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PECTINA HIDROLISADA DE POLPA CÍTRICA NA NUTRIÇÃO DE TILÁPIA DO NILO (*Oreochromis niloticus*)

Santa Maria, RS, Brasil

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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 obtenção do grau de **Mestre em Zootecnia**.

Orientador: Profº. Rafael Lazzari

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RESUMO

PECTINA HIDROLISADA DE POLPA CÍTRICA NA NUTRIÇÃO DE TILÁPIA DO NILO (*Oreochromis niloticus*)

AUTOR: Silvandro Tonetto de Freitas
ORIENTADOR: Rafael Lazzari

O estudo foi conduzido com o objetivo de testar um novo prebiótico obtido a partir da polpa cítrica, desenvolvido no laboratório de piscicultura da Universdade Federal de Santa Maria, e avaliar os efeitos de sua inclusão na dieta de tilápias do Nilo (*Oreochromis niloticus).* Durante 45 dias, 600 tilápias do Nilo (peso inicial 5,56±1,31g), foram mantidas em 20 caixas de polipropileno com volume útil de 160 litros (30 peixes/caixa), de um sistema de recirculação de água. Os animais foram alimentados três vezes ao dia com dietas experimentais isoproteicas e isoenergeticas, onde foram substituídos 0, 1,5, 2,5, 3,5 e 4,5 g kg^{-1} de celulose por pectina hidrolisada de laranja. O delineamento foi inteiramente casualisado com cinco tratamentos e quatro repetições, os dados foram submetidos a teste de normalidade e análise de regressão linear. No final do período experimental, avaliou-se os parâmetros hematológicos, zootécnicos, bromatologicos, índices digestivos, parametros metabólicos. O crescimento dos animais foi excelente mesmo não observando efeito nos resultados em nenhum nível de incorporação da pectina hidrolisada, todos os parâmetros mantiveram-se similar com a dieta controle. Conclui-se que o prebiótico não comercial pectina hidrolisada de polpa cítrica não apresentou influência no desenvolvimento dos alevinos de tilápia do Nilo na condição experimental em que os animais foram expostos, não sendo prejudicial aos mesmos.

Palavras-chave: *Oreochromis niloticus*. Aditivos. Peixe. Aquicultura.

ABSTRACT

CITRUS HYDROLYSED PECTIN IN NILO TILAPIA NUTRITION

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ADVISOR: Rafael Lazzari

The objective of this study was to test a new prebiotic obtained from the citrus pulp, developed in the fishery laboratory of the Federal University of Santa Maria, and to evaluate the effects of its inclusion in the diet of Nile tilapia (Oreochromis niloticus). For 45 days, 600 Nile tilapia (initial weight 5.56 ± 1.31g) were kept in 20 polypropylene boxes with a useful volume of 160 liters (30 fish / box), of a water recirculation system. The animals were fed three times daily with isoprotein and isoenergetic experimental diets, where 0, 1.5, 2.5, 3.5 and 4.5 g kg-1 cellulose were replaced with orange hydrolyzed pectin. The design was entirely randomized with five treatments and four replicates, the data were submitted to normality test and linear regression analysis. At the end of the experimental period the hematological parameters, zootechnical, bromatological, digestive indices and metabolic parameters were evaluated. The growth of the animals was excellent even though no effect was observed on the results at any level of hydrolyzed pectin incorporation, all parameters remained similar with the control diet. It is concluded that the non-commercial prebiotic citrus pulp hydrolyzed pectin had no influence on the development of the Nile tilapia fingerlings in the experimental condition in which the animals were exposed, not being harmful to them.

Keywords: *Oreochromis niloticus*. Additives. Fish. Aquaculture.

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LISTA DE ABREVIATURAS

ALB: Albumina

BHT: Butil Hidróxi Tolueno

BT: Biomassa Total

CAA: Conversão Alimentar Aparente

COL: Colesterol

CPS: Concentrado Proteico de Soja

CT: Comprimento Total

ED: Energia Digestível

GLI: Glicose

GPR: Ganho em Peso Relativo

HMG: Hemoglobina

IDS: Índice Digestivossomático

IGV: Índice de Gordura Visceral

IHS: Índice Hepatossomático

MM: Matéria Mineral

NS: Não Significativo ($p>0,05$)

PB: Proteína Bruta

PF: Peso Final

PRT: Proteínas Totais

RC: Rendimento de Carcaça

TCE: Taxa de Crescimento Específico

TGI: Triglicerídeos

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SUMÁRIO

1 INTRODUÇÃO GERAL

A aquicultura tem elevado a contribuição em relação à produção mundial de alimentos nas ultimas décadas. Nesse sentido, os últimos dados mostram que o Brasil encontra-se entre os 15 maiores produtores mundiais, com 86,4% da sua produção proveniente de água doce, totalizando 707,461 mil toneladas (FAO, 2014). O consumo de pescado mundial passou de 9,9 kg *per capita* em 1960 para 19,2 kg em 2012, esse dado mostra como a produção de pescado está sendo impulsionado pelo crescimento populacional, aumento da renda e distribuição mais eficiente de alimentos (FAO, 2014).

