**UNIVERSIDADE FEDERAL DE MINAS GERAIS**
**ESCOLA DE VETERINÁRIA**
**PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA**

# FONTES DE CARBOIDRATOS NA ALIMENTAÇÃO DE JUVENIS DE PACU (*Piaractus mesopotamicus*)

**TESE DE DOUTORADO**

**Marco Aurélio Lopes Della Flora**

**Belo Horizonte, MG, Brasil**
**2017**

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# FONTES DE CARBOIDRATOS NA ALIMENTAÇÃO DE JUVENIS DE PACU (*Piaractus mesopotamicus*)

Tese apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para obtenção do grau de Doutor em Zootecnia.

Área de Concentração: Nutrição e Alimentação Animal

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**Dedico esta tese**

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*(Albert Einstein)*

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*Não fosse por elas, eu não teria saído do lugar.*
*As facilidades nos impedem de caminhar.*
*Mesmo as críticas nos auxiliam muito".*
*(Chico Xavier)*

*"Sucesso é a soma de pequenos esforços, repetidos a todo tempo".*

*(Robert Collier)*

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# LISTA DE ABREVIATURAS

## Capítulo 1

ATP, adenosina trifosfato

Ha, hectare

NAD, nicotinamida-adenina-dinucleotídeo

Ton, tonelada

## Capítulo 2

ADC, apparent digestibility coefficient

DM, dry matter

FC, feed conversion

FW, final weight

HDL, high density lipoprotein

HSI, hepatosomatic index

LDL, low density lipoprotein

LRC, lipid retention coefficient

SDI, somatic digestive index

SGR, specific growth rate

VFI, visceral fat index

VLDL, very low density lipoprotein

WG, weight gain

## Capítulo 3

ALAT, alanine aminotransferase

ASAT, aspartate aminotransferase

CF, condition fator

CY, carcass yield

DWG, daily weight gain

FI, feed intake

FW, final weight

GDH, glutamate dehydrogenase

IQ, intestinal quotient

PRC, protein retention coefficient

RWG, relative weight gain

TAME, α-ρ-toluenesulphonyl-L-argininemethyl ester hydrochloride

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# RESUMO GERAL

O objetivo do trabalho foi avaliar o efeito de dietas com diferentes fontes de carboidratos sobre o crescimento e metabolismo de juvenis de pacu (*Piaractus mesopotamicus*). Para tal, realizou-se três experimentos a partir de três dietas contendo milho, quirera de arroz ou sorgo. No primeiro estudo, 375 juvenis, com 12,13±0,09 g foram distribuídos em 15 tanques (280L), alimentados com dieta extrusada, três vezes ao dia, até saciedade, por 60 dias. O delineamento foi o inteiramente casualizado com três tratamentos e cinco repetições. Os resultados obtidos foram submetidos a ANOVA e as médias comparadas pelo teste de Duncan (5%). No segundo estudo, animais foram submetidos à avaliação da glicemia pós-prandial, portanto, permaneceram 12 horas em jejum. A colheita do sangue ocorreu no tempo zero (antes da realimentação) e 1, 3, 6, 9 e 12 horas pós-alimentação. No terceiro estudo, 100 animais com 56,32±0,85g foram utilizados para determinar a digestibilidade das dietas experimentais em quatro tanques cilíndricos de fundo cônico (190 L), para decantação das fezes. Realizou-se a coleta de uma dieta por vez, com quatro repetições. Não houve mortalidade; o peso final, ganho de peso, ganho médio diário, ganho em peso relativo, fator de condição, consumo alimentar, taxa de crescimento específico, comprimento total, conversão alimentar, triglicerídeos, lipoproteína de densidade muito baixa, alanina aminotransferase, coeficiente de retenção proteica e digestibilidade foram semelhantes entre as dietas ($P > 0,05$). Lipoproteínas de alta densidade, proteínas totais, globulinas, proteína hepática, amônia hepática, glicose hepática, glicogênio hepático, proteína corporal, rendimento de carcaça foram mais elevados com o uso de sorgo ($P < 0,05$). A lipoproteína de baixa densidade foi maior quando do fornecimento de milho. A glicose plasmática foi mais elevada nos tratamentos com milho e quirera de arroz. O uso de sorgo levou a menores valores de índices hepatossomáticos, gordura visceral, digestivossomático, albumina, colesterol e quociente intestinal. As enzimas amilase, lipase, tripsina e maltase reduziram suas atividades nos animas alimentados com a dieta com sorgo. A dieta com quirera de arroz resultou em maiores teores de lipídios, coeficiente de retenção lipídica, aminoácidos livre no fígado, amônia hepática e glicose hepática. Para matéria seca, proteína e cinzas não houve diferença. Na curva glicêmica, verificou-se picos de glicemia com 3,5 e 4 horas após ingestão das dietas contendo sorgo e arroz, respectivamente, enquanto para o milho o maior valor foi após 12 horas da alimentação. A digestibilidade da matéria seca, proteína e lipídios não diferiu entre os tratamentos. Por conseguinte, os estudos demonstraram que milho, quirera de arroz ou sorgo na dieta de juvenis de pacu são adequadamente utilizadas pela espécie, sem qualquer efeito adverso sobre o crescimento e sobrevivência. O milho denota

um perfil metabólico de melhor aproveitamento dos carboidratos da dieta. A quirera de arroz e sorgo geram aumento no catabolismo de aminoácidos para manter os processos de produção de energia. Há aumento de produção de compostos nitrogenados de excreção tanto nos tratamentos com quirera quanto sorgo. O sorgo apresentou maior rendimento de carcaça e teor de proteína no peixe inteiro.

Palavras-chave: Amilopectina, amilose, carboidrato, milho, quirera de arroz, sorgo

# ABSTRACT

Objective of this work was to evaluate the effect of diets with different carbohydrate sources on the growth and metabolism of juvenile pacu (*Piaractus mesopotamicus*). Thus, three experiments were carried out, from three diets containing corn, broken rice or sorghum. In the first study, 375 juveniles, with 12.13 ± 0.09 g, were distributed in 15 tanks (280 L), fed an extruded diet three times a day, until satiety, for 60 days. The design was completely randomized with three treatments and five replications. Results were submitted to ANOVA and the means were compared by the Duncan test (5%). In the second study, animals were submitted to postprandial glycemic evaluation. Therefore, they remained fast for 12 hours. Blood collection occurred at time zero (before refeeding) and 1, 3, 6, 9 and 12 hours post-feeding. In the third study, 100 animals with 56.32 ± 0.85 g were used to determine the digestibility of experimental diets in four cylindrical tanks with a conical bottom (190 L) for faeces decantation, performing a collection of one diet at a time, with four replicates. There was no mortality; the final weight, weight gain, average daily gain, relative weight gain, condition factor, food intake, specific growth rate, total length, feed conversion, triglycerides and very low density lipoprotein, alanine aminotransferase, protein retention coefficient and digestibility were similar among diets ($P > 0.05$). High-density lipoproteins, total proteins, globulins, hepatic protein, hepatic ammonia, hepatic glucose, hepatic glycogen, body protein, carcass yield were higher with sorghum ($P < 0.05$). Low density lipoprotein was higher when fed corn. Plasma glucose was higher in treatments with corn and broken rice. The use of sorghum led to lower values of hepatosomatic index, visceral index, digestive index, albumin, cholesterol and intestinal quotient. The enzymes amylase, lipase, trypsin and maltase reduced their activities in the animas fed with the sorghum diet. The broken rice diet resulted in higher lipid levels, lipid retention coefficient, free amino acids in the liver, hepatic ammonia, and hepatic glucose. For dry matter, protein and ash in body composition, there was no difference. In the glycemic curve, glycemia peaks were observed with 3.5 and 4 hours after ingestion of the diets containing sorghum and rice, respectively, while for corn the highest value was after 12 hours of feeding. The digestibility of dry matter, protein and lipids did not differ between treatments. In conclusion, this studies have demonstrated that corn, broken rice or sorghum in the diet of pacu juveniles are suitably used by the species, without any adverse effect on growth and survival. Corn denotes a metabolic profile of better utilization of dietary carbohydrates. Broken rice and sorghum generate an increase in amino acid catabolism to maintain energy production processes. There is an increase in the production of nitrogen compounds of excretion in both

broken rice and sorghum treatments. Sorghum showed higher carcass yield and protein content in whole fish.

Keywords: Amylopectin; amylose; broken rice; carbohydrate; corn; sorghum

# 1. INTRODUÇÃO GERAL

O carboidrato é uma fonte de energia de baixo custo amplamente utilizado na alimentação animal. Todavia, os peixes, em especial os de habito alimentar carnívoro, não conseguem aproveitar esse nutriente com a mesma eficiência que os vertebrados terrestres de mesmo nível trófico. (SHIAU; LEI, 1999). Por exemplo, a truta (*Oncorhynchus mykiss*) e *European sea bass* (*Dicentrarchus labrax*), ambos carnívoros, possuem baixa eficiência na utilização de carboidratos, enquanto peixes onívoros, como a tilápia (*Oreochromis niloticus*), assimilam bem dietas com 41 a 56% de carboidratos (HEMRE et al., 2002), podendo, através da extrusão, melhorar ainda mais sua utilização (SVIHUS et al., 2005).

Embora nenhuma exigência em carboidratos tenha sido definida para peixes, sua ausência na dieta leva ao catabolismo de proteínas para gliconeogênese (LI et al., 2013; NRC, 2011; WILSON, 1994). Dessa forma, seu uso adequado pode ser estratégico, pois permite reduzir os custos de produção, por se tratar de uma fonte de energia de baixo custo e por apresentar "efeito poupador de proteína", reduzindo a emissão de compostos nitrogenados na água (STONE et al. 2003; WU et al. 2007). Entretanto, nem todas fontes de carboidratos são bem aproveitadas pelos peixes, seja pelo nível de fibra alimentar que as compõe ou pela presença de fatores antinutricionais.

O pacu (*Piaractus mesopotamicus*), espécie onívora, possui carne saborosa, resistência a patógenos, baixa exigência quanto à qualidade da água, baixa exigência proteica, resistência às baixas temperaturas, adaptabilidade ao cultivo em viveiros, métodos de reprodução estabelecidos; além de apreciada na pesca esportiva (Abimorad

& Carneiro, 2004; Jomori et al., 2008), despontando como uma das principais espécies produzidas no país.

O conhecimento sobre a utilização dos carboidratos pelo pacu ainda é bastante limitada e, até o presente momento, ainda não existe informações sobre o metabolismo de pacus quando alimentados com dietas contendo os principais grãos produzidos no país (milho, arroz e sorgo), sendo, sem dúvida, de relevada importância o aprofundamento acerca desse tema, já que essa espécie se caracteriza por apresentar elevada deposição de gordura na carcaça.

# 2. OBJETIVOS

*2.1 Objetivo geral*

Avaliar o crescimento, resposta metabólica, atividade enzimática, utilização de nutrientes e composição corporal de pacus alimentados com milho, quirera de arroz ou sorgo na dieta.

*2.2 Objetivos específicos*

- Avaliar a influência de fontes de carboidratos no desempenho zootécnico dos pacus;
- Analisar a digestibilidade das dietas e utilização dos nutrientes;
- Aferir a atividade das enzimas amilase, lipase, maltase e tripsina;
- Detectar se há influência das fontes de carboidratos sobre as variáveis sanguíneas do metabolismo;
- Examinar o efeito das fontes de carboidratos no rendimento de carcaça e composição química corporal.

# CAPÍTULO 1

## 3. REVISÃO BIBLIOGRÁFICA

### *3.1 Pacu (Piaractus mesopotamicus)*

O pacu (figura 1), *Piaractus mesopotamicus*, pertence à família Characidae, subfamília Myleinae, sendo originário da Bacia do Prata. Conhecido em algumas regiões como pacu-caranha, caranha ou pacu-guaçu. Apresenta o corpo orbicular, de cor acinzentada, mais intensa na região dorsal e mais amarelada na região ventral, sendo que os indivíduos mais jovens possuem reflexos violáceos na sua coloração (PROENÇA; BITTENCOURT, 1994).

É uma espécie onívora, que se alimenta principalmente de folhas, caules, flores, frutos e sementes. Em caso de necessidade e oportunidade, consome insetos, aracnídeos, moluscos e peixes. Sua alimentação sofre flutuação de acordo com a disponibilidade de alimento, em consequência de variações ambientais e da migração reprodutiva (URBINATI; GONÇALVES, 2005).

No ciclo produtivo de 2016, foi a 6ª espécie de peixes mais produzida no país (13.065,1 toneladas) (IBGE, 2017). Apresenta alto potencial zootécnico, com crescimento acelerado, rusticidade, fecundidade elevada, fácil adaptação à alimentação artificial, carne saborosa, resistência a patógenos, baixa exigência quanto à qualidade da água, baixa exigência proteica, resistência às baixas temperaturas, adaptabilidade aos sistemas de cultivo, apreciada na pesca esportiva (CASTAGNOLLI, 1992, ABIMORAD; CARNEIRO, 2004; JOMORI et al., 2008), com potencialidade de criação em regiões subtropicais.

Comercializado com peso médio de 1,3 kg, apresentam índices de produtividade, dependendo do sistema preconizado, entre 9,6 e 17,5 ton/ha/ano, em densidades variando de 1,0 a 1,5 peixe/$m^2$ (SCORVO-FILHO et al., 1998).

**Figura 1** Exemplar de *Piaractus mesopotamicus* (Fonte: meumundodaselva.blogspot.com.br)

*3.2 Quirera de arroz*

O arroz, bem como os farelos de arroz integral, parboilizado ou desengordurado e a quirera de arroz, seus subprodutos, apresentam aspectos nutricionais equivalentes ao milho, os quais podem ser empregados nas formulações de dietas para peixes. A quirera de arroz é oriunda da peneiração do grão em diâmetro de 1,6 milímetros, obtida a partir do polimento do arroz, após retirada da casca (BRASIL, 2009).

O Brasil é o 9º produtor mundial de arroz, e o maior não asiático. Sua área cultivada é de 1,9 milhão de hectares, e estimativa para safra 2017/2018 será de 11,75 milhões de toneladas com produtividade média de 5.989 kg/ha (CONAB, 2017). Em 27/10/2017 a saca de arroz irrigado custava R$35,44.

Seu conteúdo de proteínas é de 8,47%, lipídios 1,22%, amido 74,45% e energia 3821 kcal/kg (ROSTAGNO et al., 2011), e coeficiente de digestibilidade aparente da proteína de 72,8%, da energia de 76,9% para o pacu (GUIMARÃES et al., 2014).

*3.3 Sorgo*

Pertencente à família *Poaceae* e gênero *Sorghum,* o sorgo granífero, *Sorghum bicolor*, é de origem africana (FERNANDES, 2013; SCHEUERMANN, 1998), e atualmente, o 4º cereal mais produzido no país, depois do milho, trigo e arroz (CONAB, 2017).

O sorgo não apresenta proteção para suas sementes, como a palha (milho) ou as glumas (trigo e cevada), logo, sua defesa é de natureza química e se dá a partir da produção de compostos fenólicos, que os protege contra pássaros, patógenos, entre outros. Entretanto, esses compostos podem ocorrer ou não, entre esses, destaca-se o tanino condensado, substância adstringente que torna o grão menos digestível (MAGALHÃES et al., 2000).

O sorgo é classificado como granífero, forrageiro ou vassoura (RODRIGUES; SANTOS, 2011). O granífero apresenta a maior expressão econômica. Sendo utilizado como alimento humano em países da África, Sul da Ásia e América Central e na alimentação animal nos Estados Unidos, Austrália e América do Sul (RODRIGUES; SANTOS, 2011; FAO, 2014).

Sua área cultivada é de 629 mil ha, e sua estimativa para safra 2017/2018 será de 1,8 milhão de tonelada com produtividade média de 2.859 kg/ha (CONAB, 2017). Em 27/10/2017 a saca foi cotada em R$ 17,00.

Sua composição de proteínas é de 9,23%, lipídios 3,00%, amido 60,79% e energia 3928 kcal/kg (ROSTAGNO et al., 2011). O coeficiente de digestibilidade aparente para o pacu é 92,93% para proteína e de 93,36% para energia (ABIMORAD; CARNEIRO, 2004).

*3.4 Milho*

Estatísticas publicadas recentemente pela Companhia Nacional de Abastecimento (CONAB, 2017) sobre a safra 2017/2018, estima-se ao menos uma área plantada de 17,04 milhões hectares (entre as duas safras) e produção média de 92,19 milhões de tonelada.

