linhaça

FS: fibra solúvel

FT: fibra alimentar total

DSI: digestive somatic index

FW: final weight

HSI: hepatosomatic index

IBF: initial body fat

IBP: initial body protein

IQ: intestinal quotient

IW: initial weight

LF: linseed fiber

MPA: Ministério da Pesca e Aquicultura

PC: polpa cítrica

SGR: specific growth rate

VFI: visceral fat index

YA: yeast autolysate

SUMÁRIO

INTRODUÇÃO GERAL

A contribuição da aquicultura para a produção mundial de alimentos vem aumentando significativamente ao longo das últimas décadas. Atualmente o setor é responsável pelo fornecimento de aproximadamente metade do pescado destinado ao consumo humano, onde o Brasil encontra-se entre os 15 maiores produtores mundiais, sendo 86,4% da sua produção proveniente de água doce (FAO, 2014). As principais espécies cultivadas em viveiros são as tilápias, carpas, tambaquis, tambacus e pacus, sendo que a introdução de novas espécies e os avanços nas técnicas de cultivo tem contribuído para o rápido crescimento da indústria aquícola (MPA, 2012). Dada a sua importância no setor de alimentos, é reconhecido que a aquicultura precisa se tornar sustentável para que possa continuar seu crescimento de forma competitiva.

Esse constante crescimento vem acompanhado da intensificação dos sistemas de cultivo de organismos aquáticos, que traz como desvantagem maior exposição dos animais a doenças infecciosas, afetando principalmente os primeiros estágios de produção, onde os animais são jovens, e consequentemente mais susceptíveis a doenças. A forma mais frequente de controlar surtos de doenças em sistemas criatórios é através do uso de antibióticos e quimioterápicos adicionados as dietas (RICO et al., 2012; SOLEIMANI et al., 2012). Porém, o uso indiscriminado dessas substâncias leva ao surgimento de cepas de organismos resistentes e uma tendência a limitação e até mesmo proibição de seu uso.

Em alternativa, surgem estratégias como o uso de prebióticos, que são ingredientes nutricionais não digeríveis que afetam positivamente o hospedeiro, estimulando o crescimento e atividade de bactérias benéficas intestinais, resultando em melhorias a saúde (GIBSON e ROBERFROID, 1995). Uma das principais fontes de prebióticos disponíveis atualmente são as fibras alimentares (DIONIZIO et al., 2002), compostas pelas partes comestíveis das plantas ou análogos aos carboidratos, resistentes à digestão e absorção pelo intestino delgado, com fermentação parcial ou total no intestino grosso (AOAC, 1995).

O efeito da fibra alimentar na nutrição de peixes ainda é um assunto controverso. A maioria das pesquisas científicas utilizando ingredientes fibrosos não indica a composição detalhada da fibra, ou simplesmente avalia o desempenho e, com base nele, descrevem aspectos anti-nutricionais, o que torna difícil a observação dos seus efeitos benéficos, principalmente sobre a saúde animal. As informações disponíveis na literatura sobre o

possível efeito prebiótico da fibra alimentar para peixes são escassas em comparação com o conhecimento existente sobre seu potencial benéfico para humanos (RINGO et al., 2010).

O jundiá (*Rhamdia quelen*) é um peixe de couro nativo do Brasil, com grande potencial para piscicultura continental, por ser considerado espécie rústica, de fácil manejo e rápido crescimento (BALDISSEROTTO et al., 2010), além de apresentar tolerância ao frio e apetite mesmo nas épocas mais frias do ano (COLDEBELLA e RADÜNZ NETO, 2002), atraindo a atenção de produtores e pesquisadores para esta espécie. Dados do Ministério da Pesca e Aquicultura (MPA, 2012) mostram que a produção brasileira de jundiá oriunda da criação em cativeiro cresceu em torno de 40% nos últimos anos, passando de 911 toneladas em 2008, para 1274,3 toneladas em 2010. O consumo dessa espécie tem despertado interesse do mercado consumidor, pois a carne apresenta excelente qualidade e sabor, além de não possuir espinhos intramusculares (CARNEIRO e MIKOS, 2005).

Em relação a utilização de fibra alimentar na nutrição de jundiás, os estudos ficam restritos ao emprego de cascas de algodão e de soja, polpa cítrica e farelo de trigo na dieta (PEDRON, et al., 2008; RODRIGUES et al., 2012), avaliando os efeitos da fibra sob parâmetros de desempenho, e a considerando como um grupo único, sem dar atenção as distintas composições e propriedades físico-químicas dos ingredientes fibrosos estudados. Destaca-se assim, a necessidade de trabalhos que visem elucidar os efeitos proporcionados pela fibra alimentar de forma mais detalhada.

OBJETIVOS

Objetivo geral

Avaliar o potencial prebiótico da inclusão de concentrados de fibra alimentar em dietas de juvenis de jundiá (*Rhamdia quelen*) sobre aspectos de desempenho, metabolismo e indicadores imunológicos dos animais.

Objetivos específicos

- Selecionar fontes fibrosas com diferentes proporções de fibra solúvel e insolúvel;

- Concentrar a fibra alimentar das fontes selecionadas;

- Determinar a composição dos concentrados de fibra alimentar (CFAs);

- Avaliar o efeito da suplementação de dietas com os CFAs sobre desempenho produtivo, parâmetros metabólicos e indicadores imunológicos de juvenis de jundiá.

ARTIGO 1

EFEITO DA FIBRA ALIMENTAR SOBRE INDICADORES IMUNOLÓGICOS E METABÓLICOS DE JUNDIÁ*

*Artigo Científico a ser submetido à revista Fish and Shellfish Immunology

Efeito da fibra alimentar sobre indicadores imunológicos e metabólicos de jundiá

Resumo

Este trabalho teve como objetivo avaliar as respostas metabólicas e imunológicas da inclusão de fibra alimentar como agente prebiótico em dieta de jundiá. Foram preparados concentrados de fibra a partir da polpa cítrica, biomassa de levedura de cervejaria e grão de linhaça e incluídos em dietas, além de um tratamento controle e uma dieta contendo prebiótico comercial Actigen®. Durante 50 dias, 600 juvenis de jundiá foram mantidos em um sistema de recirculação de água e alimentados com as dietas experimentais, três vezes ao dia até a saciedade aparente. Ao final do experimento coletou-se sangue, fígado e muco para determinação de parâmetros metabólicos e imunológicos. O delineamento experimental o inteiramente casualizado, com cinco tratamentos e quatro repetições, os dados foram submetidos a análise de variância e as médias comparadas pelo teste de Tukey ($P<0,05$). Os níveis de colesterol, proteína total, globulina e mucoproteína, foram superiores nos animais alimentados com autolisado de levedura e fibra de linhaça na dieta. Observou-se maior quantidade de glicogênio hepático nos peixes alimentados com dieta controle e Actigen®. O conteúdo de proteína hepática foi superior ($P<0,05$) nos alimentados com dieta contendo fibra de linhaça. O autolisado de levedura e a fibra de linhaça proporcionam efeito prebiótico quando adicionados a dietas para jundiá, uma vez que beneficiam o sistema imune dos animais.

Palavras-chave: metabolismo, imunidade, fibra solúvel, fibra insolúvel, prebiótico, *Rhamdia quelen*

1. Introdução

Os sistemas de cultivo intensivos são caracterizados por altas densidades de estocagem e maior excreção de metabólitos, resultando em degradação da qualidade de água e maior estresse dos peixes. Essas condições elevam as concentrações do hormônio imunossupressor cortisol, reduzindo a resistência a infecções bacterianas e fúngicas [1]. A maneira mais comum de tratar as doenças decorrentes é o uso de quimioterápicos e antibióticos, dos quais se relatam problemas de contaminação ambiental, resistência a patógenos e acúmulo residual na carne [2, 3]. O que tem levado a um crescente interesse no uso terapêutico de antimicrobianos ambientalmente corretos, como a administração de prebióticos [4, 5, 6].

Constituintes não digestíveis como fibras, oligossacarídeos e amido resistente, fazem parte dos ingredientes usados na formulação de muitas dietas para peixes e têm demonstrado capacidade de influenciar positivamente a microbiota intestinal [7, 8, 9] atuando como prebióticos. A fibra alimentar é uma fração resistente à digestão e absorção pelo intestino delgado, com fermentação parcial ou total no intestino grosso [10]. Suas frações tem apresentado resultados positivos na modulação do sistema imunológico, morfometria intestinal, resistência a doenças e diminuição da diversidade microbiana patogênica intestinal dos peixes [11, 12, 13]. Porém, alguns pesquisadores comentam que os efeitos da fibra não estão relacionados apenas a quantidade adicionada nas dietas ou a sua solubilidade, mas também guardam identidade com a fonte de origem, o que determina a sua composição química e propriedades físico-químicas [14]. Dessa forma, efeitos diferenciados sobre o metabolismo e sistema imunológico poderão ser observados com o uso de distintas fontes de fibra, mesmo que promovam níveis semelhantes de fibra alimentar nas dietas.

