Use of Enzyme Supplementation in Practical Diets for Nile Tilapia *Oreochromis niloticus*

by

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ABSTRACT

Aquaculture industry is moving towards the use of plant based ingredients in tilapia diets, this due to decreased availability of fish meal worldwide. However, the replacement of fish meal by plant based ingredients can increase the fiber content of the diets thus impairing productivity, this mainly due to anti nutritional factors present. Among these antinutrients are the non starch polysaccharides which are part of the structural components of plant based feedstuffs. In the digestive system of fish, the enzymes necessary to digest non starch polysaccharides are scarce or even absent, reducing the ability of fish to obtain the nutrients from plant based diets. The addition of exogenous enzymes that can break down non starch polysaccharides can be an alternative to increase the digestibility of practical diets and improved performance in Nile tilapia *Oreochromis niloticus*.

A series of experiments were conducted to evaluate commercially available enzyme addition to practical tilapia diets to study growth performance and digestibility.

The first study was conducted to evaluate the performance and nutrient digestibility of Nile tilapia *Oreochromis niloticus*, when supplemented with a commercial beta mannanase enzyme. A basal diet was designed to contain 32% protein and 6% lipid using primarily plant based protein sources allowing high levels of “low” digestibility ingredients. Four levels of enzyme were evaluated (0, 0.05, 0.10, 0.20%). At the conclusion of the growth trial, performance parameters or apparent energy and net protein retention were not improved by beta mannanase addition. However, the inclusion of beta mannanase to the diet resulted in a linear increase in dry matter digestibility (P= 0.0004; R2 =0.75), energy digestibility (P=0.0003; R2 =0.74) and protein digestibility (P=0.0247; R2 =0.41). As compared to the diet without the enzyme; the supplementation of 0.1 and 0.2% of the diet significantly improved digestibility.

The second study was designed to evaluate the production performance and digestibility of Nile tilapia *Oreochromis niloticus* when supplemented with commercial protease and carbohydrases. Ten practical tilapia diets were formulated to contain 32% protein and 6% lipids. Six diets were formulated to contain low levels of fiber (LF) and included free protease (LF-FP), protected protease (LF-PP), free carbohydrase (LF-FC), protected carbohydrase (LF-PC), and a mix of free protease and carbohydrases (LF-MFPFC). Four diets were formulated to contained high levels of fiber (HF) and included a basal diet (HF) and a basal diet supplemented with free protease (HF-FP), free carbohydrase (HF-FC), and a mix of free protease and free carbohydrases (HF-MFPFC), distillers dried grains with solubles (DDGS) was used as a source of fiber in high fiber diets. The level in the diet of free protease (FP) and protected (PP) was 175 g per metric ton, the level of free carbohydrase (FC), protected carbohydrase (PC) and the mix of free protease and carbohydrase (MFPFC) was 125 g per metric ton. Under the conditions of this study, fish maintained on the high fiber diet performed slightly poorer than those on the lower fiber diet. Concerning enzyme supplements, apparent net energy retention was significantly different ($P= 0.0001$) in low fiber diets when free and protected proteases were added. However, for low and high fiber diets there were no significant differences ($P > 0.05$) in animal performance and apparent net protein retention. Overall, there were no clear advantages detected to the protected enzymes. Dry matter and energy digestibility were significantly improved by the addition of free carbohydrase and a mixture of free protease and free carbohydrase when supplemented in low and high fiber diets.

The third study investigated the production performance of Nile tilapia *Oreaochromis niloticus* when supplemented with a commercial beta xylanase and beta glucanase enzyme. Two practical basal tilapia diets formulated to contain a low level of fiber

(LF) based on soybean meal were modified by top coating liquid enzymes to produce five levels of enzyme inclusion (0.00, 0.015, 0.030, 0.045, and 0.060 g/100g). A second basal diet was formulated to contain a high level of fiber (HF), to increase the fiber content 30% distillers dried grains with solubles were used as a replacement for soybean meal, this basal diet was modified by top coating of liquid enzymes to produce five levels of enzyme inclusion (0.00, 0.015, 0.030, 0.045, and 0.060 g/100g). The inclusion of beta xylanase and beta glucanase resulted in significantly improved growth parameters, final mean weight ($P = 0.0029$), percent weight gain ($P = 0.0128$), thermal unit growth coefficients ($P = 0.0046$) with no change ($P > 0.05$) in feed conversion ratio ($P= 0.2153$), apparent net protein retention, apparent net energy retention, hepatosomatic index and intraperitoneal fat index. In general, fish maintained on the high fiber diet performed better with the addition of the enzyme.

Based on the results of these studies, exogenous enzymes such as beta mannase, protease, xylanase and betaglucanase can be used to increase digestibility of practical tilapia diets and improve performance of Nile tilapia when plant - based ingredients are the major components of the diets. However, the increase in performance is not always consistent and depends on the type of the enzyme and substrate make up of the diet.

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Chapter I

INTRODUCTION

Tilapia is the second most important cultured fish in the world after carps (Dan and Little, 2000). The growth trend of cultured *Oreochromis niloticus* has increased consistently, attributed to high resistance to diseases, ability to survive at low oxygen tensions and ability to feed on a wide range of foods. The development of commercial feeds has been traditionally based on fish meal as the main protein source because of its high protein content and balanced essential amino acid profile (Tacon et al., 1993; Watanabe, 2002; El-Saidy and Gaber, 2004). However, the global fishmeal price has increased more than two fold in recent years (FAO, 2016). In the year 2012, it was estimated that 24.3 million tonnes of aquaculture species were fed commercially manufactured aquaculture feed. Aquaculture feed production has been growing at an average annual rate of 10.3% since year 2000 and there is and expected consumption of aquaculture feeds of 65.4 million tonnes by 2020, and 87.1 million tonnes by 2025 (Tacon and Metian, 2015).

The use of alternative feed ingredients, including plant sources (Salze et al., 2010; Herath et al., 2016; Aydin et al., 2017; da Silva et al., 2017; Martins et al., 2017; Al-Thobaiti et al., 2018; Khalifa et al., 2018), animal sources (Montoya-Mejia et al., 2017; Moutinho et al., 2017; Wang et al., 2017; Devic et al., 2018), algae (Sarker et al., 2018; Simanjuntak et al., 2018; Younis et al., 2018) and restaurant food waste (Nasser et al., 2018) are viable options for decreasing fish meal use and decreasing formulation cost (Mbahinzireki et al., 2001; Thiessen et al., 2003; Ai and Xie, 2005). However, the use of plant sources can increase the fiber content of tilapia diets (Dan and Little, 2000).

