UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CENTRO DE AQUICULTURA DA UNESP

Grãos de milho de destilaria secos com solúveis em dietas para juvenis de *Piaractus mesopotamicus* (Holmberg 1987)

Kátia Rodrigues Batista de Oliveira

Jaboticabal, São Paulo

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Sumário

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Resumo

Devido ao maior interesse por biocombustíveis, indústrias brasileiras iniciaram, recentemente, a produção de etanol também a partir de grãos de milho, gerando um resíduo com potencial de uso como ingrediente em rações para animais, o DDGS (grãos secos de destilaria com solúveis). Por resultar de processos de fermentação de grãos de milho por leveduras e enzimas, este resíduo possui um elevado teor proteico e baixo teor de carboidratos solúveis, o que o torna boa fonte de proteína vegetal em rações para animais. Além do baixo custo, possíveis benefícios relacionados aos resíduos de leveduras e enzimas restantes da fermentação também contribuem para seu potencial de mercado. Desta forma, com este trabalho, objetivou-se avaliar a viabilidade de inclusão do DDGS do milho em dietas para juvenis de *Piaractus mesopotamicus* em substituição ao farelo de soja. Para tal, foram realizados três ensaios experimentais. No primeiro ensaio avaliaram-se os coeficientes de digestibilidade aparente (CDA) de nutrientes do DDGS para juvenis de *P. mesopotamicus* (13±0.3 gramas), distribuídos, em delineamento inteiramente casualizado (DIC), em seis tanques de fibra de vidro, na densidade de 35 peixes tanque $^{-1}$ em sistema de recirculação contínuo de água. A coleta das fezes foi realizada em sistema de Guelph modificado. Após obtenção dos CDAs, foram formuladas dietas contendo cinco diferentes níveis de inclusão de DDGS (0, 10, 20, 30 e 40%) utilizadas nos ensaios posteriores. O segundo ensaio consistiu na avaliação dos CDAs dos nutrientes das dietas contendo 0, 10, 20, 30 e 40% DDGS, onde juvenis de *P. mesopotamicus* (27±1.4 gramas) foram distribuídos em cinco tanques de fibra de vidro na densidade de 30 peixes tanque $^{-1}$ em sistema de recirculação de água. Utilizou-se delineamento em Quadrado Latino, 5x5 (05 dietas e 05 períodos). Concomitantemente ao segundo ensaio, e sob o mesmo sistema de recirculação, juvenis de *P. mesopotamicus* (21±0.2 gramas) foram distribuídos em 20 tanques de fibra de vidro, na densidade de 15 peixes tanque $^{-1}$, em DIC, e alimentados com as dietas por 100 dias. Neste terceiro ensaio foram avaliados parâmetros de desempenho produtivo, viabilidade econômica, atividade das enzimas digestivas e de estresse oxidativo do intestino, bem como morfologia intestinal dos juvenis. Os dados obtidos de desempenho produtivo, estresse oxidativo e morfometria intestinal foram submetidos à one-way

ANOVA e em caso de significância ($p < 0.05$) foi realizado teste de Tukey adotando-se 5% como nível de probabilidade. Dados de enzimas digestivas foram submetidos a two-way ANOVA e em caso de significância para interação foi feita uma one-way ANOVA e teste Tukey a 5%. Os valores obtidos para os CDA do DDGS confirmaram seu potencial de uso como ingrediente proteico em dietas para *P. mesopotamicus*, assim como os resultados de desempenho produtivo, onde se obteve menor valor de conversão alimentar e melhor eficiência de retenção de proteína para a dieta contendo maior nível de inclusão de DDGS (40DDGS). Os demais parâmetros de desempenho não foram afetados significativamente. A atividade das enzimas digestivas foi reduzida da porção anterior do intestino para distal e para as dietas com níveis superiores a 10% de DDGS. A inclusão de DDGS levou a redução do status oxidativo do intestino e melhoras na morfometria intestinal. Sendo assim, é possível o uso de até 40% de DDGS do milho como ingrediente proteico em dietas para juvenis de *P. mesopotamicus*, substituindo em totalidade o farelo de soja, mantendo os valores de desempenho produtivo, melhorando a saúde intestinal dos peixes bem como a capacidade de absorção e aproveitamento dos nutrientes disponibilizados na dieta.

Palavras-Chave

Biocombustíveis, DDGS, enzimas intestinais, pacu, peixes nativos.

Abstract

Due to the increased interest in biofuels, Brazilian companies started recently, the production of ethanol from corn, generating a waste with potential for use as an ingredient in animal feed, the DDGS (dried distillers grain with soluble). As its processes results from fermentation of corn grain by yeast and enzymes, this residue has high protein and low soluble carbohydrates, which makes it good source of vegetable protein for animal feed. Besides the low cost, possible benefits related to yeast residues and other enzymes from fermentation may also contribute to DDGS market potential. Thus, this work aimed to evaluate the feasibility of inclusion of corn DDGS in diets for *Piaractus mesopotamicus* juveniles to replace soybean meal. To this end, there were three experimental runs. In the first assay we evaluated the apparent digestibility coefficients (ADC) of DDGS nutrients for *P. mesopotamicus* (13 ± 0.3 grams), distributed in a completely randomized design (CRD) in six fiberglass tanks, at density of 35 fish tank^{-1} in a continuous recirculating water system. The collection of feces was carried out in modified Guelph system. After obtaining the ADCs, diets were formulated with five different levels of DDGS inclusion (0, 10, 20, 30 and 40%) used in subsequent assays. The second test was the evaluation of ADCs of nutrient in the diets containing 0, 10, 20, 30 and 40% DDGS where *P. mesopotamicus* juvenile (27 ± 1.4 g) were distributed in five fiberglass tanks at density of 30 fish tank^{-1} in a recirculating water system. We used a Square Latino design, 5x5 (05 diets and 05 periods). Concomitantly to the second test, and under the same recirculation system, *P. mesopotamicus* juveniles (21 ± 0.2 grams) were divided into 20 fiberglass tanks, at density of 15 fish tank^{-1} in CRD, and fed diets for 100 days. In this third test were evaluated growth performance, economic viability, activity of digestive enzymes and oxidative stress of the intestine and intestinal morphology of juveniles. Data obtained for growth performance, oxidative stress and intestine morphology were subjected to one-way ANOVA and in case of significance ($p < 0.05$) Tukey's test was carried out adopting a 5% probability level. Data from digestive enzymes were subjected to two-way ANOVA and in case of significance to interaction was made a one-way ANOVA and Tukey's test at 5%. The ADCs values obtained for the DDGS confirmed its potential use as a protein ingredient in diets for *P. mesopotamicus*, as well as the results of

productive performance, which showed lower value of feed conversion ratio and an improved on protein retention efficiency for the diet containing higher inclusion level of DDGS (40DDGS). The other performance parameters were not significantly affected. The activity of the digestive enzymes was reduced from anterior to distal portion of the intestine and for diets with levels above 10% DDGS. The DDGS inclusion led to reduction of oxidative status of the intestine and improvement in intestinal morphology. Thus, the use of up to 40% corn DDGS as a protein ingredient for *P. mesopotamicus* juvenile is possible, replacing in whole soybean meal, keeping the growth performance, improving fish gut health as well as the absorption and utilization of nutrients available in the diet.

Key-words

Biofuels, DDGS, intestinal enzymes, pacu, native fish

Introdução Geral

A aquicultura mundial encontra-se em fase de intenso crescimento, com produção prevista de mais de 186 milhões de toneladas para o ano de 2030 (World Bank, 2013). Este aumento de produção resulta em maior demanda por ingredientes para fabricação de rações, como farinha e óleo de peixe. Contudo, a produção destes ingredientes não tem se mostrado suficiente para atender a demanda de mercado (FAO, 2012), elevando os preços de custo e consequentemente o valor a ser pago pelo consumidor. Dessa forma, torna-se necessário a busca por ingredientes alternativos sustentáveis, que apresentem maior disponibilidade e menor custo de produção, como é o caso de alguns produtos de origem vegetal (Ayadi, 2012).

O principal ingrediente utilizado atualmente como substituto à farinha de peixe é o farelo de soja, por apresentar alto teor de proteína (48%), considerável balanço de aminoácidos (Gatlin et al., 2007), alta disponibilidade e preço razoável (Refstie et al., 2000; Thompson et al., 2008). Porém, este possui alguns fatores antinutricionais que podem prejudicar o consumo das rações e o ganho de peso dos animais (Francis et al., 2001). Existe um vasto grupo de ingredientes que possuem potencial para serem utilizados na fabricação de rações para animais como fontes proteicas alternativas (Ayadi, 2012). Os resíduos de grandes indústrias, por exemplo, tornam-se uma boa opção, visto que seu uso também permite a reutilização de produtos já descartados pelas indústrias e que possivelmente se tornariam um problema ambiental, como é o caso dos resíduos da indústria do etanol (Wyman, 1996).

O milho e a cana de açúcar são as principais matérias primas utilizadas na fabricação de etanol devido ao elevado teor de sacarose e amido, sendo a cana de açúcar utilizada principalmente no Brasil e o milho nos Estados Unidos. No entanto, no período da entressafra da cana de açúcar no Brasil as destilarias ficam com a produção limitada devido à menor disponibilidade desta matéria prima (Duarte et al., 2012). Estima-se que a produção de álcool no Brasil alcance 64 bilhões de litros em 2017, podendo até mesmo superar os Estados Unidos em exportação de etanol (Carvalho, 2009). Dessa forma, a utilização de milho como matéria prima para a produção de etanol no Brasil aparece como uma provável opção para usinas, uma

vez que possibilitaria, principalmente, uma produção de etanol mais constante ao longo do ano, permitindo assim uma maior competitividade do etanol brasileiro no mercado externo.

Quanto à produção de milho, mesmo sendo crescente e as exportações estarem em alta, ainda há uma perda de competitividade do milho brasileiro no mercado mundial devido, principalmente, às deficiências no transporte da produção até os portos de exportação (Duarte et al., 2012). Portanto, utilizar o milho como substrato para produção de etanol no Brasil torna-se uma alternativa, tanto por prover uma destinação viável ao excedente de produção, manter a produtividade das usinas na entressafra da cana, bem como por gerar resíduos com potencial de uso pelas fábricas de ração, como é o caso do DDGS.

DDGS ou grãos secos de destilaria com solúveis resultam da fermentação de grãos de milho pela adição de leveduras e enzimas. Após a fermentação, o líquido produzido é destilado e destinado à produção do etanol, enquanto o restante segue para um conjunto de centrífugas, onde se separam a parte fina, que será reaproveitada no processo ou levada para evaporação, e a parte mais grosseira, que após centrifugação originará o DDG (Disttilers Dried Grains) (Wyman, 1996). Ao adicionar a parte fina ao DDG obtém-se o DDGS (Distillers Dried Grains with Soluble) que também pode ser utilizado na alimentação animal. Portanto, as características do produto final, DDG ou DDGS, dependerão das práticas de fabricação adotadas pelas usinas de etanol.

Por perder grande parte dos carboidratos durante a fermentação, o DDGS torna-se um produto mais concentrado que o milho em proteína e baixa quantidade de amido (Jacques et al., 2003). Além de não possuir fatores antinutricionais (Lim et al., 2009), seu custo está bem abaixo quando comparado ao da soja (Ayadi, 2012), onde a tonelada de DDGS está cotada em R$ 500,00 (Fonte: Libra Etanol, 2016) e a da soja a R$ 1.060,00 (Fonte: Conab, 2016). Por ser um resíduo industrial sua composição nutricional está sujeita a grandes variações que podem estar relacionadas tanto aos métodos de produção do DDGS quanto à qualidade e composição do grão utilizado (Shurson e Alghamdi, 2008).

Cromwell et al. (1993) avaliando nove diferentes fontes de DDGS, observaram diferenças na composição nutricional, química e até mesmo física das amostras. O mesmo foi observado por Spiehs et al. (2002) onde foram avaliadas 118 amostras

de DDGS oriundos de 10 diferentes usinas de etanol durante os três anos consecutivos 1997, 1998 e 1999 e por Belyea et al. (2004) analisando a composição de 235 amostras de DDGS, produtos de uma usina de etanol no estado de Minnesota, Estados Unidos. Liu (2008) também avaliou a composição nutricional em função da distribuição dos tamanhos de partículas contidos em 11 amostras de DDGS do milho. Dentre estes autores, maiores variações na composição química das amostras de DDGS foram obtidas por Cromwell et al. (1993) contrastando com Spiehs et al. (2002), Belyea et al. (2004) e Liu (2008). Essa diferença pode estra relacionada à maior padronização dos métodos de produção de DDGS pelas usinas ao longo dos anos, visto que esta é uma necessidade do ponto de vista nutricional, uma vez que permite a otimização da inclusão do DDGS na alimentação animal.

Dentre os nutrientes contidos no DDGS a proteína apresenta variações mais bruscas, o que pode estar relacionado ao perfil de aminoácidos. Segundo Cromwell et al. (1993) e Spiehs et al. (2002) a lisina é o aminoácido que mais sofre variações dentre as diferentes amostras de DDGS, seguida pela metionina e triptofano. Durante seu processamento o DDGS passa por temperaturas muito elevadas, podendo chegar até 550°C. Estas elevadas temperaturas podem modificar a estrutura das proteínas contidas no DDGS bem como o perfil dos aminoácidos. A lisina, por exemplo, é altamente susceptível a altas temperaturas, estando sua concentração muitas vezes ligada aos tons de coloração do DDGS (Cromwell et al., 1993; Fastinger et al.,2006). Tons mais escuros indicam provável ocorrência de reação de Maillard (Stein et al., 2006) e portanto menor concentração de lisina, sendo o oposto observado para DDGS de coloração mais clara. Contudo alterações na coloração do DDGS também podem estar relacionadas a outros fatores como a cor do grão e quantidade de solúveis adicionados antes do processo de secagem final.

Quanto à composição de minerais, o DDGS possui grande quantidade de fósforo na forma disponível, o que possibilita maior absorção deste mineral pelos peixes e minimiza impactos ambientais. Possui também um elevado teor de sódio, cálcio e enxofre, podendo ter uma concentração destes minerais de até três vezes do que o observado para grãos de milho (Liu e Han, 2006). Além disto, o DDGS possui relativa quantidade de leveduras, estimada em 3.9% (Ingledew et al., 1999),

o que pode trazer benefícios tanto para a saúde da microbiota intestinal (He et al., 2013) como para o sistema imune dos animais (Lim et al., 2009).

Na aquicultura, o DDGS tem se apresentado como candidato à substituição da farinha de peixe quando utilizado em conjunto com outra fonte proteica animal (Coyle et al., 2004) e também quando em substituição ao farelo de soja (Øverland et al., 2013; Zhou et al., 2010). Para bagre do canal (*Ictalurus punctatus*) resultados positivos foram obtidos para níveis de até 30% de inclusão de DDGS (Tidwell, 1990; Webster et al., 1993; Robinson e Lee, 2008; Zhou et al., 2010). A inclusão de DDGS em dietas para tilápia do Nilo (*Oreochromis niloticus*) na faixa de 20 a 30% pode ser realizada sem causar alterações deletérias no crescimento e ou saúde do animal (Schaeffer et al., 2009). Já para truta do arco-íris (*Oncorhynchus mykiss*) é possível à substituição de até 50% da farinha de peixe nas dietas (Cheng e Hardy 2004a), sendo que 15% de DDGS foi o nível máximo incorporado com sucesso para esta espécie.

Pode ser uma eficiente fonte de proteína principalmente para espécies onívoras, uma vez que as exigências por dietas com altos níveis proteicos não são tão acentuadas como para as espécies de hábito alimentar carnívoro (Boscolo et al., 2011; Gatlin et al., 2007; Hardy, 2010). Além disso, peixes onívoros podem tolerar até 9% de fibra bruta na dieta (Rodrigues et al., 2010), minimizando as limitações ao uso do DDGS pelo seu alto teor de fibras. Portanto, a determinação dos níveis de inclusão de DDGS em dietas para animais aquáticos vai depender, dentre outros fatores, do hábito alimentar da espécie e demanda por nutrientes.