Diante do exposto, o propósito deste estudo foi de verificar o efeito da suplementação de concentrados de fibra alimentar (fibra de linhaça, polpa cítrica e autolisado de levedura)

com solubilidade e características físico-químicas diferenciadas, sobre a imunologia e metabolismo de juvenis de jundiá.

2. Material e métodos

2.1 Preparação dos concentrados de fibra alimentar

Pela origem distinta, o grão de linhaça, a polpa cítrica e a levedura de cervejaria foram selecionados como fontes de fibras para o estudo. A fim de otimizar a adição nas dietas experimentais, a fibra alimentar total destas fontes foi concentrada no mínimo em 50% formando os Concentrados de fibra alimentar (CFAs).

2.1.1 Fibra de linhaça

A mucilagem de linhaça foi obtida a partir do grão inteiro em meio aquoso na concentração de 10% p/v e temperatura entre 60 e 80ºC com agitação constante durante 150 minutos, seguindo metodologia descrita por Goulart et al. [15]. O grão demucilado foi desengordurado com solvente orgânico hexano na proporção 1:2 (p/v), realizando-se quatro lavagens de 30 minutos. A seguir o teor proteico do farelo remanescente foi reduzido por dispersão em água destilada a temperatura ambiente na proporção 1:30 (p/v), sendo levado a estufa de recirculação de ar a 55°C por 24 horas. A fibra concentrada resultante desta etapa foi misturada a mucilagem extraída na primeira etapa, obtendo-se a fibra de linhaça concentrada, que foi submetida a moagem (Marconi, modelo MA-630/1) para obtenção de partículas com tamanho médio de 590μm.

2.1.2 Polpa cítrica

A polpa cítrica foi obtida após a extração do suco de laranjas da variedade Valência. O resíduo remanescente (flavedo, albedo, sementes e membranas) foi lavado com água a temperatura ambiente, triturado em cutter (Metvisa, modelo CUT 2.5) por 20 segundos e seco em estufa de recirculação de ar a 55°C por 24 horas, seguido de moagem (Marconi, modelo MA-630/1) para obtenção de partículas com tamanho médio de 590μm.

2.1.3 Autolisado de levedura

Para obtenção do autolisado de levedura, a biomassa de cervejaria (*Saccharomyces cerevisiae*) foi centrifugada a 1200 x g por 15 minutos e em seguida lavada por três vezes com água destilada na proporção de 1:1 (p/v) para remoção de impurezas e álcool remanescente, seguido por nova centrifugação. A biomassa de levedura limpa foi submetida a autólise em banho Maria com agitação por 8 horas a 49°C, de acordo com metodologia descrita por Matiazi [16]. Posteriormente foi seco em estufa de recirculação de ar a 55°C por 48 horas e moído em triturador (Marconi, modelo MA-630/1) para obtenção de partículas com granulometria média de 590μm.

2.1.4 Análises químicas e físico-químicas

Nos concentrados de fibra alimentar (CFAs), as análises químicas de fibra alimentar total (FT), insolúvel (FI) e solúvel (FS) foram realizadas conforme o método enzimático gravimétrico número 991.43 da AOAC [17]. As análises físico-químicas de capacidade de hidratação e de ligação a gordura foram determinadas conforme metodologias propostas por Wang e Kinsella [18].

2.2 Dietas experimentais

Cinco dietas mistas foram formuladas para o experimento, compostas basicamente por farinha de peixe, amido de milho e celulose. A estas dietas adicionou-se 0,25% de prebiótico comercial a base de mananoligossacarídeo (Actigen® Alltech), fibra de linhaça, polpa cítrica ou autolisado de levedura, além de uma dieta controle sem adição de agente prebiótico (Tabela 1). Os ingredientes foram pesados e homogeneizados, posteriormente adicionou-se água, realizando-se a peletização (quatro milímetros). As dietas foram secas em estufa de recirculação de ar durante 24 horas a uma temperatura de 55ºC. Após a secagem, as dietas foram moídas e selecionadas considerando a capacidade de ingestão dos peixe.

Tabela 1

Formulação e composição centesimal das dietas experimentais (%)

	Tratamentos[1]				
Ingredientes	Controle	Actigen®	FL	PC	AL
Farinha de peixe[*]	61,10	61,10	57,00	60,00	57,80
Amido de milho	11,80	11,80	11,40	12,10	12,00
Polpa cítrica	0	0	0	17,70	0
Autolisado de levedura	0	0	0	0	13,80
Fibra de linhaça	0	0	14,6	0	0
Actigen®[2]	0	0,25	0	0	0
Celulose	10,60	10,60	0	0	0
Óleo de soja	6,00	6,00	4,20	4,60	5,50
NaCl	0,50	0,50	0,50	0,50	0,50
Melbond®[3]	2,00	2,00	2,00	2,00	2,00
Mistura vitamínica e mineral[4]	3,00	3,00	3,00	3,00	3,00
BHT[5]	0,01	0,01	0,01	0,01	0,01
Inerte[6]	4,99	4,74	7,29	0,09	5,39
Total	100	100	100	100	100
	Composição centesimal				
Proteína bruta	36,04	36,04	36,03	36,1	36,07
Energia digestível (MJ kg^{-1})[g 7]	13,43	13,43	13,42	13,40	13,43
Extrato etéreo	11,47	11,47	11,39	11,33	11,41
Fibra alimentar total	10,02	10,02	10,09	10,04	10,07
Fibra solúvel	-	-	3,12	3,73	5,09
Fibra insolúvel	10,02	10,02	6,97	6,31	4,98
Cinzas	14,36	14,11	15,84	12,27	14,06

[1]Tratamentos: Controle; Actigen®: inclusão do prebiótico comercial Actigen®; FL: fibra de linhaça; PC: polpa cítrica; AL: autolisado de levedura.
[2]Prebiótico comercial Actigen® Alltech.
[3]Aglutinante.
[4]Composição (Migfish): Ác. Folic: 299,88 mg; Ác. Ascorbic: 15.000,12 mg; Pantothenic: 3,000.10 mg; Biotin: 12.06 mg; Niacin (B3): 9,000.32 mg; Hill (B4): 103,500.00 mg; Vit.A: 1000000.00 IU; Vit. B1: 1500.38 mg; Vit. B2: 1500.0 mg; Vit. B6: 1500.38mg; Vit. D3: 240000.00 IU; Vit. E: 10000.00 mg; Vit. K3: 400.00 mg; Inositol: 9999.92 mg; Iron: 6416.80mg; Manganese: 8000.40mg; Copper: 1000.00 mg; Zinc: 13999.50 mg; Iodine: 45.36 mg; Cobalt: 60.06 mg; Selenium: 60.30 mg; Magnesium: 5.10 mg; Chlorine: 2.30 %; Sulfur: 0.01%.
[5]Butil hidróxi tolueno (BHT).
[6]Areia.
[7]Energia digestível: energia digestível calculada: [(Proteína bruta * 5,65 * 0,85) + (Gordura * 9,4 *0,9) + (Carboidratos * 4,15 * 0,7)] (adaptado de Meyer et al., 2004).
[*]Farinha de resíduos de tilápia/Copisces-Paraná/RS.

2.3 Animais e alimentação

O estudo foi conduzido no Laboratório de Piscicultura do Departamento de Zootecnia da Universidade Federal de Santa Maria (UFSM) - RS, Brasil, depois de aprovado pelo Comitê de Ética em Experimentação Animal da UFSM, sob o número 23081.009051/2014-53. Um total de 600 juvenis de jundiá com peso médio inicial de 3,54±0,53g foram distribuídos ao acaso em 20 tanques de polipropileno com 230 litros de capacidade, sendo 30 animais por tanque. Cada tanque possuía entrada e saída de água individual organizados em um sistema de recirculação de água composto por caixa de decantação, dois filtros biológicos de pedra britada e um reservatório de água com capacidade de 2000 litros, dotado de aquecimento. Durante o período experimental, os animais foram alimentados até a saciedade aparente, três vezes ao dia, as 8:00, 13:00 e 17:00 por um período de 50 dias.

2.4 Qualidade da água

Antes da primeira e última refeições (7:30 e 15:00 horas) foram retirados os resíduos de fezes dos tanques por meio de sifonagem. A temperatura da água, pH, alcalinidade, dureza, amônia total, nitrito e o oxigênio dissolvido foram monitorados e mantidos a 20,94±2,08ºC (temperatura da manhã), 22,01±1,93ºC (temperatura da tarde), 7,25±0,27, 55±13,95mg

$CaCO_3$/L, 64,83±11,28mg $CaCO_3$/L, 0,1±0,06 mg L^{-1}, 0,12±0,08 mg L^{-1} e 8,43±0,88 mg L^{-1}, respectivamente, durante o período experimental.