Consumidores têm buscado informações sobre produtos alimentares, tornando-se mais exigentes e preocupados com os alimentos que entram em suas refeições. Uma dessas preocupações refere-se ao efeito residual ocasionado pelo uso de antibióticos na nutrição animal, além do surgimento de cepas bacterianas resistentes a estas moléculas de aditivos (SILVA; NÖRNBERG, 2003). Para evitar estes problemas, nas últimas décadas, vários estudos foram conduzidos em busca de promotores de crescimento alternativos e sustentáveis, que não sejam prejudiciais aos animais e as pessoas, os quais agem auxiliando no equilíbrio benéfico da microbiota do trato gastrintestinal de animais de produção (SILVA; NÖRNBERG, 2003).

Apesar de encontrar várias marcas de prebióticos no mercado, basicamente as suas formulações baseiam-se em três grandes grupos de oligossacarídeos (frutooligossacarídeos, galactooligossacarídeos e mananoligossacarídeos), limitando a escolha do melhor ingrediente ativo, sabendo-se que o uso, características dos animais, composição da dieta e meio em que o animal se encontra pode apresentar diferentes respostas nos animais conforme o ingrediente usado (GRISDALE-HELLAND et al., 2008). Estudos têm mostrado o grande potencial que os prebióticos possuem de melhorar o aproveitamento dos nutrientes e o crescimento de várias espécies de peixes (MAHIOUS et al., 2006; STAYKOV et al., 2007; TORRECILLAS et al., 2007).

À vista disso, é importante e necessário o aumento de opções de moléculas de ação prebiótica, como a pectina hidrolisada de polpa cítrica, que é de grande relevância tanto científica como econômica, pois é um resíduo proveniente de agroindústrias. Pesquisas mostram que o seu uso contribui no desenvolvimento de bactérias acidolíticas, que por sua vez são inibidoras de agentes nocivos. (TAMURA et al., 2013).

1.1 Objetivo geral

Avaliar a utilização do prebiótico pectina hidrolisada de polpa cítrica na nutrição de Tilápias do Nilo (*Oreochromis niloticus*).

1.2 Objetivos específicos

- Avaliar parâmetros metabólicos (glicose e glicogênio) e zootécnicos em peixes alimentados com doses crescentes de pectina hidrolisada de polpa cítrica;
- Determinar a composição corporal de Tilápias do Nilo alimentadas com dietas contendo pectina hidrolisada de polpa cítrica.

2. REVISÃO BIBLIOGRÁFICA

2.1 Características da tilápia do Nilo

A tilápia do Nilo - cujo nome científico é *Oreochromis niloticus* - pertence à Ordem Perciformes, sendo esta uma espécie de peixe oriunda do continente Africano, que é econtrada nas bacias dos rios Nilo, Níger, Tchade e lagos do Centro-Oeste africano (Verani, 1980). A espécie possui diversos atributos favoráveis à piscicultura, tais como: rusticidade, facilidade na obtenção de larvas, crescimento acelerado em criação intensiva e hábito alimentar omnívoro (COWARD; BROMAGE, 2000). Ademais, as tilápias pertencem ao segundo grupo de maior relevância na aquacultura dulcícola mundial, perdendo somente para os cipriníeos (FAO, 2014).

Na década de 1950, foram publicados os primeiros estudos sobre a tilápia no Ocidente e, já naquela época, a produção desta espécie fora considerada bastante promissora para aquacultura (Croward e Bromage, 2000). Para Vicente e Fonseca-Alves (2013), dentre os peixes cultivados atualmente, a tilápia do Nilo é potencialmente bastante viável, uma vez que tem fácil reprodução, carne branca e de elevada qualidade. Economicamente, destaca-se pela produção de baixo custo, cujo cultivo pode se dar em locais com baixas temperaturas e alta salinidade.

2.2 Sistema digestório da Tilápia do Nilo

Quanto aos hábitos alimentares dos peixes, cumpre expor que este é bastante diversificado, pois eles podem ser: detritivos, herbívoros, carnívoros ou onívoros. Tal classificação ainda pode ser subdividida conforme a variedade de alimentos que compõem sua dieta. Devido a isso, os peixes dispõem de inúmeras adaptações do sistema digestório, consoante a necessidade para ingestão, digestão e absorção dos distintos tipos de alimentos (BALDISSEROTTO, 2013).

