UNIVERSIDADE ESTADUAL DE PONTA GROSSA
SETOR DE CIÊNCIAS AGRÁRIAS E DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

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ENZYME COMPLEX ON DIGESTIBILITY OF ENERGY AND NUTRIENTS IN PRACTICAL DIETS FOR NILE TILAPIA, *Oreochromis niloticus* JUVENILES

PONTA GROSSA

2018

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Dissertação apresentada como parte das exigências para obtenção do título de Mestre, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Ponta Grossa Área de Concentração: Produção Animal.

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PONTA GROSSA

2018

Ficha catalográfica

Elaborada pelo setor de tratamento da informação BICEN/UEPG

Brandes, Alberto

B817 Enzyme complexo n digestibility of energy and nutrients in practical diets for nile Tilapia, *Oreochromis niloticus* Juveniles/ Alberto Brandes. Ponta Grossa, 2018.

46 f.

Dissertação (Mestrado em Zootecnia – Área de concentração – Produção animal), Universidade Estadual de Ponta Grossa.

Orientador: Prof. Dr. Wilson Massamitu Furuya

Coorientador: Prof. Dr. Ivo Mottin Demiate

1. Tilapia. 2. Extrusão. 3. Enzimas exógenas. 4. Digestibilidade. 5. Aminoácidos. I. Furuya, Wilson Massamitu. II. Demiate, Ivo Mottin. III. Universidade Estadual de Ponta Grossa - Mestrado em Zootecnia. IV. T.

CDD : 639.3

Ficha catalográfica elaborada por Maria Luzia F. Bertholino dos Santos– CRB9/986

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

TERMO DE APROVAÇÃO

ALBERTO BRANDES

ENZYME COMPLEX ON DIGESTIBILITY OF ENERGY AND NUTRIENTS IN PRACTICAL DIETS FOR NILE TILAPIA, *Oreochromis niloticus* JUVENILES

Dissertação aprovada como requisito parcial para obtenção do grau de Mestre no Curso de Pós-Graduação em Zootecnia – Mestrado em Zootecnia, Setor de Ciências Agrárias e Tecnologia da Universidade Estadual de Ponta Grossa, no dia 28 de setembro de 2018, pela seguinte banca examinadora:

Prof. Dr. Wilson Massamitu Furuya-UEPG
(Orientador)

Profª. Drª. Lilian Dena dos Santos-UFPR

Profª. Drª. Cheila Roberta Lehnen-UEPG

Ponta Grossa, 28 de setembro de 2018.

AGRADECIMENTOS

Agradeço aos meus pais, Ana e Otto, pela educação que me deram, não medindo esforços para que eu sempre tivesse o melhor possível, sempre me apoiando para o crescimento pessoal e profissional;

À minha esposa, Mônica, pelo amor, paciência e por estar sempre ao meu lado;

Ao meu orientador Prof. Dr. Wilson Massamitu Furuya, pela oportunidade, pelos ensinamentos e confiança durante o período de mestrado;

Ao meu co-orientador Prof. Dr. Ivo Mottin Demiate pela sua contribuição nesta etapa;

A toda equipe do grupo de pesquisa GPA – Fish Nutrition, pelo companheirismo e amizade. Por toda ajuda no período experimental e análises realizadas;

À Mariana Michelato, pelo treinamento no Ensaio de Digestibilidade, Thais e Allan, pelo auxílio na coleta de dados.

Aos alunos do Programa de Pós-Graduação em Zootecnia da UEM, pelo auxílio na análise estatística, interpretação dos dados e treinamento no ensaio de digestibilidade.

Ao professor Luiz Edivaldo Pezzato (UNESP/Botuctu, SP), pelo auxílio na realização das análises de óxido de cromo e energia bruta.

À empresa Granja Econômica Avícola Ltda, que me apoiou para que eu pudesse conciliar o trabalho na empresa com o mestrado;

Á Alltech do Brasil, pelo financiamento parcial do projeto.

À Universidade Estadual de Ponta Grossa- UEPG e aos professores do Programa de Pós-Graduação-PPZ, por toda dedicação e conhecimentos transmitidos durante as disciplinas cursadas;

A todos que de alguma forma contribuíram para que eu conclui-se mais essa etapa da minha vida.

OBRIGADO!

“Se você pensa que pode ou se pensa que não pode, de qualquer forma você está certo”

(Henry Ford)

RESUMO

O presente estudo teve por objetivo avaliar os efeitos de Allzyme SSF®, um complexo multienzimático contendo pectinase, protease, fitase, β-glucanase, xilanase, celulase e amilase nos coeficientes de digestibilidade aparente de tilápias do Nilo, *Oreochromis niloticus*. Um total de 90 peixes sadios, machos com 100 dias de idade (150 ± 20 g) foram distribuídos aleatoriamente em seis aquários de digestibilidade de 200 litros, com 15 peixes por aquário. Foi utilizada como dieta basal uma formulação comercial para tilápias contendo 33,27% de proteína bruta (21,11% de origem animal + 12,17% de origem vegetal) e 20,18 MJ/kg de energia bruta. Os tratamentos utilizados foram: dieta controle (D1) sem complexo multienzimático, D2 e D3 suplementadas com 0,0225% e 0,045% de Allzyme SSF®, respectivamente. Óxido de cromo (0,1%) foi utilizado como marcador interno. Foi observado um maior coeficiente de digestibilidade aparente de matéria seca e energia bruta nos peixes alimentados com a dieta D3 comparado com aqueles que receberam a dieta controle (P = 0,05). Peixes alimentados com D3 apresentaram maior coeficiente de digestibilidade aparente de amido, cinza e zinco comparado com aqueles alimentados com a dieta controle e D2 (P < 0,05). Peixes alimentados com as dietas D2 e D3 demonstraram coeficientes de digestibilidade aparente maiores para cálcio e fósforo em relação aos que receberam a dieta controle (P < 0,05). Não foi observado efeito do complexo multienzimático nos coeficientes de digestibilidade aparente de lipídeos (P = 0,811). A suplementação com complexo multienzimático na dieta D3 melhorou os coeficientes de digestibilidade aparente de lisina, (P = 0,022), histidina (P = 0,001) comparado com os peixes alimentados com a dieta controle. Coeficientes de digestibilidade aparente maiores para leucina, treonina, triptofano e valina foram observados em peixes alimentados com dietas D2 e D3, comparado com aqueles que receberam a dieta controle (P = 0,05). Excreção menor de nitrogênio e fósforo foi observada nos peixes alimentados com dietas suplementadas com complexo multienzimático comparado com os que receberam a dieta controle. Como conclusão, a suplementação com 0,045% de Allzyme SSF® pode ser uma ferramenta útil para melhorar os valores nutricionais dos alimentos e dar suporte a uma criação mais sustentável de tilápia do Nilo.

Palavras- chave: aminoácidos, digestibilidade, enzimas exógenas, extrusão, tilápia.

ABSTRACT

The present study was aimed to evaluate the effects of Allzyme SSF®, a multienzyme complex with pectinase, protease, phytase, β-glucanase, xylanase, cellulase, and amylase, on the apparent digestibility coefficients for Nile tilapia, *Oreochromis niloticus*. A total of 90 healthy, 100-day-old, all-male fish (150 ± 20 g) were randomly allotted into six-200-L digestibility aquaria at stocking density of 15 fish per aquarium. A typical commercial tilapia feed formulation containing 33.27% of crude protein (21.11% from animal protein source + 12.16% from plant protein source), and 20.18 MJ/kg of gross energy was used as basal diet. The dietary treatments assigned were: control diet (D1) without multienzyme supplementation, D2 and D3 supplemented with 0.0225% and with 0.045% of Allzyme SSF®, respectively. Chromic oxide at 0.1% was used as inert marker. Higher apparent digestibility coefficients of dry matter and gross energy were observed in fish fed diet D3 compared to those fed the control diet ($P < 0.05$). Fish fed diet D3 showed higher apparent digestibility coefficients of starch, ash and zinc compared to those observed in fish fed control and diet D2 ($P < 0.05$). Fish fed diet D2 and D3 demonstrated higher apparent digestibility coefficients of calcium and phosphorus than fish fed the control diet ($P < 0.05$). No effect of multienzyme complex supplementation on apparent digestibility coefficients of lipids was observed ($P = 0.811$). Dietary multienzyme complex supplementation in diet D3 improved apparent digestibility coefficients of lysine ($P = 0.022$), histidine ($P = 0.001$) compared to fish fed D1. Higher apparent digestibility coefficients of leucine, threonine, tryptophan and valine were observed in fish fed diet D2 and D3, compared to those fed the control diet ($P < 0.05$). Lower nitrogen and phosphorus excretions were observed in fish fed diets supplemented with the multienzyme complex compared to the control diet. In conclusion, a dietary supplementation of 0.045% of Allzyme SSF® may be used as useful tool to improve nutritive value of feeds and support the sustainability of Nile tilapia culture.

Keywords: amino acids, digestibility, exogenous enzymes, extrusion, tilapia.

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REVISÃO DE LITERATURA

1 INTRODUÇÃO

A tilápia do Nilo é o segundo peixe mais cultivado no mundo (FAO, 2016) e a sua produção quadriplicou na última década devido sua facilidade de cultivo, boa aceitação pelos consumidores e preços de mercado estáveis (WANG; LU, 2016). A produção tende a continuar crescendo para atender o aumento na demanda por peixe pela sempre crescente população humana (HAYGOOD; JHA, 2016).

As tilápias tem hábito alimentar onívoro, e comparado a outras espécies de peixes, utilizam de forma eficiente uma grande variedade de ingredientes vegetais como fonte de energia e proteína (GUIMARÃES et al., 2009; MAINA et al., 2002), benefício que contribui para o aumento da produção de tilápias (PORTZ; LIEBERT, 2004).

A farinha de peixe sempre foi a escolha como principal fonte de proteína na formulação de dietas para peixes por diversas razões, entre elas o alto teor de proteína, excelente perfil de aminoácidos, alto coeficiente de digestibilidade dos nutrientes, ausência de fatores anti-nutricionais, custo relativamente baixo e disponibilidade (GATLIN et al., 2007). Os crescentes preços e baixa na oferta de farinha de peixe tem restringido o desenvolvimento da aquicultura. Para reduzir essa dependência, as fontes vegetais ganham cada vez mais força como ingredientes alternativos (SHI et al., 2016).