2.5 Coleta de dados e variáveis analisadas

Após os 50 dias de alimentação os peixes foram submetidos a jejum de 18 horas, sendo colhidas amostras de sangue de três animais por unidade experimental, por punção do vaso caudal com seringas previamente heparinizadas e posterior obtenção do plasma. Foram avaliados os seguintes parâmetros metabólicos plasmáticos: glicose, colesterol, proteínas totais circulantes e albumina. Para determinação de hemoglobina coletou-se amostras de sangue total de dois animais por unidade experimental, por punção do vaso caudal com seringas contendo Anticoagulante Universal Doles®. Todas as análises foram realizadas através de kits comerciais da marca Doles®. Destes peixes coletou-se o tecido hepático para posterior determinação de proteína [19], glicose e glicogênio [20].

Foram coletados muco de três peixes por unidade experimental através de raspagem, afim de determinar os níveis de mucoproteína (glicoproteína) através de kit comercial Bioclin®. Esta metodologia tem como princípio, precipitar as proteínas em solução de ácido perclórico, resultando numa fração glicoproteica denominada de seromucóide ou mucoproteínas. Estas são precipitadas no filtrado com ácido fosfotúngstico e posteriormente dissolvidas e dosadas através do conteúdo em tirosina.

2.6 Análise estatística

O delineamento experimental foi inteiramente casualizado, com cinco tratamentos e quatro repetições. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey. As diferenças foram consideradas significativas ao nível de $P<0,05$.

3. Resultados

3.1 Composição e propriedades dos CFAs

Todos os concentrados de fibra alimentar apresentaram teores de fibra alimentar total (FT) superiores a 50% (Figura 1). As proporções das frações de fibra solúvel/insolúvel foram de 0,44 para FL, 0,58 para PC e 1,02 para AL, sendo inversamente proporcionais.

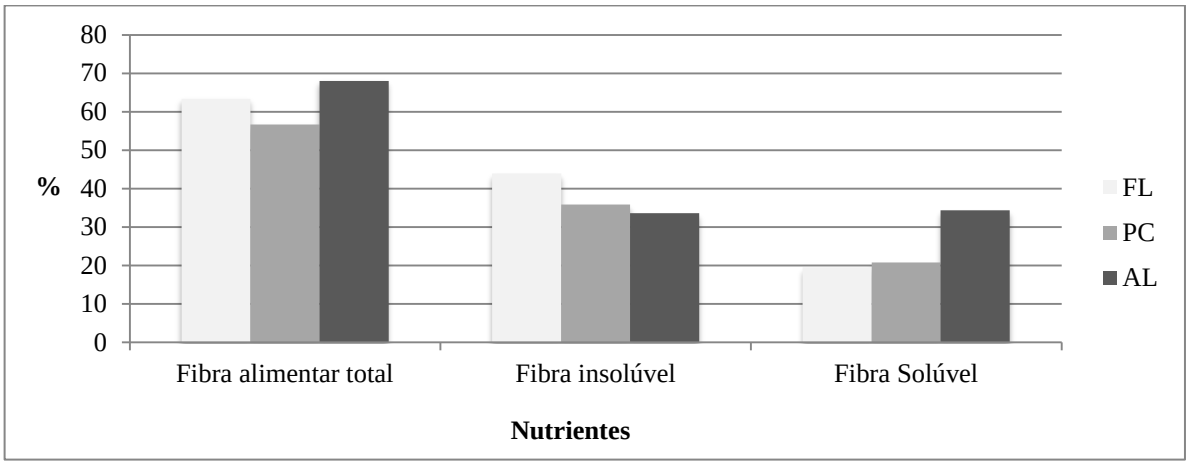

Fig. 1. Composição dos concentrados de fibra alimentar

FL: fibra de linhaça; PC: polpa cítrica; AL: autolisado de levedura.

Os CFAs e o prebiótico comercial mostraram diferenças significativas ($P<0,05$) quanto as características físico-químicas de capacidade de hidratação e de ligação a gordura. A capacidade de hidratação das dietas foi influenciada significativamente pela adição dos respectivos CFAs (Tabela 2), porém o mesmo comportamento não foi evidenciado para a capacidade de ligação a gordura.

Tabela 2

Capacidade de hidratação (CH) e de ligação a gordura (CLG) dos ingredientes e dietas experimentais

	Ingredientes[1]			
	Actigen®	FL	PC	AL
CH	2,16±0,04[d]	11,37±0,004[a]	5,22±0,04[b]	2,64±0,01[c]
CLG	0,79±0,01[c]	1,60±0,18[a]	1,20±0,01[b]	0,99±0,03[bc]

	Dietas[2]				
	Controle	Actigen®	FL	PC	AL
CH	1,51±0,03[b]	1,48±0,05[b]	1,76±0,05[a]	1,61±0,04[ab]	1,21±0,007[c]
CLG	0,89±0,01	0,87±0,02	0,89±0,01	0,87±0,06	0,86±0,03

[1]Ingredientes: Actigen®: prebiótico comercial; FL: fibra de linhaça; PC: polpa cítrica; AL: autolisado de levedura. [2]Dietas: Controle; Actigen®; FL: fibra de linhaça; PC: polpa cítrica; AL: autolisado de levedura. CH: capacidade de hidratação: (g água/g amostra); CLG: capacidade de ligação a gordura (g gordura/g amostra). Média ± desvio padrão. Letras diferentes nas linhas da tabela representam diferença significativa pelo teste de Tukey (P<0,05).

3.2 Parâmetros sanguíneos

A suplementação com os CFAs afetou significativamente (P<0,05) os parâmetros plasmáticos de colesterol, proteína total e globulina (Tabela 3). Porém, a glicose plasmática, a albumina e a hemoglobina não foram alteradas.

Tabela 3

Parâmetros sanguíneos de jundiás (*Rhamdia quelen*) alimentados com dietas contendo Concentrados de fibra alimentar

	Tratamentos[1]				
	Controle	Actigen®	FL	PC	AL
Glicose	73,07±17,49	78,11±18,27	77,99±10,21	76,30±16,99	74,90±19,28
Colesterol	75,32±11,37[b]	85,29±12,49[ab]	95,52±23,48[a]	87,66±23,11[ab]	93,59±22,44[a]
Proteínas totais	2,72±0,32[b]	3,09±0,48[ab]	3,24±0,38[a]	3,22±0,83[a]	3,28±0,39[a]
Albumina	0,24±0,08	0,29±0,09	0,28±0,06	0,30±0,07	0,26±0,08
Globulina	2,48±0,35[b]	2,80±0,43[ab]	2,96±0,45[a]	2,91±0,81[ab]	3,02±0,47[a]
Hemoglobina	4,76±0,60	5,08±1,16	5,37±0,87	5,32±0,85	4,42±0,82

[1]Tratamentos: Controle; Actigen®; FL: fibra de linhaça; PC: polpa cítrica; AL: autolisado de levedura. Glicose: (mg/dL); Colesterol: (mg/dL); Proteínas totais: (g/dL); Albumina: (g/dL); Globulina: (g/dL); Hemoglobina: (g/dL). Letras diferentes nas linhas da tabela representam diferença significativa pelo teste de Tukey (P<0,05).

A utilização de fibra de linhaça, autolisado de levedura ou polpa cítrica na dieta resultou em maiores valores (P<0,05) de proteína total circulante quando comparados a dieta controle (Tabela 3). A produção de colesterol e de globulinas foi superior (P<0,05) para os animais alimentados com dietas contendo fibra de linhaça ou autolisado de levedura, em relação a dieta controle (Tabela 3).

3.3 Produção de mucoproteína

Dietas com fibra de linhaça ou autolisado de levedura levam a maior produção de mucoproteína pelos peixes (P<0,05) em relação a dieta controle (Figura 2).

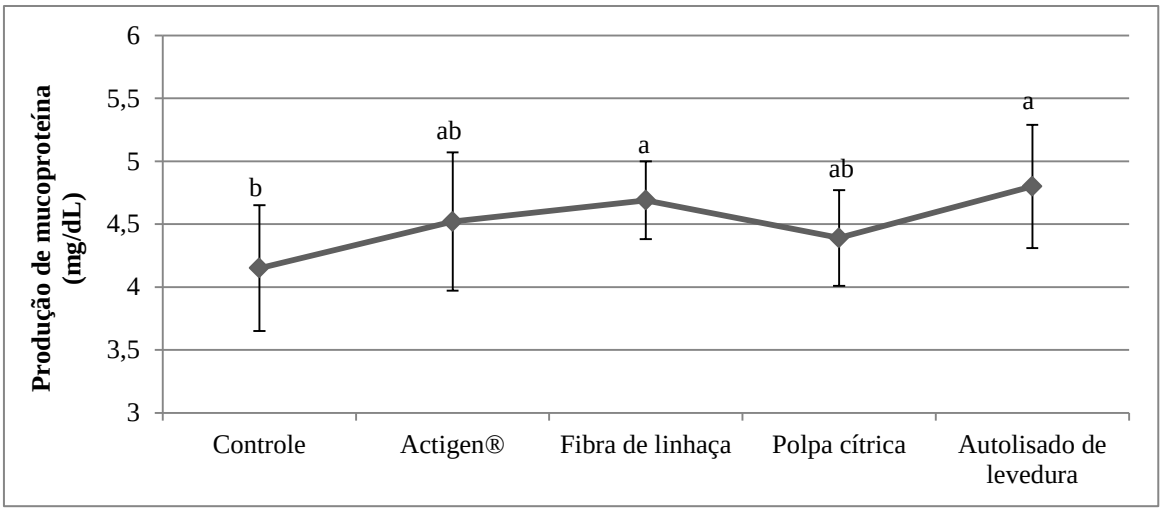

Fig. 2. Produção de mucoproteína (mg/dL) pelos peixes alimentados com as dietas experimentais.