Na visão de Baldisserotto (2013), as alterações tanto na estrutura anatômica do intestino, como em seu comprimento dependem diretamente dos hábitos alimentares da espécie. Nesse sentido, os peixes carnívoros têm o intestino curto, enquanto que os herbívoros possuem intestino longo. Já a espécie em análise, a tilápia do Nilo, apresenta um hábito alimentar onívoro, contando com intestino longo em relação ao tamanho do seu corpo, o que facilita o processo de absorção de nutrientes.

Os processos digestórios começam na boca e na cavidade faringeana do peixe, que ocorre a partir da redução mecânica das partículas do alimento, visando a superfície de contato dos fragmentos alimentares e melhorando a digestão enzimática (Cyrino e Fracalossi, 2013). Além disso, o estômago dos peixes é responsável pelo armazenamento e degradação física e enzimática inicial do alimento deglutido (GROSELL et al., 2011). O baixo pH e a pepsina desnaturam a maioria das proteínas da dieta nas espécies com estômago funcional, quebrando as ligações peptídicas e liberando pequenas cadeias polipeptídicas para o final da digestão no intestino (Lovell, 1998). Segundo Cyrino e Fracalossi (2013), é no intestino proximal que acontece a maior parte dos nutrientes são absorvidos, íons e água provenientes da dieta, como também de nutrientes em menor quantidade (monossacarídeos, aminoácidos e ácidos graxos).

2.3 Prebióticos e Pectina

Prebióticos são substâncias não digestíveis que fomentam o desenvolvimento e a atividade de bactérias benéficas no trato gastrointestinal. Dessa forma, eles proporcionam melhora na saúde do hospedeiro, além de atuar no sistema imunológico (SCHWARZ et al, 2010).

A metabolização dos prebióticos ocorre, principalmente, por *Lactobacillus* e *Bifidobacterium,* os quais possuem a capacidade de dificultarem a propagação de agentes patogênicos no trato digestório, sendo responsáveis também pelo estímulo de crescimento da mucosa intestinal e reduzindo o deslocamento de endotoxinas nos enterócitos, aumentando a produção de ácidos graxos de cadeia curta, proporcionando benefícios à saúde do animal (GULLÓN et al., 2011; SONG et al., 2014).

FOS são compostos de frutose com β (2-1) ligações glicosídicas e uma D-glicose terminal. Estes compostos são fermentados pela microbiota benéfica presente no intestino, resultando na produção de ácidos graxos de cadeia curta e lactato, que, além de ser um fonte de energia para o hospedeiro, reduz o pH no intestino e controla a proliferação de microorganismos patógenos (ROBERFROID et al., 2010). MOS são isolados da parede celular de levedura, como *Saccharomyces cerevisiae*, e têm fermentabilidade intestinal inferior em comparação com FOS (ZENTEK et al., 2002). Estes compostos têm a capacidade de reduzir bactérias patogênicas no intestino e modular o sistema imunológico. MOS também regula o crescimento de bactérias patogênicas intestinais por estar presente em certos sites de células epiteliais na mucosa intestinal, impedindo a colonização de bactérias patogênicas nesses locais (SWANSON et al., 2002).

Além destes, outras fontes vem sendo avaliadas, entre elas a pectina. Pectina é um polissacarídeo heterogenêo complexo, sendo encontrado na parede celular primária de grande parte das plantas. Além disso, ela possui variações nas quantidades de açucares neutros, xilose, ramnose, arabinose, galactose e glicose nas cadeias laterais (ROUND et al., 2010; WIKIERA et al., 2015; ZHANG et al., 2015). Ademais, a Pectina contém propriedades tecnológicas (estabilizante, espessante e emulsificante) e propriedades fisiológicas como interferência na saciedade, controle de peso, perfil lipídico e glicêmico (GUNNESS e GIDLEY, 2010; WANG et al., 2014; ESPINAL-RUIZ et al., 2014).

O uso de 10 e 20 g kg^{-1} de MOS em dietas para robalo (*Dicentrarchus labrax*), não mostrou alterações na composição corporal de (TORRECILLAS et al., 2007). Dietas contendo 2 g kg^{-1} de MOS comparadas com a dieta basal de trutas arco-íris, tiveram melhor respaldo no crescimento, eficiência alimentar e sobrevivência (STAYKOV et al., 2007). Testando dietas contendo 5 g kg^{-1} de inulina mais vitamina C em tilápias do Nilo encontrou-se resultados satisfatórios para o peso final dos indivíduos (IBRAHEM et al., 2010). Já a inclusão de 20 g kg^{-1} de inulina em dietas para larvas de *Psseta máxima* quando comparada a suplementação com 20 g kg^{-1} de oligofrutose não teve efeito sobre o crescimento do animais (MAHIOUS et al., 2006).