Piaractus mesopotamicus é uma espécie de peixe tropical, endêmico das planícies alagadas da região Centro-Oeste do Brasil. Pertencente à família *Myleinae* é um peixe de hábito alimentar onívoro-frutívoro, alimentando-se principalmente de folhas, caules, flores, frutos e sementes. Comumente conhecido como pacu ou pacu-caranha, esta espécie apresenta relativa facilidade de cultivo em cativeiro devido a sua rusticidade, boa adaptabilidade e capacidade de ganho de peso (Nunes, 2006). Apreciado pela culinária devido à boa qualidade e sabor da carne, também é explorado na pesca esportiva (Jomori et al., 2003). Pesquisas relacionadas à nutrição do pacu vêm sendo conduzidas visando melhorar a eficiência de criação desta espécie e alguns dados já se encontram disponíveis, como exigência proteica (Batista et al., 2000;. Abimorad et al., 2008), exigência de

lisina (Bicudo et al., 2009) e coeficientes de digestibilidade aparente para alguns ingredientes (Abimorad et al., 2008).

Tendo em vista a importância do pacu na aquicultura brasileira, faz-se necessário maior incentivo e investimento nas pesquisas utilizando ingredientes proteicos alternativos à farinha de peixe e farelo de soja, gerando informações que visem melhorias na eficiência de criação desta espécie. Dessa forma objetivou-se avaliar o valor nutritivo do DDGS do milho e a influência de seu uso em substituição ao farelo de soja, em dietas para juvenis de pacu (*Piaractus mesopotamicus*) sobre os parâmetros de desempenho produtivo, atividades das enzimas digestivas e de estresse oxidativo do intestino, microbiologia intestinal e viabilidade econômica.

Para fins didáticos, este projeto foi dividido em dois capítulos, ambos escritos em formato de artigos para serem prontamente submetidos à publicação científica. O primeiro capítulo “Effect of corn DDGS on growth performance, feed utilization and digestibility of *Piaractus mesopotamicus* juveniles” refere-se a uma avaliação prévia do potencial de uso do DDGS do milho em dietas para o pacu, através da avaliação dos parametros de digestibilidade, desempenho produtivo e viabilidade econômica. O segundo capítulo “Effect of corn DDGS on digestive enzymes, oxidative stress and intestine morphology of *Piaractus mesopotamicus* juveniles” trata-se de uma avaliação a nível fisiológico dos possíves efeitos provocados pela inclusão de DDGS do milho nas dietas para o pacu em remoção total ao farelo de soja, através da determinação da atividade das enzimas digestivas, status oxidativo e morfologia intestinal. Ao final, correlacionado os dois capítulos apresentados, torna-se possível uma análise mais ampla e completa do potencial de uso do DDGS do milho em dietas para pacu, como produtividade, viabilidade econômica e ambiental, bem como possíveis efeitos na saúde intestinal dos peixes.

Manuscript 01:

Effect of corn DDGS on growth performance, feed utilization and digestibility of *Piaractus mesopotamicus* juveniles

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Abstract

DDGS (Distiller's Dried Grains with Soluble) is a potential ingredient for soybean meal replacement in animal diets due to reductions on production costs. The aim of this study was to evaluate the use of DDGS as a substitute of soybean meal in diets for *P. mesopotamicus* juveniles. Three experimental trails were performed, being the digestibility of the ingredient (DDGS) the first one, with 210 *P. mesopotamicus* juveniles (13±0.3 grams) randomly allocated in six tanks (35 fish tank^{-1}), fed a diet containing 30% DDGS, 69.5% referential diet and 0.5% chromic oxide, for seven days. Fecal samples were collected and analyzed due to estimate Apparent Digestibility Coefficient (ADC) for protein, energy, dry matter, lipid and phosphorus. After obtaining data, diets were formulated to contain different levels of DDGS inclusion (0, 10, 20, 30 and 40%). The second trial consisted in determine the ADCs for the diets mentioned above. Thus, *P. mesopotamicus* juveniles (27±1.4grams) were stocked in five tanks (30 fish tank^{-1}) in Latin square design 5 x 5 (five treatments and five experimental periods). During each period, fish were fed with experimental diets, added 0.5% chromic oxide, for seven days and feces were collected. The third experiment was a growth trial with 300 *P. mesopotamicus* juveniles (21±0.2 grams) randomly distributed in 20 fiber glass tanks, divided in five treatments (0, 10, 20, 30 and 40% DDGS) and four replicates, feeding experimental diets for 100 days. Growth performance, economic viability, feed utilization and

digestibility were evaluated. All data were subjected to a one-way ANOVA analysis of variance to determine significant ($p \leq 0.05$) differences among treatments and subsequently Tukey's test. The lower ADC was obtained for dry matter (62.7 %) and the higher for protein (94.8 %) and phosphorus (91 %). The ADCs for dry matter and energy reduced significantly ($p < 0.05$) with DDGS inclusion. The opposite was true for lipid that decreased from 94.1% for 40DDGS diet to 87.8 % for the control without DDGS inclusion. Also, it was observed a decrease ($p < 0.05$) in total phosphorus release in the water with increasing levels of DDGS in the diets. Feed conversion ratio and efficiency protein ratio were positively affected by DDGS inclusion ($p < 0.05$). The other growth parameters did not differ ($p > 0.05$) between treatments. Dietary costs of weight gain were reduced ($p < 0.05$) in 26% with DDGS inclusion and soybean meal replacement. Thus, it is possible the inclusion of 40% of corn DDGS as a plant protein source in diets for *P. mesopotamicus* in total replacement of soybean meal without provide negative effects on fish production.

Key-words

DDGS, industry residues, native fish, pacu, plant protein, phosphorus availability

Introduction

Residues represent a challenge for industries since they have not more propose in the productive chain, generating difficulties especially related with storage and discard. In the United States of America, where the corn is the major source of starch for ethanol production, the amount of product and residues remained are relatively close. Each ton of corn grain produces 465 liters of ethanol and 300 kg of residue (DDGS) (Wyman, 1996). To evaluate the potential reuse of residues from industries is an alternative that would benefit both, industries and environment. The evaluation of the viability of these residues as ingredients in animal feed appears to be an alternative solution.

Distiller's dried grain with soluble is the residue derived from the fermentation of grains by the action of enzymes and yeasts on ethanol production (Wyman, 1996). Its chemical composition may vary according to the grain source and methods of fermentation (Liu, 2009; Lim et al., 2011), but basically consists of 28 - 33% of crude protein, 3.5- 12.8% of lipid, 5.4 to 10.6% of crude fiber, 2.8 to 9.8% of ash, 0.5-1.1% of lysine and 0.5-0.8% of methionine (Ayadi et al., 2012).

DDGS apparent digestibility coefficients have being established for vary terrestrial animals species with commercial interest as broilers (Liu, 2011), swine (Urriola and Stein, 2010; Yang et al., 2010; Pedersen et al., 2007) and rabbits (Youssef et al., 2012). Nevertheless, some aquatic animals are also being focus of the studies regardless DDGS digestibility like Pacific white shrimp, *Liponeaus vannamei* (Lemos et al., 2008), channel catfish, *Ictalurus punctatus* (Li et al., 2011), meagre, *Argyrosomus regius* and European seabass, *Dicentrarchus labrax* (Magalhaes et al., 2015) and sunshine bass, *Morone chrysops x M. saxatilis* (Thompson et al., 2008).

In aquaculture, the use of corn DDGS has been focus of research since the 80s (Lovell, 1980). For catfish (*Ictalurus punctatus*) studies have shown that DDGS can replace soybean meal up to 35% without the addition of lysine (Webster et al., 1991; Webster et al, 1992; Webster et al, 1993) and up to 70 % with lysine inclusion (Webster et al.1991). Recent studies reaffirm the potential of DDGS uses in diets for several species with importance in the aquaculture such as *Ictalurus punctatus*

(Li et al, 2011.), *Oreochromis niloticus* (Suprayudi et al., 2015) and *Oncorhynchus mykiss* (Welker et al., 2014; Øverland et al., 2013).

Piaractus mesopotamicus is a native Characid from rivers and floodplains of the Brazilian Midwest, commonly known as pacu or pacu-caranha. It is one of the most important native species for national aquaculture, characterized by omnivorous food habit, high growth rates, good meat quality and good acceptance by the consumer (Urbinati & Gonçalves 2005). Given Brazil's potential in grain production, the growing interest in biofuel and the benefits of using DDGS in animal feed, it is necessary to develop studies that evaluate the use of the DDGS, produced in Brazil, in diets for native fish species with commercial interest.

Thus, the objective of this study was to evaluate the inclusion of different levels of corn DDGS replacing soybean meal in diets for juvenile *P. mesopotamicus* on growth performance, economic viability, feed efficiency and digestibility.

Material and Methods

1. DDGS Digestibility trial

A reference diet was formulated to contain 32% of crude protein, 17.6 kj g^{-1} of crude energy and 0.5% of chromic oxide III. The test diet was formulated containing 70% of the reference diet and 30% of DDGS. Corn DDGS was supplied by Libra Etanol LTDA, São Jose do Rio Claro, Mato Grosso, Brazil. All the ingredients used to compound experimental diets were grounded (1mm) and mixed on their respectively proportions. The mixtures underwent to extrusion process (Extrutech, 2mm) at Fish Nutrition Laboratory from Aquaculture Center of Sao Paulo State University (CAUNESP), Jaboticabal, Sao Paulo, Brazil. Diets were dried at 50°C for 12 hours and stocked at -20°C. Diets proximate composition is shown in Table 1.

The digestibility trial was performed at Aquaculture Nutrition Laboratory from College of Food Engineer and Animal Science of Sao Paulo University, Pirassununga, Sao Paulo, Brazil. Apparent digestibility coefficients of DDGS nutrients were measured by the indirect method, following methodology describe

on NRC, 2011. For that, 210 *P. mesopotamicus* juveniles (13.6±0.3 grams mean weight) were distributed in six rectangular tanks (50 L), at density of 35 fish tank^{-1}. Tanks were disposed in a recirculated system supplied with aeration and temperature kept constant by heathers (26°C). Each tank was considered as an experimental unit, arranged in a completely randomized design. Water quality was maintained by the use of supplemental aeration (central line and air diffusers), mechanical and biological filtration.

Fish were fed during seven days, twice daily (09:00 and 17:00 hours) with experimental diets and feces were collected using Guelph modified system. After being collected, feces passed through centrifugation process (1800 xg, 10 min), -20°C storage and afterward, freezing dryer prior to chemical analysis. Apparent digestibly coefficients (ADCs) of protein, dry matter, energy, lipid and phosphorus of the experimental diets were calculated, according to NRC, 2011, as follows:

$$ADCdiet = 100 \times \left(1 - \frac{Cr_2O_3\ diet}{Cr_2O_3\ feces} \times \frac{Nutrient\ or\ Energy\ feces}{Nutrient\ or\ Energy\ diet}\right) \quad (1)$$

The apparent digestibility coefficients of protein, dry matter, energy, lipid and phosphorus of the test ingredient (DDGS) were calculated according to NRC, 2011:

$$ADC\ ti = ADC\ td + \left[(ADC\ td - ADC\ rd)x\ \left(\frac{0.7\ x\ Dref}{0.3x\ D\ test\ ingredient}\right)\right] \quad (2)$$

Where ti = test ingredient; td = test diet; rd = reference diet; D ref. is the % nutrient (or kj g^{-1}) of reference diet (dry matter basis) and D test ingredient is the % nutrient (or kj g^{-1}) of test ingredient (dry matter basis).

2. Growth and Digestibility Trail

After obtaining the apparent digestibility coefficients of DDGS nutrients, five diets with increasing levels of DDGS inclusion (0, 10, 20, 30 e 40%) were formulated to be, on digestible basis, isoproteic (29% of digestible protein) and isoenergetic (13.4 kj g^{-1} of digestible energy). Due to formulate the experimental diets, it was used the apparent digestibility coefficients of soybean meal, fishmeal, wheat meal,

corn, rice bran, poultry meal (Abimorad & Carneiro 2004) and corn gluten meal (Fabregat et al., 2008) previously obtained for *P. mesopotamicus.*

A mixture based on soybean meal, soybean oil and corn was formulated to attain DDGS protein and energy contents. As DDGS was being included on the diet, the mixture was being replaced, until reach 40% of DDGS inclusion, where all soybean content was removed. All the ingredients used to compound the experimental diets were grounded (1mm), mixed on its respectively proportions and the mixture extruded (Extrutech, 2mm) at Fish Nutrition Laboratory at Sao Paulo State University (ESALQ), Piracicaba, Sao Paulo, Brazil. Diets were dried at 50°C during 12 hours and then stocked at -20°C. The proximate composition of the ingredients and experimental diets are shown in Table 2 and Table 3, respectively.

The growth trial was conducted at Aquaculture Nutrition Laboratory of College of Food Engineer and Animal Science from Sao Paulo University, Pirassununga, Sao Paulo, Brazil. *P. mesopotamicus* juveniles, coming from commercial fish farming, were stocked and acclimatized for 30 days, feeding a commercial diet (Pira 32, Guabi 32% of crude protein). To set up the trail, fish were weighed (mean weight 21±0.2 grams) and redistributed in 20 fiber glass tanks (100 L), at density of 15 fish $tank^{-1}$. Tanks were disposed in a recirculated system supplied with aeration and temperature kept constant by heathers. Each tank was considered as an experimental unit, arranged in a completely randomized design with five treatments (0, 10, 20, 30 and 40% of DDGS) and four replicates.

Tanks were covered with netting to prevent fish from jumping out. Also, a plastic hideout was set up on the border of the tank due to reduce stress stemming from feedlot. Water quality was maintained by the use of supplemental aeration (central line and air diffusers), mechanical and biological filtration. Water temperature was controlled with a heat exchanger (26°C) and measured twice daily. Dissolved oxygen and pH were monitored daily using a multiparameter Horiba (model U – 10). Total ammonia and nitrate by the use of commercial kits (Labcon Test Fresh Water Toxic Ammonia and Labcon Test Nitrito NO_2^-). Fish were fed with experimental diets during 100 days, twice daily (9:00 and 17:00), until apparent satiation.

At the same time that it was being performed the growth trial, another experimental trial was being conducted to determinate the digestibility of DDGS

diets, with the same *P. mesopotamicus* livestock and at the same recirculated system. However, it was added to the experimental diets 0.5% of chromic oxide. The apparent digestibility coefficients for protein, energy, lipid, dry matter and phosphorus of the experimental diets were measured by the indirect method, following methodology describe on NRC, 2011. 150 *P. mesopotamicus* juveniles (27.09 ± 1.4 grams mean weight) were distributed in five fiber glass tanks (100 L), at density of 30 fish tank^{-1}. Each tank was considered an experimental unit and the experiment was lain out in Latin square design 5x5 (five treatments and five experimental periods).

Fish were fed during seven days, twice daily (09:00 and 17:00 hours) with the experimental diets. Feces were collected using Guelph modified system. After collection, feces passed through centrifugation process (1800 *x*g, 10 min), -20°C storage and afterward, lyophilized prior to chemical analysis. The apparent digestibly coefficients (ADCs) of the experimental diets were calculated as exemplified in Formula 1. Right before and immediately after each feces collection period, it was taken a sample of 200 ml of water due to analyze dissolved and total phosphorus released in the water by fish excretions. Dissolved and total phosphorus were determinate according to methodology described on AOAC (2000).