O *United States Department of Agriculture* (USDA) estima que a produção global do cereal deva ser de 1,0 bilhão de tonelada, volume recorde, superando a safra 2015/16 que foi de 961,1 milhões de toneladas (FIESP, 2016). Em 27/10/2017 a saca foi cotada em média por R$23,00.

Os teores de proteína, lipídios, amido e energia são 8,26%, 3,61%, 62,48%, 3925 kcal/kg (ROSTAGNO et al., 2011), e coeficiente de digestibilidade aparente da proteína de 84,38%, da energia de 86,69% para o pacu (ABIMORAD; CARNEIRO, 2004).

*3.5 Estrutura dos carboidratos*

Carboidratos são compostos orgânicos formados por carbono, hidrogênio e oxigênio, fracionados em três grupos. O primeiro deles é o monossacarídeo, ou açúcares simples; podem conter três (triose), quatro (tetrose), cinco (pentose) e seis carbonos (hexose). Essa última engloba a frutose, a galactose e a glicose. Os oligossacarídeos contêm de dois a dez monossacarídeos e são resultantes da hidrólise parcial dos polissacarídeos. Os polissacarídeos são compostos por um grande número de monossacarídeos, como por exemplo o amido (LOVELL, 1998; NELSON; COX, 2011; WEBSTER; LIM, 2002).

O principal carboidrato digestível presente nos alimentos utilizados nas formulações de dietas para peixes é o amido (RAWLES; LOCHMANN, 2003), o qual é composto por duas cadeias moleculares distintas. A amilose (Figura 2) é uma cadeia

linear de 200 a 20.000 unidades de glicose, unidas entre si por ligações glicosídicas do tipo α-1,4, por outro lado, a amilopectina (figura 3) é ramificada, e além das ligações α 1,4, é constituída por cadeias curtas formadas por aproximadamente 30 unidades de glicose unidas à cadeia principal por ligações α 1,6 (KUAKPETOON; WANG, 2007). Esses autores esclarecem também que a organização dessas cadeias ainda não está bem elucidada.

A amilose é responsável pela propriedade gelificante do amido, enquanto que a amilopectina é responsável pela sua viscosidade (ISI, 2017). Segundo Rawles e Lochmann (2003), as diferenças estruturais que caracterizam os amidos podem estar associadas aos diferentes graus de digestão das fontes de carboidratos pelos animais.

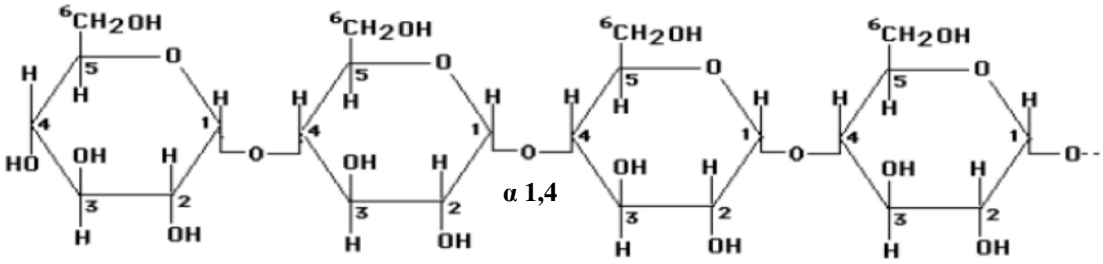


**Figura 2** Conformação de uma molécula de amilose (Fonte: FIGUEIREDO; GUERREIRO, 2017)

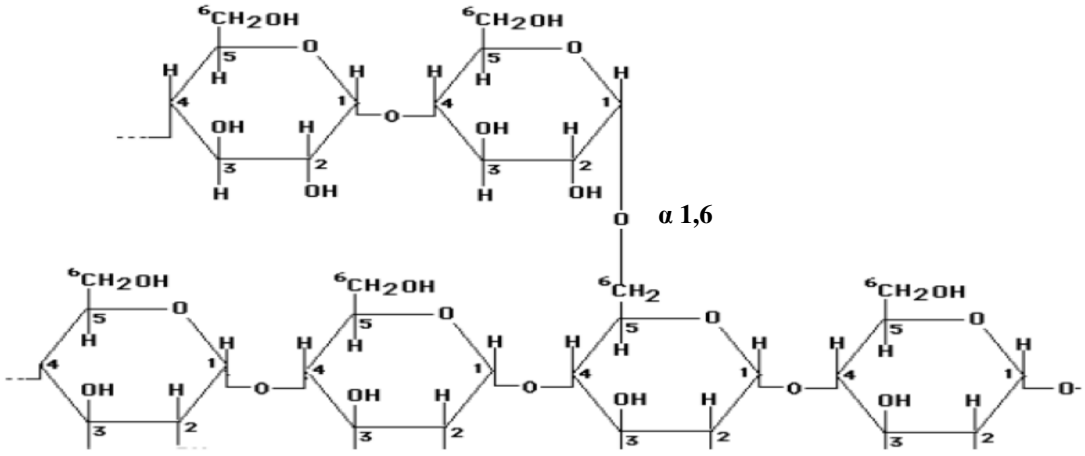

**Figura 3** Conformação de uma molécula de amilopectina (Fonte: FIGUEIREDO; GUERREIRO, 2017)

*3.6 Complexidade molecular*

A maioria dos peixes possui as enzimas que hidrolisam os polímeros de glicose formados a partir de ligações α-glicosídicas (α 1,4 e α 1,6). Contudo, em geral, faltam enzimas que clivam as ligações β-glicosídicas ou α-galactosídicas de polissacarídeos não amiláceos, como a celulose, hemicelulose, pectinas e gomas, tornando-os indisponíveis (KAUSHIK, 2001; SINHA et al., 2011).

A digestibilidade aparente e a absorção intestinal, para a maioria das espécies de peixes, diminuem à medida que se aumenta a complexidade molecular (glicose > dextrina > amido), porém, o uso de ingredientes puros não é viável economicamente (CUI et al., 2010; NRC, 2011; PIEPER; PFEFFER, 1980). A complexidade da fonte de carboidratos na dieta também afeta a dinâmica de absorção dos monossacarídeos e consequentemente dos níveis glicêmicos no plasma, exigindo do animal ajustes diferenciados para regular o metabolismo da glicose (ENES et al., 2010; REN et al., 2015; TAN et al., 2006). Essa variação nos ajustes metabólicos foi descrita por Enes e colaboradores (2010) em um estudo com *gilthead sea bream* (*Sparus aurata*). Verificou-se que açúcares simples aumentam a atividade de enzimas glicolíticas e reduzem a atividade de enzimas gliconeogênicas de forma mais marcante do que os carboidratos complexos.

O bagre do canal (*Ictalurus punctatus*) utiliza mono e dissacarídeos (glicose, maltose, frutose, sacarose) ineficientemente como fontes de energia, quando comparados às fontes mais complexas como dextrina e amido de milho regular (WILSON; POE, 1987). De maneira semelhante, a utilização de amido e da glicose foi estudada por Lin et al. (1997) para o híbrido de tilápia-do-Nilo (*Oreochromis niloticus x O. aureus*), esses

autores encontraram melhor utilização do amido de milho regular para crescimento, eficiência alimentar e taxa de eficiência proteica. Resultados similares foram encontrados por Lee et al. (2003) para o *Paralichthys olivaceus*, e por Lee e Lee (2004) para o *Platichthys stellatus*, nos quais o melhor desempenho com dietas contendo dextrina, quando comparada com outra fonte mais simples de carboidratos como glicose.

Por outro lado, esturjão branco (*Acipenser transmontanus*) alimentado com glicose, frutose, maltose, sacarose, lactose, dextrina, amido de milho cru ou celulose apresentou melhores médias de crescimento e retenção de energia nos animais alimentados com açúcares simples como maltose ou glicose (HUNG et al., 1989). Resultados semelhantes foram encontrados por Hung e Storebakken (1994), em truta arco-íris, *Oncorhynchus mykiss*, em que a utilização foi mais eficiente para maltose e glicose.

*3.7 Origem botânica*

Dependendo da fonte da planta, os constituintes do amido são altamente variáveis em relação a sua estrutura primária, forma do grânulo de amido (esférica, lenticular, poliédrica e irregular), tamanho (~1-100 μm de diâmetro), distribuição (unimodal ou bimodal), tipo (simples ou composto) e proporção amilose a amilopectina (SVIHUS et al., 2005). Essas características podem influenciar os valores nutricionais das fontes de amido aplicadas à alimentação dos peixes (GLENCROSS et al., 2012).

O tamanho do grânulo de amido e a estrutura de ramificação determinam a área de superfície disponível e os locais de clivagem para a ação das enzimas digestivas (DONA et al., 2010). Por exemplo, o tamanho do grão de amido do trigo é de 22 μm, do milho é 35 μm e da batata é entre 40 e 100 μm e seus respectivos valores de digestibilidade para truta arco-íris foram de 58, 34 e 5% (BERGOT, 1993). Esse autor avaliou também,

na mesma espécie, a digestibilidade do amido de milho ceroso (99:1), do nativo (75:25) e do com alta amilose (30:70), verificou valores de 56, 34 e 24 %, respectivamente, com diminuição das proporções amilopectina:amilose.

Outros aspectos importantes também devem ser considerados com relação à origem botânica, como por exemplo, a presença de inibidores da α-amilase, como taninos, polissacarídeos não amilácos  fitatos em alguns cereais, como trigo, centeio, triticale e sorgo (NRC, 2011) e o impacto das fontes de amido sobre a qualidade física do "pellet" na confecção da ração (AH□HEN et al., 2014; SÖRENSEN et al., 2010).

*3.8 Digestão e absorção do amido*

A amilose e amilopectina são hidrolisadas pela α-amilase a oligossacáridos (dextrinas, maltotriose e maltose). Esses resíduos são ainda hidrolisados por outras enzimas na borda em escova (dissacaridases ou glicosidases) em monossacáridos, os quais são transportados através das vilosidades (KROGDAHL et al., 2005; NRC, 2011).

O transporte transcelular de glicose do lúmen intestinal para a corrente sanguínea ocorre através de transportadores específicos nas membranas dos enterócitos. O transporte pode ser ativo, quando dependente de sódio (SGLT1), ou facilitado, independente de sódio (GLUT2) na membrana basolateral (BAKKE et al., 2011; COLLIE; FERRARIS, 1995).

Os peixes são capazes de ativar o transporte de nutrientes ao longo de todo o comprimento do intestino, no entanto, regiões proximais geralmente contribuem mais do que as regiões distais (BAKKE-MCKELLEP et al., 2000; FERRARIS; AHEARN, 1984; KAMALAM et al., 2013) e os transportadores de glicose mostram características variáveis ao longo do trato intestinal (AHEARN et al., 1992). Da mesma forma, a

atividade de carboidrases também reduz das partes proximais para as mais distais do intestino (KROGDAHL et al., 2005; STONE, 2003).

Em peixes carnívoros, o trato gastrintestinal é geralmente curto, simples e menos volumoso, apto para o processamento de uma dieta altamente digestível, com alto teor de nutrientes, rica em proteínas e baixos níveis de carboidratos (BUDDINGTON et al., 1997). Para esses peixes, os principais entraves relatados para a digestão de amido e absorção de glicose são os baixos níveis de atividade de α-amilase e dissacaridases (HIDALGO et al., 1999; KUZ'MINA et al., 2008; UGOLE; KUZ'MINA, 1994), inibição de enzimas digestivas pelos altos níveis de carboidratos na dieta (BUDDINGTON; HILTON, 1987; KROGDAHL et al., 2005; SPANNHOF; PLANTIKOW, 1983) e baixa taxa / capacidade de absorção intestinal de glicose (BUDDINGTON et al., 1987). Outra razão pode ser a baixa diversidade bacteriana ou ausência de bactérias amilolíticas na microbiota intestinal (KARASOV et al., 2011; RAY et al., 2012).

Em comparação com a tilápia, onívora, a atividade total de carboidrases em peixes carnívoros como o salmão-do-Atlântico (*Salmo salar*), a truta arco-íris, a *European sea bass* e *gilthead sea bream* são 9, 22, 31 e 33%, respectivamente (PAPOUTSOGLOU; LYNDON, 2005). Particularmente, a baixa atividade da amilase no salmão-do-Atlântico pode ser devida à supressão de sete aminoácidos no sítio ativo de ativação da enzima, o que poderia prejudicar a ligação ao substrato (FROYSTAD et al., 2006).

Outros pontos relevantes com relação ao aproveitamento de carboidratos é que quando em níveis elevados pode ocorrer a inibição da atividade da amilase devido à adsorção do amido com a enzima. A possível presença de inibidores de amilase e o trânsito intestinal acelerado também podem influenciar (SPANNHOF; PLANTIKOW, 1983). Ainda, a baixa taxa de absorção de glicose pode ser explicada pela menor

densidade de transportadores e, em parte, pela menor quantidade de tecido, no caso de carnívoros (COLLIE; FERRARIS, 1995).

Cabe salientar que carnívoros selvagens, não mudam o padrão da dieta constantemente, como onívoros (KARASOV et al., 2011). Assim, devido a esta dieta natural com baixo teor de carboidratos, eles aparentemente perderam ou não desenvolveram capacidade de modular adaptativamente as características digestivas em resposta às alterações na composição da dieta (BUDDINGTON; HILTON, 1987; BUDDINGTON et al., 1997).

*3.9 Proporção amilose:amilopectina na alimentação de peixes*

As pesquisas acerca desse tema não reportam consenso comum, sendo portanto necessários estudos mais elaborados para compreender o comportamento e possíveis efeitos no crescimento, metabolismo e fisiologia de peixes a partir das fontes energétivas disponíveis, a fim de maximizar o uso dos nutrientes, minimizando os custos com alimentação.

Analisando-se os poucos estudos a esse respeito, identifica-se que o aproveitamente da amilose e amilopectina irá depender da fonte de proteína utilizada e fisiologia do trato digestivo, além disso, fatores como nível de carboidratos na dieta, complexidade molecular, extrusão ou não da dieta, e também a evoluçao natural de cada espécie, seja quanto ao hábito alimentar, ou capacidade adaptativa.

Trutas alimentadas com amido de milho ceroso apresentaram melhor desempenho em relação aos animais alimentados com amido de milho normal (PFEFFER et al., 1991). Similarmente, Enes et al. (2006) encontraram alta digestibilidade de amido de milho ceroso comparado ao amido de milho normal para robalo europeu (*Dicentrarchus labrax*). Juvenis da tilápia-do-Nilo alimentados com dietas contendo diferentes

quantidade de amilose/amilopectina (0,54/38,72; 6,90/32,63; 10,29/29,49; 20,42/18,61; 27,35/11,05 e 28,39/9,76) tiveram maior crescimento, melhores valores de digestibilidade e atividades mais elevadas de protease, lipase e amilase no tratamento 6,90/32,63. Pior crescimento dos peixes foi observado no tratamento com alta amilose, os quais apresentaram a menor concentração de glicose circulante (CHEN et al., 2013).

Por outro lado, "European sea bass" alimentados com amido de milho regular, amido de milho ceroso, celulose e outros polissacarídeos não amiláceos, não apresentaram efeitos das dietas no desempenho produtivo (GATESOUPE et al., 2014). O mesmo foi verificado para o jundiá, peixe onívoro, quando alimentados com dietas contendo arroz com diferentes concentrações de amilose (0, 16 e 26%). Contudo, nas variáveis bioquímicas, menor quantidade de amilose proporcionou maior mobilização de triglicerídeos séricos, diminuição na deposição de glicogênio hepático e aumento no metabolismo de aminoácidos e lactato no músculo, sugerindo gliconeogênese (PEDRON et al., 2011).

### *3.10 Vias Metabólicas*

#### 3.10.1 Glicólise

O carboidrato contido nos alimentos, como o amido, é digerido e absorvido sob a forma de glicose. Para oxidação completa da glicose é necessário o envolvimento de três vias metabólicas: Via glicolítica, ciclo de Krebs e cadeia respiratória (SALWAY, 2009). Contudo, a via glicolítica não é exclusividade da glicose, outros carboidratos também encontram seus destinos catabólicos na glicólise, após transformação em intermediário glicolítico. Entre eles estariam os dissacarídeos maltose, lactose, trealose e sacarose; e os monossacarídeos frutose, manose, galactose (NELSON; COX, 2011).

A glicólise (Figura 4) é a via central do catabolismo da glicose, ocorre no citosol das células. Nela, a glicose (6 carbonos) é degradada a duas moléculas de 3 carbonos, o piruvato, através de um processo de 10 etapas que pode ser dividido didaticamente em duas fases (preparatória e compensação), apresentando saldo de dois ATP e dois NADH reduzidos, os quais são destinados à cadeia respiratória (NELSON; COX, 2011).