3 MANUSCRITO

USE OF CITRUS HYDROLYSED PECTIN IN NILE TILAPIA NUTRITION

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Abstract

The objective of this study was to evaluate the effects of a new prebiotic obtained from citrus rind on Nile tilapia (*Oreochromis niloticus*) diet. The study was accomplished in the Fishery Laboratory of the Federal University of Santa Maria. A total of 600 Nile tilapia (initial weight 5.56 ± 1.31g) were kept for 45 days in 20 polypropylene boxes with a useful volume of 160 liters (30 fish / box) of a water recirculation system. The fishes were fed three times a day with isoprotein and isoenergetic experimental diets, where 0, 1.5, 2.5, 3.5 and 4.5 g kg^{-1} of cellulose were replaced by orange hydrolyzed pectin. The study followed a randomized design with five treatments and four replications, each replication was composed by 30 fishes. According to the results, after 45 days, there was no difference between the diet without orange hydrolyzed pectin and diets containing hydrolyzed pectin levels ranging from 0 to 4.5 g kg^{-1}. In conclusion, all treatments resulted in adequate fish growth and size, showing that prebiotic citrus rind hydrolyzed pectin had no negative effect on the development of Nile tilapia fingerlings, which can potentially be used to replace isoprotein and isoenergetic diets, reducing production costs.

Keywords: *Oreochromis niloticus*, Additives, Fish, Aquaculture.

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Introduction

The prebiotics are encompassed within a complex group of non-starch polymers and oligosaccharides that, when combined with other components, are resistant to the action of enzymes in the gastrointestinal tract of non-ruminant animals, with the main role of their use by bacteria in the fermentation. a positive response on the balance of intestinal microflora (Bach Knudsen, 2001; Montagne et al., 2003; Theuwissen and Mensink, 2008).

In this sense, it is worth mentioning the generation of volatile fatty acids, which can be used as a source of energy causing beneficial changes to the animals, making the nutrients more usable and improving the performance of several fish species such as turbot (*Psetta maximum*) (Mahious et al. 2006; Staykov et al. 2007; Torrecillas et al. 2007; Burr et al. 2008; Grisdale-Helland et al. 2008).

The metabolism of prebiotics is mainly due to Lactobacillus and Bifidobacterium, which are able to inhibit the proliferation of pathogens in the digestive tract, being also responsible for the growth stimulus of the intestinal mucosa and reducing the displacement of endotoxins in enterocytes, increasing the production of fat acids (Gullón et al., 2011, Song et al., 2014).

Many commercial prebiotics found today that are available for use in fish farming are composed of oligosaccharides of galactose, fructose or mannose, which represents a small range of choices of a better ingredient, knowing that the results are influenced, in levels of addition, time of use, diet composition, characteristics of animals and production environment (Torrecilas et al., 2007; Soleimani et al., 2012; Ganguly et al., 2013).

Brazil is responsible for 50% of the world production of orange juice (Neves et al., 2010), generating a large amount of waste (each 100kg of oranges used in juice production results in about 50kg of rind), which has been mostly used in ruminant feeding (Alexandrino et al., 2007). Therefore, citrus waste obtained after juice extraction has a great potential for commercial production of pectic compounds, mainly galacturonic acid (Mohnem, 2008).

Pectin polysaccharides are complex carbohydrates found in the primary and secondary cell walls in plants, with varying amounts of neutral sugars, xylose, rhamnose, arabinose, galactose and glucose (Round et al., 2010; Wikiera et al. al., 2015; Zhang et al., 2015). It has the technological properties (stabilizer, thickener and emulsifier) and physiological properties such as interference in satiety, weight control, lipid and glycemic profile (Gunness and Gidley, 2010; Wang et al., 2014; Espinal-Ruiz et al., 2014).

Since pectin polysaccharides have high viscosity in solution, it can result in difficulties during its incorporation in great quantity in formulations, causing modifications in technological and sensorial characteristics of the food. Therefore, pectin hydrolysis appears as an alternative to reduce viscosity problems, which can be used to extend its applicability. A few studies have shown changes in citrus pectin physicochemical properties due to hydrolysis, which can affect the ideal amounts of pectin to be incorporated into fish feed. Therefore, the objective of this study was to evaluate the effects of a new prebiotic obtained from citrus rind on Nile tilapia (*Oreochromis niloticus*) diet.

Material and Methods

Biological assay

The study was accomplished at the Fishery Laboratory at the Federal Univerity of Santa Maria. The study was analyzed and approved by the Ethics and Animal Welfare Committee of the Federal University of Santa Maria (protocol nº1359100517). The water recirculation system used in the experiment was composed of a motor pump (1/2 hp), 20 experimental units with 160 L of useful volume and with individual input and output of water, with mechanical and biological filtration. Six hundred Nile tilapia (*Oreochromis niloticus*) fingerlings with a mean weight of 5.56 ± 1.31 g (30 fish / tank) were distributed in the experimental units, where the experimental diets were adapted for seven days after the initial biometry. In the period of 45 experimental days, the fish were fed three times a day (9:00 a.m., 1:30 p.m. and 5:00 p.m.) to apparent satiation.