3. Sampling

At the end of the growth trial, fish were fasted for 24 hours, anesthetized in benzocaine (50 mg L^{-1}) and slaughtered through spinal cord section. After that, each fish was weighted and gutted to obtaining the following parameters:

$$Weight\ Gain = Final\ Body\ Weight(FBW) - Initial\ Body\ Weight(IBW) \quad (3)$$

$$Specific\ Growth\ Rate\ (SGR) = [(\log FBW - \log IBW)/\ Period\ (days)] \times 100 \quad (4)$$

$$Average\ Body\ Weight\ (ABW) = (IBW + IBW)\ /\ 2 \quad (5)$$

$$Viscera\ Somatic\ Index\ (VSI) = (Viscera\ Weight\ /FBW) * 100 \quad (6)$$

$$Hepatic\ Somatic\ Index\ (HSI)\ =\ (Liver\ Weight\ /\ FBW) * 100 \quad (7)$$

$$Carcass\ Yield\ (CY)\ =\ (Carcass\ Weight\ /\ FBW)\ *\ 100 \quad (8)$$

$$Feed\ Intake\ =\ Total\ Feed\ Intake\ (g\ dry\ matter) \quad (9)$$

$$Feed\ Conversion\ Ratio\ (FCR)\ =\ Dry\ Feed\ Intake\ /\ Wet\ Weight\ Gain \quad (10)$$

$$Protein\ Efficiency\ Ratio\ (PER) = Wet\ Weight\ Gain/Crude\ Protein\ Intake \quad (11)$$

4. Chemical Analyses

Chemical analyses of ingredients, diets and feces were conducted at Aquaculture Laboratory from Animal Science and Food Engineer Faculty of Sao Paulo University, Sao Paulo, Brazil, following AOAC (2000) methodology. The ingredients and diets were first of all, plated in a forced air drying oven (105°C) to dry matter determination and feces were lyophilized. Samples were analyzed for crude protein (N x 6.25) by Kjeldahl method; ash by incineration in muffle (450°C for 16h) and crude lipid through extraction with petroleum ether using a Soxtec system. Chromium oxide and phosphorus were determined by absorbance in spectrophotometer (770 and 350nm, respectively), after acid digestion process. Gross energy was estimated by the use of the Atwater general factor system (FAO, 2012; NRC, 2011) based on the heats of protein, lipid and carbohydrate (including fiber) combustion.

5. Economic Viability

The evaluation of economic viability of the use of corn DDGS in diets for *P. mesopotamicus* juveniles was analyzed following Hoffman (2006) methodology and Gameiro (2009) suggestions. Data about the costs of the dietary ingredients was collected from the last 10 years (2005 to 2015) from the Instituto de Economia Agricola (IEA – APTA, Brazil) due to determinate the total cost of the experimental diets, using the following formulas:

$$Total\ Dietary\ Cost\ (TDC) = (\%Ingredient\ in\ diet * Ingredient\ Cost)/\ 100) \quad (12)$$

$$Weight\ Gain\ Cost\ (WGC)\ =\ TDC\ *\ FCR \quad (13)$$

6. Statistical Analyses

All data were subjected to a one-way analysis of variance to determine significant ($p \leq 0.05$) differences among the treatment means. Tukey's test was used when appropriate to distinguish significant differences between treatment means. All statistical analyses were conducted using SPSS 22.0 software package for Windows.

Results

DDGS and Experimental Diets Digestibility

The apparent digestibility coefficients (ADC) of corn DDGS nutrients and experimental diets containing the different levels of DDGS inclusion are shown in Table 4. The ADC of the test ingredient (DDGS) was higher for protein (94.8%), phosphorus (91.0%) and lipid (88.8%), followed by energy and dry matter that presented the lowest value of ADC, 66.3 and 62.7% respectively.

The different levels of DDGS included in the experimental diets for *P. mesopotamicus* juveniles caused modifications on the ADC of the most nutrients evaluated, excepted for protein. The values of ADC found for dry matter was significantly ($p < 0.05$) lower for diet 30DDGS comparing to the other treatments reaching 62.9 %. The diet with 40 % of DDGS inclusion (40DDGS) presented values of ADC for dry matter similar to the 30DDGS.

The same ongoing was observed for energy, where the ADC was reduced ($p < 0.05$) from 79.1 % to control and to 69.4 % for the 30DDGS diet. Energy ADC increased ($p < 0.1$) in 40DDGS diet, however the values obtained were not different ($p > 0.05$) from the control, 10DDGS and 20DDGS diets. The lipid ADC ranged from 87.8 for the control to 94.1% for the 40DDGS diet, being the last one the highest value of ADC obtained among all treatments. Even though no differences ($p > 0.05$) were obtained between the other treatments, all diets with DDGS, independent of the level of inclusion, presented ADC for lipid statistically different from the control ($p < 0.05$).

The values of dissolved and total phosphorus released in the water during the feces collection period, are shown in Table 5. The amount of total phosphorus varies ($p < 0.05$) among treatments. It was observed that the value of total phosphorus decreased as DDGS was included in the diets, ranging from 0.61% for control to 0.37% for the 40DDGS diet. Dissolved phosphorus did not differ significantly ($p > 0.05$) between treatments.

Growth and Economic Viability

Fish accepted the diets without difficulty and also there was no evidence in all treatments of disease or mortality during the trial. Growth parameters and feed utilization efficiency of the fish are presented in Table 6. There were variation ($p < 0.05$) between treatments for feed conversion ratio (FCR) and protein efficiency ratio (PER). The inclusion of DDGS in the diets caused a slighted decrease ($p < 0.05$) on FCR, turning 1.14 from control to 1.00 for 30DDGS diet and 1.02 for 40DDGS. Diets 30DDGS and 40DDGS were not different between them. The PER of 30DDGS was significantly higher ($p < 0.05$) than control and 20DDGS diets. The fish that received 10DDGS and 40DDGS diets did not show difference on PER ($p > 0.05$).

Results obtained for specific growth rate (SGR), viscero somatic (VSI), hepato somatic (HSI) indexes and carcass yield (CY) did not vary ($p > 0.05$) between the treatments. The same was true for weight gain (WG) that did not vary significantly ($p >0.05$) between treatments.

Dietary costs are presented in Table 7. It was observed a significant reduction ($p < 0.05$) on the cost of weight gain (R$ kg $^{-1}$ of weight gain) as DDGS was being included in the diets and soybean meal being replaced. Diets have the cost of weight gain varying from R$2.21 for control to R$1.64 for the 40DDGS diet, without in soybean meal, what represents a reduction of 26% of costs.

Discussion

DDGS Digestibility

The success of fish rearing mostly depends on quality of the food supplied in fish farms. Thus, exhibit nutrients availability of dietary ingredients appears as an important practice to allow the maximum expression of animal productive performance and reduce nutrients waste through fish excretions (Lee, 2002). The availability of nutrients can be obtained through determination of ingredients apparent digestibility coefficients (Smith et al 1995; NRC, 2011), practice that is recognized as the first step to evaluate the potential use of ingredients in animal feed production (Allan et al., 2000). Thus, corn DDGS apparent digestibility coefficients (ADC) were firstly determined to pacu (*Piaractus mesopotamicus*) juveniles in the present study.

The ADC of protein found for DDGS (94.8%) remained higher than the obtained for some ingredients majority used as protein source in *P. mesopotamicus* diets such as poultry meal (83.4%), soybean meal (81.1%) (Abimorad, 2004), corn gluten meal (78.6%) (Fabregat et al., 2008), fish meal (84.6%) and yeast extract (81.5%) (Abimorad et al., 2008). Also, the ADC of protein contained in DDGS for pacu was higher than the value of 86.2% reported for channel catfish, (Li et al., 2011) and 64.2% for sunshine bass (Thompson et al., 2008). Therefore it is possible to emphasize the potentiality of DDGS uses as protein source in *P. mesopotamicus* diets.

However, even though DDGS has shown great digestible protein value, it is required more detailed studies evaluating DDGS amino acids profile and digestibility for pacu. Magalhaes et al. (2015) found that the ADCs of amino acids in diets with DDGS inclusion trend to be lower than those obtained for fishmeal-based diet for meagre (*Argyrosomus regius*) and seabass (*Dicentrarchus labrax*), what is probably related with the lower availability of amino acids in DDGS when compared to fishmeal.

Although high contents of fiber and nitrogen free extract in diets can lead in a decrease of protein digestibility (Lech and Reich, 2012), Magalhaes et al. (2015) also did not find differences between protein digestibility coefficients of diets with

DDGS inclusion and control, for both fish species, with ADCs ranging from 89 to 91% for meagre and 91.9 to 92.9% for seabass. Results obtained by Magalhaes et al. (2015) for protein digestibility of DDGS diets were similar to those obtained in the present study, whereas increasing levels of DDGS inclusion also did not reflect in modifications on diets protein ADCs for *P. mesopotamicus*.

Furthermore, the digestibility of protein obtained for pacu juveniles in diets with DDGS, independent of inclusion level, were higher than the observed for other animals feeding DDGS diets such as pigs (Ren et al., 2011) and broilers (Liu, 2011). Ren et al. (2011) evaluating diets with three different sources of corn DDGS from ethanol production observed values of 72.9, 50.0 and 51.4% for protein apparent ileal digestibility for growing pigs, while Liu (2011) observed values of 85% for total tract and 74% for ileal apparent digestibility coefficients in broilers feeding diets with 20% of corn DDGS without xylanase addition.

Trough dry matter ADCs investigation it is possible to get a general estimative about the digestibility of a particular ingredient by indicating the amount of *non-digestible* nutrients presents in it. As well obtained for *P. mesopotamicus,* low dry matter digestibility in corn DDGS was reported for the majorly of the aquatic species studied until present moment (Magalhaes et al., 2015; Seo et al., 2011; Li, 2011; Chan et al., 2004). Farther, Cheng and Hardy (2004) and Li (2011), corroborating with the present study, observed a reduction on the digestibility of dry matter and energy of diets with increasing levels of DDGS inclusion. Authors correlated the results obtained with the high amount of fiber present in DDGS, what was confirmed through the analysis of neutral detergent fiber (NDF) promoted in this study. Krogdahl et al. (2005) also mentioned high amounts of cellulose and hemicellulose, such arabinoxylans, as an adverse feature of DDGS used in animal feed.

Among factors capable for induce changes on dietary nutrients digestibility, fiber is the biggest issue when talking about DDGS. In fish, there are two ways of accessing some of the dietary fiber, by acid hydrolysis in the stomach or through microbial enzymes activity in intestine (Kaushik, 2001). High fiber content may provide modifications in nutrients metabolism reducing the availability of dietary nutrients in many ways, either by changing the ADCs of other dietary ingredients

(Liu, 2011) or inducing endogenous losses (Back Knudsen and Hansen, 1991; Noblet and Perez, 1993).

Previous studies have been reported low digestibility coefficients of diets, not only for dry matter but also for other dietary nutrients, with increasing levels of fiber such those obtained for rainbow trout (Hilton et al., 1983), common carp (Kirchgessner et al., 1986), red drum (McGoogan and Reigh, 1996) and rockfish (Lee, 2002). The use of large amounts of fiber in diets would leads to reduction on gut retention time and digestive enzyme contact with intracellular contents (Vanderoof, 1998) which impairs the capability of absorbing nutrients available from dietary ingredients (Enes et al., 2011; Fontoulaki et al., 2005; Stone et al., 2003). Additionally, high fiber can lead in an increase of microbial activity and substrates from fermentation, what would end up in endogenous losses.

All energy released by DDGS comes, basically, from lipids since starch is degraded in the fermentation process (Han and Liu, 2010). Nevertheless, in the present study, the source of corn DDGS incorporated in the diets had lipids level (4.0 %DM) not as expressive as the reported by literature that shown values ranging from 9.0 to 12.0% (Feedstuff, 2016; USGC, 2012). During the ethanol production, the oil restrained in the wet cake can be extracted due to generate corn oil as another ethanol co-product. So, variations in lipid present in DDGS will be dependent of the process adopted by ethanol plants behind DDGS production.

Adding to that, the starch remained in the DDGS used in this experiment (5.3 % DM) does not have a remarkable contribution as energy source. Therefore, most of the energy coming from the DDGS used in the present study is probably arising from the others nutrients like fiber and protein. Due to that, the low value of apparent digestibility coefficient obtained for energy for *P. mespotamicus* either in DDGS (66.3%) or in the experimental diets with different levels of DDGS inclusion may be explained by over quantity of nutrients with low energy digestibility such as fiber components (neutral detergent fiber), what contrast with others nutrients with higher digestible value like starch and lipid (Stein and Bohlke, 2007; NRC, 2011). Thereby, even though *P. mesopotamicus* is a species that tolerate high levels of fiber in the diet (Rodrigues et al., 2010) there was not effectiveness in the use of the energy coming from the fiber content in DDGS.

The ADC of DDGS obtained for lipid in this study (88.8%) was lower than the ADCs found for corn (91.1%), soybean meal (93.1%) and fish meal (94.1%) for *Oreochromis niloticus* juveniles (Furuya et al., 2001), an omnivorous fish species with similar food habit than *P. mesopotamicus*. Also, pacu showed a lower ADC for lipids in DDGS in the present study than the 93.8 % obtained for channel catfish (Li, 2011), however higher than the 68.7% found for sunshine bass (Thompson et al., 2008). Although, lipid content in the sources of DDGS used were higher for the studies cited above, 13 % (dry matter basis) for sunshine bass and 7 % (dry matter basis) for catfish than the 4% of lipids of the DDGS source used in the present study for pacu.

Increasing amounts of lipid content in diets may lead in higher digestibility of this nutrient, due to delay in release gastric fluids making available more time for dietary lipids digestion (Quigley & Meschan, 1941; Windell et al., 1969). As obtained in the present study, increase in lipids digestibility of diets with rising lipid content is also reported for *Oncorhynchus mykiss* (Takeuchi et al., 1978), *Oreochromis niloticus* x *Oreochromis mossambicus* (De Silva et al., 1991), *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999) and *Seabastes schlegeli* (Lee, 2002).

Corn oil, as the lipid source in DDGS, has on its composition higher amounts of unsaturated fatty acids than saturated (NRC, 2011). Adding the fact that unsaturated fatty acids are better digested than saturated (Ng, 2003; Bahurmiz and Ng, 2007), it is possible to relate the increasing digestibility of lipid in DDGS diets with fatty acids profile of the experimental diets. As soybean oil and corn oil have similar saturated (SFA) and unsaturated (UFA) percentages (14 – 16% of SFA and 86 – 84% of UFA) (NRC, 2011; Martin et al., 2008) modifications on the amount of those two ingredients in experimental diets did not bring any damage on lipids digestibility due to changing SFA/UFA ratio. Thus, increasing values of lipid digestibility of diets with higher levels of DDGS inclusion may be related with the equally increase in dietary lipids content.

High amounts of fiber can also cause decrease on lipid digestibility by endogenous losses (Back Knudsen and Hansen, 1991; Noblet and Perez, 1993), however, the increasing on fiber content in DDGS diets in this study was not achievable of damage lipid diets digestibility. Otherwise, low ADCs of lipids were reported for rainbow trout (Chan et al., 2004), sunshine bass (Thompson et al.,

2008), meagre and seabass (Magalhaes et al., 2015) feeding diets with corn DDGS inclusion.

In the present study, diets were extruded due to follow fish feed commercial production process, allowing to get results as closest to real fish culture as possible. The influence of feed processing methods on ingredients digestibility was reported by Wilson and Poe (1985) for corn grains. They found higher corn energy ADC for extruded diets than pelleted, but no differences were found for soybean meal and wheat meal. However, Allan and Booth (2004) observed higher dry matter, protein and energy digestibility of soybean meal in extruded diets than pelleted diets, and the opposite for canola meal. Thus, the possibility of changings on nutrients digestibility of dietary ingredients caused by the type of processing method used in feed production is not completely discarded.

Digestible phosphorus refers to the portion absorbed by the gastrointestinal tract. This portion can be compounded by non-phytic or phytic phosphorus, being the last one available after hydrolysis process by intrinsic phytase (Bünzen, 2008; Cao et al., 2007). Thus, the high digestible phosphorus content found in the DDGS may be explained by its high bioavailability. Fermentation processes and heating, which is subjected DDGS, may have resulted in an increase of the hydrolysis of phytate molecules (Kim et al., 2008), component non digestible for animals.

The high available phosphorus content in DDGS can also be observed through measurements of body retention and the amount of phosphorus release in the water. Cheng & Hardy (2004) and Overland et al. (2013) could observed that increase amounts of DDGS in diets for Nile tilapia and rainbow trout, respectively, ended up in higher phosphorus retention in the body comparing to control diets without DDGS inclusion.

Increase in nutrient retention leads in smaller quantity of nutrient released in water as waste (Bureau & Hua, 2010; Prachom et al., 2013). In the present study, it was evaluated the discharge of phosphorus in the water, and results obtained are in agreement with Cheng & Hardy (2004) and Overland et al. (2013). This reaffirm that the inclusion of DDGS in fish diets provides higher amounts of digestible phosphorus leading in better body retention and less release of this mineral. Also, according to Conama 357/2005, only the experimental diets with DDGS inclusion

were in the range of phosphorus concentration recommended for aquaculture water that accepts values above 0.05 mgP L^{-1}.