The use of plant sources as an strategy to feed Nile tilapia is a viable option because they can tolerate higher dietary fiber and carbohydrate concentrations than most other cultured fish

(Elsayed and Teshima, 1992). However, plant based feed ingredients also contain anti-nutritional compounds which cannot be digested and have the potential to impair the digestion processes of aquatic animals (Hsiao et al., 2006). As described by Ebringerová (2005), plant ingredients in their structure are composed of cellulose, hemicellulose and lignin which are part of the cell wall. Among the hemicelluloses are the xyloglycans (xylans), beta glucans and manno glucans (mannans). Xylans are the most abundant hemicellulose type in the plant kingdom followed by beta glucans and mannans, they are part of the non starch polysaccharides (NSP) fraction in plant based feed ingredients. Non starch polysaccharides are one of the anti- nutritional factors present in plant based feed ingredients. The NSP fraction generally remains undigested, as the enzymes to hydrolyze the glycosidic bonds are scarce or non-existing in the gastrointestinal tract of fish (Sinha et al., 2011), and the ability to use NSP by the fish depends on the nature of the microbial population residing in the gut (Sinha et al., 2011). The NSP fraction may influence the gut morphology, physiology and mucus layer, affecting the endogenous secretion of water, proteins, electrolytes and lipids. These changes can lead to a reduced nutrient digestibility (Sinha et al., 2011). Supplementation with enzymes is considered effective to eliminate the anti-nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved fish performance (Lin et al., 2007) and health of the intestine (Castillo and Gatlin, 2015). Exogenous proteases can compensate for the deficiency of endogenous enzymes especially for young animals and assist in the breakdown of proteins improving digestibility (Shi et al., 2016) and carbohydrases are used to assist in the breakdown of hemicelluloses which are part of the cell wall (Ebringerová, 2005).

The use of soybean meal and full fat soybeans has been increasing in fish diets, which has also led to an increase in the beta mannan content of the diets. Beta mannan can impair

animal performance (Choct et al., 1996). As described by Hsiao et al. (2006), soybean meal contains at least 1.0% beta mannan and this level can increase up to 1.6% in a non dehulled soybean meal. In Nile tilapia, inclusion of beta mannanase improved growth, feed efficiency and feed conversion ratio and also increased the intestinal enzyme activity (Chen et al., 2016). Ng and Chong (2002) reported that addition of pure beta mannanase to a 40% palm kernel meal diet did not improve growth and feed utilization of tilapia. In contrast, Yigit et al. (2014) reported that rainbow trout supplemented with beta mannanase at two different levels (1 g kg^{-1} and 2 g kg^{-1}) to a control diet including soybean meal did not affect the growth parameters, feed efficiency and digestibility.

The addition of exogenous proteases and carbohydrase have been studied in fish diets. In rainbow trout, addition of protease to canola, pea - based diets resulted in significant improvements in apparent digestibility of crude protein, energy, lipid and dry matter (Drew et al., 2005). Dalsgaard et al. (2012) supplemented protease to soybean meal containing diets for rainbow trout reported a significant increase in the apparent digestibility of protein, lipid, phosphorus and dry matter. Farhangi and Carter (2007) fed juvenile rainbow trout with diets supplemented with protease and carbohydrases alone or in combination to de-hulled, lupin-based feeds. No effects on performance were observed, however the mixed enzyme significantly improved the protein efficiency ratio, and the apparent digestibility of dry matter, protein and gross energy. In contrast, Yigit et al. (2014) reported that rainbow trout supplemented with a mix of beta mannanase and alpha galactosidase at two levels (1 g kg^{-1} and 2 g kg^{-1}) to a control diet including soybean meal did not affect the growth parameters, feed efficiency and digestibility. In addition, (Yigit and Keser, 2016) found no differences in growth parameters, feed conversion ratio, dry matter , protein or lipid digestibility with enzyme supplementation when diets containing canola meal and

supplemented with cellulase, phytase, pectinase or an enzyme mix were fed to rainbow trout fry (*Oncorhynchus mykiss*).

Digestibility of crude protein and crude lipid were significantly improved in juvenile Gibel carp fed incremental increases in dietary protease up to 300 mg/kg (Liu et al., 2018). Carter et al. (1994) reported a positive effect on performance and feed efficiency when diets fed to Atlantic salmon smolt (Salmo salar L) were supplemented with a combination of proteolytic enzymes and carbohydrases to a diet containing 340 g/kg soybean meal. No increase was observed in the apparent digestibility of nitrogen and carbon.

In tilapia (*Oreochromis niloticus* × *Oreochromis aureus*), the digestibility of dry matter and crude protein was increased by the supplementation of protease (Li et al., 2016). Lin et al. (2007) reported that the addition of a commercial enzyme complex of neutral protease, beta glucanase and xylanase improved growth performance but no effect was detected in apparent digestibility of protein, lipid and gross energy in *Oreochromis niloticus* × *Oreochromis aureus*. Adeoye et al. (2016) fed Nile tilapia with probiotics, a mix of enzymes (containing phytase, protease and xylanase), and the combination of enzymes and probiotic. Tilapia fed diets supplemented with enzymes plus probiotics performed better than tilapia fed only the probiotic supplemented diets in terms of final body weight, feed conversion ratio and protein efficiency ratio. *Oreaochomis mosambicus* fed a kikuyu based diet were supplemented with a multi-enzyme complex composed of cellulase, xylanase and phytase improved fish growth, better feed conversion ratio values, increased protein efficiency and digestibility and increased activity of fish enzymes up to 0.5 g per kg addition to the diet (Hlophe-Ginindza et al., 2016). Inclusion of beta mannanase improved growth, feed efficiency and feed conversion ratio and increased intestinal enzyme activity in Nile tilapia (Chen et al., 2016). Red hybrid tilapia fed diets containing 40%

palm kernel meal did not improve growth and feed utilization when a combination of protease, cellulase, glucanase, pectinase and pure mannanase were added to the diets (Ng and Chong, 2002). Caspian Salmon fed two multienzyme complexs which consisted of a combination of protease, lipase, phytase, alpha amilase, cellulase, amiloglucosidase, beta glucanase, pentosonase, hemicellulase, xylanase, pectinase, acid phosphatase, acid phytase and endo- beta mannanase, amylase, xylanase, cellulose and alpha galactosidase improved growth and feed utilization when enzymes were included in the diet in a multi enzyme complex at levels of 0.5 g kg^{-1} and 2.5 g kg^{-1} (Zamini et al., 2014).