Diets

All diets were formulated according to nutritional requirements for Nile tilapia fingerlings (Furuya, 2010). The diets were maintained isonutritivas. The treatments were replacements of cellulose in the diet by 0, 1.5, 2.5, 3.5 and 4.5 g kg^{-1} of orange hydrolyzed pectin.

The ingredients of the diets were weighed in different containers, mixed and pelletized according to each treatment. The pellets were dried in an air circulating oven at 50°C for 24 hours, the size of the pellets was adjusted according to the development of the Nile tilapia fingerlings. The ingredients and composition of the experimental diets are shown in Table 1.

The hydrolyzed pectin of citrus rind used for incorporation in the experimental diets was obtained from the acid extraction method of Moura, F.A. (2015).

Experimental design

The experimental design was completely randomized with five treatments and four replicates, totaling 20 experimental units.

Water quality

Periodic analyzes were performed with a colorimetric kit to determine the physical and chemical parameters that influence water quality. The dissolved oxygen (7.76 ± 0.68 mg L^{-1}), pH (7.14 ± 0.30 mg $CaCO_3$ L^{-1}), hardness (34.7 ± 25.12 mg $CaCO_3$ L^{-1}), (0.32±0.22 mg L^{-1}) and nitrite (0.12±0.24 mg L^{-1}) the temperature was measured by a mercury bulb thermometer (24.3±1.42°C in the morning and 25.2±1.36°C in the afternoon). The water samples were always collected at the entrance of the biofilter.

Collection of data and parameters evaluated

Before starting the biological assay, 20 fishes were collected for analysis. After 45 experimental days, the data were collected in order to evaluate the performance of the animals. Biometry was performed after the fish passed a fasting period of 12 h and sedated with benzocaine at the concentration of 100 mg L^{-1}. Eight fish per treatment were euthanized by benzocaine overdose (250mg L^{-1}) for whole fish analysis. Eight fish per treatment were euthanized by benzocaine overdose (250mg L^{-1}) for the calculation of digestive indices and carcass yield, from which was also collected the liver for metabolic analysis and initial portion of the intestine (10cm) for analysis of enzymes and total intestine protein.

Zootechnical parameters: After collecting fish data and analyzing diet consumption, the following values were obtained:

- Biomass: BT = (weight of all animals within the replicate);
- Apparent feed conversion: CAA = ((total consumption) / (final biomass - initial biomass));
- Relative weight gain (%): GPR = ((final weight - initial weight) / (initial weight) * 100;
- Final weight: PF (g),
- Total length: CT (cm);

- Specific growth rate (% / day): TCE = (ln (final weight) - ln (initial weight)) / days) * 100;
- Carcass yield (%): RC = (weight of the digestive tract / weight of whole fish) * 100);
- Hepatosomatic index (%): IHS = (liver weight / weight of whole fish) * 100;
- Visceral fat index (%): IGV = (weight of visceral fat / weight of whole fish) * 100.

Hematologic parameters: Blood of two animals was collected per replicate using syringes prepared with sodium heparin, by means of puncture in the caudal vein. Using plasma obtained shortly after centrifugation of the blood (3000rpm / 10min), the levels of albumin, cholesterol, glucose, total proteins and triglycerides were determined using a biochemical analyzer with commercial colorimetric kits and a technique proposed by Labtest.

Bromatological parameters: Samples collected from whole fish were analyzed for moisture, ash and crude protein, following the methodologies recommended by AOAC (1995). Fat determination was by the method of Bligh and Dyer (1995).

Metabolic parameters: In the liver, glycogen and glucose levels were quantified (Park and Johnson 1949).

Statistical analysis

The data were submitted to normality test of the residues by Shapiro Wilk. All variables presented normal distribution and were submitted to linear regression analysis at 5% level of significance.

Results and Discussion

The levels of hydrolyzed pectin tested showed similar responses in all parameters analyzed in Nile tilapia fingerlings.

The results of glucose (GLI), albumin (ALB), cholesterol (COL), total protein (PRT) and triglycerides (TGI) did not influence the results regarding the different levels of citrus pulp hydrolysed pectin tested in the diets for tilapia of the Nile (Table 2). Hematological parameters are considered to be very valuable as reference for fish health evaluation (Houston 1997; Asadi et al., 2006), having variable factors that may compromise its maintenance, such as species, size, age, physiological status, environmental conditions and diet (Lim *et al.* 2000; Irianto e Austin, 2002; Osuigwe *et al.* 2005).

Albumin is the fraction of the total proteins found in the blood system, responsible for the loading and storage of numerous varieties of ions and organic molecules (Bruschi et al., 2013). Sado (et al., 2008) reported that 0.2-0.1% MOS in the diet had no effect on total plasma protein in Nile tilapia. According to Hoseinifar et al. (2010), in relation to oligofructose supplementation, there was a reduction in cholesterol levels, comparing to the control diet, with no change in glucose levels. According to these results, some other experimental practices report that dietary inulin did not change cholesterol levels in rainbow trout (Akrami et al., 2007).