A maioria das atividades enzimáticas que ocorre na glicólise é reversível. Todavia, as reações irreversíveis (hexoquinase, fosfofrutoquinase e piruvatoquinase) são as que atuam como reguladoras alostéricas, a fim de manter os níveis de ATP praticamente constantes. O estudo das atividades dessas enzimas regulatórias no fígado permite avaliar se o alimento ou o nutriente testado foi aproveitado na produção de energia. Permitem avaliar as condições nas quais se encontra o animal, refletindo seu estado nutricional, até mesmo predizem a rota metabólica preferencial ou regulada para manter os processos biológicos das células (ROTTA, 2003).

Após a glicose ser transportada para o interior da célula, por transportadores específicos, sendo esses estimulados pela insulina, ela é fosforilada pela glicoquinase e/ou hexoquinase, posteriormente convertida em frutose-6-fosfato. O ATP é o doador de fosfato nas duas fosforilações. A seguir, a frutose-1,6-bisfosfato é hidrolisada, para liberar duas moléculas com três carbonos cada, a dihidroxiacetona-fosfato e o gliceraldeído-3-fosfato. A dihidroxiacetona-fosfato é isomerizada em uma segunda molécula de gliceraldeído-3-fosfato. Até esse ponto duas moléculas de ATP foram "gastas" e o retorno positivo para o "investimento" ocorrerá nas próximas reações (MELO et al., 2016).

Cada molécula de gliceraldeído-3-fosfato é oxidada e fosforilada por fosfato inorgânico, para formar 1,3-bisfosfoglicerato. A libertação de energia ocorre quando as duas moléculas de 1,3-bisfosfoglicerato são convertidas em duas moléculas de piruvato.

A maior parte dessa energia é conservada pela fosforilação acoplada de quatro moléculas de ADP para ATP (NELSON; COX, 2011).

O produto líquido são duas moléculas de ATP por molécula de glicose, uma vez que houve “investimento” de 2 ATP na fase preparatória da glicose. A energia também é conservada na fase de “pagamento” na formação de duas moléculas de NADH por molécula de glicose. O destino piruvato depende do tipo de célula e das circunstâncias metabólicas (MELO et al., 2016).

Nelson e Cox (2011) salientam que a quebra da glicose é a única fonte de energia para uma série de tecidos e células, como: eritrócitos, medula renal, cérebro (em condições normais) e esperma.

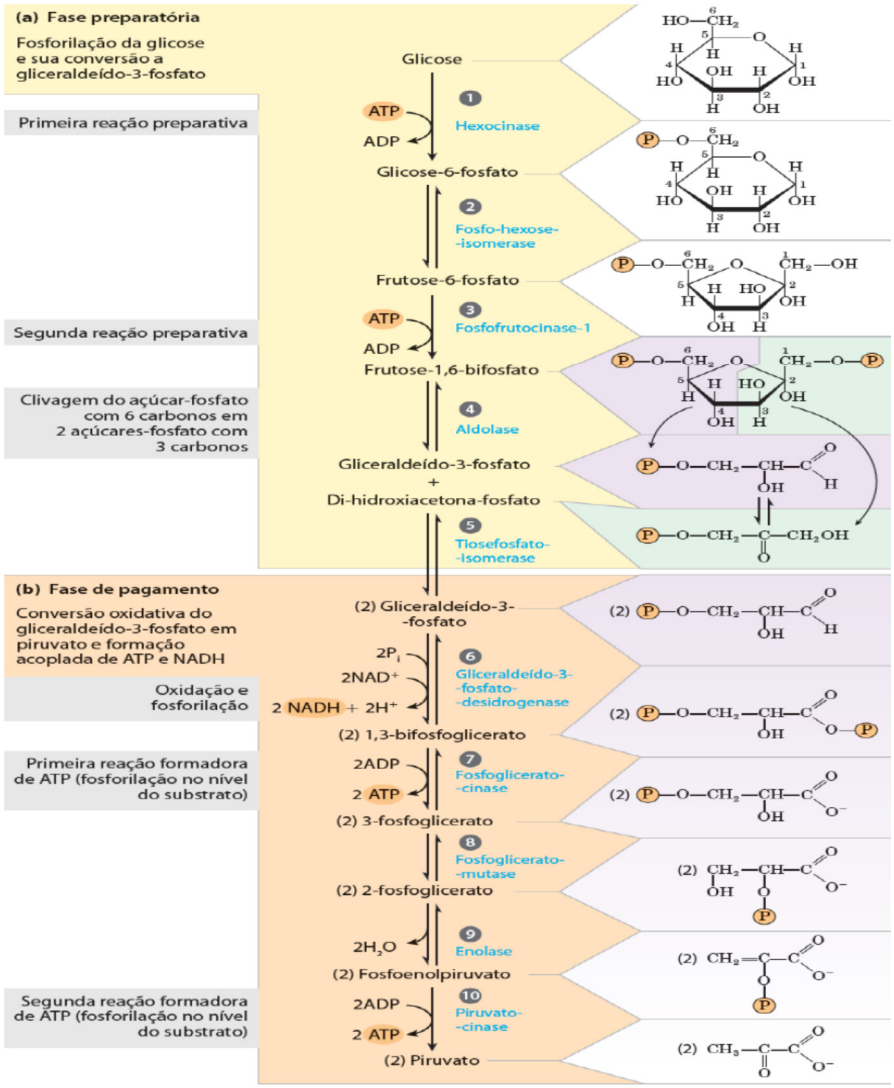


**Figura 4** Representação da via da glicólise (Fonte: NELSON; COX, 2011)

3.10.2 Gliconeogênese

O Glicogênio hepático é a primeira e mais importante reserva mantenedora dos níveis glicêmicos durante o período de jejum, entretanto, quando exaurida essa reserva, a síntese de glicose precisa ocorrer a partir de precursores não oriundos de carboidratos. Esse processo ocorre principalmente no fígado, mas durante jejum prolongado também é ativo no córtex renal, com finalidade de satisfazer as necessidades de glicose do organismo em situações onde os carboidratos dietéticos não são suficientes. Assim, esse processo ocorre durante o jejum, quando o glicogênio hepático está sendo esgotado (RIBEIRO et al., 2012; SALWAY, 2009).

Esta é uma via anabólica, com intuito de obter glicose *de novo* para fornecimento de energia. Gliconeogênese e glicólise não são simplesmente o inverso uma da outra, apesar de compartilharem a maioria das enzimas. Há pontos distintos, os quais estão justamente nas enzimas reguladoras, que são pontos irreversíveis da glicólise (Figura 5). Nesses pontos, a piruvato carboxilase, a fosfoenolpiruvato carboxiquinase, a frutose 1-6 bifosfatase e a glicose-6-fosfatase refletem a utilização de compostos não glicídicos, os quais têm o lactato, piruvato, glicerol e aminoácidos desaminados como precursores gliconeogênicos (NELSON; COX, 2011; ROTTA, 2003).

Essa via fornece glicose para células, como eritrócitos e neurônios, que não são capazes de utilizar lipídios como fonte de energia. A manutenção da glicemia pela gliconeogênese apresenta um alto consumo energético, sendo 2 ATP por piruvato, 1 GTP para cada piruvato, 1 NADH por piruvato e nenhuma produção de ATP nas reações que o consomem na glicólise. E isso equivale a 12 ATPs por glicose (NELSON; COX, 2011). O Acetil-CoA, derivado de ácidos graxos, jamais pode ser convertido à glicose, contribuindo apenas no fornecimento de energia e NADH.

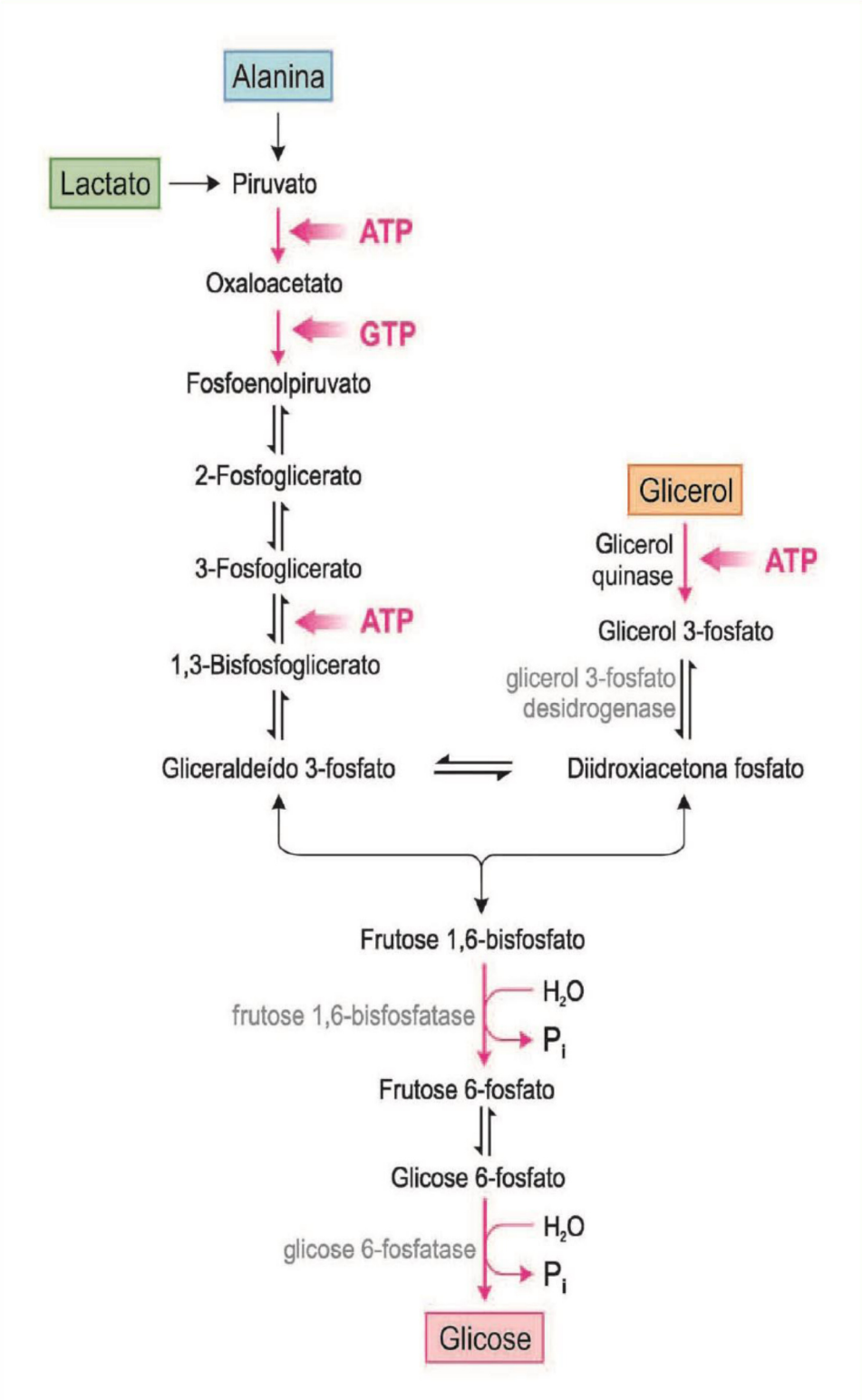


**Figura 5** Representação da via da gliconeogênese (Fonte: NELSON; COX, 2011)

### 3.10.3 Via das Pentoses

Na busca de melhorar a produção de peixes para consumo humano, principalmente na redução de gordura presente na carne, outra via metabólica, denominada via das pentoses ou desvio das pentoses, é considerada importante nas pesquisas de nutrição (MELO et al., 2016).

Essa via não é produtora de ATP, mas sim de NADPH, agente redutor utilizado para biossíntese de ácidos graxos e esteroides (colesterol e seus derivados), além da biossíntese de nucleotídeos. É uma via alternativa de oxidação de glicose-6-fosfato, que leva à produção de 3 compostos, a ribose-5-fosfato, para sintetizar ácidos nucleicos, CO2 e o NADPH (Figura 6). A energia originada da oxidação da glicose é armazenada sob a forma de NADPH, e não de ATP, como na glicólise. A via das pentoses é mais ativa quando as taxas glicêmicas na circulação são altas. Como resposta, os níveis altos de insulina resultantes acarretam, no tecido adiposo, em aumento da permeabilidade à glicose e, no fígado em intensa síntese de glicoquinase. Essas duas condições propiciam a síntese de ácidos graxos. A atividade das enzimas Glicose 6-P desidrogenase e 6-P Gluconato desidrogenase são importantes nesse processo de formação de NADPH, que irá auxiliar na via de produção de ácidos graxos e, consequentemente, triglicerídeos, além da ribose, para síntese de nucleotídeos como DNA e RNA (ROTTA, 2003).

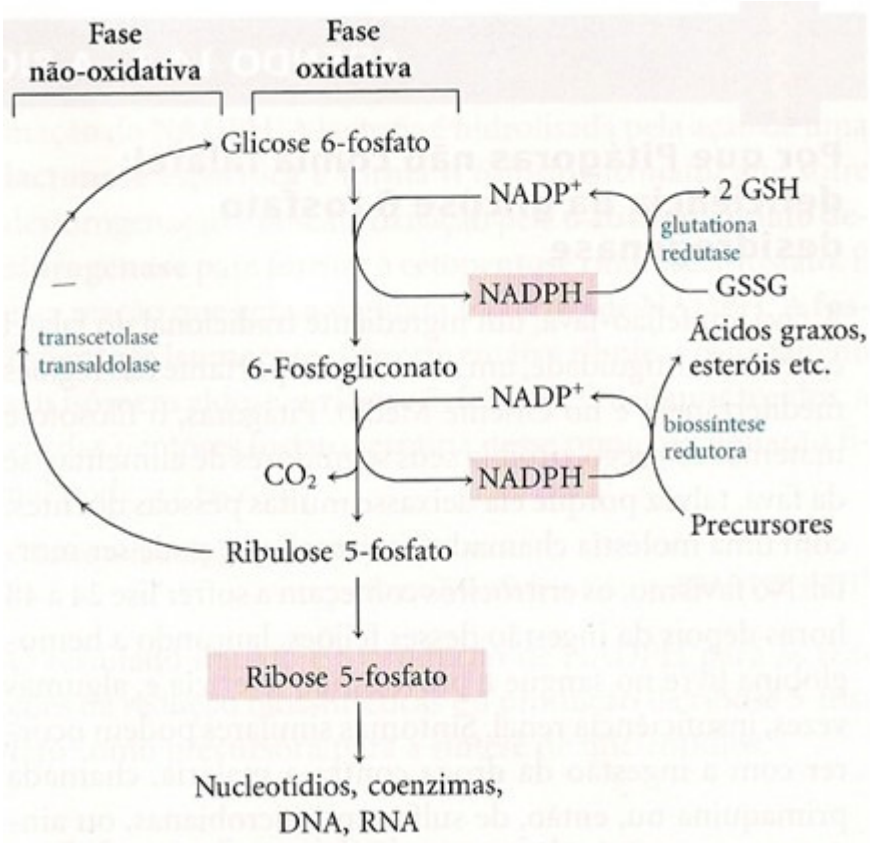


**Figura 6** Representação da via das Pentoses (Fonte: NELSON; COX, 2011)

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# CAPÍTULO 2

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# Growth and energetic metabolism of *Piaractus mesopotamicus* fed with different carbohydrate sources in the diet

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Carbohydrate sources for *Piaractus mesopotamicus*

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**Abstract**

This work aimed to evaluate the effect of different starch sources on the performance and metabolism of *Piaractus mesopotamicus*. Three isonitrogenous (300 g $kg^{-1}$ crude protein) and isolipidic (55 g $kg^{-1}$ crude lipid) diets containing carbohydrate sources (corn, broken rice or sorghum) varying between 284 and 316 g $kg^{-1}$ were formulated. Each experimental diet was tested in five replicate groups (25 fish per tank), three times a day during a period of 60 days. The growth variables (final weight, weight gain, specific growth rate, total length, feed conversion, survival, triglycerides and very low density lipoprotein were similar ($P > 0.05$). Cholesterol was lower in sorghum, and high density lipoproteins and hepatic glycogen were higher with the use of sorghum ($P < 0.05$). Low density lipoprotein was higher when feeding with corn. Plasma glucose was higher in treatments with broken rice and corn. Sorghum led to lower values of hepatosomatic, visceral and digestive somatic indices. Enzymes activities of amylase, lipase and maltase reduced their activities in fish fed with sorghum. Broken rice resulted in higher lipid content and lipid retention coefficient in whole fish. Dry matter and ash there was no difference. Glycemic curve, glycemia peaks were observed with 3.5 and 4 hours after ingestion of diets containing sorghum and broken rice, respectively, while for corn the highest value was after 12 hours. Therefore, these sources did not affect the growth and utilization of diets by *P. mesopotamicus*, however, different metabolic behaviors occurred, either from the starch content in diet, the starch composition of each ingredient, or the pro-nutritional effect of sorghum.