Uma variedade de fatores anti-nutricionais são encontrados nos ingredientes de origem vegetal, entre eles o fitato, polissacarídeos não amiláceos (PNAs), inibidores de proteases e lectinas, que podem limitar a utilização destes ingredientes pelos peixes, pois estes não produzem enzimas que degradam PNAs e fitato. (ADEOLA; COWIESON, 2011; FRANCIS; MAKKAR; BECKER, 2001; SOETAN; OYEWOLE, 2009). Por outro lado, é importante dizer que os inibidores de proteases e lectinas, presentes principalmente nos grãos de leguminosas, são desativados durante processos térmicos como a extrusão, empregados no beneficiamento destes ingredientes e na fabricação de rações (FRANCIS; MAKKAR; BECKER, 2001). Desta forma para aumentar os coeficientes de digestibilidade aparente dos

ingredientes de origem vegetal, enzimas exógenas têm sido utilizadas pela indústria de rações para peixes.

As enzimas exógenas têm sido adicionadas com intuito de melhorar o aproveitamento das frações da dieta cujas enzimas endógenas não são capazes de hidrolisar, consequentemente trazendo efeitos positivos no desempenho de crescimento (CAO et al., 2008; LIEBERT; PORTZ, 2007). Além de tornar a aquicultura uma atividade mais ecologicamente sustentável, reduzindo a excreção de fósforo (P) e nitrogênio (N) na água (FORSTER et al., 1999).

As enzimas mais utilizadas na aquicultura são as fitases e carboidrases. A fitase atua na liberação do fósforo fítico, diminuindo o custo da ração e o impacto ambiental do fósforo excretado nas fezes, além de exercer efeitos positivos na digestibilidade de outros nutrientes. Já as carboidrases são utilizadas com foco no ganho energético e melhor utilização da proteína, lipídeos e aminoácidos (ADEOLA; COWIESON, 2011).

O uso de complexos enzimáticos tem por objetivo melhorar a digestibilidade dos ingredientes pelo complemento que pode haver entre elas. Quando um carboidrato não digestível é hidrolisado, outros nutrientes como proteínas e lipídeos são liberados e ficam disponíveis para a ação de outras enzimas (ADEOLA; COWIESON, 2011).

2 ENZIMAS EXÓGENAS

Na década de 1950, cientistas já adicionavam proteases e amilases em dietas para animais e observaram benefícios em sua produtividade. Desde então, o uso de enzimas exógenas tem sido uma das áreas mais estudadas na produção animal (ADEOLA; COWIESON, 2011). Os primeiros registros de uso comercial de enzimas são de 1984 na Finlândia, onde enzimas derivadas da indústria cervejeira foram utilizadas para melhorar significativamente a qualidade nutricional de rações a base de cevada (BEDFORD; PARTRIDGE, 2001).

A busca por alternativas à farinha de peixe como fonte proteica na aquicultura, e a necessidade de reduzir possíveis causas de poluição da água, são os principais motivos que levam a utilização de enzimas exógenas na nutrição de peixes (ADEOLA; COWIESON, 2011).

Os efeitos da adição de enzimas podem ser avaliados pela determinação dos coeficientes de digestibilidade aparente (CDA) dos ingredientes (ADEOLA; COWIESON,

2011). A determinação dos CDA de ingredientes e/ou da dieta é uma etapa primordial na formulação e fornecimento de dietas balanceadas aos peixes (CHO; KAUSHIK, 1990).

Os CDA de um ingrediente ou dieta podem ser calculados por dois métodos, o método direto, obtido pela diferença entre a quantidade de nutriente/energia presente no alimento e a quantidade presente nas fezes (LOVELL, 1998), ou pelo método indireto, que é mais comumente utilizado em ensaios com peixes, onde um material inerte externo é adicionado à dieta (marcador externo) ou medindo um componente inerte da própria dieta (marcador interno). A digestibilidade é calculada comparando a concentração do marcador na dieta e nas fezes em relação a um nutriente especifico (GODDARD; MCLEAN, 2001).

Desta forma, ensaios de digestibilidade de energia/nutrientes da dieta são uma importante ferramenta utilizada para avaliar a eficiência da inclusão de enzimas nas dietas. Sendo as enzimas mais utilizadas na nutrição de peixes, em ordem de importância, a fitase, carboidrases e proteases.

2.1 FITASE

O ácido fítico, também conhecido como fitato é a forma de armazenamento de fósforo nas plantas, corresponde a até 80% do conteúdo total de fósforo e não está disponível para os peixes, pois como os demais animais não ruminantes, não secretam a enzima fitase, a qual é necessária para uma hidrolise eficiente do fitato durante a digestão (CAO et al., 2007; FRANCIS; MAKKAR; BECKER, 2001). O fitato pode ainda se complexar com minerais di e trivalentes como o Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{3+} e Fe^{3+} resultando na indisponibilidade destes nutrientes (D'MELLO et al., 1991).

Para atingir os requerimentos de fósforo nas dietas, os nutricionistas usualmente fazem a suplementação com P inorgânico, muitas vezes em excesso. Este P indisponível na forma de fitato e o excesso de P inorgânico acabam sendo excretados na água, causando a proliferação de algas, e consequentemente induzindo uma condição de flutuação nos níveis de oxigênio dissolvido (JACKSON; LI; ROBINSON, 1996). Com o aumento da pressão ambiental sobre a produção aquícola, a indústria precisa diminuir a excreção de P na água. O caminho para esta mudança está na diminuição da suplementação com fontes de P inorgânico, pois diminuindo a concentração de P na dieta, teremos uma menor excreção (CHENG et al., 2016).

As fitases são produzidas por uma grande variedade de espécies de bactérias, fungos e leveduras, as quais degradam o ácido fítico em mio-inositol-penta, tetra, tri, di e monofosfatos sequencialmente. Este processo além disponibilizar o P, neutraliza os efeitos negativos do ácido fítico sobre outros nutrientes nos animais não ruminantes (MITCHELL et al., 1997).

Nos últimos anos muitos estudos têm sido conduzidos avaliando a incorporação de fitases na produção aquícola. Dietas para tilápias do Nilo, *Oreochromis niloticus*, suplementadas com fitase resultam em melhores taxas de conversão alimentar e eficiência de utilização da proteína, efeito atribuído a melhor utilização dos nutrientes, antes sequestrados pelo fitato que são liberados com a adição da fitase (ADEOYE et al., 2016a). CAO et al., (2008) reportou uma melhor digestibilidade e desempenho de crescimento em tilápias do Nilo alimentadas com dietas a base de ingredientes vegetais suplementadas com fitase, resultados que estão de acordo com os previamente encontrados por PORTZ; LIEBERT, (2004) e FURUYA et al., (2001). Segundo ADEOYE et al. (2016) a melhor utilização de nutrientes e performance de crescimento pode também estar relacionado a uma maior densidade de microvilosidades intestinais encontradas em peixes suplementados com fitase.

Avaliando os efeitos da adição de fitase em dietas para bagre amarelo, *Pelteobagrus fulvidraco*, CHENG et al. (2016) reportou maiores coeficientes de digestibilidade aparente para P e N quando as dietas foram suplementadas com 1.000 IU/kg de fitase. A melhor digestibilidade de N e P também resultou em uma diminuição de 25 e 30% de excreção destes nutrientes respectivamente. Resultados semelhantes também foram reportados para truta-arco-íris, *Oncorhynchus mykiss* (DALSGAARD et al., 2009; FORSTER et al., 1999; VIELMA et al., 1998), carpa comum, *Cyprinus carpio* (NWANNA; SCHWARZ, 2007), carpa prussiana, *Carassius auratus gibelio* (LIU; SU; LUO, 2012) salmão do atlântico, *Salmo salar* (SAJJADI; CARTER, 2004) e pargo japonês, *Pagrus major*, (LAINING et al., 2012).

A fitase não é termoestável, devendo ser utilizada evitando excesso de calor durante os processos de produção de ração (VIELMA; RUOHONEN; PEISKER, 2002). Ela é desativada em temperaturas acima de 65°C, e como a temperatura geralmente passa de 65°C durante o processamento (CAO et al., 2007) a fitase pode ser utilizada fazendo um pré-tratamento dos ingredientes utilizados na fabricação da ração (CAO et al., 2008) ou aplicada via spray sobre os pellets após o processamento (VIELMA et al., 2004).

Os estudos citados evidenciam que a utilização da fitase em dietas a base de ingredientes de origem vegetal melhoram a utilização do P. Dessa forma reduzindo a

necessidade de suplementação com fontes inorgânicas de P, levando a uma redução na excreção de P no meio ambiente pela aquicultura.

2.2 CARBOIDRASES

Carboidrases são todas as enzimas que catalisam a redução no peso molecular de polímeros de carboidratos. Carboidrases exógenas tem por objetivo a quebra de polissacarídeos não amiláceos (PNA) e são utilizadas essencialmente para reduzir a ineficiência de utilização de nutrientes e combater os efeitos nocivos à saúde associada a dietas ricas em PNAs. Como os PNAs são compostos por diferentes estruturas, diversas enzimas tem sido desenvolvidas com o objetivo de degradar esse grupo de antinutrientes, entretanto, duas proteínas lideram o mercado de carboidrases, a xilanase e a glucanase (ADEOLA; COWIESON, 2011).

Enzimas responsáveis pela digestão dos PNAs como a β-glucanase e β-xilanase são escassas ou ausentes nos peixes (KUZ'MINA, 1996). Dessa forma os PNAs não são digeridos e acabam afetando negativamente o desempenho dos peixes. Os efeitos adversos estão associados com o aumento da viscosidade da digesta, efeitos fisiológicos e morfológicos no trato digestivo, interação com o epitélio, formação de muco e modulação da flora intestinal (SINHA et al., 2011).

O modo de ação das enzimas exógenas adicionadas para degradar os PNAs está relacionado com a ruptura da parede celular, garantindo acesso das proteases e amilases pancreáticas ao conteúdo celular, permitindo uma melhor digestão do amido e proteínas (SINHA et al., 2011). Os efeitos anti-nutritivos de uma dieta de alta viscosidade podem estar em grande parte relacionados aos efeitos na microbiota e não na taxa de digestão propriamente dita. (BEDFORD; COWIESON, 2012).