The results of final weight (PF), relative weight gain (GPR), specific growth rate (TCE), feed conversion (CAA), somatic digestive index (SDI), somatic hepato index (IHS) and visceral fat index (IGV), showed no influence on the different levels of prebiotics substituted in the diet (Table 3). And, for the bromatological parameters, no significant effect was also found between the different levels of hydrolyzed pectin of citrus pulp added in the diets. (Table 4), and digestive indices (IDS), hepatosomatic (IHS) and visceral fat (IGV), did not receive interference from the diet with inclusion of hydrolyzed pectin, maintaining levels similar to the control diet (Table 5).

The biotechnical and bromatological results were satisfactory among the different levels of citrus pulp hydrolyzed pectin in the diet and the control diet, the Nile tilapia fingerlings had an excellent performance during the experimental period comparing to Mombach et al., (2015) who used non-hydrolyzed orange pectin in diets for Nile tilapia fingerlings where they found an apparent feed conversion and specific growth rate similar to that of this study (Table 3 and 4).

The prebiotic based on non-hydrolyzed pectin has a predominance of molecules in the composition of α-D- (1,4) - galacturonic acid, with the hydrolysis pectin altered its degree of fermentability in relation to its natural state and affected the development of the treatment (Mombach et al, 2015). It is important to note that Leenhouwers et al. (2006) stated that the gastrointestinal structure of the fish is altered according to the diet provided, increasing the area of contact with the food and increasing the digestibility and absorption of the nutrients without influencing the somatic digestive index IDS), somatic hepatic index (IHS), and visceral fat index (IGV), although these results showed higher values for IDS, lower for IHS and IGV than those found by Mombach et al. there was a probable increase in thickness of the gastrointestinal tract.

No influence was found for glycogen and glucose in the liver at different levels of citrus pulp hydrolysed pectin tested (Table 6). Knowing that energy generation is among the main metabolic responses given to prebiotic intake due to fermentation, which in turn is responsible for the formation of short chain fatty acids (AGCC), the energy value formed is variable according to the structure and degree of fiber fermentability. In the present study, treatments maintained stable levels of glycogen and glucose found in the liver of animals. Comparing with the results found by Mombach et al. (2015), it was observed that those found in this research were similar for glycogen, since glucose values show a different response, lower values.

It is concluded from the results presented that the hydrolyzed pectin of citrus pulp has no effect on the response of its use to the growth of Nile tilapia fingerlings.

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Table 1 - Ingredients and composition close to the diets supplied to the Nile tilapia.

Diets	PHL				
Ingredients (g kg^{-1})	0	1,5	2,5	3,5	4,5
Fish meal[1]	310	310	310	310	310
Maize starch	300	300	300	300	300
Cellulose	60	58,5	57,5	56,5	55,5
CPS 60%[2]	200	200	200	200	200
Soy Oil	30	30	30	30	30
Vitamin and mineral blend[3]	30	30	30	30	30
Iodized sodium chloride	5	5	5	5	5
Inert	64,8	64,8	64,8	64,8	64,8
BHT[4]	0,2	0,2	0,2	0,2	0,2
PHL	0	1,5	2,5	3,5	4,5
Composition of diets (g kg^{-1})					
Moisture[5]	48,80	50,00	48,60	51,00	50,00
Crude protein[5]	31,24	30,67	30,63	30,89	30,91
ED (MJ kg^{-1})[6]	12,70	12,18	12,64	12,16	12,66
Fat[5]	72,10	66,00	72,00	64,00	67,50
Mineral matter[5]	177,20	180,30	178,00	178,80	182,00
Ca[7]	18,32	18,32	18,32	18,32	18,32
P[7]	9,63	09,63	09,63	09,63	09,63
Insoluble fiber[5]	145,60	145,80	136,00	143,10	139,10
Soluble fiber[5]	30,20	30,70	39,10	38,20	27,00

[1]Fish meal;
[2]Soy protein concentrate 60%CP;
[3]Vitamin and mineral blend - composition / kg of product: Folic Acid: 299.88 mg; B.C. Ascorbic acid: 15,000.12 mg; B.C. Pantothenic: 3,000.10 mg; Biotin: 0.06 mg; Niacin (B3): 9,000.32 mg; Choline (B4): 103,500.00 mg; Vitamin A: 1,000,000.00 IU; Vitamin B1: 1,500.38 mg; Vitamin B2: 1,500.00 mg; Vitamin B6: 1,500.38 mg; Vitamin D3: 240,000.00 IU; Vitamin E: 10,000 mg; Vitamin K3: 400.00 mg; Inositol: 9.999, 92 mg; Iron: 6.416, 80 mg; Manganese: 8,000.40 mg; 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; Chloro: 2.30%; Sulfur: 0.01%;
[4]Antioxidant Butyl Hydroxy Toluene;
[5]Composição analisada (Fisheries laboratory/UFSM);
[6]ED = calculated digestible energy: [(Crude protein * 5.65 * 0.85) + (Fat * 9.4 * 0.9) + (Carbohydrate * 4.15 * 0.7)] (adapted from Meyer et al. ., 2004);
[7]Values calculated based on the composition of the ingredients.