KEYWORDS: Amylopectin; amylose; broken rice; carbohydrate; corn; sorghum

**Abbreviations:** ADC, apparent digestibility coefficient; DM, dry matter; FC, feed conversion; FW, final weight; HDL, high density lipoprotein; HSI, hepatosomatic index; LDL, low density lipoprotein; LRC, lipid retention coefficient; SDI, somatic digestive index; SGR, specific growth rate; VFI, visceral fat index; VLDL, very low density lipoprotein; WG, weight gain.

## Introduction

The pacu fish (*Piaractus mesopotamicus*) is one of the most studied and reared species in Brazil and in other South American countries. This omnivorous fish is important in farming systems because it adapts easily to low-cost feed, grows fast under intensive farming (1.3 kg within the first 12 months), produces high-quality meat and can be exploited by the sport fishing industry, resistant to low temperatures, being adapted to subtropical climates (Jomori et al., 2003; Urbinati and Gonçalves, 2005; Abimorad et al., 2007). Despite the numerous studies on pacu feeding and nutrition, there is a large field to be investigated in terms of developing nutritionally complete diets that are truly assimilated.

Starch is the main digestible carbohydrate found in most cereals used in formulations for fish, being a cheap source. It is a complex carbohydrate composed of amylose, amylopectin and starch resistant (Singh et al. 2010).

Amylose is a linear polymer of α-1,4-linked D-glucose units, while amylopectin is highly branched, consisting of linear chain of glucose residues with α-1,6 branching points (Vandeputte et al. 2003; Chen et al. 2009). The amylose content in the starch granule ranges from 20 to 30%, while the amylopectin represents about 70-80% of the starch, depending on the ingredient studied (Gallant et al. 1992; Kotarski et al. 1992).

The differences in starch moiety chemical configuration are correlated with distinct rates of digestion and absorption, that may require different glycemic and lipid metabolism regulation (Granfeldt et al. 1994). In mammals, for example, amylopectin is hydrolyzed faster than amylose, resulting in increased glycemia (Regmi et al. 2010; Zijlstra et al. 2012). On the other hand, for fish, the scanty works have shown

controversial results (Rawles and Lochmann 2003; Enes et al. 2006; Enes et al. 2008; Pedron et al. 2011; Chen et al. 2013).

From the information available, its seems that fish (at least carnivorous species) does not handle dietary carbohydrate as well as terrestrial vertebrates. There are some hypotheses that seek to elucidate the poor use of carbohydrates by fish. According to Cowey and Walton (1989) the rainbow trout have a limited efficiency of glucose phosphorylation compared to common carp. Mommsen and Plisetskaya (1991) believed that amino acids would be more efficient at stimulating insulin release than glucose. For Párrizas et al. (1994), brown trout (*Salmo trutta fario*), gilthead sea bream (*Sparus aurata*), tilapia (*Tilapia mossambica*), and carp (*Cyprinus carpio*) have a low number of insulin receptors in the muscle when compared to mammals. According to Wright et al. (1998), tilapia would not have glucose transporters (GLUT 4) in the muscle. Finally, Panserat et al. (2001) emphasized that rainbow trout could present an imbalance between glycolysis and gluconeogenesis, thus contributing to the deregulation of glycemic indices.

To the current date, there was no previous knowledge of the relevance of the starch physicochemical configuration in carbohydrate-rich ingredients for *Piaractus mesopotamicus* metabolism. Therefore, objective of this study was to evaluate how corn, broken rice or sorghum, affect nutrient digestibility, growth performance and energy metabolism of pacu.

## Material and methods

### *Ethical principles*

The experimental procedures were conducted according to the ethical principles of animal experimentation adopted by the Federal University of Minas Gerais (UFMG), Committee on Ethics in Animal Use, according to protocol Nº 22/2015.

*Experimental diets*

Three isoproteic (30% crude protein) and isoenergetic (12.15 MJ $kg^{-1}$ digestible energy) experimental diets were formulated, varying only the carbohydrate sources used (grain corn, broken rice or grain sorghum) between 284 and 316 g $kg^{-1}$ (Table 1). Chromium oxide was added (0.1%) to the diets as an inert marker to determine the apparent digestibility coefficients (ADCs).

The ingredients were milled (< 0.5 mm), weighed, mixed and the diets extruded (3 mm). After extrusion, the oil was added and the diets were dried in a temperature controlled forced draft chamber (35 °C) for 48 hours and stored in freezer (-18 °C) until use. A completely randomized design with three treatments and five replications was adopted.

Samples of each experimental diets were taken to determine crude protein (Marconi distiller, model MA 036), ash (Muffle Sanchis, model BTT) and soluble, insoluble and total dietary fiber (AOAC 2000), lipids (Bligh and Dyer 1959), total starch (AOAC 2000, modified by Walter et al. 2008), amylose and amylopectin (Martinéz and Cuevas 1989), chromium (Bremer Neto et al. 2003) and total tannins (performed in CBO Laboratory, Valinhos, SP, Brazil according with routine protocols).

*Fish housing and experimental conditions*

The study was conducted at the Laboratório de Piscicultura da Universidade Federal de Santa Maria (UFSM) - RS, Brazil. *P. mesopotamicus* juveniles were purchased in a commercial hatchery (Ijuí, RS, Brazil) and acclimatized to the laboratory conditions for two weeks. Groups of 25 fish (12.13±0.09 g) were accounted, weighed and housed in 15 polypropylene tanks (280 L usable volume) connected to a water recirculation system with independent water inlet and outlet, biological filtration, supplementary aeration and

temperature. During the experiment, the water quality was monitored daily. The temperature was kept at 25.76±1.72 ºC, dissolved oxygen at 6.53±0.32 mg $L^{-1}$ (both variables monitored with digital oximeter, model 550A, YSI-Yellowsprings, OH, EUA), the pH was 7.52±0.18 (Servylab digital pHmeter, model mPA-210, São Leopoldo, RS), total ammonia at 0.1±0.02 ppm and alkalinity in 37.93±1.02 mg $L^{-1}$ $CaCO_3$ (both by colorimetric kit Alfakit®, Florianopolis, SC).

Fish were fed three times a day (8:00 a.m., 12:00 p.m. and 4:00 p.m.) to satiety, during 60 days. Leftovers were removed 15 minutes after each feeding, oven dried and weighed to evaluate the feed intake.

*Growth variables*

Biometric evaluations were performed at the beginning and end of the experiment. For this, the animals were fasted for 24 hours, and then anesthetized (100 mg of benzocaine $L^{-1}$), counted, weighed (Mars, 0.01 g, model AS2000C) and measured (digital caliper Digimess, model 100.178BL) to calculate the following variables: final weight (FW, g); weight gain (WG, g) = final weight - initial weight; specific growth rate (SGR, % day $^{-1}$) = (ln final weight - ln initial weight)/days * 100; total length (cm); feed conversion (FC) = feed intake/WG; and survival (%).

*Blood components*

At the end of the 60 days, the fish were fasted (24 hours), three animals per experimental unit were anesthetized (100 mg of benzocaine $L^{-1}$), randomly captured, and blood collection was performed by caudal puncture. The samples were centrifuged (Servylab, model 3PL) at 3200 rpm for 10 minutes.

The following analyzes were performed: glucose and triglyceride from the plasma; cholesterol and high density lipoprotein (HDL) in the serum, using a commercial

kit (Doles®). From these analyzes, very low density lipoprotein (VLDL = triglycerides/5) and low density lipoprotein (LDL = total cholesterol - (HDL+VLDL) were calculated according to Friedewald et al. (1972).

*Somatic indices and tissue collection*

After blood collection, the fish were euthanized (spinal cord section) for removal and weighing of the liver, visceral fat and intestine (Bel scale, 0.001 g, model M214A) to obtain the following indices: hepatosomatic index (HSI) = (liver weight/body weight) * 100; visceral fat index (VFI) = (fat weight/body weight) * 100; and somatic digestive index (SDI) = (tract weight/body weight) * 100.

After dissection, the liver and intestine were quickly stored in -20°C for further analysis of glycogen and digestive enzymes.

*Determining glycogen and digestive enzymes*

The hepatic glycogen (µmol glucose/g tissue) was quantified by the methodology proposed by Bidinotto et al. (1997). The samples were weighed, placed in test tubes, 1 mL of KOH and 3 mL of ethanol were added to precipitate the glycogen and finally centrifuged at 3500 rpm for 10 minutes. The supernatant was withdrawn, and processed for glucose determination according with Park and Johnson (1949).

For the enzymatic assays, intestine samples were homogenized (Turrax Marconi, model MA 102) in buffer solution pH 7,0 (0.02 M Tris/0.01 M phosphate), centrifuged at 3500 rpm for 10 minutes and the supernatant used to determine amylase activity (Bernfeld 1955), lipase (Gawlicka et al. 2000) and maltase (Corrêa et al. 2007). For these enzymes, 2.5% starch, p-nitrophenyl meristat (0.4 mM) and 5% maltose, respectively, were used as substrates. Starch hydrolysis, after incubation in a water bath (Marconi, model MA093) in the determination of amylase, was performed by the method of Park and Johnson

(1949). In maltase analysis, the glucose released during the reaction was dosed with a commercial glucose-oxidase kit (Doles®). The enzymes activities were expressed in milligrams of protein. Protein content was measured by the method of Bradford (1976), absorbance of 595 nm, using bovine serum albumin (1 mg $mL^{-1}$) as standard.

*Body composition*

After 60 days of experiment, the whole body composition was evaluated in another 15 animals from each experimental group. The dry matter determination, was carried out using a forced draft oven (Biopar, model S80S) at 105 ºC, ash content was determined after combustion in a muffle furnace at 550 ºC for four hours (AOAC 2000). Lipids were extracted and quantified by the method described by Bligh and Dyer (1959). The lipid retention coefficient (LRC, % = (lipid gain/ingested lipid) * 100) was calculated from initial and final lipids concentrations.

*Postprandial plasma glucose*

The 12 animals were maintained in the tanks for another week and submitted to the same daily management. For the glycemic evaluation, at the end of this week, after the last feeding of the day (4:00 p.m.), the animals remained 12 hours fasting. Each collection was performed on ten animals per treatment. Blood collection occurred at time zero (before re-feeding) and 1, 3, 6, 9 and 12 hours post-feeding. A digital blood glucose meter (Accu-Check Active®) was used to monitor the glycemia of the animals.

*Digestibility of the experimental diets*

For the digestibility assay, 100 animals with 56.32±0.85 g were stored in four cylindrical tanks with of conical bottom (190 L) and falcon tube attached at the lower end for decanting the faeces. The tanks were kept in a water recirculation system under the

conditions described for the growth experiment. The experimental design was a completely randomized design with three treatments and four replications.

The animals were fed from 7:30 to 10:30 a.m. with 30-minute intervals between each meal. After feeding, the tanks were cleaned, and then, the faeces were collected throughout the day, every two hours.

For each diet, an adaptation period of five days was carried out, for later collection. The faeces were centrifuged, dried in an oven (De Leo, model 197) at 55ºC and stored. In these samples were analyzed: chromium, protein and lipids, according to the methodologies already mentioned. The apparent dry matter digestibility coefficient was calculated according to the recommendations of Maynard et al. (1979):

$$\text{ADC DM (\%)} = \left[1 - \left(\frac{\%Cr_2O_{3d}}{\%Cr_2O_{3f}}\right)\right] \times 100$$

For protein and lipids, it was used the formula proposed by Cho and Slinger (1979):

$$\text{ADC} = 100 - \left[100 \left(\frac{\%Cr_2O_{3d}}{\%Cr_2O_{3f}}\right) \times \left(\frac{\%N_f}{\%N_d}\right)\right]$$

Where: ADC = Apparent digestibility coefficient; $Cr_2O_{3\,d}$ = % chromium oxide diet; $Cr_2O_3$ f = % chromium oxide faeces; $N_d$ = Nutrients in diet and $N_f$ = Nutrients in faeces.

*Statistical analysis*

The analyzes were performed by using the statistic software R (R Core Team, 2015). Initially the data were submitted to the outlier identification (using 2*standard deviation as criterion), normality test (Shapiro-Wilk), homogeneity of variances (Levene) and variance analysis (ANOVA). The means were compared by the Duncan test, when significant ($P < 0.05$). For data that did not satisfy these assumptions, the non-parametric Kruskal-Wallis test was applied.

## Results

*Variables of growth*

There was no mortality during the experiment. For the final weight (34.79±7.65; 36.22±4.39; 31.85±3.15 g), weight gain (22.45±7.44; 24.25±4.30; 19.72±1.44 g), specific growth rate (1.69±0.34; 1.84±0.19; 1.60±0.78% $dia^{-1}$), total length (12.42±0.81; 12.55±0.52; 12.05±0.51 cm) and feed conversion (1.58±0.22; 1.52±0.11; 1.68±0.12) no significant effects ($P > 0.05$) were recorded between treatments with corn, broken rice or sorghum in diet, respectively.

*Blood biochemistry and hepatic glycogen*

For triglycerides (2.46±0.66; 2.20±0.66; 1.84±0.80 mmol $L^{-1}$) and VLDL (0.49±0.15; 0.48±0.16; 0.36±0.11 mmol $L^{-1}$), the different diets (corn, broken rice or sorghum, respectively) did not produced significant variations ($P > 0.05$). The others results of blood and liver variables are shown in figure 1. Cholesterol (a), LDL (c) and glucose (d) were lower in animals fed with sorghum in the diet, and did not differ among the other treatments. HDL (b) was higher in fish fed with sorghum and lower when using corn ($P < 0.05$). For hepatic glycogen (e), the inverse of glucose was verified, with higher values in sorghum ($P < 0.05$) and smaller, but similar, between rice and corn ($P > 0.05$).

*Somatic indices*

Diets influenced all of the somatic indices evaluated (fig. 2). Fish fed with sorghum had the lowerst HSI, VFI and SDI values ($P < 0.05$). Treatments with broken rice and corn did not differ ($P > 0.05$).

*Digestive enzymes*

The digestive enzymes activity was affected by the carbohydrate source in the diet. The enzymes amylase, lipase and maltase reduced their activity in the animals fed with the diet in which sorghum was included ($P < 0.05$), being similar ($P > 0.05$) between corn and broken rice (fig. 3).

*Body composition*

For dry matter (29.82±2.71; 31.03±1.58; 30.35±2.29%) and ash (12.91±2.32; 13.67±1.47; 12.19±1.18%) there was no significant difference between diets with corn, broken rice or sorghum, respectively ($P > 0.05$). After the end of the experimental period, it was verified that the broken rice diet resulted in higher lipid contents and LRC in the whole fish ($P < 0.05$), presented in figure 4.

*Postprandial glycemia*

The glycemia peaks were observed 3.5 and 4 hours after ingestion of the diets containing sorghum and rice, respectively (Fig. 5). However, in fish fed with the corn-based diet, the peak was not observed, but the highest glucose value was detected after 12 hours of feeding. In the time zero, the fish of the treatment with rice presented smaller value among the treatments; intermediate value for sorghum and higher glycemia for corn. The animals treated with broken rice showed higher glycemic levels after the third hour, with a significant decline in the posterior collections. Sorghum caused intermediate glycemia and with more constant values over time, mainly between 9 and 12 hours. Corn, despite higher glycemia at the extremes evaluated (time 0 and 12 hours), presented lower glycemia soon after the first hour after feeding, until around the tenth hour.

*Digestibility of the experimental diets*

The different starch sources in the diet (corn, broken rice or sorghum) of *P. mesopotamicus* juveniles did not affect the digestibilities of dry matter (69.27±2.88; 67.90±7.71; 60.80±3.53%), protein (88.37±0.60; 85.73±3.38; 87.87±2.07%) and lipids (86.34±0.66; 83.27±3.79; 85.49±2.09%) ($P > 0.05$).

**Discussion**

The energetic ingredients used in the formulations of the test-diets presented different content of total starch, amylose and amylopectin. Therefore, changes in the biochemical, digestive, enzymatic, glycemic and body composition variables of *P. mesopotamicus* juveniles were verified, however, without influencing the growth and digestibility of the diets.