Média ± desvio padrão. Letras diferentes na figura representam diferença significativa pelo teste de Tukey (P <0,05).

3.4 Parâmetros hepáticos

As dietas controle e Actigen® promoveram maior reserva de glicogênio hepático. Já os animais suplementados com fibra de linhaça mostraram maior conteúdo de proteína hepática (P<0,05) em comparação ao demais tratamentos. A suplementação com os CFAs ou com o prebiótico comercial não alterou a concentração de glicose hepática (Tabela 4).

Tabela 4

Intermediários metabólicos hepáticos de jundiás (*Rhamdia quelen*) alimentados com dietas contendo Concentrados de fibra alimentar

Tratamentos	Glicose	Glicogênio	Proteína
Controle	168,34±63,02	8,55±3,91^{a}	6,39±0,94^{b}
Actigen®	164,38±59,04	7,27±2,82^{a}	7,27±1,27^{ab}
Fibra de linhaça	128,43±41,94	5,28±2,01^{bc}	8,78±2,10^{a}
Polpa cítrica	156,07±51,47	4,93±1,95^{bc}	6,95±1,38^{b}
Autolisado de levedura	163,14±51,62	4,07±1,84^{c}	7,40±1,13^{ab}

Glicose: µmol glicose/g tecido; Glicogênio: µmol glicose/g tecido; Proteína: mg proteína/g tecido. Média ± Desvio padrão. Letras diferentes nas colunas da tabela representam diferença significativa pelo teste de Tukey (P<0,05).

4. Discussão

Por muitos anos a fibra alimentar foi considerada um diluidor de energia, com efeitos indesejáveis de sua inclusão nas dietas para peixes. Mas estudos recentes tem demonstrado que em níveis adequados essa fração atua como promotor de crescimento, melhorando a resposta imunológica dos animais [11, 12, 13]. Essas respostas podem ser efetivamente alteradas não somente pelos níveis de fibra da dieta, mas também por variações nas suas características físico-químicas (capacidade de hidratação, capacidade de ligação a gordura,

entre outras). Nosso estudo demonstra que a concentração de fibra originou produtos (CFAs) com teor de fibra alimentar semelhante, mas de solubilidade e características físico-químicas distintas, que poderão ser explorados de forma direcionada para promoção de saúde dos animais.

Normalmente a maior capacidade de hidratação das fibras é relacionada a sua solubilidade, que é influenciada pela sua composição e estrutura química. Os concentrados de fibra de linhaça e de polpa cítrica apresentaram teor de fibra solúvel semelhante, mas diferença de aproximadamente duas vezes em suas respectivas capacidades de hidratação. A fração solúvel da fibra de linhaça (mucilagem) é composta de polissacarídeos formados por seis diferentes monossacarídeos [21] estruturados em cadeia altamente ramificada, capaz de reter grande quantidade de água por unidade molecular. Já a fração solúvel da polpa cítrica é formada de moléculas distintas de pectinas, compostas por no mínimo 65% de ácidos galacturônicos e dezessete diferentes monossacarídeos [22], estruturados de forma menos ramificada do que a mucilagem, o que explica sua menor capacidade de hidratação.

Os teores crescentes de fibra solúvel nas dietas contendo os CFAs não influenciaram a glicemia dos animais (Tabela 3). Contrapondo a estes resultados, estudos indicam que o maior consumo de fibra solúvel leva a aumentos na viscosidade e no tempo de trânsito do conteúdo luminal, reduzindo a velocidade e quantidade de glicose absorvida pelo organismo [23, 24]. O aumento da viscosidade normalmente está associado a capacidade de hidratação do alimento, que no presente estudo foi maior para as dietas contendo concentrados de fibras de linhaça e de polpa cítrica (Tabela 2). Embora as variações na capacidade de hidratação observadas nas dietas testadas possam ter refletido na viscosidade da digesta, os efeitos na digestão e absorção de nutrientes não foram expressivos a ponto de causar variações na glicemia.

Nossos resultados mostram que os peixes suplementados com autolisado de levedura ou com fibra de linhaça na dieta apresentaram maior colesterol plasmático em relação ao

controle (Tabela 3). Essa observação pode estar relacionada a fermentação das fibras e aumento na produção de ácidos graxos de cadeia curta (AGCC) no cólon, em especial o acetato, que promove intensificação na síntese de colesterol [25,26]. Estudos apontam o colesterol como precursor de hormônios relacionados a proteção contra estresse e síntese de vitamina D [27, 28]. Por este motivo sua elevação plasmática tem sido usada como indicador de saúde e de melhora na imunidade dos peixes [29, 30, 31].

O efeito imunomodulador proporcionado pela suplementação das dietas com os CFAs foi evidenciado pelo aumento dos níveis de proteína total e globulina plasmática (Tabela 3), parâmetros que estão associados a resposta imune inata e indicam maior resistência imunológica dos animais [32]. As proteínas totais e suas frações são importantes na avaliação da condição de saúde dos animais, pois atuam na regulação da resposta inflamatória e resposta a infecções [33, 34]. Globulinas são essenciais para a manutenção do sistema imunológico, já que são fonte de quase toda a proteína imunologicamente ativa do sangue [35, 36].

Os estudos encontrados na literatura não exploram o efeito da suplementação da fibra alimentar sobre os níveis proteína e globulinas plasmáticas, mas sim de carboidratos de uma forma geral, os quais promovem resposta imunológica positiva nos animais [37, 38]. Além disso, Dugenci et al. [39], relatam aumento de proteínas totais em truta arco-íris alimentadas com dietas contendo imunoestimulantes. Segundo Choudhury et al. [35], os valores de globulina plasmática de peixes alimentados com esse tipo de substâncias, são sempre mais altos que o controle. Essa afirmação corrobora com os resultados encontrados no presente estudo e sugerem que quando adicionadas a dieta, o autolisado de levedura e a fibra de linhaça beneficiem o sistema imune de jundiá.

O efeito positivo da suplementação da dieta com CFAs também foi observado na produção de mucoproteínas (Fig. 2) que são responsáveis pela formação da camada protetiva de muco nos peixes. Sabe-se que o muco é composto principalmente por água e

glicoproteínas, fazendo parte da defesa inata e que atua impedindo a aderência dos patógenos a pele em função de sua espessura e composição, mas principalmente por ser produzido e descartado continuamente pelo animal [40-44]. O contato direto dos peixes com o ambiente faz com que estejam permanentemente expostos a perigos externos, como patógenos e poluentes [45, 46]. Assim constituintes da alimentação que proporcionem incrementos na produção de muco pelos peixes podem beneficiar seus mecanismos de defesa contra patógenos, caracterizando-se como um importante promotor do sistema imunológico.

Embora não sejam encontrados estudos que avaliem o efeito da fibra alimentar sobre a produção de muco pelos peixes, Sheikhzadeh et al. [47] estudaram o efeito de fermentado *Saccharomyces cerevisiae* bobre crescimento e componentes imunológicos do muco da pele de truta arco-íris (*Oncorhynchus mykiss*), concluindo que a adição do mesmo promove efetivamente melhoras no desempenho e parâmetros imunológicos não-específico do muco. Em trabalho com a mesma espécie Yarahmadi et al. [13] destacam que a fibra alimentar pode ser considerada um suplemento dietético útil para a regulação positiva de genes relacionados a imunidade, estimulação da resposta imune e aumento na resistência a doenças, ressaltando que o modo de ação das fibras sobre o sistema imunológico ainda não foi estabelecido. Ambos os trabalhos amparam a ideia do potencial da fibra alimentar como agente prebiótico em dietas e seus efeitos benéficos sobre o sistema imunológico de peixes.

Nossos resultados mostram que os peixes que receberam a dieta controle apresentaram menores reservas de proteína hepática (Tabela 4) e redução de proteínas totais no plasma (Tabela 3), o que pode indicar menor status imunológico dos animais. O fígado é responsável pela síntese de componentes da resposta imune inata e adaptativa. As proteínas plasmáticas, de sabida importância na imunidade animal [32, 33, 34], são sintetizadas no fígado, sendo que maiores concentrações de proteína hepática indicam aumento de proteínas totais circulantes no plasma e melhorias no sistema imunológico dos animais. A redução da proteína hepática

também pode indicar que os peixes estão utilizando proteína para suprir suas necessidades energéticas de modo a manter a glicemia.