The performance response by addition of carbohydrase is the current subject of study in different species of fish. Some studies have not shown an effect to carbohydrase supplementation. Dalsgaard et al. (2012) found no differences in feed conversion ratio or fish performance in rainbow trout when supplementing beta-glucanase, xylanase and protease alone or in combination to diets with high inclusion levels of soybean meal, sunflower meal or rapeseed meal. Farhangi and Carter (2007) fed juvenile rainbow trout a de-hulled lupin-based diet, supplemented with protease and carbohydrases alone or in combination. No effects on performance were observed among the lupin containing diets, but the mixed enzyme de-hulled lupin – based diet had significantly higher protein efficiency ratio. In rainbow trout, average weight gain and thermal unit growth coefficient were unaffected by diets containing soybean meal up to 20% incorporating an enzyme complex containing xylanase, amylase, cellulase, protease and beta glucanase activity to the level of 1 g kg^{-1} or 2.5 g kg^{-1} (Ogunkoya et al., 2006). Rainbow trout (*Oncorhynchus mykiss*) fry diets containing canola meal and supplemented with cellulase, phytase, pectinase or an enzyme mix showed no difference in weight gain and feed conversion ratio with enzyme supplementation (Yigit and Keser, 2016).

In contrast, several studies have shown positive effects on growth performance. Diets with the addition of 400 mg of xylanase and 800 mg of glucanase and the combination had a significant increase ($P = 0.003$) in specific growth rate of Japanese seabass *Lateolabrax japonicus* (Ai et al., 2007). Atlantic salmon smolt (Salmo salar L) fed a combination of proteolytic enzymes and carbohydrases to a diet containing 340 g/kg soybean meal reported a positive effect on final mean weight and feed conversion ratio (Carter et al., 1994). Also in Atlantic salmon, Jacobsen et al. (2018) indicate that diets containing soybean meal and a combination of xylanase and phytase had significantly higher final weight and thermal growth coefficient than the diet without enzyme addition. *Labeo rohita* diets were supplemented with xylanase and phytase in non fermented and fermented rice bran based diets, the authors reported percent weight gain and protein energy retention in the fish were significantly improved and feed conversion ratio was significantly lower ($P=0.0001$) in the diet containing exogenous enzymes when compared to the non supplemented diets (Ranjan et al., 2018). Lin et al. (2007) reported that supplementation of a commercial enzyme complex of neutral protease, beta glucanase and xylanase improved final mean weight as enzyme inclusion increased from 0.0 to 1.0 g kg^{-1} ($P < 0.05$) in *Oreochromis niloticus* × *Oreochromis* aureus. Adeoye et al. (2016) reported that Nile tilapia fed diets containing a combination of probiotic and mix enzymes (containing xylanase, protease and phytase), had higher final mean weight, lower feed conversion ratio and better protein efficiency ratio. *Oreaochomis mosambicus* were fed a multi-enzyme complex composed of cellulase, xylanase and phytase added to a kikuyu based diet at a rate of 0, 0.25, 0.5, 0.75 and 1 g kg^{-1}. Fish fed the multi enzyme complex at a level of 0.5 g kg had better fish growth, lower feed conversion ratio values, and had higher protein efficiency (Hlophe-Ginindza et al., 2016). Nile tilapia (*Oreochromis niloticus*) were fed a soybean meal based diets supplemented with an enzyme complex containing beta glucosidase, pectinase,

xylanase, endoglucanase, amylase, protease and phytase, fish fed diets with enzyme complex added on top post-extrusion at a level of 800 ppm showed significantly higher final mean weight and weight gain (Martins et al., 2018).

Given the potential use of enzymes in different fish species to break down the non starch polysaccharide fraction in fish diets, it is important to us to explore the possible use of commercial enzymes in Nile tilapia diets. Therefore, the purpose of this study was to evaluate the efficacy of using four commercial enzyme supplementations in practical diets for juvenile Nile tilapia (*Oreochromis niloticus*).

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Chapter II

EFFICACY OF USING SUPPLEMENTAL BETA MANNANASE ENZYME IN PRACTICAL DIETS FOR NILE TILAPIA *Oreochromis niloticus*

Abstract

The study was conducted to evaluate the production performance and digestibility of Nile tilapia *Oreochromis niloticus*, when supplemented with a commercial beta mannanase enzyme. A basal diet was designed to contain 32% protein and 6% lipid using primarily plant-based protein sources allowing high levels of "low" digestibility ingredients. The basal diet was modified to produce four levels (0.00, 0.05, 0.10, 0.20%) of enzyme supplementations using corn starch as a filler. The diets were offered to sex- reversed juvenile tilapia (mean initial weight 7.45 ± 0.06 g) over a 70-day growth period. Four replicate groups of 20 fish per aquaria were offered the test diets at near satiation levels. At the conclusion of the growth trial, there were no significant ($P > 0.05$) differences in final mean weight, percent weight gain, thermal unit growth coefficients, survival, feed conversion ratio, apparent net protein retention, and apparent net energy retention. The inclusion of beta mannanase to the diet resulted in a linear increase in dry matter digestibility ($P= 0.0004$; $R2 =0.75$), energy digestibility ($P=0.0003$; $R2 =0.74$) and protein digestibility ($P=0.0247$; $R2 =0.41$). As compared to the basal diet, the supplementation of 0.1 and 0.2% of the diet significantly improved digestion above that of the basal diet. Results demonstrate that the inclusion of beta mannanase improved digestibility of practical tilapia diets but did not alter production performance, nutrient retention or body tissue composition.

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is the second most important cultured fish in the world after carp (Dan and Little, 2000). The growth trend of cultured *Oreochromis niloticus* has increased consistently attributed to high resistance to diseases, ability to survive at low oxygen tensions and ability to feed on wide range of foods. The development of commercial feeds has been traditionally based on fish meal as the main protein source because of its high protein content and balanced essential amino acid profile (Tacon et al., 1993; Watanabe, 2002; El-Saidy and Gaber, 2004). However, the global fishmeal price has increased more than two fold in recent years due to decrease availability (FAO, 2016). Aquaculture feed production has been growing at an average annual rate of 10.3% since year 2000 and there is and expected consumption of aquaculture feeds of 65.4 million tonnes by 2020, and 87.1 million tonnes by 2025 (Tacon and Metian, 2015).

Use of alternative feed ingredients to decrease the use of fish meal in tilapia diets is a viable option for cost reduction (Mbahinzireki et al., 2001; Thiessen et al., 2003; Ai and Xie, 2005), however, this also increases the fiber content of tilapia diets (Dan and Little, 2000).

Nile Tilapia can tolerate higher dietary fiber and carbohydrate concentrations than most other cultured fish (Elsayed and Teshima, 1992), however plant based feed ingredients also contain anti-nutritional compounds which cannot be digested and have the potential to impair the digestion processes of aquatic animals (Hsiao et al., 2006). Non starch polysaccharides (NSP) are one of the anti nutritional factors present in plant based feed ingredients (Kokou and Fountoulaki, 2018). Beta mannans are divided in two groups, galactomannans and glucomannans (Ebringerová, 2005), and they are a fraction of the non starch polysaccharides found in the plant ingredients used in fish diets such as soybean meal. As described by Hsiao et al. (2006) soybean meal contains at least

1.0% beta mannan and can increase up to 1.6% in a non-dehulled soybean meal. As use of soybean meal and full fat soybean have been increasing in fish diets the concentration of beta mannan content of the diets has also increased. Beta mannan can impair animal performance (Choct et al., 1996). The nonstarch polysaccharide fraction generally remains undigested, as the enzymes to hydrolyze the glycosidic bonds are scarce or non-existing in the gastrointestinal tract of fish (Sinha et al., 2011). Also, the non starch polysaccharide fraction may influence gut morphology, physiology and mucus layer, affecting the endogenous secretion of water, proteins, electrolytes and lipids. These changes can lead to a reduced nutrient digestibility (Sinha et al., 2011). Supplementation with enzymes is considered effective to eliminate the anti-nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved fish performance (Lin et al., 2007).