Table 2 - Zootechnical parameters of Nile tilapia fingerlings fed with hydrolyzed pectin from citrus pulp.

Level (PHL)	0	1,5	2,5	3,5	4,5	
Variables/factor[2]						
FW (g)	43,04±0,99	40,39±0,85	40,61±0,49	41,35±1,25	41,18±1,81	NS
RWG (%)	681,90±22,06	628,60±27,73	619,80±10,13	649,30±21,47	634,70±15,61	NS
SGR (% dia^{-1})	4,57±0,06	4,41±0,08	4,38±0,03	4,47±0,06	4,43±0,04	NS
FC	1,08±0,05	1,12±0,03	1,10±0,02	1,10±0,03	1,09±0,07	NS

[1]Values expressed as mean±standard deviation. [2]Variables - FW: final weight; RWG: relative weight gain; SGR: specific growth rate; FC: feed conversion. NS: not significant ($p > 0.05$).

Table 3 - Metabolic parameters in the plasma and liver of Nile tilapia fingerlings fed diets containing hydrolyzed pectin of citrus pulp.

Level (PHL)	0	1,5	2,5	3,5	4,5	
Plasma[2]						
GLU (mg dL^{-1})	63,43±11,67	54,25±19,17	54,25±19,17	54,25±19,17	59,87±11,7	NS
ALB (g dL^{-1})	0,56±0,24	0,82±0,5	1,05±0,61	1,00±0,64	0,8±0,44	NS
COL (mg dL^{-1})	103±16,43	106,12±14,33	108,75±12,85	103,37±19,62	101±12,28	NS
PRT (g dL^{-1})	2,45±0,15	2,47±0,3	2,92±1,17	2,64±0,26	2,61±0,48	NS
TGI (mg dL^{-1})	174,71±87,52	133,62±61,06	118,25±28,67	173,62±100,12	148,62±53,71	NS
Fígado						
Glicogênio (μmol)	5,82±2,04	4,46±2,00	6,75±0,84	5,17±2,39	6,56±1,52	NS
Glicose (μmol)	29,32±4,36	25,13±7,22	35,06±4,93	30,88±7,76	30,81±6,49	NS

[1]Values expressed as mean±standard deviation. [2]Variables - GLU: blood glucose; ALB: albumin; COL: cholesterol; PRT: total proteins; TGI: triglycerides; NS: not significant ($p > 0.05$).

Table 4 - Somatic indices and body composition of Nile tilapia fingerlings fed with hydrolyzed pectin from citrus pulp.

Level (PHL)	0	1,5	2,5	3,5	4,5	
Variables/factor[2]						
DSI (%)	6,25±1,78	6,18±1,07	6,50±0,83	6,41±1,29	6,21±1,82	NS
HSI (%)	1,55±0,38	1,38±0,22	1,53±0,15	1,65±0,35	1,40±0,30	NS
VGI (%)	0,41±0,36	0,35±0,28	0,40±0,24	0,30±0,22	0,43±0,34	NS
DM (%)	23,67±0,91	23,86±1,36	23,29±1,47	24,15±1,87	24,43±1,21	NS
CP (%)	13,23±1,51	13,79±0,81	13,52±0,77	13,39±1,55	13,39±0,62	NS
Fat (%)	3,64±0,74	3,93±0,97	3,71±0,55	3,87±1,97	4,03±0,40	NS
MM (%)	4,28±0,47	4,63±1,43	3,90±1,14	4,00±0,96	4,24±1,04	NS

[1]Values expressed as mean±standard deviation. [2]Variables – DSI: digestive somatic index; HSI: hepatic somatic index; VFI: viceregal fat index; DM: dry matter; CP: crude protein; Fat: total fat; MM: mineral matter. NS: not significant ($p > 0.05$).

4 CONCLUSÃO GERAL

Conclui-se que os níveis testados de pectina hidrolisada de polpa cítrica aplicado em dietas para tilápia do Nilo mostraram-se similares em todos os parametros analisados nas condições experimentais descritas.