Nos animais alimentados com dietas contendo CFAs, a manutenção da glicose plasmática provavelmente tenha ocorrido às custas das reservas de glicogênio hepático (Tabela 4), o que aponta maior demanda energética destes animais para produção dos indicadores imunológicos avaliados. Essa condição pode ser consequência de uma preferência dos animais por utilizarem a energia armazenada na forma de glicogênio para manter seu status imunológico na condição de jejum. Segundo Klasing [48], o sistema imune é prioritariamente atendido por nutrientes prontamente disponíveis como o glicogênio, sendo que dietas que maximizem o desempenho, geralmente proporcionam substrato adequado para o sistema imune funcionar satisfatoriamente.

Nossos resultados demonstram que o autolisado de levedura e a fibra de linhaça agem como prebióticos em dietas para jundiás, uma vez que trazem benefícios ao sistema imune, promovendo efeitos equivalentes ou superiores aos obtidos pela administração do prebiótico comercial testado (Tabela 3 e Fig. 2). Mais pesquisas devem ser realizadas para relacionar a fibra alimentar a efeitos prebióticos, como a modulação da microbiota intestinal, aumento das vilosidades intestinais e resistência a doenças.

5. Conclusão

Nossos resultados demonstram efeitos positivos da fibra de linhaça e do autolisado de levedura sobre parâmetros imunológicos e metabólicos de juvenis de jundiá, evidenciando o potencial de utilização dos mesmos em dietas para a espécie.

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ARTIGO 2

DIETARY FIBER IN THE NUTRITION OF SILVER CATFISH: PREBIOTIC OR ANTINUTRIENT?*

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Dietary fiber in the nutrition of silver catfish: prebiotic or antinutrient?

Abstract

This study was conducted to evaluate the prebiotic potential of dietary fiber as a growth promoting agent in a juvenile silver catfish diet (*Rhamdia quelen*) regarding performance, deposition of nutrients and production of digestive enzymes. The treatments were: control diet, diet containing commercial prebiotic Actigen®, inclusion of citrus pulp, yeast autolysate and linseed fiber. During 50 days, 600 juvenile silver catfish (3.54±0.53 g) were kept in a water recirculation system and fed three times a day with the experimental diets. At the end of the experiment, the growth parameters carcass yield, nutrient deposition, chemical composition and digestive enzymes were evaluated. The experimental design was a randomized, with five treatments and four replications, the data were subjected to analysis of variance and means were compared by Tukey test ($P<0.05$). Fish fed diets containing yeast autolysate and linseed fiber showed superior performance ($P<0.05$) to the other treatments tested, as well as higher values of crude protein and fat deposited in the body. Animals fed diets containing citrus pulp showed lower performance and nutrient deposition. Carcass yield, digestive rates and production of digestive enzymes were not affected by the tested fibers. In conclusion, the results have showed that yeast autolysate and linseed fiber provide better performance and nutrient deposition by animals, demonstrating the potential prebiotic effect of the use of these ingredients in the nutrition of juvenile silver catfish.

Keywords: fish, *Rhamdia quelen*, nutrition, growth promoter, soluble fiber, insoluble fiber

Abbreviations: AFC, apparent feed conversion; YA, yeast autolysate; CF, condition factor; CP, citrus pulp; DSI, digestive somatic index; HIS, hepatosomatic index; IQ, intestinal quotient; LF, linseed fiber; SGR, specific growth rate; VFI, visceral fat index.

1. Introduction

Historically, the importance of the proper quantification of the fiber fraction of food, as well as its nutritional, digestive and metabolic effects, has been delegated to the background in fish nutrition. Most approaches emphasize the negative aspects of its presence in the diet (Wilson, 1995; Tripathy and Misha, 2007) without considering that, when in adequate amounts, its effects can be as important as those of the commercial prebiotics currently applied as promoters of the development of beneficial microbiota in the digestive tract, which reflect positively on the performance and body composition of animals (Schneeman, 1998; Cummings et al., 2004; Knudsen, 2001; Montagne et al., 2003; Eshaghzadeh et al., 2014).

The results of Yarahmandi et al. (2014) demonstrated that using fiber sources for fish is promising and should be explored further in order to optimize animal performance and immune response. Fiber sources are typically plentiful and discarded by the Brazilian agribusiness, taking massive densities of nutrients of significant nutritional application and causing considerable environmental impacts due to improper waste disposal. However, these fiber sources can be extensively exploited as eco-friendly growth promoters in fish nutrition, contributing to the production of fish with greater food security and, at the same time, acting as a mitigating agent.

Brazilian fish farming is developing rapidly, turning to breeding native species such as catfish (*Rhamdia quelen*), which are typically more susceptible to the stresses resulting from high density stock breeding compared to other species consolidated in the international market, such as carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*). This fact requires nutritional strategies that assist in maintaining the health of animals and promoting growth, even under adverse conditions, and the use of prebiotics in feed agents may be promising.

In this context, the addition of dietary fiber in the diet should be studied in order to elucidate its effects on fish performance, since there are abundant sources of fiber being discarded by Brazilian agricultural industries. The aim of this study was to evaluate the effect of the nutritional dietary fiber derived from agroindustrial disposal on productive performance, nutrient deposition and activity of digestive enzymes the juvenile silver catfish.

2. Material and methods

2.1 Preparation of dietary fiber concentrates

Citrus pulp, linseed fiber and yeast autolysate were selected as sources of fiber for the study because they contain different proportions of soluble and insoluble fiber. In order to optimize their addition in the experimental diets, total dietary fiber from these sources was concentrated to obtain ingredients with at least 500 g/kg total dietary fiber.

Citrus pulp was obtained after extracting juice from oranges of the Valencia variety. The remaining residue (flavedo, albedo, seeds and membranes) was washed with tap water at room temperature, crushed in a cutter (Metvisa, model CUT 2.5) for about 20 seconds and dried in a forced air oven at 55 °C for 24 hours followed by milling in a grinder (Marconi, model MA-630/1) to obtain particles with average granulometry of 590μm.

To obtain yeast autolysate, beer yeast (*Saccharomyces cerevisiae*) biomass was centrifuged at 1200 x g for 15 minutes and then washed three times with distilled water at a concentration of 1:1 (w/v), followed by recentrifugation in order to remove impurities and the remainder alcohol. A clean yeast biomass was subjected to an autolysis process in a water bath, stirring for 8 hours at 49 °C, according to the method described by Matiazi (2006), dried in forced air oven at 55 °C for 48 hours and then milled in a grinder (Marconi, model MA-630/1) to obtain particles with average granulometry of 590μm.

Linseed fiber was obtained from whole linseed grains in an aqueous solution at a concentration of 10% w/v and temperature of 60 to 80 ºC and constant stirring for 150 minutes, following the experimental method described by Goulart et al. (2013). The ground grain with mucilage removed was degreased by organic solvent hexane 1:2 (w/v) with four washes of 30 minutes. Then, the remaining bran protein content was reduced by dispersing in water at room temperature at a ratio of 1:30 (w/v). The remaining fiber concentrate in this step was mixed and the mucilage removed from the first stage, yielding the concentrated linseed fibers with average particle size of 590μm.

2.2 Experimental diets

Five mixed feeds were formulated for the experiment, containing fish meal and maize starch, added with 2.50 g/kg of commercial prebiotics based on mannan-oligosaccharides (Actigen® Alltech) or citrus pulp (CP), yeast autolysate (YA), linseed fiber (LF) and one control diet (Table 1). The ingredients were weighed and mixed until homogeneous, then water was added and pelleting (four millimeters) was performed. They were dried in an oven with forced air circulation for 24 hours at a temperature of 55 ºC. After drying, the diets were milled and selected considering the intake condition of fish.

2.3 Fish culture and feeding trial

The study was conducted in the Laboratory of Fisheries of the Department of Animal Science, Federal University of Santa Maria (UFSM) - RS, Brazil, after being approved by the UFSM's Ethics Committee on Animal Trials under number 23081.009051/2014-53. A total of 600 juveniles silver catfish, corresponding to 30 animals per experimental unit, with initial mean weight of 3.54±0.53 g, were distributed into 20 polypropylene tank of 230 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. In the experimental period, the animals were fed to apparent satiation three times a day at 8:00, 13:00 and 17:00 for 50 days.

2.4 Water quality

During the experimental period, the water quality parameters were monitored and maintained as follows: morning temperature 20.94±2.08ºC; afternoon temperature 22.01±1.93ºC; dissolved oxygen: 8.43±0.88 mg L^{-1}; pH: 7.25±0.27; total ammonia: 0.1±0.06 mg L^{-1}; nitrite: 0.12±0.08 mg L^{-1}; alkalinity: 55.6±13.95 mg $CaCO_3$/L; and hardness: 64.83±11.28 $CaCO_3$/L. According to Baldisserotto and Silva (2004), these parameters are within the optimum range for the culture of *Rhamdia quelen*.