A eficiência da utilização de carboidrases em dietas para tilápias ainda é questionada, alguns estudos mostram não haver benefício em sua inclusão. Em um experimento realizado por ADEOYE et al. (2016) tilápias suplementadas com carboidrases tiveram desempenho de crescimento similar com aquelas que receberam a dieta controle, o que está de acordo com YIGIT; OLMEZ, (2011) que reportaram que a inclusão de carboidrase não resultou em melhora no desempenho de crescimento e coeficiente de digestibilidade de nutrientes em tilápias.

2.3 PROTEASES

A digestão da proteína é realizada pela ação de enzimas proteolíticas endógenas no trato digestivo, envolvendo pepsina, tripsina, quimotripsina, elastase, bem como aminopeptidases e carboxipeptidases. Os animais não digerem e absorvem toda a proteína e aminoácidos presentes na dieta. A fração não digerida serve como substrato para fermentação microbiana no intestino grosso nos animais monogástricos, que pode levar a desordens digestivas. A suplementação com proteases exógenas pode melhorar a digestibilidade através de maior solubilização e hidrólise das proteínas dietéticas, e reduzindo o substrato para fermentação microbiana indesejável (PHILIPPS-WIEMANN, 2018).

Nos últimos anos as proteases têm atraído maior atenção pelo aumento do preço e a disponibilidade de ingredientes proteicos (LI et al., 2016). Além da questão ambiental, uma formulação reduzindo os níveis de proteína dietética tem impacto direto na redução de excreção de nitrogênio (PHILIPPS-WIEMANN, 2018).

LI et al. (2016) avaliando os efeitos da protease em tilápias do Nilo, *Oreochromis niloticus,* reportou maior ganho de peso e utilização de nutrientes com a suplementação da enzima em dieta com baixa inclusão de farinha de peixe. Em outro estudo com tilápias do Nilo, (ADEOYE et al., 2016) não foram encontradas diferenças significativas de desempenho de crescimento e utilização de nutrientes, entre peixes que receberam a dieta com suplementação de protease em relação aos que receberam a dieta controle, os autores concluíram que não há benefício quando os níveis de proteína da dieta excedem os requerimentos de manutenção e crescimento dos peixes.

Estudos também foram realizados com truta-arco-íris, *Oncorhynchus mykiss* (DALSGAARD et al., 2012; DREW et al., 2005) e carpa negra, *Mylopharyngodon piceus* (CHEN et al., 2009). Estes resultados mostram um melhor desempenho de crescimento e digestibilidade aparente dos nutrientes, quando as dietas foram suplementadas com proteases.

2.4 COMPLEXOS MULTIENZIMÁTICOS

Complexos enzimáticos são utilizados com o intuito de combinar a ação das enzimas, a fim de melhorar a utilização das dietas e possibilitar o uso de uma maior variedade de ingredientes, como os subprodutos, permitindo formulações de baixo custo sem prejudicar o desempenho de crescimento. O uso da xilanase por exemplo, aumenta a permeabilidade da camada de aleurona dos cereais, expondo o ácido fítico, porém, a xilanase sozinha não aumenta o coeficiente de digestibilidade do P, mas quando a xilanase é combinada com a fitase pode haver efeito mutuamente benéfico (ADEOLA; COWIESON, 2011; MAAS et al., 2018a). Estudos com suplementação de fitases para peixes são amplamente difundidos, comparado aos realizados com carboidrases. E cada vez mais a combinação destas enzimas vem ganhando espaço nas pesquisas (MAAS et al., 2018).

GUIMARÃES et al. (2009) avaliou a inclusão de diferentes níveis de complexo enzimático contendo carboidrase, protease e lipase em dieta a base de milho e farelo de soja para tilápias do Nilo. A adição do complexo enzimático melhorou significativamente o coeficiente de digestibilidade aparente da proteína bruta, do extrato etéreo, do carboidrato e da energia bruta. Resultados semelhantes aos encontrados por LIN; MAI; TAN, (2007) que relacionaram o melhor desempenho dos peixes que receberam dietas suplementadas com complexo enzimático à uma melhora nas funções digestivas, tanto a nível pancreático como intestinal.

EL-SAYED et al. (2014) avaliou o efeito de um complexo enzimático contendo pectinase, protease, fitase, β-glucanase, xilanase, celulase e amilase em dietas com níveis crescentes de substituição do milho por casca de romã seca no desempenho de crescimento de tilápias do Nilo, *Oreochromis niloticus.* Os resultados mostram que o milho pode ser substituído em até 10% pela casca de romã com a inclusão do complexo multi enzimático, sem prejudicar o desempenho e saúde dos peixes. MOURA et al. (2015) avaliou o mesmo complexo enzimático em dietas onde 5, 10 ou 20% da proteína oriunda do farelo de soja foi substituída pelo farelo de crambe, *Crambe abyssinica,* com ou sem o complexo enzimático. Os autores reportam que a substituição de 10% da proteína com a inclusão do complexo enzimático manteve os resultados iguais a dieta controle, efeito atribuído ao aumento da digestibilidade dos nutrientes e redução dos fatores antinutricionais.

A suplementação com complexo enzimático se mostrou eficiente também em outras espécies de peixes. A adição de complexo enzimático contendo xilanase e glucanase melhorou os coeficientes de digestibilidade aparente de matéria seca, lipídeos, proteína, energia e alguns aminoácidos essenciais de juvenis de peixe pregado, *Scophthalmus maximus*, alimentados com dieta rica em PNA's. Além disso a suplementação com as enzimas aumentou a atividade enzimática digestiva, modulando a microbiota, aumentando sua diversidade e concentração (DIÓGENES et al., 2018a). Resultados significativos foram encontrados ainda para carpa comum, *Cyprinus carpio*, carpa capim, *Ctenopharyngodon idella* (ŞARA et al., 2012) e truta-arco-íris, *Oncorhynchus mykiss* (DALSGAARD et al., 2012).

O uso de enzimas exógenas de forma individual, ou combinadas ganham cada vez mais força, embasadas na sustentabilidade, meio ambiente e redução de custos nas formulações. Resultados e oportunidades promissores encontrados em outras espécies de animais não ruminantes, mostram que ainda podemos avançar no entendimento dos benefícios que as enzimas exógenas podem trazer para a aquicultura (CASTILLO; GATLIN, 2015).

3 REFERÊNCIAS BIBLIOGRÁFICAS

ADEOLA, O.; COWIESON, A. J. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. **Journal of Animal Science**, v. 89, n. 10, p. 3189–218, 2011.

ADEOYE, A. A. et al. Supplementation of formulated diets for tilapia (*Oreochromis niloticus*) with selected exogenous enzymes: Overall performance and effects on intestinal histology and microbiota. **Animal Feed Science and Technology**, v. 215, p. 133–143, 2016.

BEDFORD, M. R. MICHAEL R.; PARTRIDGE, G. G. **Enzymes in farm animal nutrition**. CABI Pub, 2001.

BEDFORD, M. R.; COWIESON, A. J. Exogenous enzymes and their effects on intestinal microbiology. **Animal Feed Science and Technology**, v. 173, n. 1–2, p. 76–85, 2012.

CAO, L. et al. Application of microbial phytase in fish feed. **Enzyme and Microbial Technology**, v. 40, n. 4, p. 497–507, 2007.

CAO, L. et al. Effects of pretreatment with microbial phytase on phosphorous utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). **Aquaculture Nutrition**, v. 14, n. 2, p. 99–109, 2008.

CASTILLO, S.; GATLIN, D. M. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. **Aquaculture**, v. 435, p. 286–292, 2015.

CHEN, J.-M. et al. Effect of adding neutral protease to diets on growth performance, digestion and body composition of fingerling black carp (*Mylopharyngodon piceus*). **Acta Hydrobiologica Sinica**, v. 33, n. 4, p. 726–731, 5 ago. 2009.

CHENG, N. et al. The sparing effect of phytase in plant-protein-based diets with decreasing supplementation of dietary NaH2PO4for juvenile yellow catfish *Pelteobagrus fulvidraco*. **Aquaculture Research**, v. 47, n. 12, p. 3952–3963, 2016.

CHO, C. Y.; KAUSHIK, S. J. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). **World Rev Nutr Diet**, v. 61, p. 132–172, 1990.

D'MELLO, J. P. F. et al. **Toxic substances in crop plants**. 1. ed. Woodhead Publishing, 1991.

DALSGAARD, J. et al. Effect of supplemented fungal phytase on performance and phosphorus availability by phosphorus-depleted juvenile rainbow trout (*Oncorhynchus mykiss*), and on the magnitude and composition of phosphorus waste output. **Aquaculture**, v. 286, n. 1–2, p. 105–112, 2009.

DALSGAARD, J. et al. Effects of exogenous enzymes on apparent nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed diets with high inclusion of plant-based protein. **Animal Feed Science and Technology**, v. 171, n. 2–4, p. 181–191, 2012.

DIÓGENES, A. F. et al. Exogenous enzymes supplementation enhances diet digestibility and digestive function and affects intestinal microbiota of turbot (*Scophthalmus maximus*) juveniles fed distillers' dried grains with solubles (DDGS) based diets. **Aquaculture**, v. 486, p. 42–50, 2018.

DREW, M. D. et al. Effect of adding protease to coextruded flax:pea or canola:pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). **Animal Feed Science and Technology**, v. 119, n. 1–2, p. 117–128, 2005.

EL-SAYED, B. M. et al. Effect of Partial Replacement of Yellow Corn by Pomegranate Peel with or Without Allzyme SSF on Growth Performance and Health Status of *Oreochromis*

niloticus. **World Journal of Fish & Marine Sciences.** v. 6, n. 2, p. 182–189, 2014.

FAO. **The State of World Fisheries and Aquaculture (SOFIA)**. Rome: 2016.

FORSTER, I. et al. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11 °C freshwater. **Aquaculture**, v. 179, n. 1–4, p. 109–125, 1999.

FRANCIS, G.; MAKKAR, H. P. S.; BECKER, K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. **Aquaculture**, v. 199, n. 3–4, p. 197–227, 2001.

FURUYA, W. M. et al. Fitase na alimentação da tilápia do Nilo (*Oreochromis niloticus*). Desempenho e digestibilidade. **Revista Brasileira de Zootecnia**, v. 30, n. 3 suppl 1, p. 924–929, 2001.

GATLIN, D. M. et al. Expanding the utilization of sustainable plant products in aquafeeds: A review. **Aquaculture Research**, v. 38, n. 6, p. 551–579, 2007.