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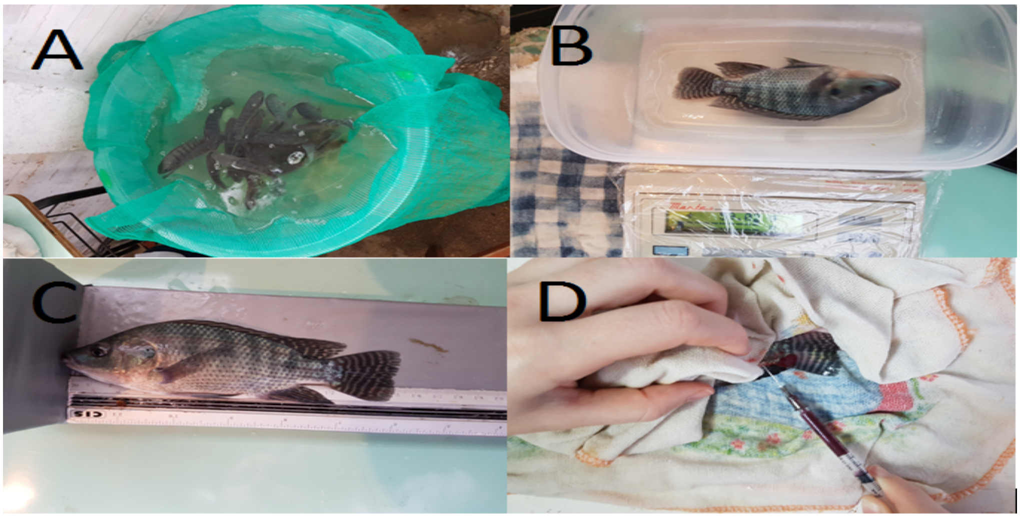
ANEXOS

Anexo 1 – Sistema de recirculação de água onde foi conduzido o ensaio biológico

Anexo 2 – Alevinos de tilápia do Nilo durante a biometria final (A: Sedação; B: Pesagem; C: Medição; D: Coleta de Sangue



Anexo 3 - Analises de farinha de peixe e concentrado proteico de soja

	Farinha de peixe	CPS 60%
Umidade (%)	5,59	6,29
Matéria seca (%)	94,41	93,71
Proteína Bruta (%)	58,20	60,79
Matéria mineral (%)	24,50	6,17
Lipídeos (%)	10,26	1,51
FDN (%)	0	15,2047
Amido (%)	0	0
Energia digestível calculada (k/cal)	3700	3120
Cálcio (%)	5,64	0,42
Fósforo (%)	2,54	0,88

Anexo 4 - Normas para a submissão de trabalhos na Revista Tropical Animal Health and Production

Instructions for Authors
AUTHORSHIP POLICY
Authorship should incorporate and should be restricted to those who have contributed substantially to the work in one or more of the following categories:
• Conceived of or designed study
• Performed research
• Analyzed data
• Contributed new methods or models
• Wrote the paper
It is the responsibility of the corresponding authors that the names, addresses and affiliations of all authors are correct and in the right order, that institutional approvals have been obtained and that all authors have seen and agreed to a submission. This includes single authorship papers where appropriate. If at all in doubt please double check with eg. Supervisors, line managers department heads etc.
TYPES OF ARTICLES
Manuscripts should be presented preferably in Times New Roman font, double spaced, using A4 paper size. Please use the automatic page and line numbering function to number the pages
and lines in your document and number the lines in a single continuous sequence.
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Regular Articles: Articles should be as concise as possible and should not normally exceed approximately 4000 words or about 8 pages of the journal including illustrations and tables. Articles should be structured into the following sections;
(a) Abstract of 150-250 words giving a synopsis of the findings presented and the conclusions reached. The Abstract should be presented as a single continuous paragraph without subdivisions.
(b) Introduction stating purpose of the work
(c) Materials and Methods
(d) Results
(e) Discussion (conclusions should be incorporated in the discussion!)
(f) Acknowledgements
(g) Statement of Animal Rights
(h) Conflict of Interest Statement
(i) References
Short Communications and Technical Notes: Short Communications and Technical Notes should
not normally exceed approximately 2000 words or about 4 pages of the journal, including illustrations, tables and references. An abstract of 150-250 words should be included and a minimum number of sub-headings may be included if it adds clarity to the article.
Short Communications report original scientific data.
Technical Notes describe innovative methodologies.
Reviews: Review articles will be welcomed. However, authors considering the submission of review articles are advised to consult the Editor-in-Chief in advance.
Correspondence: Letters on topics relevant to the aims of the Journal will be considered for

publication by the Editor-in-Chief who may modify them.
It is the authors responsibility to ensure that submitted manuscripts comply with journal format
as indicated in the current instructions to authors and free sample articles on the springer.com journal homepage.
ETHICAL STANDARDS
Manuscripts submitted for publication must contain a statement to the effect that all human and
animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.
It should also be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted.
These statements should be added in a separate section before the reference list. If these statements are not applicable, authors should state: The manuscript does not contain clinical studies or patient data.
The editors reserve the right to reject manuscripts that do not comply with the above-mentioned
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