Differently from piscivorous fish, omnivorous species, such as *P. mesopotamicus,* usually feed on a broad selection of alimentary items and can adapt their digestive physiology accordingly in response to dietary changes through several mechanisms including structural modifications, modulation of absorptive dynamics (Wagner et al. 2009), enzymatic activity and nutrient transport capacity of post-digestive tract (Bakke et al. 2010). In this way, fish can maintain synergistic digestion, absorption and conversion of nutrients from food without necessarily interfering in the growth.

The lack of significant differences in growth parameters may be a reflex of an efficient physiological adjustment of digestive function to the experimental diets. In addition to this physiological adjustment, other factors, such as good acceptance of the different diets by the animals and nutritional balance of the formulations may also be accountable for the similarity in performance.

During the execution of the experiment no mortality occurred and the growth variables were satisfactory. In a research carried out with the same species, Sanchez et al. (2016) found that, when feeding the animals with diets containing 31.48% of corn or 35.18% of sorghum, weight gain, feed conversion and specific growth rate were also unaffected by the carbohydrate sources, similar to what happened in the present study, reinforcing the ability of this species to accept different carbohydrate sources. For juveniles of *Rhamdia quelen* and *Cyprinus carpio*, both omnivorous, the inclusion of corn, oats or defatted rice bran also did not affect the length, specific growth rate, apparent feed conversion, protein efficiency rate and weight gain (Corrêia et al. 2012). Studies conducted by Pedron et al. (2011) with rice varieties for *R. quelen*; by Enes et al. (2008) with native waxy maize starch to the *Sparus aurata*, marine carnivorous, and by Rodrigues et al. (2012) with ground corn and broken rice for the omnivorous *R. quelen* and *Oreochromis niloticus* also corroborate with the results of the present study. Thus, it is evident that using different carbohydrate sources for several species of fish of different alimentary habits not negatively affecting the performance of the animals.

In fact, the absence of statistical differences in digestibility assay performed in the present study is an indicative of the ability of *P. mesopotamicus* to digest, with similar efficiency, starch presenting different molecular configurations. The presence of tannin in sorghum may compromise growth performance, due to depression in food palatability, reduction of voluntary intake or digestion of proteins, carbohydrates, lipids and decreased calcium absorption (Chang et al. 1994). However, none of the negative effects were found in this study. Abimorad and Carneiro (2004) determined the digestibility coefficients of protein and energy fractions of foods for *P. mesopotamicus*. Sorghum, broken rice, corn and wheat bran resulted in similar ADCs. On the other hand, Guimarães et al. (2014)

verified lower ADCs of energy, protein and dry matter for sorghum in relation to corn, and both were lower than that of broken rice, in *Colossoma macropomum* feeding. Although they did not analyze the level of tannin in the diets, the authors attributed the lower digestibility of sorghum to the presence of antinutritional factor.

In addition, the results of this study are consistent with those obtained by Rodrigues et al. (2012) and Gominho-Rosa et al. (2015) for *R. quelen* and *O. niloticus*, in which broken rice and ground corn had equivalent ADCs within each species, however, *O. niloticus* presented higher efficiency of feed utilization than *R. quelen*. Rice varieties with different ratios of amylose and amylopectin were also digested by *R. quelen* (Pedron et al. 2011), an omnivorous species such as *P. mesopotamicus*.

As for performance and digestibility, triglyceride and VLDL were not affected by the starch source. These plasma concentrations were smaller in corn, possibly because of their higher amount of amylose. The slower absorption of glucose in a diet with higher amylose content may have prevented hyperlipidemia due to the lack of glucose availability, thus resulting in a lower synthesis of hepatic triglyceride from this substrate. On the other hand, sorghum presented the highest proportion of amylopectin, which could induce higher circulating levels of triglycerides and cholesterol. However, phytosterols, policosanols (Carr et al. 2005; Hoi et al. 2009) and proanthocyanidins (condensed tannins) (Althwab et al. 2015) compounds present in this ingredient have preventive properties of cellular oxidation, inflammation, hyperlipidemia (Chung et al. 1998; Shim et al. 2013; Lee et al. 2014), possibly modifying blood levels. These effects will be proportional to the concentration of these compounds in the ingredient, level of inclusion in the diet, varieties of a cultivar and species of fish studied. However, tannin may cease to be a pro-nutrient when in excess.

For Weisburger and Chung (2002) and Auger et al. (2003), the tannins present hypocholesterolemic effect due to their antioxidant action, since they block the initiation and propagation of free radicals that induce oxidation of LDL. In addition, tannins inhibit the activity of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase, necessary for cholesterol biosynthesis (Chang et al. 2001), which catalyzes the reduction of HMG-CoA to form mevalonate, point of control in cholesterogenesis (Boucher et al. 1998). This may have been the key to reducing circulating cholesterol levels in fish that were fed with sorghum as an energy source in the diet. Because LDL is responsible for the transport of cholesterol from the liver to the tissues, the reduction in their circulating levels is possibly a reflection of cholesterol drop in sorghum-treated animals.

HDL was higher when using sorghum. Tannin reduces the activity of hepatic lipase, which is involved in the processes of uptake and degradation of HDL (Tebib et al. 1994). This fact may be due to the higher activity of this enzyme in the other treatments, which would help explain the lower HDL values.

Hepatic glycogen was higher for animals fed with diets containing sorghum. Amongst the liver functions, its storage capacity of reserve substances, both in the form of glycogen and lipids (Enes et al. 2008), stands out. In general, because it is easily digested, diets with higher levels of amylopectin will result in elevated hepatic glucose uptake, leading to a significant deposition of glycogen or fat; the same occurs in diets with high levels of starch (Rawles et al. 2008; Moro et al. 2016).

The treatments with sorghum and broken rice showed higher amounts of amylopectin; However, broken rice diet also contained higher total starch content and carbohydrate: lipid ratio of 7.5, while in sorghum it was 4.5; what associates with the fact that the animals fed with broken rice had a higher hepatosomatic index, but lower

glycogen, indicating a greater deposit of fat in the liver. Works comprising *R. quelen* (Moro et al. 2010), onivorous fish, and *Salminus brasiliensis* (Moro et al. 2016), carnivorous, showed that the carbohydrate:lipid ratio from 4.6:1 and 3.3:1, respectively, caused a decrease in hepatic glycogen concentration, thus with metabolic overload due to this fact. Fish fed with corn in the diet had lower plasma glucose levels over the analyzed period of 12 hours, without a pronounced glycemic peak. Perhaps the animal did not absorb enough glucose to supply the cellular demand and still have left for storage, justified by the smaller hepatic glycogen and higher plasma glucose.

The animals remained fasted for 12 hours, were fed again, and after other 12 hours they did not return to the initial glycemic levels. This was due to the circadian rhythm. *P. mesopotamicus* is a species with diurnal habits, therefore, in the collection of time zero (4:00 a.m.), the animals presented lower locomotor activity, with that, they demanded less nutrients to maintain their activities. On the other hand, at 4:00 p.m. (final collection) the required nutrient flow was substantially increased to cover all energy maintenance and production costs.

Fish fed with diets containing sorghum had a reduction in the activity of amylase, lipase and maltase enzymes. It is known that condensed tannins inhibit the activity of a number of enzymes, such as: cellulases, pectinases, amylases, lipases, proteolytic enzymes and alpha-galactosidases (Chung et al. 1998). This seems to be the main factor that influenced the enzymatic activity of *P. mesopotamicus*. However, this reduction does not necessarily result in negative aspects to performance, as verified in the present study.

Body lipids and lipid retention coefficient were lower in sorghum treated animals. These variables can be explained by the reduction of lipid absorption dynamics, with a decrease in lipase activity. This profile of lipid deposition is also highlighted by Aiura

and Carvalho (2007), Hossain et al. (2001) and Pinto et al. (2001) in fish fed with diets containing tannin.

*P. mesopotamicus* efficiently utilized the nutrients of the evaluated sources. The lower enzymatic activity verified in the animals receiving sorghum diet had no negative impact on nutrient digestibility and also promoted a lower fat deposition in carcass and viscera. Although leaner carcass is a desirable trait, this effect must be evaluated throughout longer periods, especially near market size, to ensure that final growth is not adversely affected during a complete production cycle and that the fat lowering effect is maintained.

In addition, sorghum is not traditionally used in human food, unlike the other ingredients tested. It should also be noted that this grain is on average 34.8% cheaper than corn (average price for the month of August 2017). Thus, sorghum becomes an alternative to increase the production and quality of the fish, being able to reduce costs with feeding.

## Conclusion

The growth of *Piaractus mesopotamicus* and digestibility are not affected by the chemical composition of starch from the carbohydrate sources tested. On the other hand, its energetic metabolism is influenced by diet. Sorghum provides better energetic-protein dynamics resulting in lower retention of lipids in the whole fish and less deposit of fat in the viscera.

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**Table 1** Formulation and proximal composition of the experimental diets

| INGREDIENTS (g $kg^{-1}$) | Corn | Broken rice | Sorghum |
|---|---|---|---|
| Soybean meal | 484.6 | 490.2 | 480.5 |
| Fish meal | 50.0 | 50.0 | 50.0 |
| Grain sorghum | - | - | 284.7 |
| Grain corn | 300.6 | - | - |
| Broken rice | - | 316.0 | - |
| Soybean oil | 20.0 | 20.0 | 20.0 |
| Crystalline cellulose | 30.9 | 35.2 | 29.5 |
| Dicalcium phosphate | 27.5 | 25.5 | 27.0 |
| DL-methionine | 0.2 | - | 0.2 |
| Chromic oxide | 1.0 | 1.0 | 1.0 |
| L-lysine | 2.2 | 1.7 | 2.3 |
| Inert | 77.8 | 55.2 | 99.6 |
| Mineral and vitamin supplement[a] | 5.0 | 5.0 | 5.0 |
| Antioxidant[b] | 0.2 | 0.2 | 0.2 |
| PROXIMAL COMPOSITION (g $kg^{-1}$) | | | |
| Crude protein[c] | 306.6 | 308.7 | 316.0 |
| Digestible energy (MJ $Kg^{-1}$)[d] | 121.6 | 121.6 | 121.5 |
| Insoluble fiber[c] | 129.8 | 120.3 | 128.3 |
| Soluble fiber[c] | 38.4 | 32.9 | 38.5 |
| Total starch[c] | 246.1 | 318.4 | 251.0 |
| Amylose[c] | 110.4 | 144.7 | 90.1 |
| Amylopectin[c] | 135.7 | 173.6 | 160.9 |
| Amylose: amylopectin[c] | 0.81 | 0.83 | 0.56 |
| Lipids[c] | 55.2 | 42.4 | 55.0 |
| Ash[c] | 151.7 | 168.5 | 152.4 |
| Tannin[c] | - | - | 2.4 |
| Lysine[d] | 16.4 | 16.4 | 16.4 |
| Methionine[d] | 3.8 | 3.9 | 3.8 |
| Calcium[d] | 11.0 | 10.6 | 10.9 |
| Phosphorus[d] | 7.0 | 7.0 | 7.0 |

[a] Mineral and vitamin supplement composition $kg^{-1}$: Selenium, 75 mg; iron, 15 g; copper, 1,250 mg; manganese, 3,750 mg; zinc, 17.5 g; cobalt, 50 mg; iodine, 100 mg; niacin, 8,750 mg; folic acid, 625 mg; panthotenic acid, 7,500 mg; biotin, 50 mg; vitamin C, 37.5 g; choline, 100 g; Inositol, 12.5 g; vitamin A, 1,750,000 UI; vitamin D3, 375,000 UI; vitamin E, 20,000 UI; vitamin K, 3,500 mg, vitamin B1, 2,000 mg; vitamin B2, 2,500 mg; vitamin B6, 2,500 mg; vitamin B12, 5;000 mcg;
[b] Butyl-Hydroxy-Toluene;
[c] Analyzed values;
[d] Values were estimated based on ingrediets composition and inclusion levels.

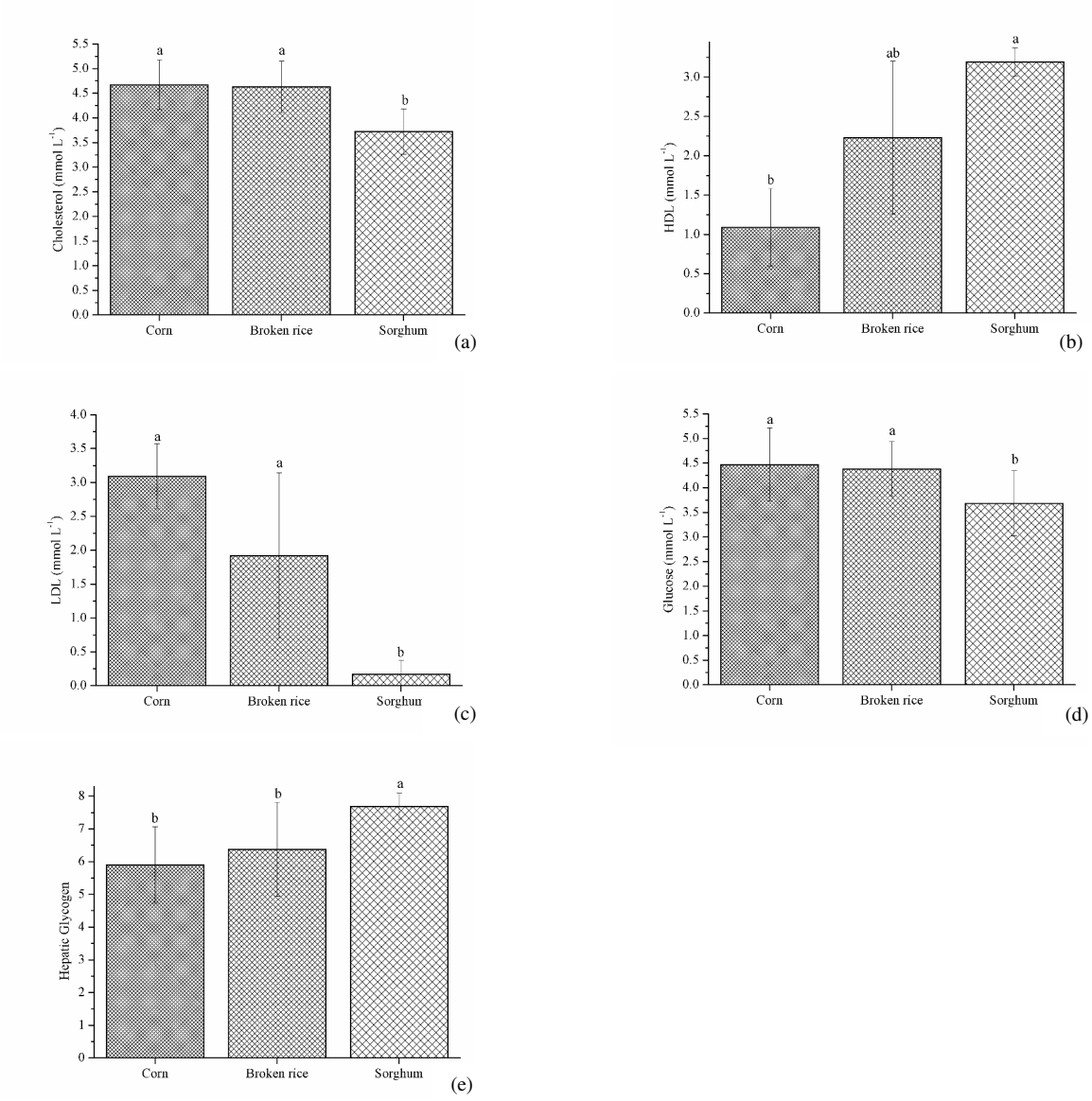


**Figure 1** Cholesterol (P=0.011) (a), HDL (P=0.001) (b), LDL (P=0.002) (c), glucose (P=0.022) (d) and hepatic glycogen (values expressed in µmol glucose/g tissue) (P=0.008) (e) of *Piaractus mesopotamicus* juveniles (results represent means ± SD, n=15) fed with different starch sources in the diet. Different letters in "a, d and e" indicate significant differences between treatments by the Duncan test ($P < 0.05$). Different letters

in “b and c” indicate significant differences between treatments by Kruskal-Wallis test ($P < 0.05$).