Apparent phosphorus digestibility contained in the DDGS was considerably high (91%) when compared to ingredients such as soybean meal (35.1%), fishmeal (27.1%), bone meal (54.6%), corn meal (7.3%) and wheat bran (30.5%), obtained for tilapia (*Oreochromis niloticus)* fingerlings (Miranda et al., 2000). Furuya et al. (2001) also obtained ADCs for corn (45.1%), wheat bran (29.5%), soybean meal (47.1%) and fishmeal (49.8%) for tilapia below values found in the present study. Furthermore, phosphorus ADC for DDGS obtained in the present study was even higher than that observed for catfish (Li et al., 2015) and pigs (Almeida et al., 2012; Rojas et al., 2013). Whereby corn DDGS has higher phosphorus content availability compared to other ingredients commonly used in diets for aquaculture, its use is also recommended as a strategy for the development of a complete and appropriate aqua feed, allowing the reduction of phosphorus excretion to the environment.

It is important to emphasize that possible variations on the quality and nutrient composition of the DDGS, between different facilities or even the same facility (Spiehs et al., 2002; Kleinschmitt et al. 2007) may occur, basically, by differences between plants and process methods (Ortin and Yu, 2009; Liu, 2011). Therefore, the success of DDGS inclusion on fish diets will be dependent of the protein and amino acid requirements of the fish, the type of feed ingredient being replaced and the source and composition of the DDGS.

Growth and Economic Viability

Changes on dietary protein source can interfere on fish growth performance due to variations on nutritional values, nutrients digestibility and amino acid composition (Anderson et al., 1992). Thus, to obtain a considerable growth performance it become necessary to check some nutritional aspects of the new ingredient being added, mainly when changing a protein source in fish diets.

In agreement with the present experiment, a decreased on feed conversion ratio (FCR) was also observed for rainbow trout (143 grams mean initial weight) feeding diets with 50% of DDGS inclusion as substitute of a mixture with plant protein ingredients (Øverland et al., 2013) without significant effects on weight gain

and feed intake. Distinctly, it was reported improvements on weight gain for hybrid catfish feeding diets with DDGS inclusion (Zhou et al., 2010). Robinson & Li (2008) observed that the lower FCR found for channel catfish feeding 30% DDGS diet was not due to improvements on feed intake but by an increase in fish weight gain.

Li et al. (2010) suggested that the high lipid level of the diet containing 30% DDGS was partially responsible for the increase in feed efficiency ratio in catfish juveniles. Also, improvements on feed utilization were reported for catfish feeding diets with DDGS, DS (distillers' solubles) and EDS (distillers' solubles from corn endosperm) when compared with HPDDG (high protein distillers dried grain with solubles) diet, probably due to the absence of the soluble portion in HPDDG (Li et al., 2010). Thus, it is possible to affirm that DDGS may have some components that facilitated the digestibility of the diet or nutrient absorbance.

Some studies related with the inclusion of brewer's yeast in fish diets shown improvements on feed efficiency ratio for hybrid striped bass, *Morone chrysops x Morone saxatilis* (Li and Gatlin, 2006) and sea bass, *Dicentrarchus labrax* (Oliva-Teles and Gonçalves, 2001). That may be related with the nucleotides, β glucans and oligosaccharides present in yeast cells that can cause modifications on intestine morphometry of some fish species, increasing the area of nutrients absorption (Burrells et al., 2001; Santin et al., 2001; Yang et al., 2007; Dimitroglou et al., 2009) and consequently the use of the dietary nutrients. As DDGS is composed by considerable amounts of yeast cells (Ingledew, 1999; Zohu et al. 2010) it is possible that its components had acted modifying *P. mesopotamicus* intestine, improving the absorption of nutrients present in the experimental diets.

The inclusion 50% of dried brewer's yeast (*Saccharomyces cerevisiae*) in replacement of fishmeal resulted in improvements on feed efficiency, growth performance and reduction on nitrogen release for pacu, *P. mesopotamicus* (initial mean weight 26.6 ± 1.7 grams) (Ozorio et al., 2010). Adding to that, Ozorio et al. (2010) observed high digestibility of nutrients and amino acids in experimental diets, suggesting high digestibility of this ingredient for pacu and its benefic interference on the digestibility of other dietary ingredients. Thus, considering results obtained in the present study it is possible to affirm that yeast present in DDGS may have exerted positive influence on fish growth performance, feed utilization and nutrients digestibility.

Instead of Shelby et al. (2008) did not find differences for weight gain, feed intake and feed efficiency ratio on Nile tilapia fed 30 or 60% DDGS diets with lysine addiction, compared to the control. The same behavior was described for catfish feeding diets with 40% of DDGS inclusion (Lim et al., 2009). No differences in body weight gain was observed for yellow perch, a carnivorous specie, feeding diets with increased DDGS levels (Schaeffer et al., 2011) however weight gain and FCR showed better results for the fishmeal based diet than the others with DDGS inclusion. Furthermore, the success of DDGS use in fish diets may be dependent on the combination of dietary ingredients and fish food habit.

Improvements on feed utilization in the present study reflected on economic viability, where inclusion of DDGS promotes reduction of 26% on dietary cost per weight gain for *P. mesopotamicus* fed 40% DDGS diets. Once feed contributes with 60 to 80% of total production costs (Campos et al., 2007; Rola and Hasan, 2007) turns priority the use of low-cost ingredients in aqua feed, as long as do not cause losses on animal production.

Conclusion

This is the first work that investigated the inclusion of corn DDGS in total soybean meal replacement in diets for *P. mesopotamicus*, and the data obtained indicate that, the use of 40% DDGS in the diet did not provide negative consequences for growth and digestibility of the juveniles, although improved feed utilization and reduced dietary costs and phosphorus release in culture water. Thus it is possible the inclusion of corn DDGS in levels up to 40% in the diets for *P. mesopotamicus* juveniles in total replacement of soybean meal.

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Tables Index

Tables

Table 1: Chemical and percentage composition of reference and test diets

	Reference Diet	Test Diet
Ingredients (%)		
DDGS	-	30.0
Poultry meal	14.5	10.1
Fishmeal	6.0	4.2
Soybean meal	30.0	20.9
Wheat meal	24.0	16.7
Corn	15.0	10.5
Rice bran	9.0	6.3
BHT[1]	0.1	0.0
Chromium oxide III	0.5	0.5
Vit. And Min. Premix[2]	1.0	0.7
Total	100.0	100.0
Chemical Composition		
Dry Matter	93.5	94.5
Crude Protein (% DM)	36.2	35.5
Gross Energy (kj g^{-1})	18.8	19.2
Crude Lipid (% DM)	3.4	4.5
Phosphorus (% DM)	1.0	0.8
Digestible Protein (% DM)	33.0	30.9
Digestible Energy (kj g^{-1})	14.5	14.0

[1] BHT (Butyl hydroxytoluene);

[2] Vitamin and Mineral Premix: vitamin A - 500.000 UI; vitamin D3 - 250.000 UI; vitamin E - 5.000 mg; vitamin K3 - 500 mg; vitamin B1 - 1.500 mg; vitamin B2 - 1.500 mg; vitamin B6 - 1.500 mg; vitamin B12 - 4.000 mg; folic acid - 500 mg; pantothenate Ca - 4.000 mg; vitamin C - 10.000 mg; biotin - 10 mg; Inositol - 1.000; nicotinamide - 7.000; choline - 10.000 mg; Co - 10 mg; Cu - 1.000 mg; Fe - 5.000 mg; I - 200 mg; Mn - 1500 mg; Se - 30 mg; Zn - 9.000 mg[3.] (Agromix LTDA, Sao Paulo, Brazil)

Table 2: Proximate analyses of the experimental dietary ingredients[1]

	DDGS	SBM	CM	WM	RB	CG	FM	PM
Chemical Composition								
Dry Matter	92.9	88.9	89.3	90.3	86.9	92.7	97.7	97.3
Crude Protein (% DM)	33.6	44.8	8.4	14.8	13.2	71.5	58.0	48.9
Gross Energy (MJ kg^{-1})	20.9	17.1	16.2	16.7	19.4	21.1	14.6	13.1
Crude Fiber (% DM)*	-	7.6	2.2	9.8	9.0	2.2	1.5	1.5
Lipid (% DM)	4.0	2.0	4.0	2.9	15.2	1.9	3.4	15.2
Ash (% DM)	2.0	7.8	1.3	5.5	10.2	2.2	27.5	15.0
Phosphorus (% DM)	0.2	0.6	0.3	0.9	1.6	0.4	3.0	2.7
Starch (%DM)	5.3	-	-	-	-	-	-	-

[1] DDGS: Corn Distiller's Dried Grains with Soluble (Libra Etanol, Mato Grosso, Brazil); SB: Soybean meal, CM: Corn Meal, WM: Wheat Meal, RB: Rice Bran (Cargill, Sao Paulo, Brazil); CG: Corn Gluten, FM: Fish meal (In Vivo, Sao Paulo, Brazil); PM: Poultry Meal (Agromix, Sao Paulo, Brazil)

* DDGS: neutral detergent fiber (NDF) = 56.4% (DM basis);

Table 3: Composition and proximate analyses of experimental diets with increasing levels of DDGS inclusion

	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS
Ingredients					
DDGS	0.0	10.0	20.0	30.0	40.0
Soybean meal	23.1	17.3	11.5	5.8	0.0
Corn	25.4	21.8	18.1	14.5	10.8
Soybean oil	3.4	2.8	2.3	1.7	1.1
Poultry meal	6.6	6.6	6.6	6.6	6.6
Fish meal	11.0	11.0	11.0	11.0	11.0
Corn Gluten	8.3	8.3	8.3	8.3	8.3
Wheat meal	13.0	13.0	13.0	13.0	13.0
Rice bran	7.7	7.7	7.7	7.7	7.7
Lysine[1]	0.5	0.5	0.5	0.5	0.5
BHT[2]	0.1	0.1	0.1	0.1	0.1
Vit. and Min. Premix[3]	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0
Chemical Composition					
Dry Matter	93.1	91.6	93.4	91.3	93.8
Crude Protein (% DM)	32.8	32.1	32.7	32.1	32.7
Gross Energy (kj g $^{-1}$)[*]	18.6	19.1	19.4	19.6	19.9
Crude Lipid (% DM)	3.2	5.2	5.8	7.1	7.6
Ash (% DM)	8.2	7.8	6.9	6.9	6.6
NDF	24.9	28.1	32.8	38.7	44.2
Starch	22.8	19.6	18.1	15.7	14.3
Digestible Protein (% DM)	29.5	27.6	29.4	28.2	29.1
Digestible Energy (kj g $^{-1}$)	14.7	14.7	14.9	12.5	14.5
DP/DE [**]	2.0	1.9	2.0	2.3	2.0

[1] Lysine: Anjinomoto LTDA, Sao Paulo, Brazil
[2] BHT (Butyl hydroxytoluene);
[3] Vitamin and Mineral Premix: vitamin A - 500.000 UI; vitamin D3 - 250.000 UI; vitamin E - 5.000 mg; vitamin K3 - 500 mg; vitamin B1 - 1.500 mg; vitamin B2 - 1.500 mg; vitamin B6 - 1.500 mg; vitamin B12 - 4.000 mg; folic acid - 500 mg; pantothenate Ca - 4.000 mg; vitamin C - 10.000 mg; biotin - 10 mg; Inositol - 1.000; nicotinamide - 7.000; choline - 10.000 mg; Co - 10 mg; Cu - 1.000 mg; Fe - 5.000 mg; I - 200 mg; Mn - 1500 mg; Se - 30 mg; Zn - 9.000 mg[3.] (Agromix LTDA, Sao Paulo, Brazil)
[*] Values calculated according to FAO (2012) and NRC (2011)
[**] DP/DE: digestible protein/digestible energy ratio

Table 4: Apparent digestibility coefficients (%) of corn DDGS and experimental diets nutrients for *P. mesopotamicus* juveniles[1]

	DDGS	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	p-value	SEM
Dry matter	62.7	73.9^{a}	71.7^{a}	71.7^{a}	62.9^{b}	67.4^{ab}	0.002	1.1
Crude Protein	94.8	89.8	88.6	90.1	87.9	88.9	0.61	0.3
Energy	66.3	79.1^{a}	77.0^{a}	76.8^{a}	69.4^{b}	73.0^{ab}	0.002	0.9
Crude Lipids	88.8	87.8^{b}	91.5^{ab}	92.6^{ab}	92.5^{ab}	94.1^{a}	0.047	0.7
Phosphorus	91.0	58.1	59.2	62.5	56.3	60.1	0.73	1.2

[1]Values presented as means (n = 60) and pooled standard error of the mean (SEM). Means with different letters represents significant differences between treatments ($p < 0.05$);

Table 5: Dissolved Phosphorus (DP) and Total Phosphorus (TP) in mg L^{-1} present in the water during feces collection of *P. mesopotamicus* fed diets with distinct levels of corn DDGS inclusion

Diet	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	p-value	SEM
DP	0.034	0.04	0.035	0.034	0.042	0.83	0.002
TP	0.061^{a}	0.059^{ab}	0.055^{ab}	0.045^{ab}	0.037^{b}	0.01	0.003

[1]Values presented as means (n = 25) and pooled standard error of the mean (SEM). Means with different letters represents significant differences between treatments ($p < 0.05$)

Table 6: Growth parameters and feed utilization efficiency of *P. mesopotamicus* juveniles fed experimental diets[1]

Diet	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	p-value	SEM
Final body weight (g)	101.1	100.1	120.3	113.2	115.1	0.69	3.5
Weight gain (g)	79.4	79.0	100.0	92.2	94.7	0.44	0.2
Specific Growth Rate	1.6	1.6	1.8	1.7	1.7	0.43	0.04
Feed Intake (g %DM)	90.5	81.7	107.2	92.7	97.1	0.75	0.3
Feed conversion ratio	1.14^{a}	1.04^{ab}	1.07^{ab}	1.00^{b}	1.03^{ab}	0.04	0.01
Protein efficiency ratio	2.7^{b}	3.1^{ab}	2.8^{b}	3.2^{a}	3.0^{ab}	0.006	0.1
Carcass Yield (%)	90.3	93.7	93.3	85.9	87.5	0.08	1.1
HSI (%)	1.6	1.5	1.4	1.4	1.4	0.78	0.05
VSI (%)	8.8	8.1	10.3	9.3	9.1	0.52	0.4

[1]Values presented as means (n = 60) and pooled standard error of the mean (SEM). Means with different letters represents significant differences between treatments ($p < 0.05$)

*

Table 7: Dietary costs[*] and costs of weight gain of diets with increasing levels of corn DDGS in soybean meal replacement for *P. mesopotamicus* juveniles[1]

	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	p-value	SEM
Diet Cost (R$ kg $^{-1}$)	1.93	1.85	1.77	1.69	1.60	0.82	0.03
Cost of Weight Gain**	2.21	1.92	1.89	1.69	1.64	0.00	0.05

[1]Values presented as means (n = 60) and pooled standard error of the mean (SEM). Means with different letters represent significant differences between treatments ($p < 0.05$)
[*]Costs of ingredients based on IEA – APTA (2005 to 2015)
[**] Cost of Weight Gain (R$ kg^{-1} gain) = Diet Cost * FCR (feed conversion ratio)

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Effect of corn DDGS on digestive enzymes, oxidative stress and intestine morphology of *Piaractus mesopotamicus* juveniles

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Abstract

To evaluate the effects of corn DDGS inclusion and soybean meal replacement in diets for *Piaractus mesopotamucus* on intestine enzymes, oxidative status and intestine morphology, fish with 21±0.2 grams of mean weight were stocked in 20 fiber glass tanks (130L) at density of 15 fish tank^{-1} fed test diets for 100 days. Due to compound the treatments were formulated five diets with equal protein and energy content and increasing DDGS levels (0, 10, 20, 30 and 40%). Each tank was considered as an experimental unity, in a completely randomized design (five treatments and four replicates). At the end of the trial, all fish were euthanized, weighted and gutted. Intestines from three fish per tank were collected and divided in three portions: anterior, mid and posterior, frozen in liquid nitrogen and stored at -80°C for enzymatic analysis. It was evaluated the activity of total protease, amylase, lipase, trypsin and chymotrypsin (digestive enzymes), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD) and lipid peroxidation (LPO) (oxidative stress enzymes). To proceed with histological analyzes, the intestines from two fish from each tank were removed. Samples from middle intestine were collected, immersed in Bouin solution and stored in alcohol 70% prior to histological slides confection to later descriptive analysis. Data was subjected to a one-way ANOVA analysis of variance to determine significant ($p \leq 0.05$) differences among

treatments and subsequently Tukey's test. A two-way ANOVA analysis was applied to determinate significant ($p \leq 0.05$) interactions for enzymes activity between treatments and intestine portions. The inclusion of corn DDGS and soybean meal replacement caused a decrease ($p < 0.05$) on lipase, amylase, trypsin and chymotrypsin activities. The activity of total protease was higher ($p < 0.05$) for 30DDGS diet in the mid intestine portion. There were also differences for the enzymes activities between intestine portions, where the anterior showed higher values ($p < 0.05$) than distal portion, except for proteases. The activity of G6PD was smaller ($p < 0.05$) for diets 30DDGS and 40DDGS; also LPO had lower values for diets with DDGS inclusion than the control whereas CAT increased ($p < 0.05$) in diets with DDGS inclusion. The results indicate that the inclusion of corn DDGS in *P. mesopotamicus* diets may improve fish intestine health and the capability of absorption and utilization of the nutrients.