When monogastric animals are offered diets high in beta-mannan concentration, improvements in growth have been found for those receiving diets with beta mannanase. Considerable work has been conducted by the poultry industry, which currently uses these supplements. Exogenous beta mannanase enzymes have been reported to facilitate a reduction in the degree of polymerization of feed, decreasing its viscosity and liberating carbohydrate oligomers, thus enhancing nutrient utilization in chickens (Rao et al., 2014). Digestibility of energy yielding nutrients, such as starch and fat, were increased by beta mannanase supplementation since non starch polysaccharides impair the ability for nutrient absorption by decreasing enzyme accessibility to substrates in male broilers (Williams et al., 2014; Latham et al., 2018). Enzyme use also can improve nitrogen and amino acid utilization by increasing the access to proteins for digestive proteases in broilers (Tahir et al., 2008) and the addition of beta mannanase decreases the immune-related signal caused by beta mannans (Arsenault et al., 2017).

In Nile tilapia, inclusion of beta mannanase improved growth, feed efficiency and feed conversion ratio and also increased the intestinal enzyme activity (Chen et al., 2016). Ng and Chong (2002) reported that addition of pure beta mannanase to a 40% palm kernel meal diet did not improve growth and feed utilization of tilapia. Yigit et al. (2014) reported that rainbow trout supplemented with beta mannanase at two different levels (1 g kg^{-1} and 2 g kg^{-1}) to a control diet including soybean meal did not affect the growth parameters, feed efficiency and digestibility. Caspian Salmon improved growth and feed utilization when betamannase was included in the diet in a multi enzyme complex at levels of of 0.5 kg^{-1} and 2.5 2 g kg^{-1} beta mannanase (Zamini et al., 2014).

Based on previous findings using beta mannanase, the purpose of this study was to evaluate the efficacy of using a commercial beta mannanase enzyme on growth performance, nutrient retention and nutrient digestibility in practical diets for juvenile tilapia (*Oreochromis niloticus*).

2. Materials and Method

2.1. Experimental diets and feeding trial

A practical basal diet was designed to contain 32% protein and 6% lipid using primarily plant-based protein sources allowing high levels of undigested materials (Table 1). The tilapia diets were designed to contain 6% meat and bone meal and 2% menhaden fish meal to ensure palatability of the diets. The test diets were formulated to meet the nutritional requirements of the Nile tilapia (NRC, 2011). The basal diet was modified to produce four levels (0.00, 0.05, 0.10 and 0.20 g/100g) of enzyme supplements using corn starch as a filler. The test diets were prepared at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground dry ingredients and oil were weighed and then mixed using a food mixer (Hobart Corporation, Troy, Ohio, USA) for 15

minutes. Boiling water was then blended into the mixture at ~20% in order to attain an appropriate consistency for pelleting. Diets were then extruded through a 3-mm diameter die in a meat grinder, air dried at < 50°C to a moisture content of 8-10 % and stored at room temperature. A sample of each feed was collected and analyzed for proximate composition as per AOAC (1995) procedures by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, USA (Table 2).

Thermostable beta-mannanase (CTCzyme, CTC Bio Inc., Seoul, Korea) was produced from Bacillus subtilis fermentation. One Beta-D-Mannanase unit is defined as the amount of enzyme which liberates 1 micromole of reducing sugar equivalent to D-mannose per minute at 50◦C and pH 6.0.

2.2. Culture methods

Juvenile sex- reversed Nile tilapia (mean initial weight 7.45 ± 0.06 g) were randomly stocked into 75-L aquaria which are a component of a 2,500-L indoor recirculation system at 20 fish per aquarium at the E.W. Shell Fisheries Center, Auburn, Alabama. Each diet was randomly assigned to the tanks and offered to fish in four replicate aquaria for the duration of a 70-day growth trial. Samples of fish from the initial stocking were retained for later protein retention analysis.

Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using air stones in each aquarium and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen and water temperature were measured twice a day using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, Ohio) while pH, total ammonia nitrogen and Nitrite-N were measured once per week. Photoperiod was set at 14 h light and 10 h

dark.

Diets were offered to fish at 3.0-6.0% BW daily, according to fish size and divided into two equal feedings each day. Test diets were applied two times per day 0800 and 1600 h for a 70-day experimental period. Fish were weighed every week for the first two weeks and every other week thereafter. Daily feed rations were calculated based on % body weight. The ration was adjusted each week based on growth and observation of the feeding response. At the end of the growth trial, fish were counted and group weighed to determine weight gain, survival and feed conversion ratio. At the conclusion of the trial, four fish were randomly collected from every aquarium and frozen at 20 °C for later biochemical analysis. These whole-body fish samples were homogenized and sent to Midwest Laboratories (Omaha, NE, USA) for conducting dry matter, crude protein, crude lipid, and ash analyses.

Growth performance indexes including weight gain, feed conversion ratio (FCR), survival, apparent net protein retention, apparent net energy retention, and thermal unit growth coefficient were computed using the following calculations:

a) Weight gain (g) = Average final weight (g) – Average initial weight (g)

b) Feed conversion ratio (FCR) = dry feed intake / wet weight gain.

c) Survival (%) = (Initial fish number – Final fish number)/Initial fish number × 100

d) Apparent net protein retention (ANPR, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.

e) Apparent net energy retention (ANER, %) = (final weight × final energy content) - (initial weight × initial energy content) × 100 / energy intake.

f) Thermal-unit growth coefficient (TGC) = (final weight$^{1/3}$ - initial weight$^{1/3}$) / (temperature × day) × 100.