GODDARD, J. S.; MCLEAN, E. Acid-insoluble ash as an inert reference material for digestibility studies in tilapia, *Oreochromis aureus*. **Aquaculture**, v. 194, n. 1–2, p. 93–98, 2001.

GUIMARÃES, I. G. et al. Digestibilidade aparente de rações contendo complexo enzimático para tilápia-do-nilo. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 61, n. 6, p. 1397–1402, 2009.

HAYGOOD, A. M.; JHA, R. Strategies to modulate the intestinal microbiota of Tilapia (*Oreochromis sp*.) in aquaculture: A review. **Reviews in Aquaculture**, p. 1–14, 2016.

JACKSON, L. S.; LI, M. H.; ROBINSON, E. H. Use of microbial phytase in channel catfish Ictalurus punctatus diets to improve utilization of phytate phosphorus. **Journal of the World Aquaculture Society**, v. 27, n. 3, p. 309–313, 1996.

KUZ'MINA, V. V. Influence of age on digestive enzyme activity in some freshwater teleosts. **Aquaculture**, v. 148, n. 1, p. 25–37, 1996.

LAINING, A. et al. Dietary inorganic phosphorus or microbial phytase supplementation improves growth, nutrient utilization and phosphorus mineralization of juvenile red sea bream, *Pagrus major*, fed soybean-based diets. **Aquaculture Nutrition**, v. 18, n. 5, p. 502–511, 2012.

LI, X. Q. et al. Effects of temperature and feed processing on protease activity and dietary protease on growths of white shrimp, *Litopenaeus vannamei*, and tilapia, *Oreochromis niloticus* × *O. aureus*. **Aquaculture Nutrition**, v. 22, n. 6, p. 1283–1292, 2016.

LIEBERT, F.; PORTZ, L. Different sources of microbial phytase in plant based low phosphorus diets for Nile tilapia *Oreochromis niloticus* may provide different effects on phytate degradation. **Aquaculture**, v. 267, n. 1–4, p. 292–299, 2007.

LIN, S.; MAI, K.; TAN, B. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus x O. aureus*. **Aquaculture Research**, v. 38, n. 15, p. 1645–1653, 2007.

LIU, L. W.; SU, J.; LUO, Y. Effect of partial replacement of dietary monocalcium phosphate with neutral phytase on growth performance and phosphorus digestibility in gibel carp, *Carassius auratus gibelio* (Bloch). **Aquaculture Research**, v. 43, n. 9, p. 1404–1413, 2012.

LOVELL, T. **NUTRITION AND FEEDING OF FISH**. 2nd. ed. New York: Springer science + Business media, LLC, 1998.

MAAS, R. M. et al. The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. **Aquaculture**, v. 487, p. 7–14, 2018.

MAINA, J. G. et al. Digestibility and feeding value of some feed ingredients fed to tilapia *Oreochromis niloticus* (L .). **Aquaculture Research**, v. 33, p. 853–862, 2002.

MITCHELL, D. B. et al. The phytase subfamily of histidine acid phosphatases: Isolation of genes for two novel phytases from the fungi *Aspergillus terreus* and *Myceliophthora thermophila*. **Microbiology**, v. 143, n. 1, p. 245–252, 1997.

MOURA, G. DE S. et al. Crambe meal in diets supplemented with enzyme complex solid state fermentation (SSF) for Nile tilapia. **African Journal of Agricultural Research**, v. 10, n. 4, p. 289–294, 2015.

NWANNA, L. C.; SCHWARZ, F. J. Effect of supplemental phytase on growth, phosphorus digestibility and bone mineralization of common carp (*Cyprinus carpio L*). **Aquaculture Research**, v. 38, n. 10, p. 1037–1044, 2007.

PHILIPPS-WIEMANN, P. Enzymes in Human and Animal Nutrition. In: ROMANO, N.; KUMAR, V. (Eds.). . **Enzymes in Human and Animal Nutrition**. Elsevier Inc., 2018. p. 279–297.

PORTZ, L.; LIEBERT, F. Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fed plant-based diets with graded levels of microbial phytase. **Journal of Animal Physiology and Animal Nutrition**, v. 88, n. 9–10, p. 311–320, 2004.

SAJJADI, M.; CARTER, C. G. Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar L.*) fed a canola-meal-based diet. **Aquaculture**, v. 240, n. 1–4, p. 417–431, 2004.

ŞARA, A. et al. The Influence of Multienzymatic Complex Allzyme SSF on Production Performances of Common Carp (*Cyprinus carpio l .*) and Grass Carp (*Ctenopharyngodon idella v .*) Juveniles after Introduction of Alfalfa Meal in Feed. **Animal Science and Biotechnologies**, v. 45, n. 2, p. 93–96, 2012.

SHI, Z. et al. Effects of protease supplementation in low fish meal pelleted and extruded diets on growth, nutrient retention and digestibility of gibel carp, *Carassius auratus gibelio*. **Aquaculture**, v. 460, p. 37–44, 2016.

SINHA, A. K. et al. Non-starch polysaccharides and their role in fish nutrition - A review. **Food Chemistry**, v. 127, n. 4, p. 1409–1426, 2011.

SOETAN, K. O.; OYEWOLE, O. E. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. **African Journal of Food Science**, v. 3, n. 9, p. 223–232, 2009.

VIELMA, J. et al. Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). **Aquaculture**, v. 163, n. 3–4, p. 309–323, 1998.

VIELMA, J. et al. Top-spraying soybean meal-based diets with phytase improves protein and mineral digestibilities but not lysine utilization in rainbow trout, *Oncorhynchus mykiss* (Walbaum). **Aquaculture Research**, v. 35, n. 10, p. 955–964, 2004.

VIELMA, J.; RUOHONEN, K.; PEISKER, M. Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss*. **Aquaculture**, v. 204, n. 1–2, p. 145–156, 2002.

WANG, M.; LU, M. Tilapia polyculture: a global review. **Aquaculture Research**, v. 47, n. 8, p. 2363–2374, 2016.

YIGIT, N. O.; OLMEZ, M. Effects of cellulase addition to canola meal in tilapia (*Oreochromis*

niloticus L.) diets. **Aquaculture Nutrition**, v. 17, n. 2, 2011.

CAPÍTULO 2

ENZYME COMPLEX ON DIGESTIBILITY OF ENERGY AND NUTRIENTS IN PRACTICAL DIETS FOR NILE TILAPIA, *Oreochromis niloticus* JUVENILES

ABSTRACT

The present study was aimed to evaluate the effects of Allzyme SSF®, a multienzyme complex with pectinase, protease, phytase, β-glucanase, xylanase, cellulase, and amylase, on the apparent digestibility coefficients for Nile tilapia, *Oreochromis niloticus*. A total of 90 healthy, 100-day-old, all-male fish (150 ± 20 g) were randomly allotted into six-200-L digestibility aquaria at stocking density of 15 fish per aquarium. A typical commercial tilapia feed formulation containing 33.27% of crude protein (21.11% from animal protein source + 12.16% from plant protein source), and 20.18 MJ/kg of gross energy was used as basal diet. The dietary treatments assigned were: control diet (D1) without multienzyme supplementation, D2 and D3 supplemented with 0.0225% and with 0.0450% of Allzyme SSF®, respectively. Chromic oxide at 0.1% was used as inert marker. Higher apparent digestibility coefficients of dry matter and gross energy were observed in fish fed diet D3 compared to those fed the control diet ($P < 0.05$). Fish fed diet D3 showed higher apparent digestibility coefficients of starch, ash and zinc compared to those observed in fish fed control and diet D2 ($P < 0.05$). Fish fed diet D2 and D3 demonstrated higher apparent digestibility coefficients of calcium and phosphorus than fish fed the control diet ($P < 0.05$). No effect of multienzyme complex supplementation on apparent digestibility coefficients of lipids was observed ($P = 0.811$). Dietary multienzyme complex supplementation in diet D3 improved apparent digestibility coefficients of lysine ($P = 0.022$), histidine ($P = 0.001$) compared to fish fed D1. Higher apparent digestibility coefficients of leucine, threonine, tryptophan and valine were observed in fish fed diet D2 and D3, compared to those fed the control diet ($P < 0.05$). Lower nitrogen and phosphorus excretions were observed in fish fed diets supplemented with the multienzyme complex compared to the control diet. In conclusion, a dietary supplementation of 0.045% of Allzyme SSF® may be used as useful tool to improve nutritive value of feeds and support the sustainability of Nile tilapia culture.

Keywords: amino acids, digestibility, exogenous enzymes, extrusion, tilapia.

1. INTRODUCTION

Tilapias are the second most farmed fish in the world, only behind carps, playing an important role in aquaculture of tropical and subtropical countries. In Brazil, Nile tilapia, *Oreochromis niloticus* is the most farmed freshwater fish species whose culture expanded from extensive earth ponds to intensive production in tanks (FAO, 2016). This species is omnivorous (OSO; AYODELE; FAGBUARO, 2006) and utilizes efficiently vegetable feedstuffs as energy and protein sources (GUIMARÃES et al., 2008; MAINA et al., 2002; VIDAL et al., 2017).

In recent years, many research efforts have focused on the replacement of fish meal protein with other less expensive plant-based and co-products to elaborate low cost diets for Nile tilapia (VIDAL et al., 2015). Vegetable feeds contain several antinutritional factors such as lectins and protease inhibitors, which are sensitive to processing temperature during extrusion. However, phytic acid is thermoresistant and non-starch polysaccharides cannot be degraded because fish cannot excrete non-starch polysaccharides enzymes (SOETAN; OYEWOLE, 2009).

Extrusion is defined as a high temperature and short time continuous processing operation which is widely applied by the fish feed industry (KAMARUDIN et al., 2018). During extrusion cooking, starch and protein are subjected to high temperature, pressure and mechanical shearing forces and cause chemical and structural changes such as protein denaturation, inactivation of many antinutritional factors (Rojas and Stein, 2017) and decreased microbial counts (OKELO et al., 2006) with significant impact on starch gelatinization (SAMUELSEN; MJØS; OTERHALS, 2013) and nutritional value of feeds. Physical quality of fish feed can be assessed by properties like Floatability, bulk density, strength at rupture and durability (DE CRUZ; KAMARUDINA; SAADA, 2015; SØRENSEN et al., 2009; CIAN et al., 2017. In addition, aquafeeds with high residual enzyme activity have been elaborated adding the enzymes before the extrusion process (CIAN et al., 2018).