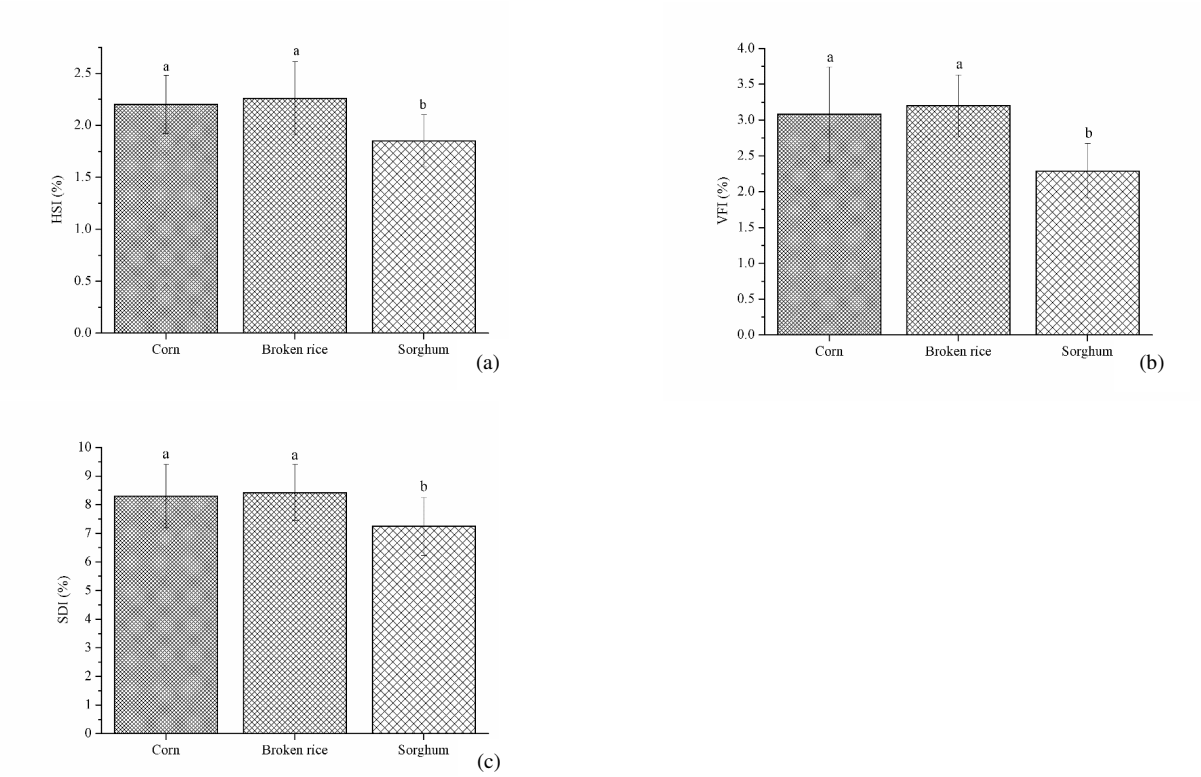


**Figure 2** Hepatosomatic index (P=0.041) (a), visceral fat index (P=0.014) (b) and somatic digestive index (P=0.032) (c) of *Piaractus mesopotamicus* juveniles (results represent means ± SD, n=15) fed with different starch sources in the diet. Different letters indicate statistic difference by the Duncan test ($P < 0.05$)

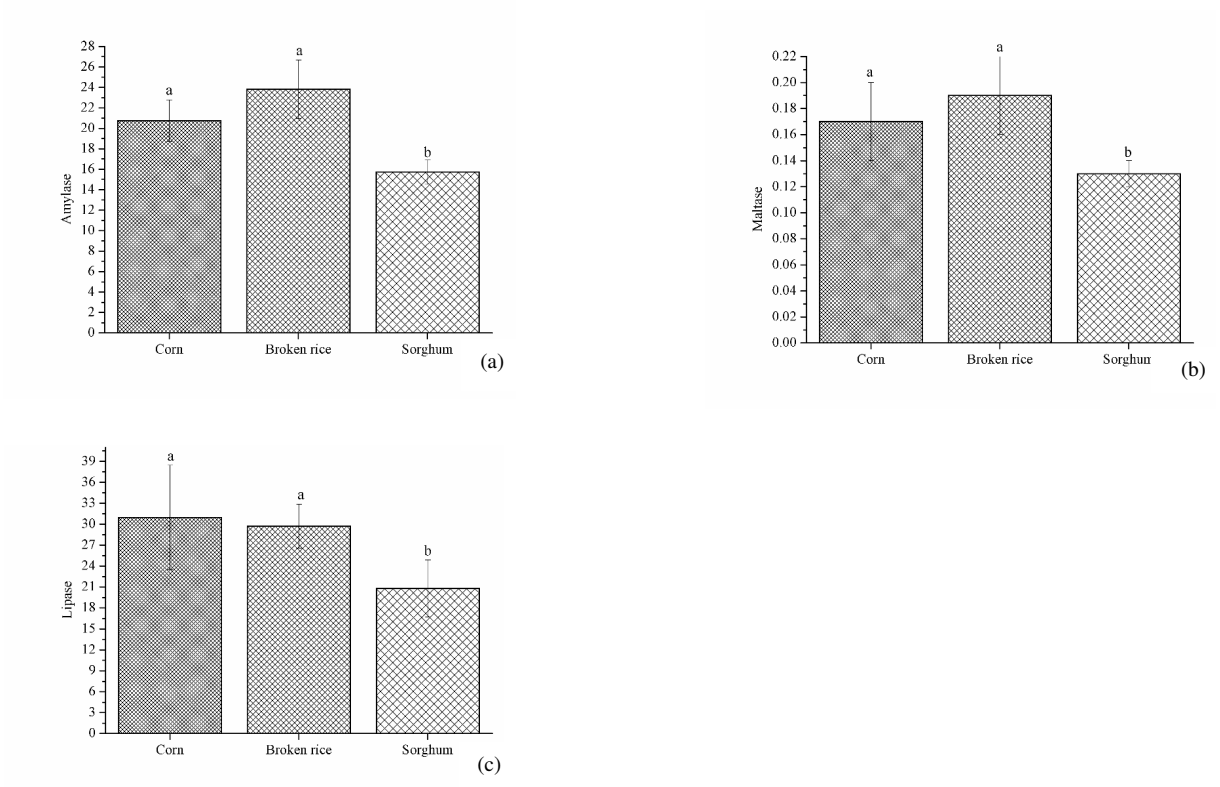


**Figure 3** Effect of the starch source on the amylase (P=0.019) (a), maltase (P=0.054) (b) and lipase (P=0.013) (c) activity of *Piaractus mesopotamicus* juveniles (results represent means ± SD, n=15), after 60 days of feeding. Different letters in "a"indicate significant differences between treatments by Kruskal-Wallis test ($P < 0.05$). Different letters in "b and c" indicate significant differences between treatments by Duncan test ($P < 0.05$). (a) Values expressed in µmol hydrolyzed glucose/minute/mg protein; (b) Values expressed in µmol glucose/mg protein; (c) Values expressed in UI/minute/mg protein

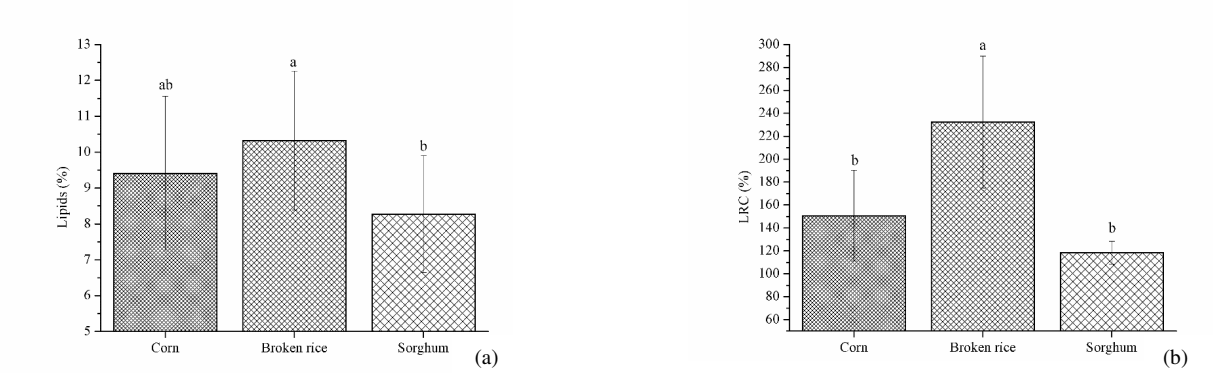


**Figure 4** Body lipids (P=0.056) (a) and lipid retention rate (P=0.002) (b) of *Piaractus mesopotamicus* juveniles (results represent means ± SD, n=15) fed with different starch sources diet. Different letters indicate statistic difference by the Duncan test ($P < 0.05$)

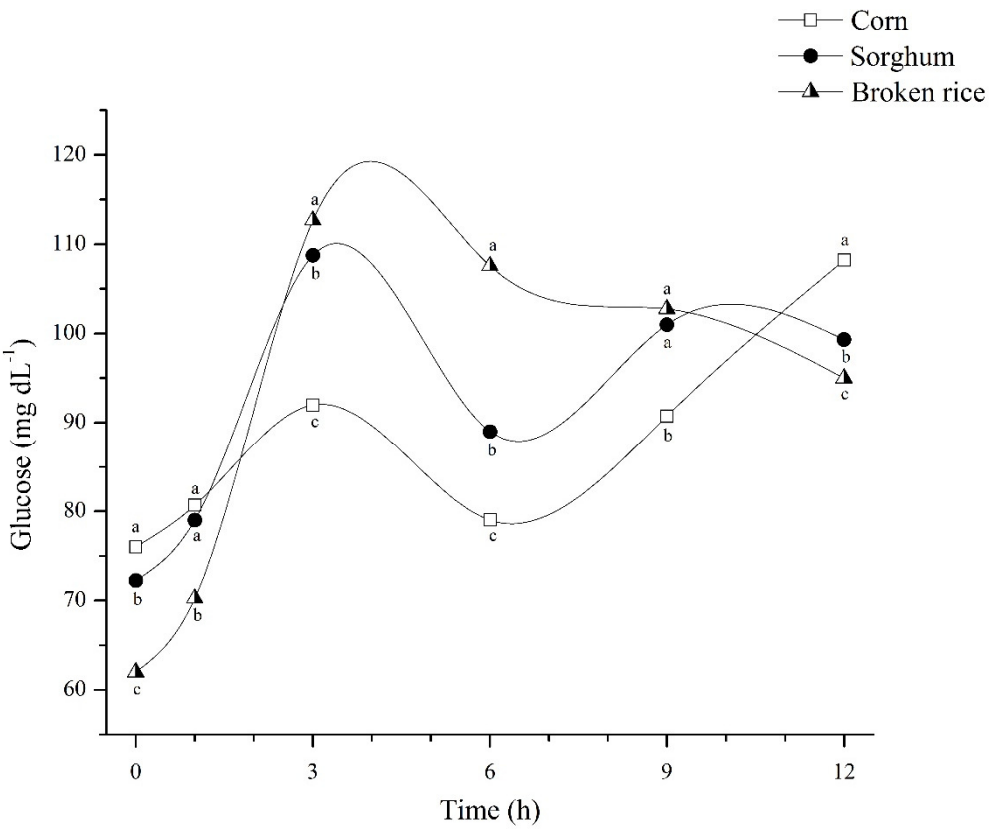


**Figure 5** Postprandial glycemia of *Piaractus mesopotamicus* juveniles for 12 hours after feeding with different starch sources in the diet. Values are averages of 10 fish per treatment. Different letters, within each collection time, indicate statistical difference by the Duncan test ($P < 0.05$)

# CAPÍTULO 3

Artigo nas normas da Aquaculture Research.

# Starch sources in the diet generate different responses in proteic metabolism, nutrient utilization, and enzymatic activity of *Piaractus mesopotamicus*

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**Abstract**

The effect of dietary carbohydrate source on growth, blood chemistry, hepatic metabolism, nutrient retention, body composition and digestive enzyme activities, responses was evaluated in juvenile *Piaractus mesopotamicus*. Three isonitrogenous (300 g $kg^{-1}$ crude protein) and isolipidic (55 g $kg^{-1}$ crude lipid) diets containing carbohydrate sources (corn, broken rice or sorghum) varying between 284 and 316 g $kg^{-1}$ were formulated. Each experimental diet was tested in five replicate groups (25 fish per tank), three times a day during a period of 60 days. Growth variables, alanine aminotransferase activity and protein retention coefficient were similar ($P > 0.05$) among treatments. Serum albumin, intestinal quotient and trypsin activity were lower in fish fed with sorghum diet. Total protein, globulin, hepatic protein, hepatic ammonia, hepatic glucose, body protein and carcass yield were also significantly higher in this group. Broken rice diet resulted in lower total protein, globulin, hepatic protein, body protein and carcass yield; and highest hepatic free amino acid, ammonia, glucose, intestinal quotient and trypsin. In fish fed with corn in diet showed lower values for total protein, globulin, hepatic protein, free amino acid, ammonia, glucose, carcass yield and intestinal quotiente. Highest albumin, carcass protein and trypsin. The study demonstrated that corn, broken rice or sorghum in the diet of *Piaractus mesopotamicus* juveniles did not interfere in the growth variants. The use of corn denotes a metabolic profile of better exploration of dietary carbohydrates. The use of broken rice and sorghum generates an increase in amino acid catabolism to maintain energy production processes. There is an increase in the production of nitrogen compounds of excretion in both broken rice and sorghum treatments. Sorghum showed higher carcass yield and protein content in whole fish.

Keywords: Amylopectin; amylose; broken rice; carbohydrate; corn; sorghum

## Introduction

The pacu fish (*Piaractus mesopotamicus*) is one of the most studied and reared species in Brazil and in other South American countries. This omnivorous fish is important in farming systems because it adapts easily to low-cost feed, grows fast under intensive farming (1.3 kg within the first 12 months), produces high-quality meat and can be exploited by the sport fishing industry, resistant to low temperatures, being adapted to subtropical climates (Jomori, Carneiro, Malheiros & Portella 2003; Urbinati & Gonçalves 2005; Abimorad, Carneiro & Urbinati 2007). Despite the numerous studies on pacu feeding and nutrition, there is a large field to be investigated in terms of developing nutritionally complete diets that are truly assimilated.

Fish do not present a nutritional requirement for carbohydrates (NRC 2011), but this nutrient is often used as a source of energy, mainly due to the low cost and the protein sparing effect, as demonstrated by some studies (Hidalgo, Sanz, Garcia Gallego, Suarez & De La Higuera 1993; Shiau & Peng 1993; Erfanullah & Jafri 1995; Shiau 1997; Stone, Allan & Anderson 2003; Mohanta, Mohanty & Jena 2007). The adequate supply of carbohydrates in the diet is important because it reduces protein catabolism, for either energy or gluconeogenesis, which decreases protein retention and increases ammonia excretion into the environment (Cowey & Walton 1989).

Starch is the main digestible carbohydrate found in most cereals used in formulations for fish, being a cheap source. It is a complex carbohydrate composed of amylose, amylopectin and starch resistant (Singh, Dartois & Kaur 2010).

Amylose is a linear polymer of α-1,4-linked D-glucose units, while amylopectin is highly branched, consisting of linear chain of glucose residues with α-1,6 branching points (Chen et al. 2009; Vandeputte, Vermeylen, Geeroms & Delcour 2003). The amylose content in the starch granule ranges from 20 to 30%, while the amylopectin represents about 70-80% of

the starch, depending on the ingredient studied (Gallant, Bouchet, Buleon & Perez 1992; Kotarski, Waniska & Thurn 1992).

The differences in starch moiety chemical configuration are correlated with distinct rates of digestion and absorption, that may require different glycemic and lipid metabolism regulation (Granfeldt, Liljeberg, Drews, Newman & Björck 1994). In mammals, for example, amylopectin is hydrolyzed faster than amylose, resulting in increased glycemia (Regmi, Matte, Van Kempen & Zijlstra 2010; Zijlstra, Jha, Woodward, Fouhse & Van Kempen 2012). On the other hand, for fish, the scanty works have shown contradictory results (Rawles & Lochmann 2003; Enes, Panserat, Kaushik & Oliva-Teles 2006; Enes, Panserat, Kaushik & Oliva-Teles 2008; Pedron et al. 2011; Chen, Ji-Dan Ye & Wang 2013).

From the information available, its seems that fish (at least carnivorous species) does not handle dietary carbohydrate as well as terrestrial vertebrates. There are some hypotheses that seek to elucidate the poor use of carbohydrates by fish. According to Cowey and Walton (1989) the rainbow trout have a limited efficiency of glucose phosphorylation compared to common carp. Mommsen and Plisetskaya (1991) believed that amino acids would be more efficient at stimulating insulin release than glucose. For Párrizas, Baños, Baró, Planas and Gutiérrez (1994), brown trout (*Salmo trutta fario*), gilthead sea bream (*Sparus aurata*), tilapia (*Tilapia mossambica*), and carp (*Cyprinus carpio*) have a low number of insulin receptors in the muscle when compared to mammals. According to Wright, O'Hali, Yang, Han and Bonen (1998), tilapia would not have glucose transporters (GLUT 4) in the muscle. Finally, Panserat, Plagnes-Juan, Kaushik (2001) emphasized that rainbow trout could present an imbalance between glycolysis and gluconeogenesis, thus contributing to the deregulation of glycemic indices.