Key Words

Intestine enzymes, DDGS, yeast, grain fermentation, intestine health, antinutritional factors

Introduction

Digestive enzymes play an important role on ingredients digestibility making available nutrients to animal utilization. Factors such as dietary components have the capability of modify the activity and profile of these digestive enzymes and being dependent on the quality and quantity of ingredients as well as the nutrient profile of diet formulation (Ugolev and Kuz'mina, 1994; Lundstedt et al., 2004; Fountoulaki et al., 2005).

The study of the modulation action of dietary ingredients and nutrients on the digestive enzymes activities is of primordial importance to understand the mechanisms behind the effects of its inclusion and to strength the basic knowledge of its effects on feed utilization and growth performance of fish (Sunde, 2001; Li et al 2014; Krogdahl et al., 2015). Indeed, it has been conducted several studies to evaluate the effect of the dietary ingredients and nutrient levels on the activity of gastrointestinal enzymes in different fish species, such as pintado, *Pseudoplatystoma corruscans (*Lundstedt et al., 2004), matrinxa, *Brycon cephalus* (Vieira et al., 2005), pacu, *Piaractus mesopotamicus* (Bidinotto et al., 1997; Honorato et al., 2009) and Atlantic salmon, *Salmo salar* (Krogdahl et al., 2015).

Corn distiller's dried grains with soluble (DDGS) are the dry portion of corn grain resulted after its fermentation, by addition of enzymes and yeast, to produce ethanol and carbon dioxide. After fermentation the remaining product pass through a set of centrifuges and drying processes, originating the DDGS (Wyman, 1996). DDGS has moderated protein content (33%), low starch content (less than 3%) and lack anti-nutritional factors (Jacques et al., 2003). Also, part of the enzymes and yeast used during fermentation remain in the final product (Lim et al., 2009).

Few studies have been focused on the effects of DDGS inclusion in diets on animal intestinal nutrients metabolism such as digestive enzymes activity, oxidative status and intestine morphology. Magalhaes et al. (2015) studying two different DDGS source for European seabass (*Dicentrarchus labrax*) and meagre (*Argyrosomus regius*) observed that both DDGS sources were well digested by the fish, however, none effects were observed for animal's digestive enzymes activity. Rahman et al. (2015) evaluating the inclusion of rice DDG in diets for Olive Flounder

juveniles (*Paralichthys olivaceus*) did not observed differences for liver antioxidant enzymes and gastrointestinal tract digestive enzymes. However, fish response to DDGS inclusion in diets may vary according to DDGS composition, dietary ingredients characteristics, fish species and food habit.

Pacu, *Piaractus mesopotamicus*, is a Characin, native of rivers, floodplains, lakes and flooded forests from Brazil Midwest. It is one of the most important indigenous species for aquaculture in Brazil, being characterized as omnivorous feeding species (Trophic Level of 2.0, based on food items; Fishbase; www.fishbase.org). Pacu has been recognized as one of the best candidates for a sustainable aquaculture production, due to the very high growth rates, reaching 1.2 kg in one year, associated with the good meat quality and good consumer acceptance (Urbinati & Gonçalves 2005).

The aim of this study was to evaluate the effects of dietary replacement of soybean meal by corn DDGS on *P. mesopotamicus* intestine digestive and oxidative stress enzymes and intestine morphology.

Material and Methods

1. Experimental Trial

Five diets with increasing levels of DDGS inclusion (0, 10, 20, 30 e 40%) were formulated to be, on digestible basis, isoproteic (29% of digestible protein) and isoenergetic (13.4 kj g^{-1} of digestible energy). Due to formulate the experimental diets, based on digestible values, it was used the apparent digestibility coefficients (ADC) of corn DDGS (previously determined), soybean meal, fishmeal, wheat meal, corn, rice bran, poultry meal (Abimorad & Carneiro 2004) and corn gluten meal (Fabregat et al., 2008) previously obtained for *P. mesopotamicus* juveniles.

A mixture based on soybean meal, soybean oil and corn was formulated to attain DDGS protein and energy contents. As DDGS was being included on the diet, the mixture was being replaced, until reach 40% of DDGS inclusion, where all soybean content was removed. All the ingredients used to compound the experimental diets were grounded (1mm), mixed on its respectively proportions and

the mixture extruded (Extrutech, 2mm) at Fish Nutrition Laboratory at Sao Paulo State University (ESALQ), Piracicaba, Sao Paulo, Brazil. Diets were dried at 50°C during 12 hours and then stocked at -20°C. The proximate composition of the experimental diets is shown in Table 1.

The trial was conducted at Aquaculture Nutrition Laboratory of Food Engineer and Animal Science Faculty from Sao Paulo University, Pirassununga, Sao Paulo, Brazil. *P. mesopotamicus* juveniles, coming from commercial fish farming, were stocked and acclimatized for 30 days, feeding a commercial diet (Pira 32, Guabi, 32% of crude protein). To set up the trail, fish were weighted (mean weight 21±0.2 grams) and redistributed in 20 fiber glass tanks (100 L), at density of 15 fish tank $^{-1}$. Tanks were disposed in a recirculated system supplied with aeration and temperature kept constant by heathers (26°C). Each tank was considered as an experimental unit, arranged in a completely randomized design with five treatments (0, 10, 20, 30 and 40% of DDGS) and four replicates.

Tanks were covered with netting to prevent fish from jumping out. Also, a plastic hideout was set up on the border of the tank due to reduce stress stemming from feedlot serving as refuge. Water quality was maintained by the use of supplemental aeration (central line and air diffusers), mechanical and biological filtration. Water temperature was controlled with a heat exchanger and measured twice daily. Dissolved oxygen, total ammonia, nitrate and pH were monitored daily using a multiparameter Horiba (model U – 10). Fish were fed with experimental diets during 100 days, twice daily (9:00 and 17:00h), until apparent satiation.

2. Sampling

At the end of the feeding trial, fish were fasted for 24 hours. After that, twelve fish from each treatment (three animals from each replicate) were randomly sampled, anesthetized in benzocaine (50 mg L^{-1}) and euthanized through spinal cord section and dissected on chilled trays. The intestines were carefully removed, freed from the adjacent adipose tissue and divided into three portions (anterior, mid and distal), according to Bertin (1958). Distal intestine was distinguished from the medium intestine by the increased diameter, darker mucosa, and annular rings. The anterior and mid potions were obtained by the division of the remaining intestine in

two identical portions. Each portion was individually stored in Eppendorfs and immediately frozen by immersed in liquid nitrogen and stored at -80°C until the enzymatic analyses.

Samples from mid intestine were collected from two fish per treatment, gently washed with saline water (0.9%) to biological fragments removal, fixed in Bouin solution for 12 hours, passed by series washes and stored in alcohol 70% until histological processing.

3. Digestive Enzymes Analyses

The enzymatic activity measurement was performed at Nutrition and Immunobiology Research Group (NUTRIMU), at Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) and Faculty of Science, University of Porto, Porto, Portugal.

First, each intestine portion was weighted and the pH was measured. After that, samples were homogenized in ice (1:5 dilution) with an Ultra Turrax, centrifuged at 3,300 *g,* for 30 min at 4°C and the supernatant collected and stored at -80°C, until analyses. All enzyme activities were determined using a PowerWavex microplate scanning spectrophotometer (Bio-Tek Instruments, USA).

Total proteases activity was measured by the casein-hydrolysis method according to Hidalgo et al. (1999) with 0.1M tris HCL buffer, for pH 7.8. The reaction mixture containing casein (1% w/v; 0.125 ml), buffer (0.125 ml) and homogenate supernatant was incubated for 1 hour at 37°C and stopped by adding 0.6 ml trichloroacetic acid (8% w/v) solution. After being kept for 1 h at 2°C, samples were centrifuged at 1800 g for 10 min and the supernatant absorbance was read at 280 nm against blanks. A control blank for each sample was prepared adding the supernatant from the homogenates after incubation. Tyrosine solution was used to establish a calibration curve. One unit of enzyme activity was defined as the amount of enzyme needed to catalyze the formation of 1.0 µmol of tyrosine per min.

Amylase (E.C.3.2.1.1) activity was measured using a Spinreact kit (ref. 41201), adapted for fish intestine samples. It comprises in the hydrolysis of 2-chloro-4-nitrophenyl-α-D-maltotrioside by α-amylase; this reaction releases 2-chloro-4-

nitrophenol (CNP) and forms 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and Glucose (G). The rate of 2-chloro-4-nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of α-amylase present in the sample:

$$10\ CNPG_3 \xrightarrow{Amylase} 9\ CNP + 1\ CNPG_2 + G_3 + G$$

The reaction mix cocktail, consisted of amylase reagent (2-chloro-4-nitrophenyl-α-D-maltotrioside, $CNPG_3$) and sample homogenate was adjusted to our samples. This mixture was incubated at 37 ºC during 30 seconds and absorbance (ΔDO/min) was read at 1 minute intervals during 3 minutes at 405 nm and 37°C.

Lipase (EC 3.1.1.3) activity was measured using a Spinreact Kit (Ref. 1001274). This is a new procedure at the NUTRIMU laboratory, so it was needed to validate and adjust this method to fish intestine samples. Validation was accomplished by ensuring linearity of lipase activity in the same sample with different dilutions and comparing it to the quantification of the calibrator activity using the molar extinction coefficient of the reaction product (Methylresorufin) and the theorical activity expected in the kit calibrator. In this method, the pancreatic lipase, along with the colipase, desoxycholate and calcium ions, hydrolyses the substrate 1-2-O-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin)-ester.

1-2-O-dilauryl-rac-glycero-3-glutaric-(6'-methylresorufin)-ester $\xrightarrow{Lipase}$

1-2-O-dilauryl-rac-glycerol + Glutaric-6'-methylresorufin-ester (no stable) $\xrightarrow{OH^-}$

Glutaric acid + Methylresorufin

The rate of methylresorufin formation was quantified photometrically and it is proportional to the concentration of catalytic lipase present in the sample homogenate. The reaction mix cocktail, consists of R1 (TRIS pH 8.3, colipase, desoxycholate and taurodesoxycholate), R2 (tartrate pH 4.0, lipase substrate and calcium chloride ($CaCl_2$)) and sample, was adjusted to our samples. This mixture was incubated for 30 seconds and the sample absorbance (ΔDO/min) was then read at 10 seconds intervals, during 11 minutes, at 580 nm and 37°C.

Trypsin activity was measured following methodology described by Bergmeyer (1994) using 10nM TAME ester (Nα-p-toluenesulfonyl-L-arginine methyl ester) as substrate in Tris-HCl 41.4 mM and CaCl2 10.4 mM (pH 8.1). Enzyme activity was obtained through reading absorbance at 247 nm caused by toluenossulfonil-L-arginine formation. This mixture was incubated for 30 seconds and the sample absorbance (ΔDO/min) was then read at 10 seconds intervals, during 11 minutes, at 580 nm and 37°C.

Chymotrypsin activity was determinated using BTEE (N-benzoyl-L-tyrosine ethyl ester) as substrate following Hummel (1959) and Rao and Lombardi (1975) modifications. The samples absorbance (ΔDO/min) was read at 20 seconds interval, for 15 minutes, at 256 nm and 37°C. Changes on absorbance were considered due to N-benzol-L-tirosine formation.

Enzyme activity of total proteases, amylase and lipase was expressed as specific activity (units per milligram of soluble protein; one unit (U) of activity was defined as μmol of product generated per minute). Soluble protein concentration was determined using Bradford's method (1976), with bovine serum albumin solution as standard. Amylase and lipase activities were determined using the formula:

$$mU\ mg\ protein^{-1} = \frac{(\Delta\ \mathrm{DO}/\ \Delta \mathrm{t}) x\ \mathrm{Vt}\ x\ \mathrm{f}}{\mathrm{Ex}\ x\ 10^{-3}\ x\ 10^{-9}\ x\ \mathrm{Ve}\ x\ \mathrm{d}\ x\ \mathrm{P}} \qquad (14)$$

where (Δ DO/ Δ t) is the decrease or increase of optical density / minute, Vt is the total reaction volume, f is the correction factor for the dilution of the extract, Ex is the molar extinction coefficient, 10^{-3} is the conversion factor of liter to milliliter, 10^{-9} is the conversion factor from mol to nmol, Ve is the volume of added extract in ml, d is the length of the light beam through the microplate and P is the mg of protein per ml.

4. Oxidative Stress Analyses

The enzymatic activity measurement was made at Nutrition and Immunobiology Research Group (NUTRIMU) laboratory from Faculty of Science, University of Porto, Porto, Portugal. First of all, each intestine portion was weighted and pH was measured. After that, samples were homogenized (dilution of 1:5) in ice-cold buffer (100mM-Tris-HCl, 0·1mM-EDTA and 0·1% triton X-100 (v/v), pH 7·8) and 1 ml L^{-1} of PMSF (Phenylmethanesulfonyl fluoride), with an Ultra Turrax, and centrifuged at 16,000 rpm, for 30 min at 4°C. The supernatant was collected and stored at -80°C, until analyses. All enzyme activities were determined using a PowerWavex microplate scanning spectrophotometer (Bio-Tek Instruments, USA).

Superoxide dismutase (SOD) activity was measured at 550 nm by the ferricytochrome C method, using xanthine oxidase as the source of superoxide radicals (McCord & Fridovich, 1969). Catalase (CAT) activity was determined according to Aebi (1984) by measuring the decrease in H_2O_2 concentration at 240 nm. Glutathione Peroxidase (GPX) activity was assayed as described by Flohé & Günzler (1984). The GSSG generated by GPX was reduced by GR, and NADPH consumption rate was monitored at 340 nm. Glutathione reductase (GR) activity was determined at 340 nm by measuring the oxidation of NADPH as described by Morales et al (1990).

Glucose-6-phosphate dehydrogenase (G6PD) activity was assayed by measuring the reduction of NADP+ at 340 nm as previously described by Morales et al (1990). For lipid peroxidation (LPO) measurement the method described by Buege & Aust (1978), reading the absorbance at 535 nm of the reaction malondialdehyd and thiobarbituric acid was used. Soluble protein concentration was determined using Bradford's (1976) method, with bovine serum albumin solution as standard.

Enzyme activity was expressed as units (SOD and CAT) or milliunits (GPX, GR and G6PD) per mg of intestine soluble protein. Except for SOD, one unit of enzyme activity was defined as the amount of enzyme required to transform 1 μmol of substrate per minute under the assay conditions. One unit of SOD activity was defined as the amount of enzyme necessary to produce 50% inhibition of the

ferricytochrome c reduction rate. Lipid peroxidation (LPO) was expressed as nmol of malonoaldehyde per gram of intestine tissue.

5. Intestine Histology

Fish intestine samples included in paraffin were then sectioned (5µm) and stained with hematoxylin and eosin technique (Bancroft et al., 2008). Laminas were photographed using light microscope. Blinded evaluation of the histological samples was performed by disposing scores from 1 to 3, where score 1 indicates normal conditions and 3 for major deformities (Table 2). It was evaluated the following parameters used as criteria of enteritis degree: loss of the supranuclear vacuolization in the absorptive cells (enterocytes) in the intestinal epithelium, widening of the lamina propria and sub mucosa, infiltration of leucocyte in the lamina propria and sub mucosa (Krogdahl et al., 2003).The overall value of the degree of enteritis was calculated by averaging the scores of the separate parameters.