2.3. Digestibility

In order to assess the digestibility of the diets, 1% Chromic Oxide was added to a sub-sample of the diets (Table 1) used in the growth trial. Digestibility coefficients of test diets were determined using groups of 8 fish (~40 g weight). Fish were allowed to acclimate to the various test diets before starting the collection of feces. Prior to each feeding the tanks and fecal settling chambers (FSC) were cleaned. Fish were offered two feeding and all feces collected using a settling system. Samples were collected for several days until a suitable quantity was obtained for analyses (~1 g dry weight). Daily samples were pooled by tank and three replicate aquaria (n=3) were utilized for each treatment. Feces were stored in sealed plastic containers and stored in a freezer. Dry matter, crude protein, total energy were determined for the fecal and diet samples according with established procedures. Crude protein content was analyzed using the micro-Kjeldahl method (Ma and Zuazaga, 1942). Total energy content using a micro-calorimetric adiabatic calorimeter bomb using benzoic acid as standard (Model 1425, Parr Instrument Co. Moline, IL, USA). Chromic oxide content analysis followed McGinnis and Kasting (1964) procedures. Apparent digestibility coefficients of the dry matter, protein, and energy for each diet were calculated according to Cho et al. (1982) using the following formulae:

$$\text{ADMD (\%)} = 100 - [100 \times (\%\ Cr_2O_3 \text{ in feed} / \%\ Cr_2O_3 \text{ in feces})]$$

$$\text{APD or AED (\%)} = 100 - [100 \times ((\%\ Cr_2O_3 \text{ in feed\%} / \%\ Cr_2O_3 \text{ in feces}) \times (\%\ \text{nutrient feces} / \%\ \text{nutrient feed}))]$$

2.4. Statistical analyses

Statistical analyses were conducted using SAS system for windows, (V9.4. SAS Institute, Cary, NC). Initial weight, biomass, final weight, thermal unit growth coefficient, percent weight gain, feed conversion rate, dry matter, apparent nutrient retention of protein and energy, dry matter,

protein and energy digestibility coefficients were analyzed using a one-way analysis of variance to determine significant ($P < 0.05$) differences among the treatment means followed by Student-Neuman Keuls multiple range test to distinguish significant differences between treatment means. Survival was analyzed by logistic (binary) regression.

3. Results

3.1 Water quality

During the experimental period dissolve oxygen, temperature, salinity, pH, total ammonia nitrogen, and nitrite were maintained within acceptable ranges for Nile tilapia at 6.10 ± 0.86 mg L^{-1}, 29.01 ± 0.62 °C, 1.81 ± 0.58 ppt, 7.0 ± 0.57, 0.19 ± 0.05 mg L^{-1}, 0.08 ± 0.04 mg L^{-1} over the 70-day trial period.

3.2 Growth performance

Parameters of growth performance are summarized in Table 3. No significant ($P > 0.05$) differences were detected for initial mean weight, final mean weight, weight gain percentage, thermal unit growth coefficient (TGC), survival, feed conversion ratio (FCR) as different beta mannanase levels were added to the diets. Initial mean weight was not significantly different ($P= 0.51$) for the dietary treatments (7.45 ± 0.06 g). Final mean weight was unchanged ($P = 0.55$) by the addition of different levels of beta mannanase (71.94 – 77.15 g). Percent weight gain was unchanged ($P = 0.63$) by dietary treatments (876.12 - 925.01 %). Thermal unit growth coefficient (TGC) was not significantly different ($P = 0.57$) among treatments (0.108 – 0.113). Survival percentage was the same ($P = 1.0$) across treatments (91.5%). Feed conversion ratio was not affected ($P = 0.95$) by the addition of beta mannanase (1.34 – 1.37).

3.3 Nutrient retention and body composition

Apparent net protein retention (P = 0.10; 31.55 - 33.68%) and apparent net energy retention (P = 0.66; 22.39 - 23.67%) were unchanged by the inclusion of beta mannanase (table 3). Whole body fish composition is summarized in table 4. No differences were observed in crude protein (P=0.8415; 14.88 - 15.35%), dry matter (P= 0.7294; 24.9 – 26.12%), fat (P= 0.4689; 5.48 – 6.36%) and ash (P=0.7166; 3.84 – 4.25%) content of whole body fish samples among fish fed various levels of beta mannanase.

3.4 Digestibility

Dry matter digestibility (P= 0.0004; 35.69- 68.54 %, figure 1), energy digestibility (P = 0.0003; 41.28 - 71.32 %, figure 2) and protein digestibility (P= 0.0247; 68.55- 87.92 %, figure 3) were linearly improved by the addition of beta mannanase, based on regression analysis (table 5).

4. Discussion

Beta mannanase and other enzymes are used in some monogastric production systems such as poultry with good success. As the aquaculture industry moves towards the use of ingredients with higher fiber content, there is a need to address the undigestible component of the diet. Hence, the use of beta mannanase enzyme could improve the performance of tilapia diets. In the present study, dry matter digestibility, energy digestibility and protein digestibility coefficients were linearly improved by the addition of beta mannanase to the tilapia diets at level of 0.1 g kg^{-1} and 0.2 g kg^{-1}. Similarly, Farhangi and Carter (2007) indicate that apparent digestibility coefficients of dry matter, crude protein and energy were increased by enzyme addition in rainbow trout due to increases in nutrient digestibility by the stimulation of the release of bile acids improving emulsification by non starch polysaccharides (De Keyser et al., 2016). In contrast, addition of mannanase in red hybrid tilapia diets did not affect the digestibility of dry matter, energy and lipid

(Ng and Chong, 2002). Rainbow trout fed 0.1% and 0.2 % mannanase supplemented soybean meal diets did not show improved digestibility of protein, lipid and dry matter (Yigit et al., 2014). Effects of supplemental mannanase on digestibility have been reported in terrestrial animals. Sows lost less weight during gestation due to increased digestibility with beta mannanase addition (Kim et al., 2018). Growing pig's apparent total tract digestibility of dry matter was improved but not crude protein apparent tract digestibility due to beta mannase supplementation (Kim et al., 2017a). In contrast, Mok et al. (2015) reported that beta mannase inclusion had no effect on the apparent total tract digestibility of growing pigs. Tewoldebrhan et al. (2017) reported a decrease in digestibility of dry matter, organic matter and crude protein for lactating dairy cows fed 0.1% inclusion of beta mannanase, but remained unaffected by 0.2% inclusion in the diets in agreement to the results in goats fed beta mannanase at 0.06% (Lee et al., 2014). Despite that digestibility of dry matter, energy and protein has improved growth performance parameters like final mean weight, feed conversion ratio, weight gain, thermal unit growth coefficient, percent survival was not affected by the inclusion of beta mannanase. This may be due to the energy coming from available mannose and protein content of the diets were not limited in the present study, absorption and utilization of mannose as source of energy, absorption and utilization by tilapia is currently not known. Similar results were reported by Ng and Chong (2002) in which growth performance was not affected in red hybrid tilapia by diets containing 10 to 40% palm kernel meal and beta mannanase at inclusions of 0.01%, 0.05% or 0.10%. Yigit et al. (2014) reported that beta mannanase inclusion to a 44% soybean meal diet did not improved the growth performance and feed efficiency on rainbow trout. Similar findings were observed by Farhangi and Carter (2007) with inclusion of 50% dehulled lupin and enzyme supplementation. In contrast, Chen et al. (2016) observed a significant effect on growth performance in juvenile tilapia, demonstrating an increase in final mean weight and