To the current time, there is no knowledge of the metabolic study of the main conventional energy ingredients used in *Piaractus mesopotamicus* feeding. The objective of

this study was to evaluate the effect on development and proteic metabolism of this species when fed with diets containing corn, broken rice or sorghum.

## Material and methods

*Ethical principles*

The experimental procedures were conducted according to the ethical principles of animal experimentation adopted by the Federal University of Minas Gerais (UFMG), Committee on Ethics in Animal Use, according to protocol Nº 22/2015.

*Experimental diets*

Three isoproteic (30% crude protein) and isoenergetic (12.15 MJ $kg^{-1}$ digestible energy) experimental diets were formulated, varying only the carbohydrate sources used (grain corn, broken rice or grain sorghum) between 284 and 316 g $kg^{-1}$ (Table 1).

The ingredients were milled (< 0.5 mm), weighed, mixed and the diets extruded (3 mm). After extrusion, the oil was added and the diets were dried in a temperature controlled forced draft chamber (35 °C) for 48 hours and stored in freezer (-18 °C) until use.

Samples of each experimental diets were taken to determine crude protein (Marconi distiller, model MA 036), soluble, insoluble and total dietary fiber (AOAC 2000), total starch (AOAC 2000, modified by Walter, Silva & Perdomo 2008), amylose and amylopectin (Martinéz & Cuevas 1989), lipids (Bligh & Dyer 1959), ash (Muffle Sanchis, model BTT) and total tannins (performed to CBO Laboratory, Valinhos, SP, Brazil). The experimental design was a completely randomized design with three treatments and five replications was adopted.

*Fish housing and experimental conditions*

The study was conducted at the Fish Farming Laboratory of the Federal University of Santa Maria (UFSM) - RS, Brazil. *P. mesopotamicus* juveniles were purchased in a commercial hatchery (Ijuí, RS, Brazil) and acclimatized to the laboratory conditions for two weeks. Groups

of 25 fish (12.13±0.09 g) were accounted, weighed and housed in 15 polypropylene tanks (280 L usable volume) connected to a water recirculation system with independent water inlet and outlet, biological filtration, supplementary aeration and temperature. During the experiment, the water quality was monitored daily. The temperature was kept at 25.76±1.72 ºC, dissolved oxygen at 6.53±0.32 mg $L^{-1}$ (both variables monitored with digital oximeter, model 550A, YSI-Yellowsprings, OH, EUA), the pH was 7.52±0.18 (Servylab digital pHmeter, model mPA-210, São Leopoldo, RS), total ammonia at 0.1±0.02 ppm and alkalinity in 37.93±1.02 mg $L^{-1}$ $CaCO_3$ (both by colorimetric kit Alfakit®).

Fish were fed three times a day (8:00 a.m., 12:00 p.m. and 4:00 p.m.) to satiety, during 60 days. Leftovers were removed 15 minutes after each feeding, oven dried and weighed to evaluate the feed intake.

*Growth variables*

Biometric evaluations were performed at the beginning and end of the experiment. For this, the animals were fasted for 24 hours, and then anesthetized (100 mg of benzocaine $L^{-1}$), weighed (Mars, 0.01 g, model AS2000C) and accounted to calculate the following variables: final weight (FW, g); daily weight gain (DWG, g) = (final weight - initial weight)/days; relative weight gain (RWG, g) = [(final weight - initial weight)/ initial weight] * 100; condition factor (CF, %) = [(weigh/total length)$^3$] *100; feed intake (FI, g $fish^{-1}$) = (total feed intake)/ fish number.

*Blood components*

At the end of the 60 days, the fish were fasted (24 hours), three animals per experimental unit were anesthetized (100 mg of benzocaine $L^{-1}$), randomly captured, and blood collection was performed by caudal puncture.. The samples were centrifuged (Servylab, model 3PL) at 1000 x g for 10 minutes.

The following analyzes were performed: total protein and albumin in the serum using a commercial kit (Doles®) with spectrophotometer reading (Servylab, model SP-200) at 550 and 630nm, respectively. From these analyzes, globulin (total protein - albumin) was calculated.

*Tissue collection and hepatic biochemistry analysis*

After blood collection, the fish were euthanized (spinal cord section) for removal of the liver. The samples were frozen at -20 ° C for analysis of biochemical parameters.

For protein analysis, the samples were heated at 100 ºC with KOH and centrifuged (1000 x g for 10 min). Supernatant was used to determine the protein level according to the method described by Bradford (1976), using bovine serum albumin as standard.

To determine the amount of free liver amino acid, samples (50 mg) were homogenized by adding 1 mL of a phosphate buffer (20 mM, pH 7.5) and then centrifuged (1000 x g for 10 min), the supernatant extract was used to determine amino acid concentration by colorimetry (Spies 1957), using 1.5% ninhydrin solution in isopropyl alcohol as the color reagent.

To quantify the hepatic ammonia, tissue samples were homogenized by adding 10% TCA and centrifuged (1000 x g for 10 min) for protein flocculation. Hepatic ammonia was measured according to the technique described by Verdouw, Vanechteld and Deckkers (1978) protocol after ammonia reaction with phenol and hypochlorite forming a blue-colored indophenol compound.

For glucose determination, we used the methodology proposed by Park and Johnson (1949), in which liver samples (50 mg) were homogenized in 10% TCA, centrifuged (1000 g, for 10 minutes). The supernatant was used as an extract for the quantification of glucose.

The neutral extract was used to measure the hepatic transaminase concentration. Activity of the alanine aminotransferase enzyme (ALAT) was determined by using colorimetric procedures following the protocols described in the kits (Doles®).

*Determining digestive enzyme*

Three samples of fish of each tank were collected to determine the activity of trypsin. The intestines collected were homogenized in a buffer solution (10 mM phosphate/20 mM Tris). The samples were then centrifuged, and the supernatants were used in the assays as enzyme source for determining intestine trypsin enzyme. To determine the trypsin enzyme activity, TAME (α-ρ-toluenesulphonyl-L-argininemethyl ester hydrochloride) was used as substrate. The intestine extracts were incubated for 2 minutes (25 ºC) in 2 mL buffer solution (0.2 M Tris/0.01 M de $CaCl_2$, pH 8.1). The trypsin activity was expressed in µmol of hydrolyzed TAME/minute/mg of protein, absorbance of 247 nm, following the methodology described by Hummel (1959).

*Nutrient deposition and body composition*

After 60 days of experiment, the whole fish body composition was evaluated in another 15 animals from each experimental group. The body protein was carried in an digester block (Marconi, model MA 056) and nitrogen distiller (Marconi, model MA 036). With the initial (pool of 20 animals) and final protein analyzes, the protein retention coefficient (PRC, %) = (protein gain/ingested protein) * 100 was calculated.

The animals were eviscerated and mensured the length of the intestine for intestinal quotient calculation (IQ) = (length of the digestive tract/total fish length). The carcass yield was also calculated (CY, %) (eviscerated weight with head and gills / whole fish weight) * 100.

*Statistic analysis*

The analyzes were performed by using the statistic software R (R Core Team 2015). Initially the data were submitted to the outlier identification (using 2*standard deviation as criterion), normality test (Shapiro-Wilk), homogeneity of variances (Levene) and variance analysis (ANOVA). The means were compared by the Duncan test, when significant ($P < 0.05$).

For data that did not satisfy these assumptions, the non-parametric Kruskal-Wallis test was applied.

## Results

*Growth and survival*

There was no mortality during the experiment. For the final weight (34.79±7.65; 36.22±4.39; 31.85±3.15 g), daily weight gain (0.37±0.12; 0.40±0.07; 0.33±0.02 g), relative weight gain (181.07±56.82; 202.38±34.70; 162.57±12.50%), condition factor (1.79±0.05; 1.83±0.07; 1.84±0.06) and feed intake (34.27±7.30; 36.54±4.36; 33.21±1.15) no significant differences ($P > 0.05$) were recorded between treatments with corn, broken rice or sorghum in diet, respectively.

*Blood biochemistry*

Figure 1 shows the results of the blood parameters of *P. mesopotamicus* juvenile fed with different carbohydrate sources in the diet. The inclusion of different sources in the experimental diets caused change in the studied parameters (total proteins (1a), albumin (1b) and globulin (1c)) of the fish the end of the experimental period.

*Hepatic metabolism*

For alanine aminotransferase, no influence of treatments was observed (48.33±6,68; 49,16±3.73; 49,79±6.93 UI mg tissue$^{-1}$ for corn, broken rice and sorghum). On the other hand, significant differences were found for the others hepatic metabolism parameters evaluated. The hepatic protein was significantly higher in fish fed with sorghum in diet when compared to corn and broken rice (figure 2a). Diets containing sorghum and rice provided higher values of free amino acids in relation to the corn-based diet (figure 2b). Ammonia was also higher in fish fed with sorghum in the diet, and lower in animals treated with corn (figure 2c). The carbohydrate

source effect on hepatic glucose is shown in figure 2d. The Duncan test showed a significant difference ($P < 0.05$). Fish fed broken rice or sorghum in the diet had higher hepatic glucose values when compared to corn-based treatments, but did not differ from each other.

*Nutrient retention coefficient and body composition*

Protein retention coefficient of juvenile *Piaractus mesopotamicus* was unaffected (37.57±5.08, 35.40±3.42, 36.68±2.03) by carbohydrate sources (corn, broken rice or sorghum) of the diets ($P > 0.05$).

After the end of the experimental period, it was verified that the sorghum diet resulted in higher protein content in the whole fish (fig. 3a) and carcass yield (fig. 3b) ($P < 0.05$). For intestinal quotient (fig. 3c), the higher value was verified in the fish fed with broken rice in diet.

*Digestive enzyme*

The digestive enzyme activity was affected by the carbohydrate source in the diet. The trypsin reduced their activity in the animals fed with the diet in which sorghum was included ($P < 0.05$), being similar ($P > 0.05$) between corn and broken rice (fig. 4).

## Discussion

This study investigated whether carbohydrate sources interfere with the growth and metabolism of *Piaractus mesopotamicus*. Thus, changes in biochemical, blood, enzymatic, and body composition of juveniles of *P. mesopotamicus* were observed, however, not influencing growth.

The growth results at the end of the experimental period (60 days) demonstrate that the amylose:amylopectin ratios of 0.81; 0.83 and 0.56 present in present in diets based on corn, broken rice and sorghum, respectively, did not alter the variables of final weight, daily average gain, relative weight gain, condition factor, and food intake. Research on this topic demonstrate varied results, however, it is known that the complexity and type of carbohydrate chain and

dietary habits can significantly influence the energy utilization of the diets, as well as the growth and the protein sparing effect (Lin, Cui, Hung & Shiau 1997; Hemre, Mommsen & Krogdahl 2002; Wu, Liu, Tian, Mai & Yang 2007; Young, Morris, Huntingford & Sinnott 2005).

Studies conducted with *Dicentrarchus labrax*, by Enes *et al.* (2006) no found significant differences in productive performance when fed with 10 and 20% waxy corn starch (high amylose concentration) or normal corn starch. For *Rhamdia quelen*, diets containing different rice varieties (30% inclusion) with different concentrations of amylose (0, 16 and 26%) did not result in altered growth (Pedron *et al.* 2011) either. Enes *et al.* (2008) also observed the same trend as in the present study, juveniles of *Sparus aurata* fed with diets containing 1 or 28% amylose. Results obtained by Sanchez, Nascimento and Hisano (2016) found that in the feeding *P. mesopotamicus* with diets containing corn, the weight gain, feed conversion and growth rate were also unaffected when replaced of 0; 25; 50; 75 ou 100% by sorghum.

On the other hand, high levels of amylose in starch (70%) resulted in better growth for sunshine bass (*Morones chrysops* x *M. saxatilis*) (Rawles & Lochman 2003). In juveniles of the Nile tilapia *Oreochromis niloticus* fed diets containing different ratios amylose / amylopectin (0.11, 0.24, 0.47, 0.76 and 0.98) were observed best productive responses in the fish that received the diet with the ratio of 0.24 (Chen et al. 2013).

Amylose concentrations varying from 35 to 45%, present in this study, can be efficiently used to feed *P. mesopotamicus* without negatively affecting the performance of the animals. Thus, the good acceptance of the different diets tested, the nutritional balance of the formulations, the availability of nutrients for the synthesis and the inherent characteristics of the species studied help to justify the performance similarity among the treatments.

In the liver of the animals fed with the broken rice experimental diet, it was observed a significant reduction ($P < 0.05$) of the protein, with increase of ammonia, free amino acids and glucose. This, associated to the increase in plasma glucose (article 1, figure 1d) would indicate

that the animals would be deaminating the amino acids, possibly due to the gluconeogenic state, with consequent glucose exportation to the other tissues. The lower fiber values, associated with the higher starch content, higher trypsin activity and other enzymes (article 1, figures 3a-c), contributed to a reduction in the passage rate, maximizing hydrolysis, rapid absorption and glucose utilization, and, finally, after 24 hours of fasting, it contributed to obtaining glucose from glyconeogenic substrates, such as amino acids.

On the other hand, the animals belonging to the sorghum treatment indicated that they were transaminated, since they presented higher values of protein, free amino acids, ammonia and hepatic glucose, however without raising ($P < 0.05$) the circulating glucose (article 1, figure 1d). Possibly, in this scenario, together with the highest values of protein content in whole fish, protein retention coefficient and carcass yield suggest a targeting of amino acids for muscle deposition and protein sparing effect.

In fish fed corn in the diet, there were a reduction in catabolic metabolism, lower values of protein, free amino acids and hepatic ammonia, indicating that the animals of this treatment were able to efficiently take advantage of the diet carbohydrates, sparing the protein as an energy source. Other important factors to be highlighted would be the higher amylose: amylopectin ratio of this diet compared to the broken rice treatment. A higher proportion of amylose may have promoted a slower rate of glucose digestion and absorption (article 1, figure 5) by the animals of this treatment and this may have benefit the overall glucose cellular uptake, with more steady glycemic levels and possibly more favorable hormonal responses.

With the nitrogenous compounds increase in the liver of animals fed broken rice and sorghum, the activity of alanine aminotransferase was expected to increase. However, not only this one but other enzymes are also important in the catabolism of amino acids, such as aspartate aminotransferase (ASAT) and glutamate dehydrogenase (GDH). When evaluating the protein metabolism of *R. quelen*, an omnivorous species, Melo, Lundstedt, Metón, Baanante and

Moraes (2006) verified that ASAT and GDH present a more effective role than ALAT in both fed and fasting conditions. Perhaps this is the reason why no statistical difference was detected in this variable.

Total protein and its fractions are also important in evaluating the health status of animals since they act to regulate the inflammatory response and resistance to infection. Changes in total protein concentration may be a result of variations in albumin, globulins, or both. The increase in the synthesis of some proteins is seen in cases of stress and inflammation, resulting in increased production of globulins (α and β fractions) that migrate as a response to the acute phase. Unbalance in the production of one protein may compensate that of another one. This relationship can identify abnormalities in total protein concentration, such as in the acute phase response, in which increased globulins are associated with decreased albumin concentration (Kratz, Lee-Lewandrowski & Lewandrowski 2002). Some vegetable origin ingredients are known to promote some degree of inflammatory response in intestinal tissue and it is possible that some substance present in sorghum acted as an allergen, although without a histological evaluation this cannot be confirmed.

Serum albumin level was lower ($P < 0.05$) in the fish fed sorghum. This has an essential role in the transport of fatty acids, and perhaps the reduction of trypsin activity in these animals, which is responsible for the activation of both colipase and phospholipase A, which are fundamental in the hydrolysis process of fatty acids, has implicated the lowest concentrations of circulating albumin. In addition to being the constituent of the largest protein fraction in the blood of vertebrates (about 55%), albumin is also responsible for several functions, such as osmotic pressure regulator, lipid and hormones transporter, among others (Gray & Doolittle1992).

The lower celomic fat (article1, figure 2b), higher protein biosynthesis, protein in the whole fish and protein retention coefficient resulted in higher carcass yield in the fish treated with sorghum.