6. Statistical Analyses

Intestine oxidative status and morphology data were subjected to a one-way analysis of variance to determine significant ($p \leq 0.05$) differences among the treatment means. Intestine digestive enzymes data was subjected to a two-way ANOVA (five diets x three intestine sections) and in case of significant interaction a one-way ANOVA analyses were also performed. Tukey's test was used when appropriate to distinguish significant differences between treatment means ($p < 0.05$). Normality and homogeneity of variances were tested using the Levene tests. All statistical analyses were conducted using SPSS 22.0 software package for Windows.

Results

Intestine pH and digestive enzymes

The values of total pH for *P. mesopotamicus* intestine were calculated as the average of the pH for the same intestine portion or same dietary treatment (Table 3). The pH ranged from 6.7 to 7.4 for anterior and distal portions respectively for all

the treatments. Independent of the dietary treatment, the distal intestine portion presented higher pH values ($p < 0.05$) than the anterior and mid portions. There was not observed effects ($p > 0.05$) of dietary treatment in the individual sections of intestine. Total pH of 10DDGS and 20DDGS diets were, however, lower than the obtained for 30DDGS and 40DDGS diets.

Specific activities of digestive enzymes in anterior, mid and distal intestine portions are presented on Table 4. The replacement of soybean meal by corn DDGS affected ($p < 0.05$) the activity of digestive enzymes that, moreover, differs greatly according to the intestine section.

Total protease activity ranged from 1672 to 427 mU mg^{-1} protein, being highest for the 30DDGS diet and lowest for the control diet. It was observed a trend to increase the activity of total proteases with the replacement of soybean meal with DDGS being significant ($p < 0.05$) only for the 30DDGS diet. Moreover, for the control, the total protease activity was higher in the distal than in the anterior or mid intestine, while the opposite was true for the 30DDGS diet.

The activity of lipase, amylase, trypsin and chymotrypsin reduced ($p < 0.05$) as DDGS was included on the diets. Lipase activity varies from 7.0, 1.0 and 0.6 mU mg^{-1} protein for the anterior, mid and distal intestine portion, respectively, for 40DDGS diet to 12.2, 2.7 and 1.2 mU mg^{-1} protein for the control. It was observed a slightly increase of lipase activity for 30DDGS diet in the mid intestine portion (3.5 mU mg^{-1} protein), comparing with the same portion for the control diet. Lipase activity also differs according to the intestine portion, being highest for anterior and lower for mid and distal intestine portion ($p < 0.05$).

Amylase activity was lower ($p < 0.05$) for 30DDGS and 40DDGS diets comparing to the other treatments. The control, 10DDGS and 20DDGS diets did not present differences ($p > 0.05$) for anterior and mid intestine portions, except the mid portion for 20DDGS diet that showed activity similar with the 30DDGS and 40DDGS diets. Differences between intestines portions for fish fed the same diet were observed, being noted a decrease ($p < 0.05$) in the amylase activity in mid and distal portions when compared with the anterior. The difference of amylase activity in the intestine portions was less expressive when fish were fed with 30DDGS and 40DDGS diets.

Trypsin activity varies among treatments only for the mid intestine portion, where the 10DDGS diet expressed the higher activity (6.7 mU mg^{-1} protein) comparing to the other treatments. Variations of activity between intestine segments were observed, except for 30DDGS diet. The anterior and mid intestine portions were similar and both different ($p < 0.05$) from the distal, except for 20DDGS diet where the mid intestine portion was similar to the distal.

The activity of chymotrypsin decreased ($p < 0.05$) with soybean meal replacement by DDGS, ranging from 58.2 x 10^3 for control to 26 x 10^3 mU mg^{-1} protein for 40DDGS diet in anterior intestine portion and from 4 x 10^3 to 0.9 x 10^3 mU mg^{-1} protein for distal. For mid intestine, the control and 40DDGS diet showed the same value of enzyme activity ($p > 0.05$), while 10DDGS diet presented the highest value (24 x 10^3 mU mg^{-1} protein) and the 20DDGS the lowest (9.8 x 10^3 mU mg^{-1} protein). There was also observed a decrease ($p < 0.05$) of chymotrypsin activity on the intestine portions in all treatments.

Total specific activities of digestive enzymes for each treatment were calculated as the sum of the activity expressed on the three distinct intestine portions and are presented in Table 5. Specific activity for all digestive enzymes was reduced ($p < 0.05$) with DDGS inclusion, excepted total protease that was not affected ($p > 0.05$).

The activity of lipase was higher ($p < 0.05$) for diets control, 10DDGS and 30DDGS than for 20DDGS and 40DDGS whereas the lowest activity was expressed for diet 40DDGS, reaching 8.5 mU mg $protein^{-1}$. Amylase exhibited a similar activity profile as lipase, being higher values for control, 10DDGS and 20DDGS diets and lower for 30DDGS and 40DDGS, whereas the lowest activity was expressed for diet 40DDGS (162.7 5 mU mg $protein^{-1}$). Total trypsin activity ranged from 14.4 for 10DDGS diet to 8.8 mU mg $protein^{-1}$ for 30DDGS. However, control, 20DDGS and 40DDGS diets were not different ($p > 0.05$). The total activity of chymotrypsin was higher for the control than for the diets with DDGS inclusion, attaching 44.4 x 10^3 of mU mg $protein^{-1}$, for 40DDGS diet.

The ratios of amylase/protease (A/P) and amylase/lipase (A/L) were also reduced ($p < 0.05$) with increasing levels of DDGS inclusion. A/P ratio appeared with lower values for the diets 30DDGS and 40DDGS, in agreement with the results found for total amylase activity. The same is true for A/L, which ranged from 18.4

for 30DDGS diet to 27 mU mg protein^{-1} for the 10DDGS. L/P ratio was not affected by dietary treatments ($p > 0.05$).

The distribution of total specific activities of digestive enzymes on the different portions of *P. mesopotamicus* intestine is presented on Table 6. It was observed that the most digestive enzymes evaluated have the activity decreasing ($p < 0.05$) from anterior to distal intestine portions, except for proteases that, after presents a reduction ($p < 0.05$) from anterior to mid portions had the activity growing back for the distal.

Total lipase activity ranged from 10 to 0.7 mU mg^{-1} protein for anterior and distal intestine portions, respectively, being the last one the lower value of enzyme activity found in *P. mesopotamicus* intestine. The activity of amylase showed a 30% decrease ($p < 0.05$) from the anterior to distal portion. The same behavior was observed for trypsin that presented 5.4 mU mg^{-1} protein of activity in the anterior intestine portion and 1.9 in the distal. However, the most expressive activity decrease was observed for chymotrypsin, which values ranged from 38.9 x 10^3 for the anterior to 0.007 mU mg^{-1} of protein for the distal portion.

Oxidative Stress Enzymes

The activity of the enzymes glucose-6-phosphate dehydrogenase (G6PD); glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO), related with the oxidative stress status of the intestine of *P. mesopotamicus* juveniles, is shown in Table 7. There were found differences ($p < 0.05$) between the DDGS dietary inclusion levels for the enzymes activities.

G6PD showed a decrease ($p < 0.05$) of specific activity the extent to which DDGS was included in the diet and soybean meal replaced, reaching 222.0 for the control and 159.2 UI mg of protein $^{-1}$ for the 40DDGS diet. Also, the activity of LPO reduced ($p < 0.05$) with DDGS inclusion, attaching 6.7 for the 30DDGS diet compared to 11.9 nmol malondialdehyde gram of tissue^{-1} for the control. Even though the values of treatments with DDGS inclusion were not different between them ($p > 0.05$), they were lower ($p < 0.05$) than those of the control.

Otherwise, the activity of catalase increased ($p < 0.05$) with DDGS inclusion, comparing to the control that presented the lowest value, 51.4 UI mg of protein $^{-1}$ compared to 69.7 for the 10DDGS diet that was the higher. Diets with DDGS, independent of the level of inclusion, had similar activity ($p > 0.05$). The activity of GPX, GR and SOD in *P. mesopotamicus* intestine did not change ($p > 0.05$) as DDGS was included in the diet.

Histology

The average scores of parameters used to assess gut morphology were affected by dietary treatments (Table 8). It was observed higher score values for the intestine of fish fed diet with control and 10DDGS diets, whereas diet 40DDGS presented the lowest score ($p < 0.05$). The other treatments did not differ between them ($p > 0.05$). Between the parameters evaluated reduction of damage status of the intestine was marked by reduction in width and cellularity of lamina propria and normalization of enterocytes vacuolization (Figure 01).

Discussion

Intestinal pH profile

In normal situations, intestinal pH of fish trend to increase as it distances from the stomach. This fact was true for pacu in the present study, where distal intestine had higher pH than anterior and mid sections. In controversial situations, the pH is altered by pathogen agents (Montgomery & Pollak, 1988) modifying its behavior. The intestinal pH values obtained in our study are in the range of 6.5 to 8.2, suggested by Krogdahl et al. (2015) for a variety of fish species.

Basically, there are two ways of controlling the intestinal pH of fish, one trough secretion of neutralize substances, in the proximal intestine, by the liver and pancreas (Nikolopoulou et al., 2011) and another by anion absorption that occurs majorly in the distal intestine (NRC, 2011). Diet composition may interfere on the pH of the intestine. Some dietary components such as protein, carbohydrate and salt may have distinct buffering capacity (Thompson & Weber 1979) that will exert influence on the pH of the intestinal tract. Dietary protein levels and profile exert

influence on the pH of fish intestine and chime, as observed for Rhoads et al. (1994) and Nikolopoulou et al. (2011) that are probably related with the transporters used for amino acids and peptides intestine absorption (Rotta, 2003). Adding to that fiber content can also play a role on intestinal pH since interactions between minerals and ions may happen depending on the dietary fiber source (Wong & Cheung 2005).

In the present study, the increasing levels of DDGS in experimental diets and probably modifications on amino acids profile and also the increase in fiber content may have lead in increases on pH of fish fed 30 and 40DDGS diets. Similar to this study, an alkalization of cecum and apex was observed for pigs fed 30% of DDGS diet (Wilberts et al., 2014). Contrarily, Yousseff et al. (2013) observed that increasing levels of DDGS until 15% in diets for broilers provoked an acidification of duodenum and ileum. The same was true for swine fed a diet containing 30% of corn DDGS inclusion comparing to diet with 30% of wheat middling (Moran et al., 2016).

Digestive enzymes profile

The behavior of digestive enzymes along pacu intestine observed in the present study is in accordance with Hlophe et al. (2014) for a carnivorous fish (*C. gariepinus*) and two tilapia species (*T. rendalli* and *O. mossambicus*). Hlophe et al. (2014) had shown that amylase and lipase activities were higher for the proximal intestine than the distal for all the three species, independent of food habit. The same was observed for Nile tilapia (Klahan et al., 2009) where lipase activity was higher in the proximal intestine of the fish. Lipase and amylase are produced by pancreas and released directly to the first portion of fish intestine, thus it is notable higher activities of those enzymes in this area.

Otherwise, Hlophe et al. (2014) also observed that total protease activity was higher for the distal intestine for all species evaluated. In accordance, pacu fed the control diet also presented higher activity of total protease in distal intestine. This may be related with the mechanism which protein was absorbed by the intestine. Protein can be absorbed as free amino acids in enterocytes or as peptides (bigger molecules) by pinocytosis. The first occurs basically in the proximal portion of the

intestine, whereas the second commonly happened in the distal intestine (NRC, 2011; Rotta, 2003). Thus, distribution of total protease activity in fish intestine seems to be dependent on the preferentially rote of absorption of this nutrient by the organism and even dietary protein profile.

Among the digestive enzymes, protease activity does not seem very dependent on the fish nutritional habits (Chan et al., 2004) unlikely amylase and lipase. As omnivorous specie, higher activity of total protease in pacu intestine than the other enzymes evaluated may appear as an alternative used for the fish to work around its limited capacity in utilize protein when compared with carnivorous specie (Hidalgo et al., 1999). Most of the protein used to compound the experimental diets came from vegetable sources (soybean, corn gluten meal and DDGS). Even though some plants had shown positive results as protein source in fish diets, they contain components (fiber and others cell wall components) that can difficult protein digestibility and absorption. Thus, the higher activity of total protease observed in the present study may be a mechanism used by fish organism to permit a better utilization of the protein consumed.

Not only animal phylogeny, but diet composition is also a noted factor that may bring influence on the activity of intestine enzymes (German et al., 2004; Chaudhuri et al., 2012; Saha and Ray, 1998). Even better for omnivorous species that have, naturally, higher plasticity of increasing amylase and lipase syntheses in response of higher amounts of starch and lipids in the diets (Hidalgo et al., 1999). However, even though it was observed an increase in lipid contents in experimental diets, all digestive enzymes activity decreased with DDGS inclusion, inducing the presence of some factors capable of increasing dietary nutrients utilization. Distinctly, the inclusion of 28% of rice DDG in diets for olive flounder juveniles (*Paralichthys olivaceus*) did not provide interference on the activity of trypsin, amylase and lipase (Rahman et al., 2015).

Non-starch polysaccharides (NSP), commonly present in vegetal ingredients, are molecules of sugar resistant to hydrolysis process in animals' gastrointestinal tract during dietary nutrients metabolism. Non-starch polysaccharides may vary among vegetal plants and culture conditions (Rosa & Uttapel, 2007; Caprita et al., 2010) and can be classified in soluble and insoluble. Soluble NSP is related with antinutritional factors, while the insoluble form may exert

influence on gut retention time and water absorption (Opalinski, 2006). The effects of NSP on digestion process and digestibility of dietary ingredients have been reported for a variety of fish species such as Atlantic salmon (Refstie et al., 1999), catfish, *Clarias gariepinus,* (Leenhouwers et al., 2006; Leenhouwers et al., 2007b) and Nile Tilapia, *Oreochromis niloticus,* (Leenhouwers et al., 2007a; Haidar et al., 2016). As DDGS derivate from corn, differences in NSP composition of corn and soybean meal probably interfere on nutrients digestibility and enzymes activity.

Smits and Annison (1996) reported differences between the amount and profile of NSP in corn and soybean meal. Corn showed a total of NSP equal to 8%, which 6% was on the insoluble form, while for soybean meal they reported 27% of NSP where soluble form corresponded in 6% only. As DDGS derives from corn, this proportion of soluble and insoluble NSP forms is probably maintained. Soluble NSP is reported to have higher digestibility than the insoluble form thus, it is possible that the control diet, in the present study, had higher amounts of insoluble NSP than the diets with DDGS inclusion. Higher insoluble NSP reflects on less contact between substrate and digestive enzymes in the chime due to increases on diet viscosity and reduction on gut retention time, caused by the excess of insoluble NSP (Andriguetto et al., 2002). Therefore, DDGS inclusion may have reduced the presence of indigestible NSP in fish intestine, allowing higher substrate-enzymes contact in e chime and consequently more efficiently digestibility process. This may explain the reduction on activity of all digestive enzymes studied for pacu in the present study.

Also, the weakly alkalization of fish intestine with DDGS inclusion in diets observed in the present study may provide better conditions for digestive enzymes activity, making nutrients digestible processes more efficient. Evaluating the optimum pH for digestive enzymes activities for some fish species with distinct food habit Solovyev et al. (2015) observed that, independent of the species, the optimum pH values for enzymatic activity comported the same way; higher than the range reported in fish intestine. Xiong et al. (2011) reported for *Glyptosternum maculatum*, a carnivorous fish, optimum pH for amylase and lipase activity close to neutral and alkaline conditions, whereas protease had higher activity with pH ranging from 9 to 10.

Another issue that may play a rule on digestive enzymes activity is the presence of significant amount of yeast cells in DDGS (Ingledew, 1999; Zohu et al.,

2010). Yeast as yeast cell wall components are known to beneficiate dietary nutrient digestibility (Lara-Flores et al., 2003; Guo et al., 2003; Li and Gatlin, 2005; Yang et al., 2007; Dimitroglou et al., 2009). The increase presence of these components in experimental diets as DDGS was included may have contributed with improvements on nutrients digestibility in the present study, minimizing digestives enzymes activity enforcements on dietary nutrients metabolism.