significant decrease in feed conversion when diets were supplemented with beta mannanase at 0.5 and 1.0 g kg^{-1}. Similar results are reported by Zamini et al. (2014), the addition of 0.5 g kg^{-1} and 2.5 g kg^{-1} beta-mannanase to trout feeds resulted in significant improvements in body weight gain and feed efficiency in Salmo trutta caspius. In addition, 2.5 g/kg beta-mannanase produced significantly higher growth than 0.5 g kg^{-1} beta-mannanase and control diet. Research on fish suggest that the type of enzymes and the concentration (relative to body weight) affect their response to enzyme supplementation (Lin et al., 2007). In terrestrial animals, increases in average daily gain and feed efficiency by beta-mannanase supplementation were observed in pigs (Kim et al., 2017a), Korean native goat (Lee et al., 2014) and growing heifers (Seo et al., 2016). The use of beta mannanase in diets increased body weight and improved feed conversion rate in broiler chickens (Ferreira et al., 2016) and increased performance and egg quality in laying hens (Kim et al., 2017b). Whole body composition was not affected by the addition of beta mannanase in this study, in agreement with the findings of Ng and Chong (2002) indicating that pure beta mannanase supplementation in the diets of red hybrid tilapia had no effect on the whole body composition. Farhangi and Carter (2007) in rainbow trout fed lupin and Yigit et al. (2014) indicated that addition of beta mannanase supplementation in the diets had no effect on the whole body composition of rainbow trout.

5. Conclusion

Results demonstrate that the inclusion of beta mannanase improved digestibility of practical tilapia diets and did not alter production performance, nutrient retention or body tissue composition, the use of beta mannanase in diets formulated for Nile tilapia offers an opportunity to incorporate plant based ingredient to obtain a less expensive diet without affecting performance.

Table 1. Ingredient composition (g 100 g-1 as is) of test diets formulated to contain 32% protein and 6% lipid.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Menhaden fishmeal [1]	2.00	2.00	2.00	2.00
Meat & bone meal [2]	6.00	6.00	6.00	6.00
Soybean meal [3]	39.00	39.00	39.00	39.00
DDGS [4]	30.00	30.00	30.00	30.00
Menhaden fish oil [5]	1.51	1.51	1.51	1.51
Lecithin [6]	0.50	0.50	0.50	0.50
Corn Starch [7]	1.39	1.34	1.29	1.19
Yellow Corn [8]	17.00	17.00	17.00	17.00
Mineral premix [9]	0.50	0.50	0.50	0.50
Vitamin premix [10]	0.80	0.80	0.80	0.80
Choline chloride [7]	0.20	0.20	0.20	0.20
Stay C 35% active [11]	0.10	0.10	0.10	0.10
CaP-dibasic [7]	1.00	1.00	1.00	1.00
β Mannanase[12]	**0.00**	**0.05**	**0.10**	**0.20**

[1] Menhaden Fishmeal, Omega Protein Inc., Houston, TX, USA.
[2] Meat & Bone Meal, Midsouth milling Co Memphis Tn.
[3] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[4] Distillers dried grains with solubles (DDGS) Flint Hills Resources, LLC, Wichita, KS, USA.
[5] Menhaden Fish Oil, Omega Protein Inc., Reedville, VA, USA.
[6] Enhanced D-97, The Solae Company, St. louis, MO, USA.
[7] MP Biomedicals Inc., Solon, Ohio, USA
[8] Faithway Feed Co., LLC., Guntersville, AL, USA
[9] Trace mineral (g/100g Premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.25; Ferrous sulfate, 4.0; Magnesium sulfate anhydrous, 13.86; Manganous sulfate monohydrate, 0.65; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.19; cellulose, 67.96.
[10] Vitamin (g/kg Premix): Thiamin HCl, 0.44; Riboflavin, 0.63; Pyridoxine HCl, 0.91; D-pantothenic acid, 1.72; Nicotinic acid, 4.58; Biotin, 0.21; Folic acid, 0.55; Inositol, 21.05; Menadione sodium bisulfite, 0.89; Vitamin A acetate (500,000 IU g-1), 0.68; Vitamin D3 (400,000 IU g-1), 0.12; DL-alpha-tocopherol acetate (250 IU g-1), 12.63; cellulose 955.59.
[11] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[12] CTCzyme (CTC Bio Inc., Seoul, Korea)

Table 2. Proximate composition and amino acid profile of test diets (as is basis).

	Diet 1	Diet 2	Diet 3	Diet 4
(%) β mannanase	0	0.05	0.1	0.2
Crude protein[1]	34.16	33.90	35.26	35.30
Moisture	6.53	8.00	5.12	4.60
Crude Fat	7.28	7.30	7.82	7.49
Crude Fiber	4.78	4.45	5.06	4.87
Ash	7.49	7.39	7.72	8.02
Beta Mannanase[2] activity unit/g	0.00	0.47	0.59	0.78
Alanine	1.83	1.79	1.89	1.92
Arginine	2.14	2.07	2.21	2.27
Aspartic Acid	3.28	3.16	3.30	3.38
Cysteine	0.48	0.46	0.49	0.51
Glutamic Acid	5.78	5.55	5.64	5.80
Glycine	1.71	1.74	1.88	1.90
Histidine	0.88	0.85	0.89	0.92
Hydroxylysine	0.07	0.07	0.09	0.09
Hydroxyproline	0.23	0.23	0.30	0.30
Isoleucine	1.49	1.42	1.50	1.52
Leucine	2.93	2.80	2.92	2.98
Lysine	2.05	1.97	2.06	2.11
Methionine	0.57	0.57	0.58	0.61
Ornithine	0.02	0.02	0.02	0.02
Phenylalanine	1.70	1.63	1.73	1.76
Proline	2.09	1.98	2.10	2.12
Serine	1.48	1.44	1.46	1.53
Taurine	0.02	0.02	0.03	0.03
Threonine	1.30	1.26	1.32	1.36
Tryptophan	0.44	0.41	0.38	0.46
Tyrosine	1.13	1.11	1.16	1.19
Valine	1.84	1.67	1.85	1.88

[1]Crude Protein = %N x 6.35

[2]CTCzyme (CTC Bio Inc., Seoul, Korea)

Table 3. **Growth response of juvenile tilapia (7.45 ± 0.06 g) fed for 70 d three levels of inclusion of beta mannanase.**

Diet	(%) β mannanase [+]	Final mean weight (g)	Weight gain (%)	Thermal-unit growth coefficient	Survival (%)	Feed conver-sion ratio	ANPR %	ANER %
1	0	77.15	925.01	0.1130	91.25	1.347	33.29	23.15
2	0.05	76.678	928.01	0.1129	91.25	1.365	33.68	22.39
3	0.1	71.948	876.12	0.1087	91.25	1.372	31.88	23.65
4	0.2	74.458	899.87	0.1108	91.25	1.357	31.55	23.38
PSE		2.7784	31.896	0.0023	1.6137	0.0338	0.6594	0.0080
P value		0.5507	0.6394	0.5782	1.000	0.9587	0.1024	0.6610

PSE= Pooled Standard Error, n=4. ANPR= Apparent net protein Retention. ANER= Apparent Net Energy Retention. Significance (P<0.05) based on analysis of variance followed by Student Newman Keuls grouping. [+] CTCzyme, CTC Bio, Seoul, Korea.