In addition, recently been used to elaborate aquafeeds with high residual enzyme activity, adding the enzymes before the extrusion process.

Exogenous enzymes have been proposed to increase bioavailability of energy and nutrients, and consequently result in positive effects on growth performance and environmental sustainability of fish production. The most commonly used enzymes in fish feeds is phytase, used to release P (DALSGAARD et al., 2009; NWANNA; SCHWARZ, 2007; SAJJADI;

CARTER, 2004) and exert other positive effects on energy (FORSTER et al., 1999), protein (BISWAS et al., 2007; VON DANWITZ et al., 2016) and amino acids (CHENG; HARDY, 2003) in fish diets and consequently improved growth of fish (CAO et al., 2008; LIEBERT; PORTZ, 2007) and decreased P output (FORSTER et al., 1999). Carbohydrases have been also evaluated (CASTILLO; GATLIN III, 2015) to improve energy utilization but also improve protein, lipids (MAGALHÃES et al., 2018) and amino acids utilization by fish (DIÓGENES et al., 2018b). Recently, multi-enzymes complex has been proposed to maximize digestibility and growth performance of Nile tilapia (ADEOYE et al., 2016; MAAS et al., 2018; NOVELLI et al., 2017). Therefore, applying a combinations of exogenous enzymes preparations may be potential benefits to elaborate nutritive and sustainable diets for fish.

Despite the economic and social importance of tilapia in Brazil, there is still little information that allows the use of exogenous multi-enzymes complex in extruded diets for Nile tilapia evaluating effects on amino acids availability and nitrogen and P waste output to elaborate environmental oriented diets. Therefore, the primary objective of this research was to determinate the effects of multi-enzymatic complex on apparent digestibility coefficients of Nile tilapia. The secondary objective was to estimates nitrogen and P waste load of fish fed diets without and with multi-enzymatic complex

2. MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the State University of Ponta Grossa, Paraná, Brazil, Protocol n° 55772018.

2.1 Diets

A commercial feed formulation used for tilapia in Brazilian market was used to elaborate the control diet (D1) containing 332.7 g/kg CP (21.11% from animal meal + 12.16% from plant protein) and meets the dietary requirement of Nile tilapia (NRC, 2011), as shown in Table 1. Two other test diets were formulated similar to the control but supplemented with EC at 0.0225% (D2) or 0.0450% (D3) (Table 2).

The EC (Allzyme SSF®, Alltech, Kentucky, USA) contained pectinase 4,000,000 IU/kg, protease 700,000 IU/kg, phytase 300,000 IU/kg, β-glucanase 200,000 IU/kg, xylanase 100,000 IU/kg, cellulase 40,000 IU/kg and amylase 30,000 IU/kg as determined by the manufacturer. All ingredients were finely ground into an 800-µm mesh, prior to mixing in an automatic "V" mixer (MA200; Marconi, Piracicaba, SP, Brazil). All diets were extruded at 100-105ºC in a single-screen experimental feed mill (Exteec, Ribeirão Preto SP, Brazil).

Table 1: Composition of the experimental diets (g/kg).

Ingredients	Diets[a]		
	D1	D2	D3
Corn	276.100	275.875	275.650
Wheat bran	124	124	124
Soybean meal	150	150	150
Meat and bone meal	140	140	140
Fish meal	90	90	90
Poultry by-products meal	100	100	100
Feather meal	20	20	20
Low tannin sorghum	40	40	40
Soybean oil	34.3	34.3	34.3
Blood meal	13.9	13.9	13.9
Salt	4	4	4
DL-methionine (99%)	1.8	1.8	1.8
Mineral and vitamin mix[b]	4	4	4
Antioxidant[c]	0.1	0.1	0.1
Antifungic[d]	0.8	0.8	0.8
Chromium oxide (Cr_2O_3)	1	1	1
Enzymatic complex[e]	0.0	0.225	0.45

[a]D1: control diet, without enzymatic complex supplementation; D2: diet control supplemented with .0225% of enzymatic complex and D3: diet control supplemented with .0450% of enzymatic complex.

[b]Mineral and vitamin mix supplied per kg of diet: Vitamin A (retinyl acetate), 6,000 IU; vitamin D_3, (cholecalciferol), 1,000 IU; vitamin E (DL-α-tocopheryl acetate), 60 mg; vitamin K_3 (menadione Na-bisulphate), 12 mg; vitamin B_1 (thiamine HCl), 24 mg; vitamin B_2 (riboflavin), 24 mg; vitamin B_6 (pyridoxine HCl), 20 mg; vitamin B_{12} (cyanocobalamin), 0.05 mg; folic acid, 6 mg; D-calcium pantothenate, 60 mg; ascorbic acid (ascorbyl polyphosphate), 240 mg; D-biotin, 0.24 mg; choline chloride, 325 mg; niacin, 120 mg; ferrous sulfate ($FeSO_4.H_2O.7H_2O$), 50 mg; copper sulphate ($CuSO_4.7H_2O$), 3 mg; manganese sulphate ($MnSO_4.H_2O$), 20 mg; zinc sulphate ($ZnSO_4.7H_2O$), 30 mg; potassium iodide (KI), 0.4 mg, cobalt sulphate ($CoSO_4.4H_2O$), 0.25 mg; sodium selenite (Na_2SeO_3), = 0.1 mg.

[c]Butilated hydroxylotuene (BHT)

[d]Calcium propionate

[e]Allzyme SSF®, Alltech, Kentucky, USA.

Table 2: Analyzed composition of the experimental diets (g/kg).

(to be continued)

Item	Diets[1]		
	D1	D2	D3
Dry matter	949.8	948.4	951.2
Gross energy, MJ/kg	20.17	20.20	20.18
Crude protein	332.5	332.8	332.7
Starch	260.9	263.9	267.8
Lipids	81.7	81.5	82.3
Crude fiber	28.51	28.47	28.56
Ash	90.08	90.13	90.07
Calcium	25.37	25.41	25.36
Phosphorus	15.1	15.3	15.1
Zinc, mg/kg	113.55	116.37	110.85
Essential amino acids			
Arginine	21.1	21.5	19.8
Phenylalanine	15.7	15.3	15.6
Histidine	8.6	8.8	8.4
Isoleucine	12.7	13.1	12.9
Leucine	25.9	26.5	26.0
Lysine	19.3	19.4	18.7
Methionine	6.0	5.9	5.9
Threonine	12.9	12.9	13.2
Tryptophan	3.9	3.5	4.0
Valine	16.1	16.0	15.4

Table 2: Analyzed composition of the experimental diets (g/kg).

(conclusion)

Item	Diets[1]		
	D1	D2	D3
Non-essential amino acids			
Aspartic acid	33.0	31.9	30.3
Glutamic acid	52.5	52.3	50.4
Alanine	18.9	19.5	18.8
Cysteine	3.6	3.4	3.0
Glycine	19.6	19.6	18.5
Serine	17.1	17.4	16.6
Tyrosine	9.7	10.2	10.0

[1]D1: control diet, without enzymatic complex supplementation, D2: diet control supplemented with 0.0225% of enzymatic complex and D3: diet control supplemented with 0.0450% of enzymatic complex.

Diets were extruded using a laboratory scale stand-alone single-screw extruder with a throughput of 5 kg/h (Exteec, Ribeirão Preto SP, Brazil). The extruder had a barrel length and barrel diameter of 420 mm and 19 mm, respectively with a length to diameter ratio of 22:1. A uniform pitch screw with a length to diameter ratio of 25 was used in the experiment. The maximum screw torque was 150 Nm and the compression ratio achieved inside the barrel was 3:1. The barrel zone was heated electrically with heating/cooling jacket. The barrel temperature profile was set at 80–90–100ºC while the die temperature was 105ºC. The extruder was operated at a preset feeder, screw and 4-bladed cutter speeds of 40, 120, and 300 rpm, respectively. The die had a diameter of 3 mm and pressure of 8.0–10.0 MPa.

Extrudates were oven-dried at 55ºC for less than 24 h. After that, 1 g of each replicate diet was placed inside the drying chamber of an infrared moisture-determination balance (AD-4715; A&D Weighing Co., Milpitas, CA, USA) and its moisture content was recorded. After drying, extrudates with approximately 5% of moisture content were stored in polyethylene bags until laboratorial analyses and use in the digestibility trial.

2.2 Fish experiment and samples collection

The digestibility assay was carried out at the Aquaculture Laboratory at State University of Ponta Grossa (Ponta Grossa, Paraná, Brazil). A total of 90 Nile tilapia (initial body weight 150 ± 20 g), were randomly distributed into 6-200 L conical fiberglass aquaria with 15 fish per aquaria. A constant and natural photoperiod of 12 hours light and 12 hours dark was kept during the experimental trial. Water quality parameters were monitored daily and the values of mean ± standard error of mean (SEM) were as follow: water temperature was 27.3 ± 0.4°C, total ammonia was 0.01 ± 0.001 mg/L and pH was 7.1 ± 0.1. Dissolved oxygen was kept (6.2 ± 0.2 mg/L) throughout the experimental period using blower and diffusers.

Before the beginning of the digestibility trial, fish were acclimatized for two weeks to the laboratory rearing, feed and feeding conditions. Fish were hand fed until apparent satiety a commercial extruded diet containing 320 g/kg of CP, twice daily for prior to fecal collection. The fecal collection in each digestibility aquaria followed seven days according to modified Guelph protocol previously established by (GUIMARÃES et al., 2009).

2.3 Laboratorial Analysis

Dry matter, crude protein, crude lipid and mineral matter of the experimental diets and feces composition analyses were performed according to the methodology described by

AOAC (1995). Dry matter was determined by oven-drying at 105ºC until constant weight. Crude protein (N×6,25) was determined by the Kjeldahl method after acid digestion (Tecnal, MA-036, Piracicaba, SP, Brazil). Crude fat was determined by the ether-extraction method (Tecnal, TE-044, Piracicaba, SP, Brazil). Mineral content was obtained in muffle furnace at 600°C for 5 h (Tecnal, 2000B, Belo Horizonte, MG, Brazil). Chromic oxide concentrations were determined by flame atomic absorption spectro-photometer following combustion of the sample in a muffle furnace, before and after digestion in nitric acid (AOAC, 1995). Gross energy was determined using an adiabatic bomb calorimeter (Parr, Moline, IL, USA). Amino acids analyses were performed by high-performance liquid chromatography (HPLC) (Hitachi, Tokyo, Japan), at the Ajinomoto Laboratory, Animal Nutrition Division, São Paulo, SP, Brazil, after perchloric digestion. Tryptophan was determined after alkaline-hydrolysis of the sample with lithium hydroxide.