2.5 Data collection and assessed variables

In the early and late experimental period, a biometric assessment was performed to collect data from the animals, which had fasted for 18 hours and were anesthetized with Benzocaine (100 mg/L), to estimate the following: individual weight gain (g); total length (cm); specific growth rate (SGR): [(ln (final weight) - ln (initial weight)) /days] x 100, where: ln= Neperian logarithm; condition factor (CF): weight/(total length) 3 x 100; apparent feed conversion (AFC): feed intake/weight gain and consumption (g). For the analysis of the somatic parameters, twelve animals per treatment were used for determining the digestive somatic index (DSI): (weight of the digestive tract/weight of the whole fish) x 100; hepatosomatic index (HSI): (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, twelve 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 and Dyer (1959).

The nutrients retention was calculated according to the following equations:

- Body deposition protein (g): [FW * (% FBP/100)] – [IW * (% IBP/100)];

- Body deposition fat (g): [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.

2.6 Analysis of digestive enzymes

After fifty days of treatment, three samples of fish of each tank were collected to determine the activity of trypsin and chymotrypsin enzymes. The intestines collected were 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 determining intestine trypsin and chymotrypsin enzymes. To determine the trypsin enzyme activity, TAME (α-ρ-toluenesulphonyl- L-argininemethyl ester hydrochloride) was used as substrate. The intestine extracts were incubated for two minutes in a 2-ml buffer solution of Tris/$CaCl_2$, pH of 8.1. For determining chymotrypsin, the substrate used was BTEE (benzoyl-L-tyrosine ethyl ester). The extracts were incubated for two minutes in a 2-ml buffer solution of Tris/$CaCl_2$ (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 µmol of BTEE/minute/mg protein. Readings were taken in a spectrophotometer, absorbance of 247 and 256 nm respectively, following the methodology described by Hummel (1959).

2.7 Statistical analysis

Initially, the data were analyzed for outlier identification. The experimental design was completely randomized with five treatments and four replications. The data were subjected to analysis of variance and means were compared by Tukey's test. Differences were considered significant at the level of $P<0.05$.

3. Results

3.1 Performance parameters

Diets containing yeast autolysate and linseed fiber provided greater weight gain ($P<0.05$) in animals when compared to the other treatments tested (Table 2). The total length, specific growth rate and condition factor was also higher ($P<0.05$) for fish fed yeast autolysate and linseed fiber in the diet, but not different from animals fed the diet containing Actigen® (Table 2). Food consumption was influenced by the diets, with citrus pulp causing reduced food intake by fish (Table 2).

3.2 Nutrient deposition and somatic parameters

Diets containing yeast autolysate and linseed fiber caused greater deposition of crude protein and fat in the body ($P<0.05$) of juvenile silver catfish, compared to the other treatments (Figure 1), but this was not reflected in increased deposition of visceral fat (Table 3). There was no significant difference in corporal yield, somatic and digestive parameters (Table 3).

3.3 Digestive enzymes

Table 4 shows the results of the digestive enzymes activity in silver catfish fed with dietary fiber concentrates. The addition of concentrated dietary fiber to the silver catfish diet had no influence on the activity of trypsin and chymotrypsin of these animals during the experimental period.

4. Discussion

The yeast autolysate and linseed fiber effectively acted as growth promoters in silver catfish diets, since performance was equivalent or superior to the commercial prebiotic observed (Table 2). Other studies show that the fermentable fraction of the dietary fiber has a positive effect on the production of short chain fatty acids and intestinal morphology, promoting metabolic and performance benefits on fish, in addition to providing improvements over the immune system and, consequently, resistance to diseases (Schely and Field, 2002; Yarahmandi et al., 2014).

Most commercial prebiotics of proven efficiency to fish are produced by extracting mananoligossacarids and β-glucans off the cell wall of the yeast (*Saccharomyces cerevisiae*) (Ringo et al., 2010; Sang et al., 2014; Torrecillas et al., 2014). However, our results show that the use of a less elaborate product, such as yeast autolysate, allows for a performance similar to that of prebiotics of proven efficiency. Hisano et al. (2007) evaluated the performance of juvenile Nile tilapia (2.22±0.07 g), observing a higher performance in animals supplemented with yeast autolysate when compared to whole yeast or yeast cell wall. These studies show that the use of yeast autolysate is promising and may reduce production costs because the process to obtain it is simpler than that employed for the manufacture of products made from the yeast cell wall.

Similar to that observed study, Goulart et al. (2013) reported good performance of silver catfish fed with a diet containing linseed bran, suggesting a growth promoting effect of the dietary fiber present in this ingredient. Parts of the components of the dietary fiber are used as substrate by intestinal bacteria, while the insoluble fraction presents slow or partial fermentation compared to the soluble fraction which is rapidly fermented (Puupponen-Pimiä et al., 2002). According to Lan et al. (2005), any food or dietary ingredient that reaches the large intestine intact is a potential prebiotic, but should be fermented by beneficial microorganisms in order to be effectively considered as such. Silva and Nörnberg (2003) state that the qualitative modulation of the native microbiota present in the host is the primary mode of action of prebiotics and, in some cases, the higher performance of the animals is obtained as a response. Thus, it is suggested that fermentation of dietary fiber fractions of yeast autolysate treatments and linseed fiber caused prebiotic effects, directly and positively reflecting on animal performance.

The positive effects of dietary supplementation with autolysate and linseed fiber were confirmed by increased deposition of protein and fat in fish (Figure 1). In addition to the beneficial effects of intestinal lumen, some authors have reported that the production of fatty acids may contribute as an energy source for intestinal cells, favoring the use of dietary energy for the purpose of muscle production (Merrifield et al., 2010; Wong et al., 2006; Schely and Field, 2002), which may explain the results found in this study.

In recent decades, the presence of soluble dietary fiber in the diets of non-ruminant animals has been identified with an undesirable factor for its action in increasing the viscosity of the digesta. This fact would hinder the action of enzymes and bile salts in the food bolus, reflecting negatively on animal performance (Bedford and Classen, 1992; Annison, 1993). However, the results obtained in this study are opposed to these reports, since the increased

consumption of soluble fiber (autolysate diet) or of a source with higher hydration capacity (linseed fiber) optimized animal performance.

Although the citrus pulp diet has an amount of soluble fiber and hydration capacity similar to the linseed fiber diet (Table 1), its effect on performance was not satisfactory, which can be attributed both to palatability as to the antimicrobial action of its oil (Albach et al., 1981; Celiktas et al., 2007). Similar results were reported for pacu (*Piaractus mesopotamicus*) (Fabregat et al., 2011). According to Heuzé et al. (2014) the palatability of citrus pulp is variable due to the limonin present in the seeds and hulls, which are responsible for its characteristic bitter taste, resulting in decreased intake. It is emphasized that these are secondary factors that may have masked the true effects of the fiber fraction of this ingredient, since the pectin extracted from oranges exerts a positive effects on the intestinal microbiota and immune system, causing performance benefits (Fooks et al., 1999; Salman et al., 2008; Muzzarelli et al., 2012).

Thus, it should be noted that the effects of dietary fiber in the animal body are connected not only the amount consumed, but also to its composition and its physical-chemical characteristics (Silva and Walter, 2012), as well as secondary factors present at the source.

Somatic and digestive indices linked to the trypsin and chymotrypsin activity found in the study reinforce the possibility of using the fibers studied as eco-friendly growth promoters (Table 4). Changes in these parameters may occur in response to the composition of the ingredients used in the diets in the form of adjustments to the gastrointestinal tract to increase the area of contact with the food, digestibility and absorption of nutrients (Leenhouwers et al., 2006).

The digestive capacity of an animal may be defined as the ability to secrete enzymes in the tract, capable of hydrolyzing the polymers present in the food to their respective

monomers, and the levels of these enzymes depend on the levels of nutrients present in ingested food (Stech et al. 2009). According to Vanderoof (1998), insoluble fiber maintains its integrity during the passage of the digesta through the intestine and may act as a physical barrier capable of limiting the access of digestive enzymes to the food contents, decreasing the digestion and absorption of nutrients. Furthermore, the increase in intestinal viscosity provided by the presence of soluble dietary fiber may impair the enzyme-substrate interaction and the animals would compensate such inefficiency with increased gastrointestinal organs and secretion of digestive enzymes (Ikegami et al., 1990), which was not observed in this study.

5. Conclusion

The results demonstrate that yeast autolysate and linseed fiber provide a better performance and deposition of nutrients by the animals, they do not affect the ability of digestive, showing that the fiber had no antinutritional action. This demonstrates the potential use of these prebiotic ingredients such as growth promoter in juvenile silver catfish nutrition.