The presence of protease inhibitors and lectins in soybean meal after heating treatments remain in lack. Van den Ingh et al. (1996) affirmed that it is impossible the presence of antinutritional factors after soy grain treatments. However other authors have being pointed that the presence of those antinutritional factors as responsible for reduction on feed intake and intestinal damages (Francis et al., 2001; Webster et al. 1992a). Reduction and absence of soybean meal in experimental diets may have also leaded in reduced activity of digestive enzymes, majorly chymotrypsin and trypsin due to reductions on antinutritional factors, absent in DDGS.

Intestine Oxidative Status and Morphology

Reactive oxygen species (ROS) are highly reactive molecules including free radicals, superoxide (O_2^{-}•) and hydroxyl radicals (HO•), and a non-radical, hydrogen peroxide (H_2O_2). Those components are capable to induce deleterious effects on animal tissues by modifying lipids, proteins and DNA structures (Girotti, 1998; Cabiscol et al., 2000; Salmon et al., 2004). ROS can be produced from endogenous sources, like aerobic metabolism (Datta et al., 2012) or from exogenous sources such as diet composition (Jiang et al., 2010). The imbalance between ROS formation and removal in organism (Zheng et al., 2013) characterizes an oxidative stress situation. High ROS production can lead in changes on animal organism capable of ended up in cell death and tissue injury like damages on intestine epithelium cells (Chen et al., 2009). Those modifications include changes on antioxidant enzymatic activities, damages on cells membrane through peroxidation of membrane lipids and modification of nucleic acids.

Fish organism antioxidant defense count with non-enzymatic and enzymatic actions, where the last one occurs by directly participation of the enzymes

superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Trenzado et al., 2006), as well as glucose-6-phosphate dehydrogenase (Enes et al., 2012; Munhoz & Netto, 2004). In synthesis, the anti-oxidant action of those enzyme includes the conversion by superoxide dismutase of $O_2^{-}\bullet$ in H_2O_2, compound that will subsequently be break down in water molecules by the enzymes catalase and glutathione peroxidase. Glutathione redox cycle is an important antioxidant defense mechanism. Glutathione peroxidase uses as substrate GSH (thiol tripeptide glutathione) a non-enzymatic anti-oxidant. Meanwhile, the enzyme glutathione reductase is responsible for GSH regeneration from GSSG (oxidized glutathione) reduction turning this component available for reutilization for ROS removal.

Nutrition is one of the factors capable of exert influence on oxidative status of fish intestine (Bayir et al., 2011; Morales et al., 2004; Olsvik et al., 2011; Pérez-Jiménez et al., 2009, 2012b). So, select ingredients capable of enhance antioxidant system and prevent those responsible for ROS production increment are alternatives for avoiding cells oxidative stress. Dietary changes in DDGS levels of inclusion and soybean meal replacement, in the present study, seem to have played influence on the results about pacu intestine oxidative status and morphology.

Evaluation of DDGS inclusion in diets and the effects on oxidative status of animals' intestine is a lack. Studies have been focused on influence of DDGS in liver and serum oxidative status due to the high polyunsaturated fatty acids content in DDGS. In broilers, fed 15% DDGS diets, DDGS appeared to reduce total capacity of antioxidant status and superoxidase dismutase activity in plasma and liver (Min et al., 2015). Although Hanson et al. (2015) did not find influence of DDGS-based diets on oxidative status of nursery pigs. Considering that high lipid peroxidation values are indicative of oxidative stress in fish tissues (Ferreira et al. 2005; Farombi et al. 2007), the reduction of intestine lipid peroxidation, obtained in the present study, for diets with DDGS inclusion instead of control, appears to be an indicative of some anti-oxidative agents in DDGS composition.

The effects of DDGS on tissue oxidative status may be related with its chemical composition, like the elevated amounts of non-starch polysaccharides and the presence of yeast cells components. High amounts of dietary non-starch polysaccharides (NSP) in DDGS can cause changes on fish gut microbial

population (Amirkolaie et al., 2006; Leenhouwers et al., 2007b; Dimitroglou et al., 2010) and intestine ecosystem (Sinha et al., 2011). Fish intestine microbiota has the capacity of produce hydrogen peroxide (H_2O_2), even if as a defense from pathogens (Olsen et al., 2008). Thus, it is not discarded that modifications on gut microorganism profile may play an important role on the oxidative status of fish intestine. Even though DDGS has high amounts of NSP, the reduction on oxidative status of *P. mesopotamicus* intestine suggest that, somehow, dietary inclusion of DDGS may have brought improvements on gut microbiota population and immunity, avoiding a potential oxidative stress situation. However, microbiological and immunological analyses were not preceded in the present study, being an important issue to be evaluated in later researches.

Not only NSP, but the addition of yeast (*Saccharomyces cerevisiae*) during DDGS production may have generated interferences on oxidative status of pacu intestine. *Saccharomyces cerevisiae* is known to have distinct protective oxidative stress responses in animals' organism (Jamieson, 1992; Collinson and Dawes, 1992; Flattery-O'Brien, et al., 1993; Jamieson et al., 1994; Duncan and Klesius, 1996; Wu et al., 2011; Santacroce et al., 2012) including some fish species such as rainbow trout (Nakano et al. 1999), gilthead seabream (Reyes-Becerril et al. 2008a) and sea bass (Tovar-Ramirez et al. 2010). Even its cell walls components such as mannans and glucans have been shown to bring antioxidant benefits (Krizˇkova´ et al. 2001; Jaehrig et al. 2007). Adding to that, yeast as well as its cell wall components are capable of modulating gut microbial communities (Gatesoupe, 2007; Sweetman et al., 2010) allowing changes on ROS production in fish organism.

One of the mechanisms responsible for anti-oxidant defense in animal tissues includes increasing on catalase activity stimulated by high presence of ROS in the organism (Tovar-Ramírez et al. 2010). Catalase is an enzyme that catalyzes the breaking down reaction of hydrogen peroxide (H_2O_2) to water and oxygen molecules. Corroborating with the present study, high activity of catalase was reported for different aquatic species (Tovar-Ramírez et al., 2010; Jiang et al., 2010) as an anti-oxidative defense. Enhancements on catalase activity might have suffer influence with probiotics inclusion in diets, as reported by Castex et al. (2009) that obtained higher activity of CAT for shrimp, *Litopenaeus stylirostris* fed a treated diet

with probiotic than control diet. Thus, the presence of yeast cells in DDGS may have promoted an increase in catalase activity, improving ROS removal mechanisms.

Another enzyme that is related with oxidative status in animal organism is glucose-6-phosphate dehydrogenase (G6PDH) that converts glucose-6-phosphate into 6-phosphogluconate generating NADPH. NADPH supports the action of the enzyme glutathione reductase that restores the levels of reduced glutathione in the organism (Gaetani et al., 1989). However, in the present study reduction of G6PDH does not seem to be related with anti-oxidant defense but with lipids content in diets. G6PDH is one of the major rate-limiting enzymes from pentose phosphate pathway and has high relation with lipogenesis, since its product, NADPH, is very important in biosynthesis of fatty acids and cholesterol. Once lipids contents were increasing in experimental diets as DDGS was being included, lipogenesis was reduced due to high lipids availability, what was represented by reductions on G6PDH activity.

Besides DDGS levels of inclusion, another factor that may have played an important rule on oxidative status of pacu intestine is the removal of soybean meal from experimental diets. High lipid peroxidation in the intestine of fish fed control diet may be an indicative of anti-nutritional effects of soybean meal components such as β-conglycinin, glycinin, lectin and trypsin inhibitor (Zhang et al., 2013; Brandon et al., 1991). Even after passing by heat treatment some anti-nutritional factors can still be present and active in soybean grains like saponins, non-starch polysaccharides, antigenic proteins, estrogens and some phenolic compounds (Van der Peol, 1989; Rumsey et al., 1993). Among those anti-nutritional factors, trypsin inhibitor have been shown to have a close relationship with oxidative stress in animal tissue, due to its capacity of combined with dietary trypsin and chymotrypsin This reaction originates a complex that deactivates those two digestive enzymes, forcing pancreas in increasing the synthesis and secretion of those digestive enzymes (Huisman, 1991). Synthesis and secretion of enzyme are dependent of ATP. However, ATP production includes the formation of reactive oxygen species as by-products becoming a factor capable of induce oxidative stress in animal tissues. Gu et al. (2011) concluded that anti-nutritional factors present in soybean were responsible for promoting increase in free radical levels and an imbalance between ROS production and removal in mice fed raw soybean-based diets.

Damages on gut morphology, such as inflammatory reactions, are commonly accomplished by lipid oxidation (Yoshikawa et al., 1987). It is known that soybean protein may induce inflammation in the distal portion of fish intestine (Krogdah et al., 2003; Bakke-Mckellep et al., 2007). Lectins and saponins, anti-nutritional factors present in soybean, have been shown to have the ability of produce histological abnormalities in gastrointestinal tract cells affecting mucosal-cell membrane permeability, what might facilitate the development of enteritis in some fish species (NRC, 2011). The replacement of soybean meal by corn DDGS promoted changes on pacu intestinal morphology. Improvements on intestine health status of fish fed DDGS diets, majorly highlighted for 40DDGS diet, may be related with dietary chemical composition. Increments of yeast contents, parts of DDGS composition, in the experimental diets may have had influence on gut morphology changes.

Adding to that yeast cells also have been shown to improve gut morphology of some fish species. Omar et al. (2012) observed increase in goblet cells in the posterior section of the gut of carp fed increasing quantity of yeast protein concentrate, probably due to components like β-glucans or mannam oligosaccharides. European seabass (*Discentrarchus labrax)* fed diets with increasing levels of manna oligosaccharides had the number of goblet cells in the intestine increased (Torrecillas et al., 2011). Promising results have been published referring improved morphological properties and the proliferation capacity of microvilli of the intestinal epithelium (JarmoŁowicz et al., 2012; Merrifield et al., 2010a; Sáenz de Rodrigáñez et al., 2009),

In summary, corn DDGS inclusion in diets for *Piaractus mesopotamicus* juveniles reduced intestinal digestive enzymes activity, promoted equal balance of ROS production/removal in the intestine avoiding oxidative stress on fish intestine and reduce gut morphology damage. Therefore, it is necessary deeper studies about gut microbiota profile to allow better and clarified understanding about the participation of microorganisms on nutrients metabolism and maintenance of fish gut health.

Conclusion

Inclusion of corn DDGS in diets for *Piaractus mesopotamicus* juveniles in levels up to 40% promoted improvements on intestine digestive enzymes efficiency

and morphology, as well as reductions on oxidative status, allowing high nutrient absorbance and digestion efficiency by fish organism.

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Tables

Table 1. Formulation and chemical composition of experimental diets

Diet	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS
Ingredients					
DDGS[1]	0.0	10.0	20.0	30.0	40.0
Soybean meal[2]	23.1	17.3	11.5	5.8	0.0
Corn[2]	25.4	21.8	18.1	14.5	10.8
Soybean oil	3.4	2.8	2.3	1.7	1.1
Poultry meal[4]	6.6	6.6	6.6	6.6	6.6
Fish meal[3]	11.0	11.0	11.0	11.0	11.0
Corn Gluten[3]	8.3	8.3	8.3	8.3	8.3
Wheat meal[2]	13.0	13.0	13.0	13.0	13.0
Rice bran[2]	7.7	7.7	7.7	7.7	7.7
L-Lysine[5]	0.5	0.5	0.5	0.5	0.5
BHT[6]	0.1	0.1	0.1	0.1	0.1
Vit. and Min. Premix[7]	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0
Chemical Composition					
Dry Matter	93.1	91.6	93.4	91.3	93.8
Crude Protein (% DM)	32.8	32.1	32.7	32.1	32.7
Gross Energy (kj g $^{-1}$)*	18.6	19.1	19.4	19.6	19.9
Crude Lipid (% DM)	3.2	5.2	5.8	7.1	7.6
Ash (% DM)	8.2	7.8	6.9	6.9	6.6
NDF	24.9	28.1	32.8	38.7	44.2
Starch	22.8	19.6	18.1	15.7	14.3
Digestible Protein (% DM)	29.5	27.6	29.4	28.2	29.1
Digestible Energy (kj g $^{-1}$)	14.7	14.7	14.9	12.5	14.5
DP/DE **	2.0	1.9	2.0	2.3	2.0

[1] Corn Distiller's Dried Grains with Soluble (CP 33.6% 20.9 MJkg^{-1}), Libra Etanol, Mato Grosso, Brazil;
[2] Soybean meal (CP 44.8% 17.1 MJkg^{-1}), Corn Meal (CP 8.4% 16.2 MJkg^{-1}), Wheat Meal (CP 14.8% 16.7 MJkg^{-1}), Rice Bran (CP 13.2% 19.4 MJkg^{-1}), Cargill, Sao Paulo, Brazil;
[3] Corn Gluten (CP 71.5% 21.1 MJkg^{-1}), FM: Fish meal (CP 58.0% 14.6 MJkg^{-1}), In Vivo, Sao Paulo, Brazil);
[4] Poultry Meal (CP 48.9% 13.1 MJ kg^{-1}), Agromix, Sao Paulo, Brazil;
[5] Lysine, Anjinomoto LTDA, Sao Paulo, Brazil;
[6] BHT (Butyl hydroxytoluene);
[7] Vitamin and Mineral Premix: vitamin A - 500.000 UI; vitamin D3 - 250.000 UI; vitamin E - 5.000 mg; vitamin K3 - 500 mg; vitamin B1 - 1.500 mg; vitamin B2 - 1.500 mg; vitamin B6 - 1.500 mg; vitamin B12 - 4.000 mg; folic acid - 500 mg; pantothenate Ca - 4.000 mg; vitamin C - 10.000 mg; biotin - 10 mg; Inositol - 1.000; nicotinamide - 7.000; choline - 10.000 mg; Co - 10 mg; Cu - 1.000 mg; Fe - 5.000 mg; I - 200 mg; Mn - 1500 mg; Se - 30 mg; Zn - 9.000 mg$^{3.}$ (Agromix LTDA, Sao Paulo, Brazil)
* Values calculated according to FAO (2012) and NRC (2011)
** DP/DE: digestible protein/digestible energy ratio

Table 2. Scale scoring system of intestine histology descriptive analysis with the range of tissue scores set at 1 to 3*

	Score range	
	1	to 3
Gut folds	Tall and distinct	Short, indistinct, fused
Lamina propria width and cellularity	Thin, low cellularity	Markedly widened and increased cellularity
Intraepithelial leucocytes infiltration	Low infiltration	Highly infiltrated
Enterocytes vacuolization	Well defined, regular vacuoles size	Absent or hyper vacuolated; irregular vacuoles size
Enterocytes nucleus position	Basal	Apical

*Descriptive analysis based on Krogdahl et al., 2003

Table 3. Mean values of pH from the intestine portions of *P. mesopotamicus* juveniles fed experimental diets[1].

Diets	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	Mean	p-value	SEM
Anterior	6.8	6.7	6.8	6.9	6.8	6.8^{B}	0.00	0.02
Mid	6.8	6.8	6.8	7.0	6.9	6.8^{B}	0.00	0.03
Distal	7.2	7.1	7.0	7.3	7.4	7.2^{A}	0.00	0.04
Mean	6.9^{bc}	6.87^{c}	6.86^{b}	7.1^{a}	7.0^{ab}	-	0.003	0.03

[1]Values presented as means (n = 6) and pooled standard error of the mean (SEM). In the same row, different superscript letters represent significant differences between diets and in the same column means with different capital letters represent significant differences between intestine sections ($p < 0.05$)

Table 4. Specific activities of proteases, lipase, amylase, trypsin and chymotrypsin (mU mg^{-1} protein) in different intestine sections of *P. mesopotamicus* fed experimental diets[1].