2.4 Apparent digestibility coefficients (ADC)

The ADC of dry matter, crude protein, crude fat, amino acids, gross energy, starch, ash, calcium and phosphorus were calculated as using chromic oxide as inert marker (BREMER NETO et al., 2005). The ADC were calculated as follow (MAYNARD; LOOSLI, 1969):

$$ADC\ (\%) = 100 - \left[\left(\frac{\text{g/kg } Cr_2O_3\ Diet}{\text{g/kg } Cr_2O_3\ Feces}\right) \times \left(\frac{\text{g/kg Nutrient Feces}}{\text{g/kg Nutrient Diet}}\right)\right]$$

2.5 Nitrogen and phosphorous excretion

Nitrogen and phosphorus excretions were determined as follow:

$$Ne\ (g/kg\ of\ diet) = Nd - (Nd\ x\ ADC)$$

Where, Ne = nutrient (nitrogen or phosphorus) excretion (g/kg of diet), Nd = nutrient contents in the diet (g/kg) and ADC = apparent digestibility coefficient of respectively nutrient (%/100).

2.6 Statistical Analysis

Data were analyzed as a completely randomized design with three treatments and two independent replicates. Percentage data were submitted to arc sin square root percentage transformations prior to statistical analysis. Differences among the dietary treatments were compared by Tukey´s multiple range test at a level of significance of $P < 0.05$. Variability in

the data was expressed as the pooled standard error of mean SEM. All data were analyzed using the SPSS (version 10.0) statistical package for Windows.

3. RESULTS

3.1 Apparent digestibility coefficients of dry matter, gross energy and nutrients

The effect of the multienzyme complex on the ADC of dry matter, gross energy and nutrients is shown in Table 3.

Table 3: Effect of enzyme complex supplementation on the apparent digestibility coefficients (%) of dry matter and nutrients in Nile tilapia

Parameter	Enzyme complex inclusion %			SEM[1]	*P-value*
	0	0,0225	0,0450		
Dry matter	64.79^{b}	68.28^{ab}	69.51^{a}	0.817	0.033
Gross energy, kcal/kg	68.67^{b}	71.49^{b}	81.83^{a}	2.22	0.013
Crude protein	85.61^{c}	87.38^{b}	89.86^{a}	0.677	0.001
Crude lipid	90.04	89.89	90.8	0.212	0.811
Starch	90.69^{b}	92.37^{b}	94.62^{a}	0.646	0.009
Ash	32.83^{b}	32.91^{b}	36.02^{a}	0.575	< 0.001
Calcium	25.39^{b}	27.36^{a}	28.91^{a}	0.567	0.007
Phosphorus	71.04^{b}	75.04^{a}	79.93^{a}	1.344	0.004
Zinc	25.35^{b}	35.11^{b}	46.66^{a}	3.450	0.009

[1] SEM = standard error of means

Distinct letters e the same row indicate significant differences according to Tukey´s test at $P < 0.05$.

The ADC for dry matter was higher in fish fed diet with D3 compared to those fed control diet (D1) (P = 0.033), however, no difference was observed in fish fed D1 and diet D2. Higher ADC of gross energy was observed in fish fed diet D3 compared to those fed D1 and D2 (P = 0.013). No statistical difference between the ADC of gross energy in fish fed diet control and D2. The highest ADC of crude protein was observed in fish fed diet D3 (P = 0.001). In addition, fish fed D2 showed increased ADC of crude protein compared to fish fed the control diet. Higher ADC of starch (P = 0.009), ash (P < 0.001) and zinc (P = 0.009) were observed in fish fed diet D3 compared to fish fed control and diet D2, and no differences on the ADC between fish fed control and diet D2 was observed. Same pattern was observed in ADC of mineral, which fish fed diet D2 and D3 resulted in higher ADC of calcium (P = 0.007) and phosphorus (P = 0.004) than fish fed the control and diet, and no differences on the ADC between fish fed diet D2 and D3 were observed. Crude fat was the only parameter that was not affected by dietary treatments (P = 0.811).

3.2 Apparent digestibility coefficients of amino acids

The effect of the multienzyme complex on the ADC of amino acids is shown in Table 4.

Table 4: Effect of enzyme complex supplementation on the apparent digestibility coefficients (%) of amino acids in Nile tilapia.

Item	Enzyme complex inclusion %			SEM[1]	*P-value*
	0	0,0225	0,0450		
Essential amino acids					
Arginine	94.44	95.13	95.15	0.340	0.207
Phenylalanine	90.38	90.67	91.59	0.207	0.127
Histidine	89.28^{c}	89.85^{b}	90.39^{a}	0.258	0.001
Isoleucine	89.22^{c}	90.63^{b}	91.3^{a}	0.338	0.001
Leucine	88.45^{b}	89.88^{a}	90.37^{a}	0.321	0.007
Lysine	91.23^{b}	91.58^{ab}	92.32^{a}	0.206	0.022
Methionine	93.01	91.99	92.84	0.180	0.086
Threonine	84.31^{b}	87.33^{a}	87.79^{a}	0.609	0.006
Tryptophan	88.13^{b}	90.03^{a}	90.35^{a}	0.394	0.016
Valine	85.91^{b}	87.41^{a}	87.3^{a}	0.287	0.027
Non-essential amino acids					
Aspartic acid	91.82	91.84	92.22	0.088	0.218
Glutamic acid	94.35^{b}	94.51^{a}	94.87^{a}	0.087	0.011
Alanine	85.04	86.41	86.55	0.419	0.478
Cysteine	85.65	87.39	84.16	0.695	0.253
Glycine	88.68^{b}	89.04^{ab}	89.56^{a}	0.149	0.042
Serine	90.17	90.83	91.38	0.239	0.207
Tyrosine	93.11	92.12	93.96	0.464	0.471

[1]SEM = standard error of means

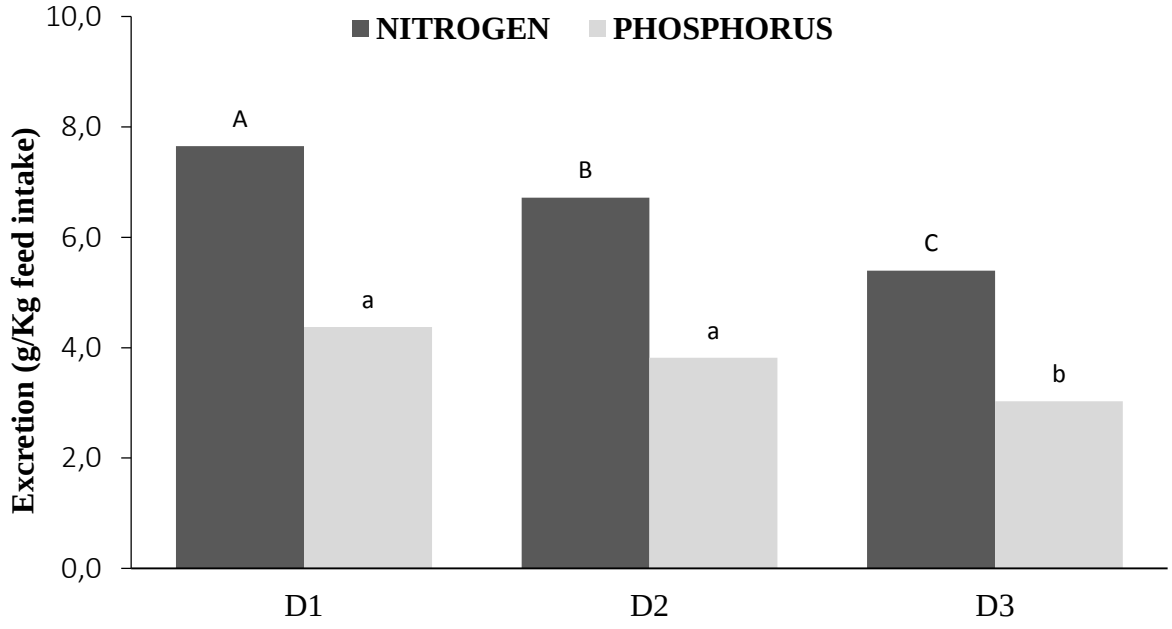
Distinct letters in the same row indicate significant differences according to Tukey´s test at $P < 0.05$.

Fish fed diet D3 showed higher ADC of lysine (P = 0.022) and glycine (P = 0.042) compared to those fed the control diet. However, no significant effect between the ADC of fish fed diet control and D2 was observed. Fish fed diet D3 demonstrated higher ADC of histidine (P = 0.001) than fish fed other diets. Higher ADC of leucine (P = 0.007), threonine (P = 0.006), tryptophan (P = 0.016), valine (P = 0.027) and glutamic acid (P = 0.011) were observed in fish fed diet D2 and D3, compared to fish fed D1. On the other hand, no difference between the ADC of fish fed diets D2 and D3 was observed. No effects of dietary treatments on ADC of arginine (P = 0.207), phenylalanine (P = 0.127), methionine (P = 0.086) aspartic acid (P = 0.218), alanine (P = 0.478), cysteine (P = 0.253), serine (P = 0.207) and tyrosine (P = 0.471) were observed.

3.3 Waste nitrogen and phosphorus output

Figure 1 shows the effects of myltienzyme complex on nitrogen and phosphorus excretion by Nile tilapia.

Figure 1: Nitrogen and phosphorus excretion (g/kg of feed intake) of Nile tilapia fed the experimental diets.



D1: control diet, without enzymatic complex supplementation, D2: diet control supplemented with 0.0225% of enzymatic complex and D3: diet control supplemented with 0.045% of enzymatic complex

Values are means ± SEM of two replicates. Distinct letters indicate significant differences according to Tukey´s test at $P < 0.05$.

The lowest nitrogen output was estimated in fish fed diet D3 compared to those fed D1 and D2 ($P = 0.050$). In addition, when the multienzyme was supplemented at 0.0225%, lower excretion of nitrogen was observed compared to observed in fish fed control diet. Fish fed diet D3 resulted in lower phosphorus output than fish fed control and D2 diet ($P < 0.050$). However, no difference on phosphorus output between fish fed control and D2 diet was observed.