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Tables

Table 1

Formulations and proximate composition of the experimental diets (g/kg)

Treatments[1]					
Ingredients	Control	Actigen®	PC	AY	LF
Fish meal[*]	610.10	610.10	600.00	570.80	570.00
Maize starch	118.00	118.00	121.00	120.00	114.00
Citrus pulp	0	0	177.00	0	0
Yeast autolysate	0	0	0	138.00	0
Linseed fibre	0	0	0	0	146.00
Actigen®[2]	0	2.50	0	0	0
Cellulose	106.00	106.00	0	0	0
Soybean oil	60.00	60.00	46.00	55.00	42.00
NaCl	5.00	5.00	5.00	5.00	5.00
Melbond®[3]	20.00	20.00	20.00	20.00	20.00
Vitamin and mineral mixture [4]	30.00	30.00	30.00	30.00	30.00
BHT[5]	0.10	0.10	0.10	0.10	0.10
Inert[6]	49.90	47.40	0.90	53.90	72.90
Total	1000	1000	1000	1000	1000
Proximate analysis					
Crude protein	360.40	360.40	361.00	360.70	360.30
Digestible energy (MJ/kg)[7]	13.43	13.43	13.40	13.43	13.42
Ether extract	114.70	114.70	113.30	114.10	113.90
Total dietary fiber	100.20	100.20	100.40	100.70	100.90
Soluble fiber	-	-	37.30	50.90	31.20
Insoluble fiber	100.20	100.20	63.10	49.80	69.70
Ash	143.60	141.10	122.70	140.60	158.40
Ca	3.36	3.36	3.31	3.22	3.18
P	1.63	1.63	1.61	1.57	1.55
Hydration capacity	1.51	1.48	1.61	1.21	1.76

[1]Treatments: Control; Actigen®: inclusion of commercial prebiotic comercial Actigen®; PC: citrus pulp; YA: yeast autolysate; LF: linseed fiber; Hydration capacity: (g water/g sample).
[2]Prebiotic comercial Actigen® Alltech.
[3] Binder.
[4]Composition (Migfish): Ác. Folic: 299.88 mg; Ác. Ascorbic: 15,000.12 mg; Pantothenic: 3,000.10 mg; Biotin: 12:06 mg; Niacin (B3): 9,000.32 mg; Hill (B4): 103,500.00 mg; Vit.A: 1,000,000.00 IU; Vit. B1: 1,500.38 mg; Vit. B2: 1,500.0 mg; Vit. B6: 1,500.38mg; Vit. D3: 240,000.00 IU; Vit. E: 10,000,00 mg; Vit. K3: 400.00 mg; Inositol: 9,999.92 mg; Iron: 6,416.80mg; Manganese: 8,000.40mg; Copper: 1,000.00 mg; Zinc: 13,999.50 mg; Iodine: 45.36 mg; Cobalt: 60.06 mg; Selenium: 60.30 mg; Magnesium: 5.10 mg; Chlorine: 2.30 %; Sulfur: 0.01%.
[5]Butil hidróxi tolueno (BHT).
[6]Sand.
[7]Digestible energy: calculated digestible energy: [(Crude protein * 5.65 * 0.85) + (Fat * 9.4 *0.9) + (Carbohydrates * 4.15 * 0.7)] (adapted Meyer et al., 2004).
[*]Waste flour tilapia/Copisces-Paraná/RS.

Table 2
Growth performance of juvenile silver catfish in the final experiment

	Treatments				
	Control	Actigen®	Citrus pulp	Yeast autolysate	Linseed fiber
Weight gain (g)	37.73±7.46^{bc}	38.22±9.08^{b}	34.43±9.64^{c}	43.16±10.62^{a}	42.63±10.52^{a}
Total length (cm)	15.32±1.14^{bc}	15.62±1.25^{abc}	15.19±1.43^{c}	15.97±1.32^{a}	15.67±1.36^{ab}
Specific growth rate (%/day)	4.51±0.08^{b}	4.64±0.04^{ab}	4.51±0.06^{b}	4.81±0.09^{a}	4.76±0.09^{a}
Condition fator	1.01±0.07^{ab}	1.03±0.08^{ab}	0.99±0.08^{b}	1.03±0.11^{a}	1.03±0.11^{a}
Apparent feed conversion	1.10±0.07	1.05±0.05	1.11±0.05	1.05±0.03	1.08±0.05
Consumption (g)	1154.60±56.42^{a}	1132.62±36.96^{a}	1025.07±31.02^{b}	1193.70±25.80^{a}	1191.35±30.88^{a}

Mean ± standard deviation. Different letters in the rows of the table represent significant difference by Tukey test ($P<0.05$).

Table 3
Corporal yield and digestive index (g/kg) of juvenile silver catfish (*Rhamdia quelen*) at the end of the biological assay

	Treatments				
	Control	Actigen®	Citrus pulp	Yeast autolysate	Linseed fiber
CY	855.10±18.10	857.20±21.50	852.10±14.80	844.60±12.30	857.90±24.20
DSI	34.20±5.90	33.80±5.70	38.40±5.30	35.40±4.50	36.40±7.40
HSI	15.80±3.00	16.40±4.40	14.90±2.90	16.10±2.90	16.00±2.30
IQ	11.70±1.90	10.60±1.60	11.00±1.30	11.80±1.60	11.50±2.40
VFI	26.30±8.60	31.00±12.90	22.50±9.50	28.40±12.30	31.00±16.60

CY: Corporal yield; DSI: Digestive somatic index; HSI: Hepatosomatic index; IQ: Intestinal quotient; VFI: Visceral fat index. Mean ± standard deviation. Different letters in the rows of the table represent significant difference by Tukey test ($P<0.05$).

Table 4

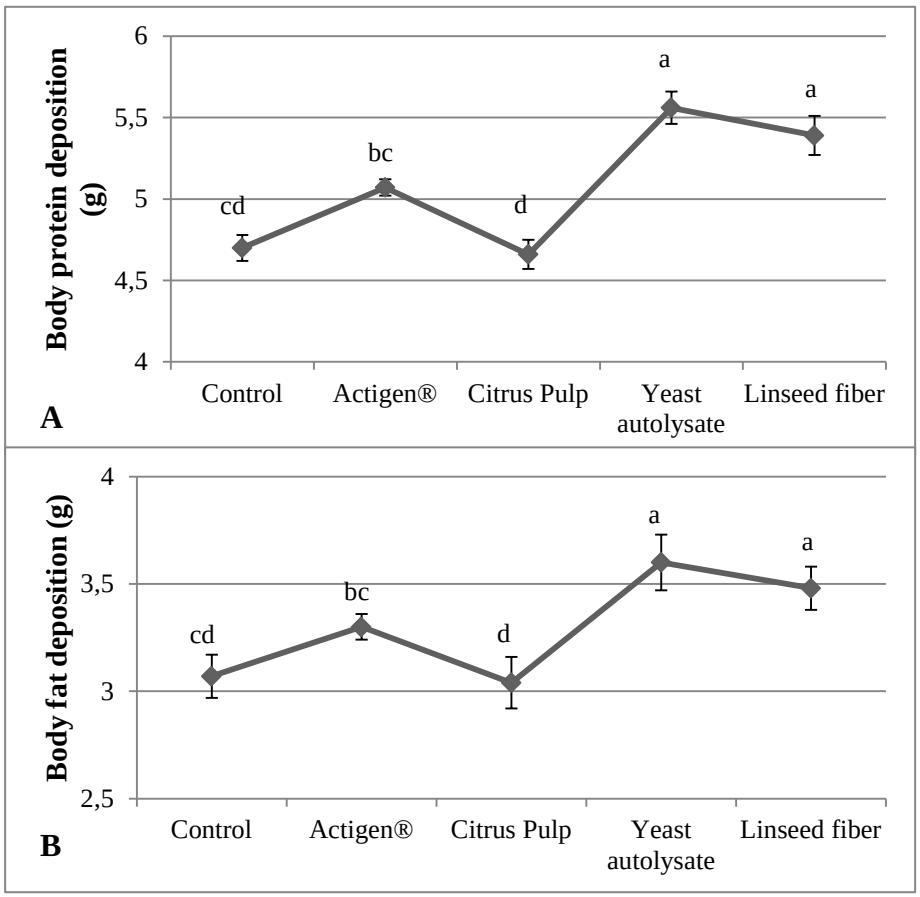
Activity of digestive enzymes of juvenile silver catfish receiving the experimental diets

Treatments	Chymotrypsin (µmol/btee/min/mg prot)	Trypsin (µmol/tame/min/mg prot)
Control	9828.70±2598.32	12.30±3.27
Actigen®	9524.28±2431.22	11.12±3.38
Citrus pulp	9948.36±2309.84	11.55±2.55
Yeast autolysate	9941.87±2286.77	11.04±2.81
Linseed fiber	9781.53±2561.49	11.85±2.32

Mean ± standard deviation. Means with different letters in the column indicate significant differences by Tukey test ($P<0.05$).

Fig.1. Body deposition of protein (g) (A) and fat (B) of juvenile silver catfish

Different letters in the figure represent significant difference by Tukey test (P<0.05).