Diet	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS
Proteases					
Anterior	538.2^{Bb}	719.7^{ab}	687.6^{b}	1671.7^{Aa}	650.0^{b}
Mid	460.0^{B}	536.8	426.8	489.3^{B}	553.2
Distal	834.0^{A}	577.2	675.6	743.3^{B}	468.9
Lipase					
Anterior	12.3^{Aa}	10.5^{Aa}	10.5^{Aa}	9.8^{Aab}	7.0^{Ab}
Mid	2.7^{Ba}	3.4^{Ba}	1.0^{Bb}	3.5^{Ba}	1.0^{Bb}
Distal	1.2^{Ba}	0.5^{Cbc}	1.1^{Bab}	0.4^{Ac}	0.6^{Babc}
Amylase					
Anterior	247.6^{Aa}	210.8^{Aa}	192.7^{Aab}	132.4^{Abc}	67.0^{Ac}
Mid	114.1^{Ba}	110.8^{Bab}	59.5^{Bc}	76.9^{Bbc}	66.7^{Ac}
Distal	53.5^{Cab}	60.9^{Cab}	67.6^{Ba}	42.6^{Bbc}	29.0^{Bc}
Trypsin					
Anterior	6.1^{A}	6.1^{A}	6.0^{A}	3.8	5.2^{A}
Mid	4.2^{ABb}	6.7^{Aa}	2.1^{Bb}	3.4^{b}	3.7^{Bb}
Distal	2.0^{B}	1.8^{B}	2.0^{B}	1.9	1.9^{B}
*Chymotrypsin**					
Anterior	58.2^{Aa}	35.7^{Abc}	45.8^{Aab}	29.0^{Abc}	25.7^{Ac}
Mid	17.3^{Bb}	23.7^{Ba}	9.8^{Bc}	13.9^{Bbc}	17.3^{Ab}
Distal	4.4^{Ba}	1.2^{Cb}	2.8^{Bbc}	1.3^{Cb}	0.9^{Bb}

Two-way ANOVA

	Diet	Section	Interaction	SEM
Proteases	$p < 0.001$	$p < 0.007$	$p < 0.002$	46.9
Lipase	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	0.5
Amylase	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	7.2
Trypsin	$p < 0.003$	$p < 0.0001$	$p < 0.002$	0.2
Chymotrypsin	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	18.7

[1]Values presented as means (n = 6) and pooled standard error of the mean (SEM).
In the same row, different superscript letters represents significant differences between diets and in the same column means with different capital letters represents significant differences between intestine sections ($p < 0.05$). Two-way ANOVA: * ($p < 0.05$). If interaction was significant, a One-Way ANOVA was performed for each factor (diet and section).

Table 5. Total specific activities of digestive enzymes (mU mg protein^{-1}) in *P. mesopotamicus* juveniles fed experimental diets[1].

Diets	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	p-value	SEM
Proteases	1832.2	1833.2	1790.0	2904.3	1672.0	0.077	157.1
Lipase	16.2^{a}	14.4^{ab}	12.5^{b}	13.6^{ab}	8.5^{c}	0.000	0.6
Amylase	415.2^{cd}	382.6^{c}	319.8^{bc}	251.9^{b}	162.7^{a}	0.000	18.2
Trypsin	12.3^{ab}	14.6^{b}	10.1^{ab}	8.8^{a}	10.8^{ab}	0.011	0.6
Chymotrypsin*	79.9^{a}	60.6^{a}	58.5^{b}	44.2^{b}	44.4^{b}	0.000	3.0
A/P[2]	0.2^{b}	0.2^{b}	0.2^{ab}	0.1^{a}	0.1^{a}	0.001	0.0
L/P[3]	0.01	0.01	0.01	0.01	0.01	0.134	0.0
A/L[4]	25.7^{bc}	27.0^{c}	26.4^{bc}	18.4^{a}	19.6^{ab}	0.003	0.1

[1]Values presented as means (n = 6) and pooled standard error of the mean (SEM). Means with different letters represents significant differences between intestine sections ($p < 0.05$).
[2]amylase/proteases ratio
[3]lipase/protease ratio
[4]amylase/lipase ratio
*Values are shown in numeric scale of thousands

Table 6. Total specific activities (mU mg^{-1} protein) of digestive enzymes in different intestine sections of *P. mesopotamicus* juveniles[1]

Intestine Section	Anterior	Mid	Distal	p-value	SEM
Proteases	853.4^{a}	493.2^{b}	659.8^{ab}	0.006	46.9
Lipase	10.0^{a}	2.3^{b}	0.7^{c}	0.000	0.6
Amylase	170.1^{a}	85.6^{b}	50.7^{c}	0.000	7.2
Trypsin	5.4^{a}	4.0^{b}	1.9^{c}	0.000	0.2
Chymotrypsin*	38.9^{a}	16.5^{b}	0.007^{c}	0.000	18.7

[1]Values presented as means (n = 6) and pooled standard error of the mean (SEM). Means with different letters represents significant differences between intestine sections ($p < 0.05$).
*values are shown in numeric scale of thousands

Table 7. Specific activities of oxidative stress enzymes G6PD, GPX, GR (mU/mg protein), SOD, CAT (UI/mg protein) and LPO (nmol malondialdehyde per g tissue) of distal portion of *P. mesopotamicus* intestines fed experimental diets[1].

Diet	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	p-value	SEM
Enzymes							
G6PD	222.0^{a}	211.3^{a}	225.2^{a}	164.4^{b}	159.2^{b}	0.000	7.0
GPX	69.6	67.7	61.8	72.6	73.7	0.875	3.6
GR	34.8	35.3	31.9	29.8	31.8	0.317	1.1
SOD	132.4	160.7	106.8	133.3	183.0	0.094	9.3
CAT	51.4^{c}	69.7^{a}	61.1^{b}	67.0^{ab}	63.2^{ab}	0.004	1.4
LPO	11.9^{a}	8.2^{b}	8.4^{b}	6.7^{b}	7.9^{b}	0.000	0.4

[1]Values presented as means (n = 6) and pooled standard error of the mean (SEM). Means with different letters represents significant differences between treatments ($p < 0.05$);
G6PD: Glucose-6-phosphate dehydrogenase; GPX: glutathione peroxidase; GR: glutathione reductase; SOD: superoxide dismutase; CAT: catalase; LPO: and lipid peroxidation.

Table 8. Descriptive histology analysis of pacu intestine fed experimental diets with increasing levels of DDGS inclusion[1].

	Control	10 DDGS	20 DDGS	30 DDGS	40 DDGS	p-value
Score	1.87±0.4^{a}	1.91±0.4^{a}	1.73±0.2 ^{ab}	1.79±0.3 ^{ab}	1.70±0.2^{b}	0.04

[1]Values presented as means ± standard deviation (n = 24). Score from 1 to 3, with 3 indicating major alterations. Mean scores calculated by averaging the scores of parameters evaluated. Means with different letters represents significant differences between treatments ($p < 0.05$)

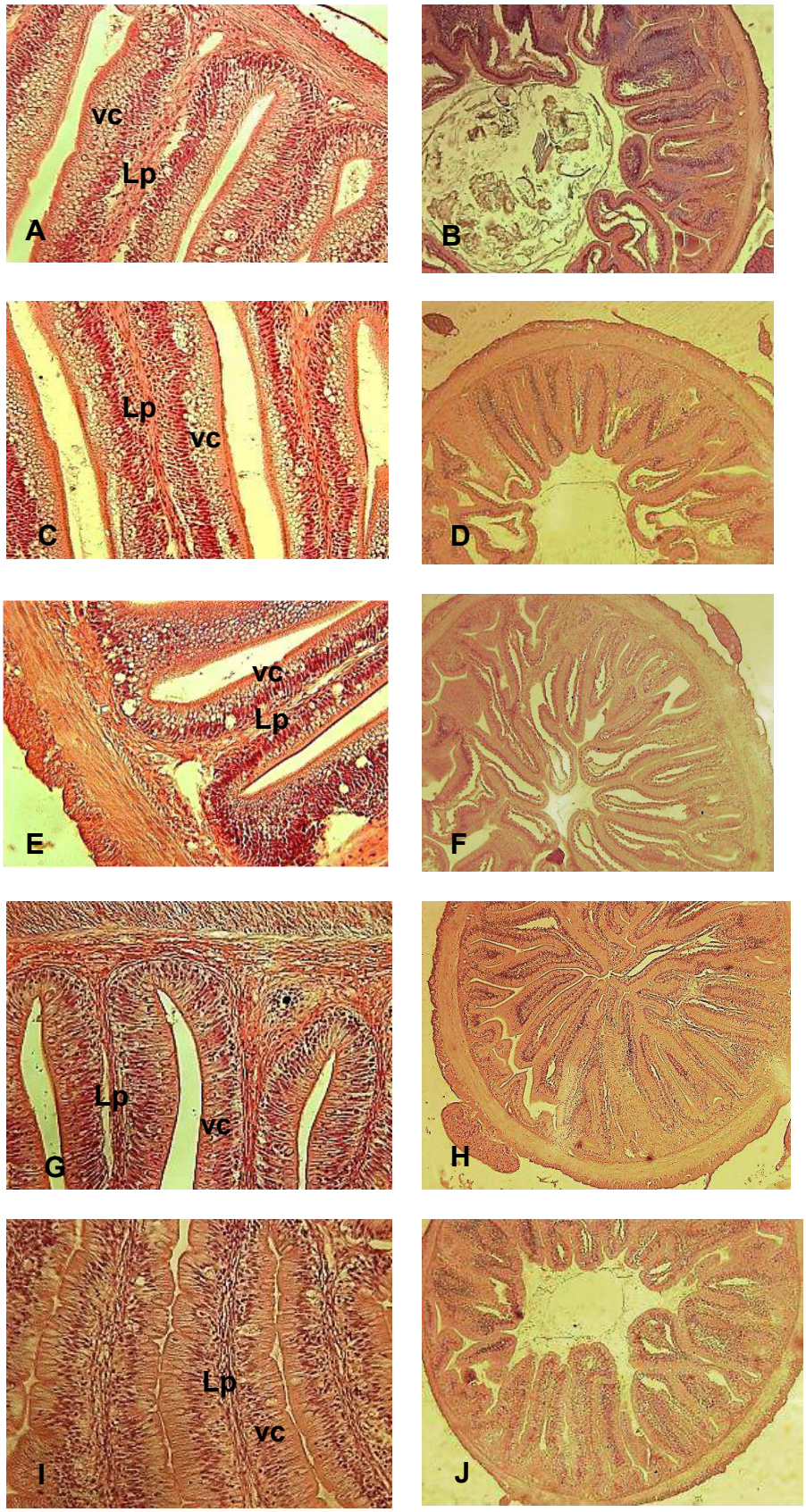

Figure 1: Histological modifications on intestines of *P. mesopotamicus* juveniles feeding diets with increasing levels of corn DDGS inclusion and soybean meal replacement. Images H&E. Objective lens: 40X (right) and 10X (left): Control (A, B), 10DDGS (C, D), 20DDGS (E, F), 30DDGS (G, H), 40DDGS (I, J). Lp, lamina propria; vc, vacuoles

Discussão Geral

Este é o primeiro trabalho a avaliar a inclusão de grãos de milho de destilaria com solúveis (DDGS) em dietas para *Piaractus mesopotamicus* e os possíveis efeitos tanto quanto aos parâmetros produtivos, como desempenho e viabilidade econômica, quanto aos parâmetros fisiológicos: digestibilidade, atividade das enzimas digestivas, status oxidativo e morfologia intestinal. Através da avaliação destes parâmetros foi possível uma maior contextualização entre os efeitos da inclusão do DDGS nas dietas e as reações fisiológicas e metabólicas provocadas no organismo dos peixes.

Grãos de milho secos de destilaria com solúveis, DDGS, apresentou-se como um candidato a ingrediente proteico na alimentação de juvenis de pacu, uma vez que apresentou coeficiente de digestibilidade aparente (CDA) para proteína superior a maioria dos ingredientes proteicos comumente utilizados na composição das rações para esta espécie (Abimorad, 2004; Fabregat et al., 2008; Abimorad et al., 2008). Além disto, o pacu mostru-se uma espécie capaz de utilizar a proteína do DDGS com eficiência, sendo o CDA para proteina maior do que o obtido para este ingrediente em outras espécies de peixe (Li et al., 2011; Thompson et al., 2008) e outras espécies animais (Ren et al., 2011; Liu et al., 2011). Contudo, foi observado que, crescentes níveis de inclusão de DDGS levaram a uma redução na digestibilidade das dietas para matéria seca e energia. Tal redução foi relacionada ao aumento no teor de fibra das dietas, resultado do elevado conteúdo deste nutriente no DDGS (FDN = 56.4%).

Fibras são componentes importantes e indispensáveis na alimentação de monogástricos por manterem a saúde intestinal e contribuírem, assim, com os processos de digestão de nutrientes e combate a patógenos. Contudo, quando presente em níveis muito elevados podem provocar prejuízos ao organismo dos animais, podendo levar a diminuição da capacidade absortiva do intestino dos nutrientes fornecidos na dieta. Este efeito abrange modificações na viscosidade e tempo de passagem do alimento pelo trato gastrointestinal (Vanderoof, 1998; Enes et al., 2011; Fontoulaki et al., 2005; Stone, 2003), atividade das enzimas digestivas (Refstie et al., 1999; Leenhouwers et al., 2006) bem como alterações na

composição da microbiota intestinal (Amirkolaie et al., 2006; Leenhouwers et al., 2007b; Dimitroglou et al., 2010).

Porém, no presente estudo, ainda que se tenha tido aumento no teor de fibra das dietas experimentais, a inclusão de DDGS do milho em até 40% na dieta em substituição total do farelo de soja propiciou efeitos positivos no organismo dos juvenis de pacu. Tais efeitos podem ser confirmados pelos dados obtidos para os parâmetros produtivos, status oxidativo e morfologia do intestino, que podem estar relacionados aos componentes de levedura presentes no DDGS.

Leveduras e seus componentes de parede celular tem sido relatados como fatores capazes de trazer melhorias na morfologia intestinal (Omar et al., 2012 Torrecillas et al., 2011; JarmoŁowicz et al., 2012; Merrifield et al., 2010a; Sáenz de Rodrigáñez et al., 2009) bem como na digestibilidade e utilização dos nutrientes fornecidos nas dietas para peixes (Lara-Flores et al., 2003; Guo and Qureshi, 2003; Li e Gatlin, 2005; Yang et al., 2007; Dimitroglou et al., 2009), Além disto, estes compostos podem também contribuir com mecanismos de defesa antioxidante (Reyes-Becerril et al. 2008; Kriz˘kova´ et al. 2001; Jaehrig et al. 2007) mantendo o balanço entre produção e remoção de radicais livres de oxigênio, que podem provocar danos nos tecidos dos animais por modificar a estrutura de lipídeos, proteínas e ácidos nucleicos (Girotti, 1998; Cabiscol et al., 2000; Salmon et al., 2004).

Atrelado aos benefícios provocados pela inclusão de DDGS nas dietas, a remoção do farelo de soja também tem participação importante nos resultados obtidos. Uma vez que não houve diferença para ganho de peso dos animais entre os tratamentos e ainda considerando-se que o preço do farelo de soja chega a ser duas vezes superior ao do DDGS, pode concluir que a confecção de dietas para juvenis de pacu utilizando DDGS em substituição total ao farelo de soja pode promover considerável redução nos custos da ração, como observado no presente experimento, uma redução de 26% no custo por ganho. Além disto, os fatores antinutricionais presentes no farelo de soja mostram-se estar relacionados à redução no consumo de alimento e danos no intestino dos animais (Francis et al., 2001; Webster et al. 1992a) podendo estes estar relacionados a morfologia

(Krogdah et al., 2003; Bakke-Mckellep et al., 2007) ou até mesmo ao estresse oxidativo do intestino (Gu et al., 2011).

Outro fato que potencializa a inclusão do DDGS do milho em dietas para animais é o elevado teor de fósforo disponível. Os processos de fermentação e aquecimento necessários para fabricação do DDGS podem resultar em aumento na hidrólise de moléculas de fitato, tornando o fósforo mais disponível para ser metabolizado pelo organismo animal (Kim et al., 2008). Desta forma, o fósforo passa a ser mais retido no organismo e menos liberado para o ambiente, o que ressalta o potencial de uso do DDGS na alimentação animal por promover também benefícios ao meio ambiente.

Sendo assim, como conclusão final, pode-se recomendar a inclusão de DDGS do milho em até 40% em dietas para juvenis de pacu, em total substituição ao farelo de soja, por promover benefícios econômicos e ambientais, além de melhorias na saúde intestinal dos peixes.

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