Fish from this treatment also had a reduction in trypsin activity. The condensed tannins present in this ingredient inhibit the activity of a number of enzymes, including cellulases, pectinases, amylases, lipases, proteolytic enzymes and alpha-galactosidases (Chung, Wong, Wei, Huang & Lin 1998). However, such suppression in enzymatic activity did not result in impairment of *P. mesopotamicus* growth. It is important to note that this species is characterized as omnivorous. In its natural environment, it feeds mainly of fruits, seeds and leaves, conferring it a physiological, anatomical and functional adaptation of the gastrointestinal tract, as well as associated organs, in order to maximize the exploration of different foods.

Adaptations of the gastrointestinal tract, as well as increased rates of cell proliferation, hyperplasia and protein synthesis, may be understood as an attempt to expand the contact area with food and consequently increase nutrient absorption, reflecting higher intestinal quotient values (Leenhouwers, Adjei-Boateng, Verreth & Schrama 2006). Changes in intestine length are related to enterocytes, as they use part of the nutrients that cross their cytosol into the bloodstream, triggering internal stimuli for greater or lesser cell multiplication, depending on the flow (Costa 2017). For the author, the intestine is as long as the greater quantity of nutrients that reach the distal portion, since they stimulate the proliferation of enterocytes in this region and, consequently, of all the anterior ones where it passed, stretching the intestine from the moment when the maximum development of villi has occurred. However, this only happens if the food ingested is slow-digesting. It is likely that the lower fiber values and higher amylose and cellulose added to the broken rice diet have increased the food bolus permanence along the tract and allowed more nutrients to pass to the final portions of the intestine, justifying the greater intestinal quotient.

## Conclusion

The study demonstrated that corn, broken rice or sorghum are equally suitable to be used in n the diet of *Piaractus mesopotamicus* juveniles without any discernible adverse effects on survival and growth. The use of corn in the diets resulted in lower hepatic ammonia levels, indicating improved protein sparing effect compared to the other ingredients tested in this study, although sorghum showed higher carcass yield and protein content in whole fish.

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**Table 1** Formulation and proximal composition of the experimental diets

| INGREDIENTS (g $kg^{-1}$) | Corn | Broken rice | Sorghum |
|---|---|---|---|
| Soybean meal | 484.6 | 490.2 | 480.5 |
| Fish meal | 50.0 | 50.0 | 50.0 |
| Grain sorghum | - | - | 284.7 |
| Grain corn | 300.6 | - | - |
| Broken rice | - | 316.0 | - |
| Soybean oil | 20.0 | 20.0 | 20.0 |
| Crystalline cellulose | 30.9 | 35.2 | 29.5 |
| Dicalcium phosphate | 27.5 | 25.5 | 27.0 |
| DL-methionine | 0.2 | - | 0.2 |
| Chromic oxide | 1.0 | 1.0 | 1.0 |
| L-lysine | 2.2 | 1.7 | 2.3 |
| Inert | 77.8 | 55.2 | 99.6 |
| Mineral and vitamin supplement[1] | 5.0 | 5.0 | 5.0 |
| Antioxidant[2] | 0.2 | 0.2 | 0.2 |
| PROXIMAL COMPOSITION (g $kg^{-1}$) | | | |
| Crude protein[3] | 306.6 | 308.7 | 316.0 |
| Digestible energy (MJ $Kg^{-1}$)[4] | 121.6 | 121.6 | 121.5 |
| Insoluble fiber[3] | 129.8 | 120.3 | 128.3 |
| Soluble fiber[3] | 38.4 | 32.9 | 38.5 |
| Total starch[3] | 246.1 | 318.4 | 251.0 |
| Amylose[3] | 110.4 | 144.7 | 90.1 |
| Amylopectin[3] | 135.7 | 173.6 | 160.9 |
| Amylose: amylopectin[3] | 0.81 | 0.83 | 0.56 |
| Lipids[3] | 55.2 | 42.4 | 55.0 |
| Ash[3] | 151.7 | 168.5 | 152.4 |
| Tannin | - | - | 2.4 |
| Lysine[4] | 16.4 | 16.4 | 16.4 |
| Methionine[4] | 3.8 | 3.9 | 3.8 |
| Calcium[4] | 11.0 | 10.6 | 10.9 |
| Phosphorus[4] | 7.0 | 7.0 | 7.0 |

[1] Mineral and vitamin supplement composition $kg^{-1}$: Selenium, 75 mg; iron, 15 g; copper, 1,250 mg; manganese, 3,750 mg; zinc, 17.5 g; cobalt, 50 mg; iodine, 100 mg; niacin, 8,750 mg; folic acid, 625 mg; panthotenic acid, 7,500 mg; biotin, 50 mg; vitamin C, 37.5 g; choline, 100 g; Inositol, 12.5 g; vitamin A, 1,750,000 UI; vitamin D3, 375,000 UI; vitamin E, 20,000 UI; vitamin K, 3,500 mg, vitamin B1, 2,000 mg; vitamin B2, 2,500 mg; vitamin B6, 2,500 mg; vitamin B12, 5;000 mcg;
[2] Butyl-Hydroxy-Toluene;
[3] Analyzed values;
[4] Calculated values.

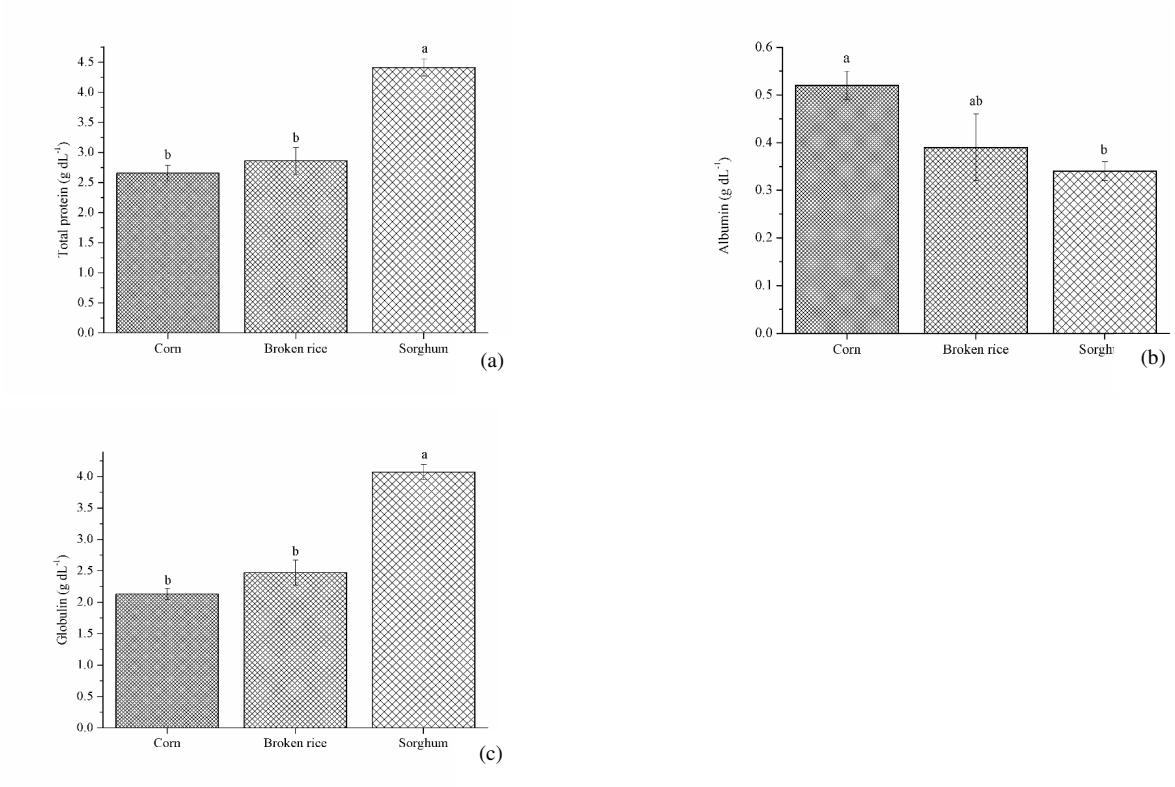


**Figure 1** Total protein (P=0.000) (a), albumin (P=0.035) (b) and globulin (P=0.000) (c) of *Piaractus mesopotamicus* juveniles (Results represent means ± SD, n=15) fed with different starch sources in the diet. Different letters in (a) and (c) indicate statistic difference by the Duncan test ($P < 0.05$), and in (b) by the non-parametric Kruskal-Wallis test

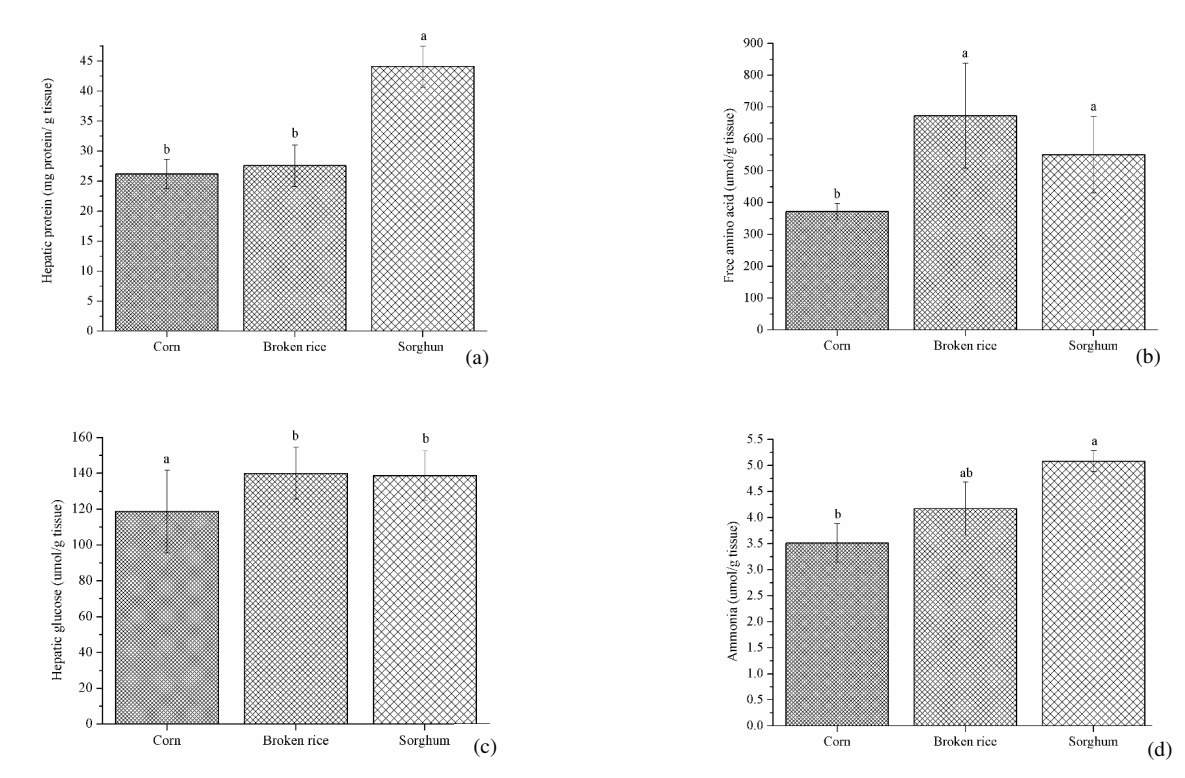


**Figure 2** Hepatic protein (P=0.001) (a), hepatic free amino acid (P=0.000) (b), hepatic ammonia (P=0.032) (c) and hepatic glucose (P=0.025) (d) of *Piaractus mesopotamicus* juvenile (Results represent means ± SD, n=15) fed for 60 days diets formulated with the inclusion of different carbohydrate sources. In (a) and (d) different letters indicate statistic difference by the Duncan test ($P < 0.05$). In (b) and (c), different letters indicate statistic difference by the non-parametric Kruskal-Wallis test ($P < 0.05$)

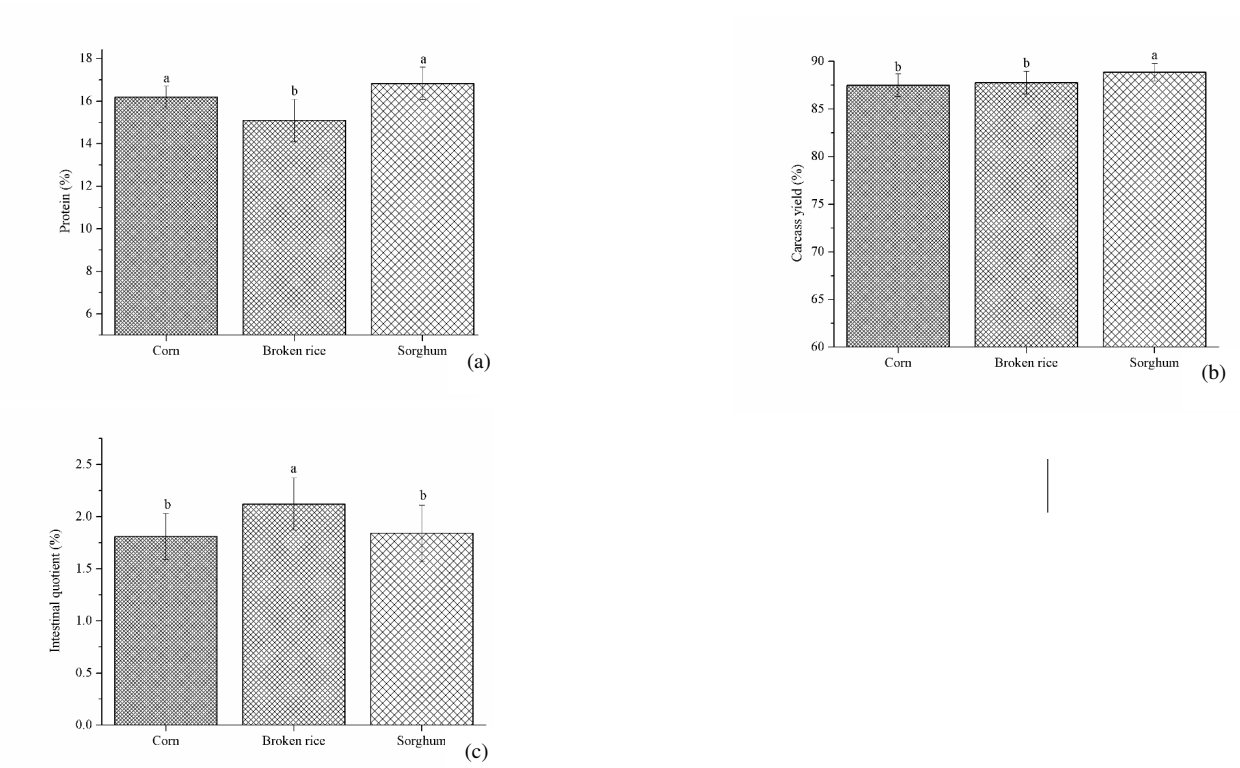


**Figure 3** Body composition of protein (P=0.000) (a) carcass yield (P=0.030) (b) and intestinal quotient (P=0.019) (c) of juvenile (Results represent means ± SD, n=15) *Piaractus mesopotamicus* at the end of the biological assay. Different letters indicate statistic difference by the Duncan test ($P < 0.05$)

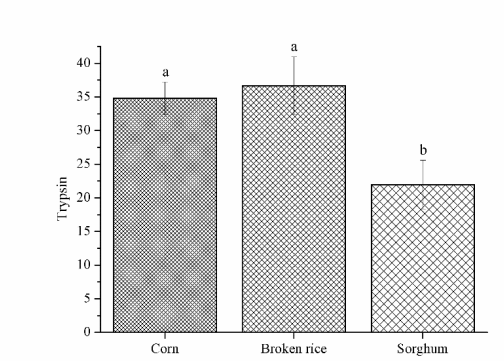


**Figure 4** Effect of the carbohydrate source on the trypsin activity (P=0.019) of *Piaractus mesopotamicus* juveniles (Results represent means ± SD, n=15), after 60 days of feeding. Different letters in the line indicate statistic difference by the Duncan test ($P < 0.05$). Value expressed in µmol TAME /min/mg protein

**Anexos**

*Submissão do artigo do capítulo 2*.

Submission ID: AQUI-D-17-00490

Growth and energetic metabolism of *Piaractus mesopotamicus* fed with carbohydrate sources in the diet

Full author list: Marco Aurélio Della Flora, M.D; Leila Picolli da Silva; Cátia Aline Veiverberg; Suziane Martinelli; Bruno Loureiro; Marina Dalcin; Ronald Luz; Paula Adriane Ribeiro

Dear M.Sc. Della Flora,

Thank you for submitting your manuscript, Growth and energetic metabolism of *Piaractus mesopotamicus* fed with carbohydrate sources in the diet, to Aquaculture International.

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