Table 4. Proximate composition (g kg-1, as is) of whole body tilapia fed three levels of inclusion of beta mannanase.

As is basis	Diet 1	Diet 2	Diet 3	Diet 4	PSE	P-value
(%) β mannanase[+]	0	0.05	0.1	0.2		
Dry matter	24.90	25.65	24.90	26.10	0.7588	0.7294
Protein	15.03	15.35	15.20	14.88	0.3776	0.8415
Fat	5.69	6.36	5.48	6.25	0.4253	0.4689
Ash	4.23	3.84	3.85	4.25	0.5262	0.7166
Calcium %	1.32	1.26	1.31	1.13	0.2290	0.9255
Copper ppm	2.45	2.30	2.50	2.38	0.2129	0.9154
Iron ppm	24.77	25.73	23.85	31.98	3.1939	0.3520
Manganese ppm	2.00	1.90	2.07	1.85	0.2600	0.9349
Magnesium %	0.04	0.04	0.04	0.03	0.0041	0.6418
Phosphorus %	0.77	0.73	0.77	0.67	0.1083	0.8898
Potassium %	0.27	0.27	0.26	0.28	0.0595	0.3804
Sodium %	0.12	0.12	0.12	0.12	0.0030	0.7256
Sulfur %	0..16	0.17	0.17	0.17	0.0030	0.1382
Zinc ppm	38.50	35.03	36.78	35.23	3.0862	0.8749

PSE= Pooled Standard Error, n=4. Significance (P<0.05) based on analysis of variance followed by Student Newman Keuls grouping. [+] CTCzyme, CTC Bio, Seoul, Korea.

Table 5. Digestibility values of dry matter, energy and protein in diets with three levels of inclusion of beta mannanase

		Digestibility		
	(%)β mannanase*	Dry matter	Energy	Protein
Diet 1	0	35.69 ± 11.42	41.28 ± 7.34	72.70 ± 8.09
Diet 2	0.05	40.88 ± 11.38	44.22 ± 11.68	68.55 ± 3.22
Diet 3	0.10	57.21 ± 6.33	59.78 ± 6.87	75.02 ± 14.19
Diet 4	0.20	68.54 ± 4.48	71.32 ± 3.64	87.92 ± 3.03
P-value		0.0004	0.0003	0.0247

Significance ($P < 0.05$) determined by regression model.
*CTCzyme, CTC Bio, Seoul, Korea

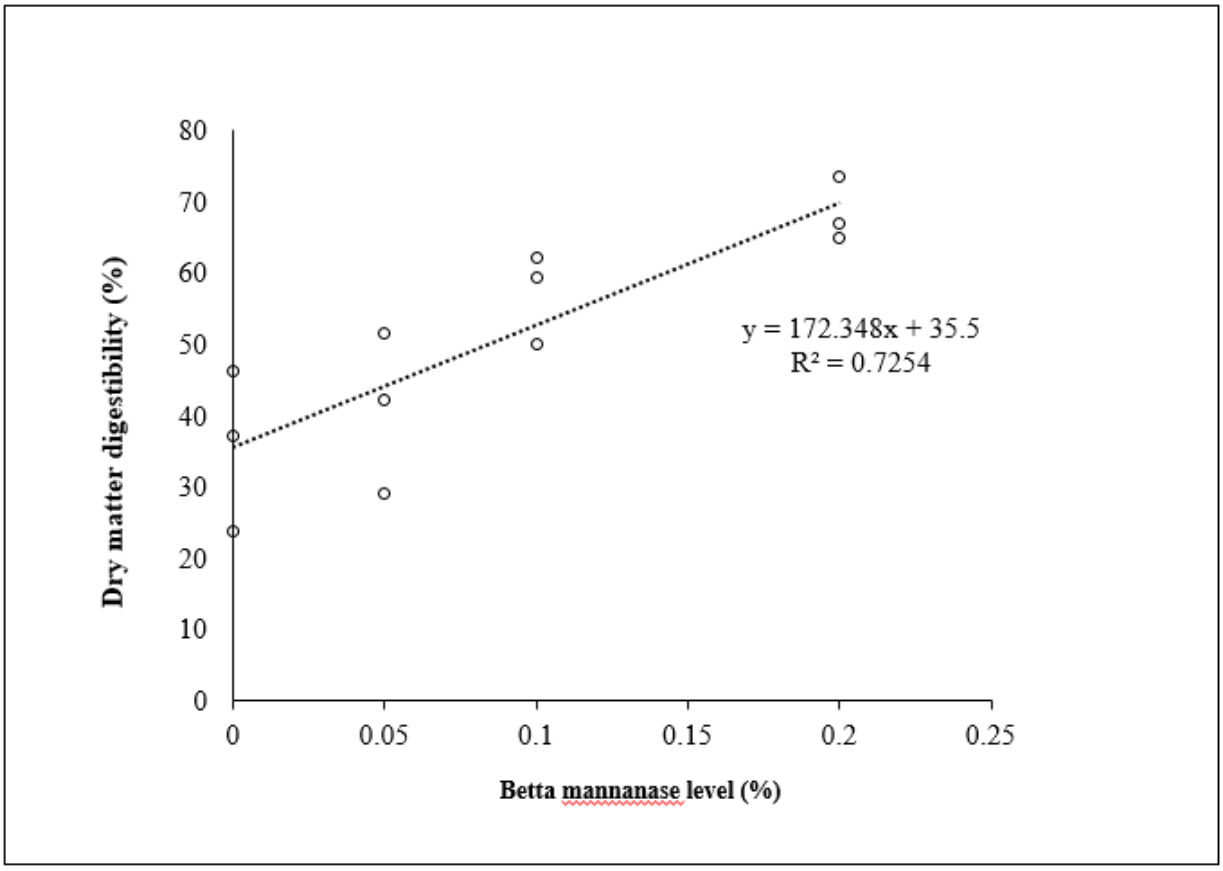

Figure 1. Regression of dry matter digestibility of Nile tilapia against dietary beta mannanase levels.