4 DISCUSSION

All diets met the dietary digestible protein value (27.25%) recommended to optimize growth and health of Nile tilapia (FERNANDES et al., 2016). However, only diets supplemented with the multienzyme complex met the dietary digestible energy (14.65 MJ/kg) requirements described by referred authors. Therefore, multienzyme complex may be a useful tool to improve the dietary GE and CP content and should be considered during feed formulation to improve cost-benefits by reducing energy and protein sources in diets for Nile tilapia.

In the present research, available Ca (0.64 to 0.91%) and P (1.07 to 2.03%) contents of the diets were found above requirements described for Nile tilapia (NRC, 2011). The high levels of Ca and P are commonly found in commercial diets for Nile tilapia because of many rendered protein meals such as meat and bone meal, fish meal and poultry by-products meal are widely used as main sources of protein. The ADC of minerals for Nile tilapia is quite variable among feedstuffs, source and level of other minerals or nutrients (GUIMARÃES et al., 2012). Minerals antagonistic interactions have been reported and may be taken into consideration when defining dietary mineral levels (NRC, 2011). Calcium and phytate are well-known inhibitors of Zn availability and diets containing high levels of fish meal or phytate require higher Zn levels (APINES et al., 2001). Similarly to observed in the present study, increased availability of Zn has been reported in rainbow trout, *Oncorhynchus mykiss* (CHENG; HARDY, 2003b) and tiger puffer, *Takifugu rubripes* (LAINING et al., 2011) fed diets supplemented with phytase. In addition, exogenous phytase has been recommended for partial replacement of inorganic Ca source, also exhibiting extra positive effects on dietary crude protein digestibility (LIU; SU; LUO, 2012).

In recent years, several ingredients from plant origin have been proposed to elaborate commercial diets for Nile tilapia in Brazil and worldwide, including corn, wheat, soybean meal and co-products. In addition to the presence of phytates, many other antinutritional factors, such

as non-starch polysaccharides have been described and negatively related to growth performance and nutrients utilization (GATLIN et al., 2007). Thus, the combination of enzymes may result in positive effects on the ADC of gross energy and nutrients. Recently, Nile tilapia fed diets supplemented with phytase and xylanase showed improved ADC of dry matter, crude lipid, crude protein and P (MAAS et al., 2018a). Combined phytase, protease and lipase resulted in positive effect on the ADC of gross energy, crude protein, crude lipid and P for Nile tilapia (GUIMARÃES et al., 2009), while blend of phytase and protease resulted in increased ADC of dry matter, gross energy, crude protein, crude lipid and availability of P and Ca for this fish species. Multienzyme complex composed by phytase, protease, xylanase and cellulase was tested in diets for Atlantic salmon, *Salmo salar*, demonstrating improved ADC of gross energy, crude protein, crude lipid and ash. Moreover, improved ADC of crude protein and higher growth performance were observed in gibel carp, *Carassius auratus* fed diet supplemented with protease (SHI et al., 2016).

Non-starch polysaccharides-degrading enzymes reduce digesta viscosity and improve nutrient digestibility, thus increasing growth and feed conversion in non-ruminants (O'NEILL; SMITH; BEDFORD, 2014) and ruminants animals (ADEOLA; COWIESON, 2011). Several cereals such as wheat, barley, oats and rye and co-products may result in highly viscous intestinal contents, and detrimental effects of energy and nutrients utilization and consequently on growth performance. Viscosity is known to be a major cause of their reduced feeding value caused by physical barrier for endogenous enzymes actions on nutrients (SINHA et al., 2011). However, the anti-nutritive effects of viscosity are mediated may be related to the effects upon the size and composition of microbial population (ADEOYE et al., 2016). Exogenous enzymes not only influence the partitioning of nutrients to the host but also produce nutrients for beneficial populations of microbiota (BEDFORD; COWIESON, 2012). Exogenous carbohydrases such as xylanase, β-glucanase and amylase have been extensively used for poultry and swine diets (O'NEILL; SMITH; BEDFORD, 2014), and recently used to improve nutritive value of aquafeeds (CASTILLO; GATLIN, 2015). Multi-carbohydrases supplementation has been proven to improve the ADC of dry matter, gross energy and crude protein for turbot, *Scophthalmus maximus* (DIÓGENES et al., 2018) and European seabass, *Dicentrarchus labrax* (MAGALHÃES et al., 2018).

Phytase is widely known to increases phosphorus availability (CAO et al., 2008; LIEBERT; PORTZ, 2005, 2007), and positive effects on growth and bone mineralization of Nile tilapia (LIEBERT; PORTZ, 2005) and other fish species such as rainbow trout (DALSGAARD et al., 2009), common carp (NWANNA; EISENREICH; SCHWARZ, 2007).

In addition, increased ADC of dry matter, crude protein (VANDENBERG; SCOTT; DE LA NOÜE, 2012; VON DANWITZ et al., 2016) and amino acids (CHENG; HARDY, 2003) have been reported. Phytase also influences the availability of various minerals (LAINING et al., 2012) and has been recommended to reduce the inclusion of inorganic minerals (CHENG et al., 2016; DALSGAARD et al., 2009; LIU; SU; LUO, 2012).

Exogenous enzymes not only increase the ADC of gross energy and nutrients but also contribute to more environmentally sustainable aquaculture, mainly by reducing N and P excretions by fish (MELO et al., 2012; ROCHA et al., 2008). This effect was demonstrated in the present study, where the multienzyme complex supplementation resulted in lower N and P excretion and by fish. Compared to observed in fish fed the control diet, fish fed diets containing 0.0225 and 0.0450% of multienzyme complex excreted 12.20% and 29.48% less N and 12.58% and 27.21% less P, respectively.

In freshwater aquaculture at the bioaccumulation of the off-flavor compounds (geosmin and 2-methylisoborneol) is responsible for “earthy” and “musty” off-flavors to the fillet, respectively, which affects negatively the product quality and results in economic consequences (DAVIDSON et al., 2014). Geosmin and 2-methylisoborneol are secondary metabolic products of certain species of cyanobacteria (blue-green algae) and actinomycete bacteria that can occur in aquatic animals, especially under circumstances in which the aquatic ecosystem has been subjected to high nutrient loading rates (TUCKER, 2010), and has been described in Nile tilapia reared under intensive system (YAMPRAYOON; NOOMHORM, 2008).

Isolated or combined enzymes have been also described to improve ADC of amino acids in many fish species. The use of multienzyme complex has been demonstrated to improve the ADC of many amino acids, such as lysine, leucine, threonine and glycine in turbot, *Scophthalmus maximus* (DIÓGENES et al., 2018). Increased ADC of arginine, aspartic acid, histidine, isoleucine, lysine and tyrosine were also described in Atlantic salmon fed combined phytase, protease, xylanase and cellulase (JACOBSEN et al., 2018). To date, this is the first report evaluating the ADC of amino acids in Nile tilapia fed extruded diets supplemented with a multienzyme complex. In this study, multienzyme complex demonstrated effective effects on ADC of dry matter, gross energy and nutrients when supplemented in diets for Nile tilapia. However, specific combined effects applied during industrial process of aquafeeds (grinding, extrusion and drying) must be taken into consideration due the effects on residual exogenous enzymes activity.

Recently, new generations of thermostable enzymes have been produced to elaborate aquafeeds with high residual enzymes to be mixed thoroughly the feed before extruding (CIAN et al., 2017) and may be advantageous, less expensive, and practical way to improve the nutritive value and high efficiency large-scale industrial feed production. In addition, many alternative and conventional ingredients have been considered to be used in aquafeeds targeted to Nile tilapia production and ADC may be variable according to feed formulation because of specific effects of enzymes according to source and levels of substrates and inter-relationships between nutrients. In conclusion, dietary supplementation with multienzyme complex (Allzyme SSF®) supplementation showed improved nutritive value and was higher when supplemented at 0.0450%.

5. REFERENCES

ADEOLA, O.; COWIESON, A. J. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. **Journal of Animal Science**, v. 89, n. 10, p. 3189–218, 2011.

ADEOYE, A. A. et al. Supplementation of formulated diets for tilapia (*Oreochromis niloticus*) with selected exogenous enzymes: Overall performance and effects on intestinal histology and microbiota. **Animal Feed Science and Technology**, v. 215, p. 133–143, 2016.

AOAC. **Official methods of analysis of AOAC International**. 16th ed. ed. Washington DC: AOAC International, 1995.

APINES, M. J. et al. Bioavailability of amino acids chelated and glass embedded zinc to rainbow trout, *Oncorhynchus mykiss*, fingerlings. **Aquaculture Nutrition**, v. 7, n. 4, p. 221–228, 2001.

BEDFORD, M. R.; COWIESON, A. J. Exogenous enzymes and their effects on intestinal microbiology. **Animal Feed Science and Technology**, v. 173, n. 1–2, p. 76–85, 2012.

BISWAS, A. K. et al. Use of soybean meal and phytase for partial replacement of fish meal in the diet of red sea bream, *Pagrus major*. **Aquaculture**, v. 267, n. 1–4, p. 284–291, 2007.

BREMER NETO, H. et al. Determinação de rotina do crômio em fezes, como marcador biológico, pelo método espectrofotométrico ajustado da 1,5-difenilcarbazida. **Ciência Rural**,

v. 35, n. 3, p. 691–697, 2005.

CAO, L. et al. Effects of pretreatment with microbial phytase on phosphorous utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). **Aquaculture Nutrition**, v. 14, n. 2, p. 99–109, 2008.

CASTILLO, S.; GATLIN, D. M. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. **Aquaculture**, v. 435, p. 286–292, 2015.

CHENG, N. et al. The sparing effect of phytase in plant-protein-based diets with decreasing supplementation of dietary NaH2PO4for juvenile yellow catfish *Pelteobagrus fulvidraco*. **Aquaculture Research**, v. 47, n. 12, p. 3952–3963, 2016.

CHENG, Z. J.; HARDY, R. W. Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). **Aquaculture**, v. 218, n. 1–4, p. 501–514, 2003.

CIAN, R. E. et al. Optimization of single screw extrusion process for producing fish feeds based on vegetable meals and evaluation of nutritional effects using a juvenile *Piaractus mesopotamicus* model. **Animal Feed Science and Technology**, v. 234, p. 54–64, 2017.

CIAN, R. E. et al. Extruded fish feed with high residual phytase activity and low mineral leaching increased *P. mesopotamicus* mineral retention. **Animal Feed Science and Technology**, v. 240, n. March, p. 78–87, 2018.