DISCUSSÃO GERAL

A possibilidade de incorporação de fibra alimentar concentrada de resíduos agroindustriais em dietas para peixes é de grande relevância econômica e científica, uma vez que busca a produção sustentável de proteína de alto valor biológico para consumo humano, sem a utilização de antibióticos, os quais podem promover o surgimento de cepas de microrganismos resistentes e deixar resíduos na carne. Aliado a isso, ainda pode-se mitigar os efeitos do descarte incorreto destes resíduos no meio ambiente, beneficiando não somente a cadeia produtiva de pescado, como as produtoras dos resíduos objetos deste estudo.

A concentração da fibra alimentar de distintas fontes de origem, resultou em produtos (CFAs) diferentes quanto as frações insolúvel e solúvel de fibra (Artigo 1, Figura 1) e quanto as propriedades físico-químicas (Artigo 1, Tabela 2). Como consequência, mesmo mantendo-se as dietas isofibrosas (Artigo 1, Figura 2, Tabela 1), a incorporação dos CFAs resultou em distintas capacidade de hidratação e proporções de fibra solúvel e insolúvel nas dietas.

O ensaio biológico mostrou que a incorporação dos CFAs proporcionaram diferenças significativas nos indicadores imunológicos avaliados (Artigo 1, Tabela 3, Figura 2), no metabolismo (Artigo 1, Tabela 4), parâmetros de desempenho (Artigo 2, Tabela 2) e deposição de nutrientes pelos animais (Artigo 2, Figura 1). As mudanças no funcionamento do sistema digestório provocadas pela ingestão de diferentes fontes de fibras podem influenciar a fisiologia, metabolismo e características do epitélio intestinal, alterando assim a absorção e níveis de nutrientes sanguíneos, modificando a composição corporal e a deposição de músculo e gordura. Salienta-se que os CFAs testados tem características peculiares, uma vez que se distinguem quanto as proporções de fibra solúvel e insolúvel e características físico-químicas, refletindo decisivamente sobre respostas dos animais.

Os peixes que receberam a dieta contendo autolisado de levedura e fibra de linhaça apresentaram maior concentração plasmática de colesterol, proteínas totais e globulinas (Artigo 1, Tabela 3), além de maior produção de mucoproteína (Artigo 1, Figura 2), quando comparados ao controle. Os mesmos tratamentos também proporcionaram melhor desempenho (Artigo 2, Tabela 2) e deposição de nutrientes (Artigo 2, Figura 1), indicando que o status imunológico resultante do consumo das dietas contendo autolisado de levedura e fibra de linhaça possibilitou melhor utilização dos nutrientes contidos nas dietas. Estes resultados podem estar relacionados a mudanças na produção de ácidos graxos de cadeia curta (AGCC) causada pela fermentação a nível intestinal da fibra alimentar contida nestes

tratamentos. Uma vez que constituem a principal fonte de energia para o colonócitos, a produção destes AGCC resultante do consumo de fibras fermentáveis proporcionam benefícios ao sistema imunológico e resistência a doenças, promovendo melhorias no desempenho dos peixes (SCHELY e FIELD 2002; YARAHMANDI et al., 2014).

A menor reserva de glicogênio hepático observada nos animais suplementados com autolisado de levedura e fibra de linhaça na dieta (Artigo 1, Tabela 4) não afetou o desempenho dos peixes (Artigo 2, Tabela 2), os quais apresentaram os melhores resultados de ganho de peso, taxa de crescimento específico, conversão alimentar aparente e deposição de nutrientes (Artigo 2, Figura 1). Essa constatação sugere que os animais estejam utilizando as reservas de glicogênio para prover os indicadores imunológicos avaliados, uma vez que encontraram-se aumentados nos animais que receberam as dietas suplementadas com esses CFAs (Artigo 1, Tabela 3, Figura 2). Essa hipótese é reforçada pelo fato de que se as demandas energéticas não estivessem sendo supridas, a menor reserva de glicogênio teria refletido em menor desempenho, o que não aconteceu no presente estudo.

Segundo Klasing (1998) a prioridade do sistema imune pelo uso dos nutrientes é baseada na observação de que uma restrição alimentar moderada é suficiente para prejudicar a taxa de crescimento, mas não prejudica os índices de imunocompetência. Assim os níveis de nutrientes que maximizam a produção geralmente proporcionam que o sistema imune funcione satisfatoriamente, uma vez que é o sistema prioritariamente atendido pelos nutrientes disponíveis. Isso justifica o fato de que apesar dos animais alimentados com dieta contendo polpa cítrica terem os piores índices de desempenho (Artigo 2, Tabela 2), baixa reserva de glicogênio hepático (Artigo 1, Tabela 4) e deposição de nutrientes (Artigo 2, Figura 1) observados no ensaio, os indicadores imunológicos não terem apresentado diferença significativa daqueles que proporcionaram os melhores resultados (Artigo 1, Tabela 3, Figura 2).

Os resultados negativos de desempenho decorrentes da suplementação com polpa cítrica podem estar relacionados ao sabor amargo resultante da presença de limonina na casca e sementes da laranja. Essa substância prejudica a palatabilidade do ingrediente (HEUZÉ et al., 2014), refletindo em alterações no consumo de alimento (Artigo 2, Tabela 2) e prejuízo ao desempenho animal.

Analisando os indicadores imunológicos avaliados isoladamente, percebe-se que para parâmetros como colesterol, proteínas totais, globulinas (Artigo 1, Tabela 3) e produção de mucoproteínas (Artigo 1, Figura 2), os animais suplementados com polpa cítrica diferiram significativamente apenas daqueles suplementados com a dieta controle, o que demonstra o

potencial desta fonte para uso como prebiótico em dietas de jundiá. Porém, analisando os resultados de ambos os artigos, fica clara a importância de avaliações de desempenho em estudos relacionados a utilização de imunomoduladores.

Segundo Silva e Nörnberg (2003), uma vez que os prebióticos atuem positivamente sobre o sistema imune dos animais, espera-se reflexos desejáveis no desempenho, porém muitas vezes a utilização destas substâncias não resulta em diferenças significativas. Os autores salientam que este fato pode estar relacionado ao efeito diluidor causado pelos componentes das dietas, ao desequilíbrio nas populações microbianas, a seletividade do prebiótico em questão ou até mesmo ao nível de estresse dos animais, uma vez que em condições não estressantes a microbiota esta em equilíbrio, refletindo respostas semelhantes, já em situações de estresse espera-se que o efeito prebiótico seja evidenciado. Esse fato expõe a necessidade de estudos que avaliem a utilização de dietas práticas em ensaios biológicos para avaliar o potencial prebiótico da fibra alimentar, com o objetivo de detectar possíveis interações entre os CFAs testados e os componentes da dieta, além de uma fase experimental onde os animais são submetidos a desafio.

O efeito prebiótico da fibra alimentar em dietas para peixes foi citado em trabalho realizado por Goulart et al. (2013), que estudaram a utilização de farelos de linhaça demucilada e *in natura* na dieta de juvenis de jundiá (*Rhamdia quelen*). Os bons resultados de desempenho obtidos pelos animais alimentados com 17% de farelo de linhaça in natura, foram relacionados a presença de fibra solúvel (mucilagem) que, segundo os autores, estimulam o crescimento e a atividade de bactérias benéficas, refletindo-se de forma desejável no desempenho animal.

Os nossos resultados demonstraram que autolisado de levedura e a fibra de linhaça agiram efetivamente como prebióticos nas dietas para jundiá, visto que parâmetros de desempenho e indicadores imunológicos foram beneficiados pela suplementação de ambas as fontes de fibra, proporcionando resultados equivalentes ou superiores ao observado pelo prebiótico comercial testado.

CONCLUSÃO GERAL

Os resultados deste estudo demonstram que a fibra de linhaça e o autolisado de levedura tem ação prebiótica quando adicionadas a dietas de juvenis de jundiás, beneficiando parâmetros imunológicos, metabólicos, de desempenho e deposição de nutricional. Evidenciando que a adição da fibra, nas quantidades testadas, não afeta a capacidade digestiva dos animais não apresentando assim efeito antinutricional.

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ANEXOS

ANEXO A – Instruções para submissão de trabalhos na revista Fish and Shellfish Immunology- Artigo 1

GUIDE FOR AUTHORS

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INTRODUCTION

Types of Papers

Research papers

Review articles

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ANEXO B – Instruções para submissão de trabalhos na revista Animal Feed Science and Technology - Artigo 2

INTRODUCTION

Types of article

1. Original Research Papers (Regular Papers)
2. Review Articles
3. Short Communications
4. Book Reviews

Original Research Papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

Review Articles should cover subjects falling within the scope of the journal which are of active current interest.

A Short Communication is a concise but complete description of a limited investigation, which will not be included in a later paper. Short Communications should be as completely documented, both by reference to the literature and description of the experimental procedures employed, as a regular paper. They should not occupy more than six printed pages (about 12 manuscript pages, including figures, tables and references).

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