DALSGAARD, J. et al. Effect of supplemented fungal phytase on performance and phosphorus availability by phosphorus-depleted juvenile rainbow trout (*Oncorhynchus mykiss*), and on the magnitude and composition of phosphorus waste output. **Aquaculture**, v. 286, n. 1–2, p. 105–112, 2009.

DAVIDSON, J. et al. Evaluation of depuration procedures to mitigate the off-flavor compounds geosmin and 2-methylisoborneol from Atlantic salmon *Salmo salar* raised to market-size in recirculating aquaculture systems. **Aquacultural Engineering**, v. 61, p. 27–34, 2014.

DIÓGENES, A. F. et al. Exogenous enzymes supplementation enhances diet digestibility and digestive function and affects intestinal microbiota of turbot (*Scophthalmus maximus*) juveniles fed distillers' dried grains with solubles (DDGS) based diets. **Aquaculture**, v. 486, p. 42–50, 2018.

FAO. **The State of World Fisheries and Aquaculture (SOFIA)**. Rome: 2016.

FERNANDES, A. C. et al. The effect of digestible protein to digestible energy ratio and choline supplementation on growth, hematological parameters, liver steatosis and size-sorting stress response in Nile tilapia under field condition. **Aquaculture**, v. 456, p. 83–93, 2016.

FORSTER, I. et al. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11 °C fresh water. **Aquaculture**, v. 179, n. 1–4, p. 109–125, 1999.

GATLIN, D. M. et al. Expanding the utilization of sustainable plant products in aquafeeds: A review. **Aquaculture Research**, v. 38, n. 6, p. 551–579, 2007.

GUIMARÃES, I. G. et al. Nutrient digestibility of cereal grain products and by-products in extruded diets for nile tilapia. **Journal of the World Aquaculture Society**, v. 39, n. 6, p. 781–789, 2008.

GUIMARÃES, I. G. et al. Digestibilidade aparente de rações contendo complexo enzimático para tilápia-do-nilo. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 61, n. 6, p. 1397–1402, 2009.

GUIMARÃES, I. G. et al. Apparent nutrient digestibility and mineral availability of protein-rich ingredients in extruded diets for Nile tilapia. **Revista Brasileira de Zootecnia**, v. 41, n. 8, p. 1801–1808, 2012.

JACOBSEN, H. J. et al. Different enzyme incorporation strategies in Atlantic salmon diet containing soybean meal: Effects on feed quality, fish performance, nutrient digestibility and distal intestinal morphology. **Aquaculture**, v. 491, p. 302–309, 2018.

LAINING, A. et al. Dietary calcium/phosphorus ratio influences the efficacy of microbial phytase on growth, mineral digestibility and vertebral mineralization in juvenile tiger puffer, *Takifugu rubripes*. **Aquaculture Nutrition**, v. 17, n. 3, p. 267–277, 2011.

LAINING, A. et al. Dietary inorganic phosphorus or microbial phytase supplementation improves growth, nutrient utilization and phosphorus mineralization of juvenile red sea bream, *Pagrus major*, fed soybean-based diets. **Aquaculture Nutrition**, v. 18, n. 5, p. 502–511, 2012.

LI, X. Q. et al. Effects of temperature and feed processing on protease activity and dietary protease on growths of white shrimp, Litopenaeus vannamei, and tilapia, Oreochromis niloticus × O. aureus. **Aquaculture Nutrition**, v. 22, n. 6, p. 1283–1292, 2016.

LIEBERT, F.; PORTZ, L. Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant

based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. **Aquaculture**, v. 248, n. 1–4, p. 111–119, 2005.

LIEBERT, F.; PORTZ, L. Different sources of microbial phytase in plant based low phosphorus diets for Nile tilapia *Oreochromis niloticus* may provide different effects on phytate degradation. **Aquaculture**, v. 267, n. 1–4, p. 292–299, 2007.

LIU, L. W.; SU, J.; LUO, Y. Effect of partial replacement of dietary monocalcium phosphate with neutral phytase on growth performance and phosphorus digestibility in gibel carp, *Carassius auratus gibelio* (Bloch). **Aquaculture Research**, v. 43, n. 9, p. 1404–1413, 2012.

MAAS, R. M. et al. The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. **Aquaculture**, v. 487, p. 7–14, 2018.

MAGALHÃES, R. et al. Improved digestibility of plant ingredient-- based diets for European seabass (*Dicentrarchus labrax*) with exogenous enzyme supplementation. **Aquaculture Nutrition**, v. 1, n. December 2017, p. 1–9, 2018.

MAINA, J. G. et al. Digestibility and feeding value of some feed ingredients fed to tilapia *Oreochromis niloticus* (L .). **Aquaculture Research**, v. 33, p. 853–862, 2002.

MAYNARD, L. A.; LOOSLI, J. K. **Animal Nutrition**. New York: 1969.

MELO, K. D. M. et al. Adição de fitase em rações para tilápia-do-Nilo Adding phytase in ration for Nile tilapia. **Revista Portuguesa de Ciências Veterinarias**, v. 111, p. 581–582, 2012.

NOVELLI, P. K. et al. Enzymes produced by agro-industrial co-products enhance digestible values for Nile tilapia (*Oreochromis niloticus*): A significant animal feeding alternative . **Aquaculture**, v. 481, p. 1–7, 2017.

NRC. **Nutrient Requirements of Fish and Shrimp**. Washington, D.C.: National Academies Press, 2011.

NWANNA, L. C.; EISENREICH, R.; SCHWARZ, F. J. Effect of wet-incubation of dietary plant feedstuffs with phytases on growth and mineral digestibility by common carp (*Cyprinus carpio L*). **Aquaculture**, v. 271, n. 1–4, p. 461–468, 2007.

NWANNA, L. C.; SCHWARZ, F. J. Effect of supplemental phytase on growth, phosphorus digestibility and bone mineralization of common carp (*Cyprinus carpio L*). **Aquaculture Research**, v. 38, n. 10, p. 1037–1044, 2007.

O'NEILL, H. V. M.; SMITH, J. A.; BEDFORD, M. R. Multicarbohydrase enzymes for non-ruminants. **Asian-Australasian Journal of Animal Sciences**, v. 27, n. 2, p. 290–301, 2014.

ROCHA, C. B. et al. Suplementação da enzima fitase e o desempenho e retenção mineral em juvenis de jundiá (*Rhamdia quelen*). **Boletim do Instituto de Pesca**, v. 34, n. 1, p. 151–157, 2008.

ROJAS, O. J.; STEIN, H. H. Processing of ingredients and diets and effects on nutritional value for pigs. **Journal of Animal Science and Biotechnology**, v. 8, n. 1, p. 1–13, 2017.

SAJJADI, M.; CARTER, C. G. Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar L.*) fed a canola-meal-based diet. **Aquaculture**, v. 240, n. 1–4, p. 417–431, 2004.

SHI, Z. et al. Effects of protease supplementation in low fish meal pelleted and extruded diets on growth, nutrient retention and digestibility of gibel carp, *Carassius auratus gibelio*. **Aquaculture**, v. 460, p. 37–44, 2016.

SINHA, A. K. et al. Non-starch polysaccharides and their role in fish nutrition - A review. **Food Chemistry**, v. 127, n. 4, p. 1409–1426, 2011.

SOETAN, K. O.; OYEWOLE, O. E. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. **African Journal of Food Science**, v. 3, n. 9, p. 223–232, 2009.

TUCKER, C. S. Off-Flavor Problems in Aquaculture. **Reviews in Fisheries Science**, n. September 2012, p. 37–41, 2010.

VANDENBERG, G. W.; SCOTT, S. L.; DE LA NOÜE, J. Factors affecting nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed a plant protein-based diet supplemented with microbial phytase. **Aquaculture Nutrition**, v. 18, n. 4, p. 369–379, 2012.

VIDAL, L. V. O. et al. Apparent protein and energy digestibility and amino acid availability of corn and co-products in extruded diets for Nile Tilapia, *Oreochromis niloticus*. **Journal of the World Aquaculture Society**, v. 46, n. 2, p. 183–190, 2015.

VIDAL, L. V. O. et al. Apparent digestibility of soybean coproducts in extruded diets for Nile Tilapia, *Oreochromis niloticus*. **Aquaculture Nutrition**, v. 23, n. 2, p. 228–235, 2017.

VON DANWITZ, A. et al. Dietary phytase supplementation in rapeseed protein based diets

influences growth performance, digestibility and nutrient utilisation in turbot (*Psetta maxima L.*). **Aquaculture**, v. 450, p. 405–411, 2016.

YAMPRAYOON, J.; NOOMHORM, A. Geosmin and Off-Flavor in Nile Tilapia (*Oreochromis niloticus*). **Journal of Aquatic Food Product Technology**, p. 29–41, 2008.

CAPÍTULO 3

CONSIDERAÇÕES FINAIS

A utilização de complexo enzimático é uma nova opção para melhoria do valor nutritivo da energia e nutrientes em dietas para organismos aquáticos. Nos últimos anos, novas tecnologias estão sendo desenvolvidas para elaboração de enzimas mais estáveis termicamente, facilitando sua inclusão em dietas para peixes.

É importante considerar os efeitos sobre o aumento do valor nutritivo e considerar as exigências nutricionais para evitar valores energéticos ou de nutrientes além das exigências, com impactos negativos sobre a resposta econômica e a sustentabilidade ambiental da criação.

Durante o processamento de dietas extrusadas, é importante considerar também os efeitos da secagem sobre os valores residuais das enzimas exógenas adicionadas no complexo enzimático, objetivando melhoria do valor nutricional e a viabilidade econômica da sua suplementação. Dessa forma, sugere-se o complexo enzimático na forma líquida, com aplicação após os processos térmicos, garantindo maiores valores residuais das enzimas adicionadas.

Em função do elevado valor proteico em dietas de tilápias, sugere-se aumento no valor de protease no complexo enzimático, objetivando aumentos nos coeficientes de digestibilidade da proteína bruta e dos aminoácidos.

Diferentemente de dietas para aves e suínos, formuladas com base em milho e farelo de soja, as dietas para tilápias são elaboradas com diversos alimentos de origem vegetal e animal. Assim, espera-se variações dos efeitos da adição do complexo enzimático sobre os coeficientes de digestibilidade em função da combinação de ingredientes utilizados.