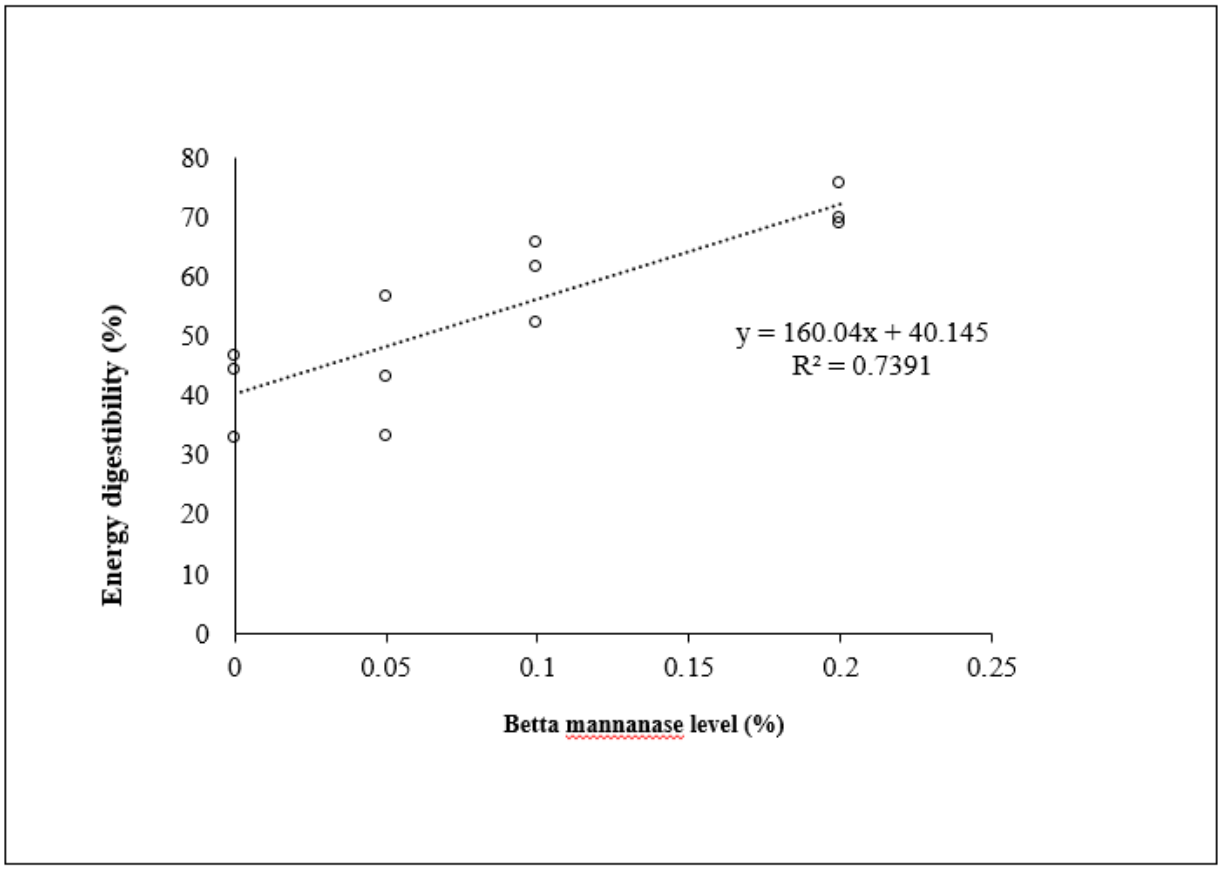

Figure 2. Regression of energy digestibility of Nile tilapia against dietary beta mannanase levels.

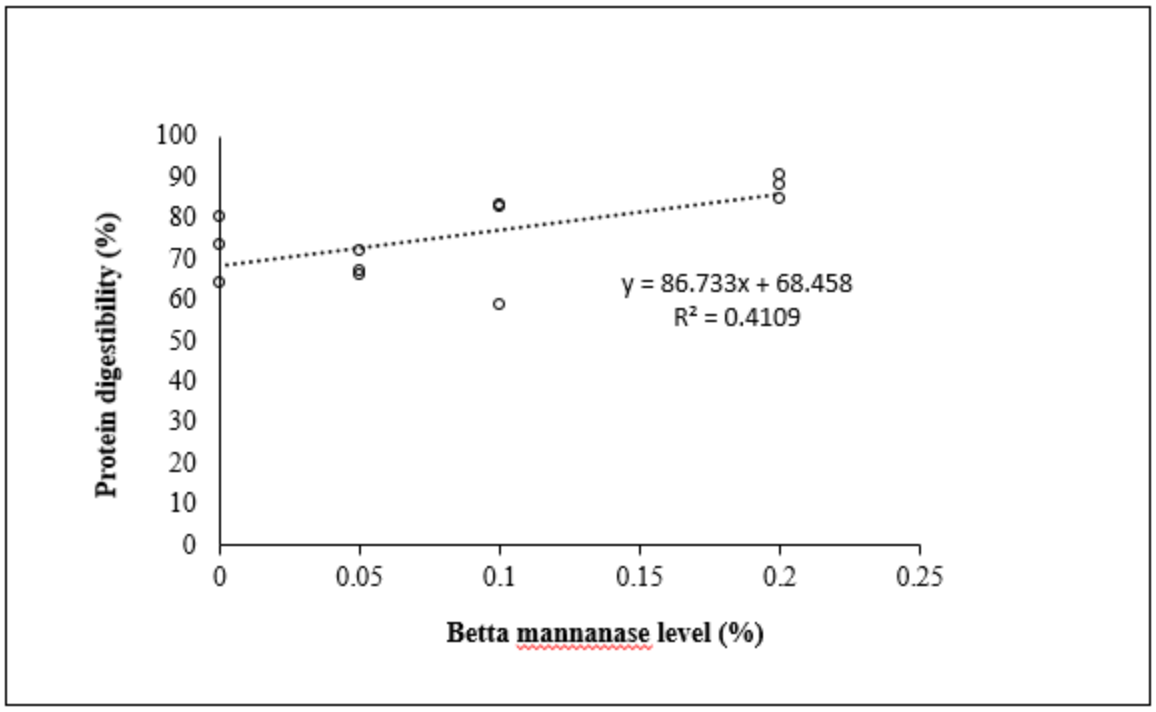

Figure 3. Regression of protein digestibility of Nile tilapia against dietary beta mannanase levels.

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Chapter III

TILAPIA GROWTH PERFORMANCE AND DIGESTIBILITY USING PROTEASES AND CARBOHYDRASES

Abstract

This study was conducted to evaluate the production performance and feed digestibility of Nile tilapia *Oreaochromis niloticus* when supplemented with commercial proteases and carbohydrases. Ten practical tilapia diets were formulated to contain 32% protein and 6% lipids. Six diets were formulated to contain low levels of fiber (LF) and included free protease (LF-FP), protected protease (LF-PP), free carbohydrase (LF-FC), protected carbohydrase (LF-PC), and a mix of free protease and carbohydrases (LF-MFPFC). Four diets were formulated to contained high levels of fiber (HF) and included a basal diet (HF) and a basal diet supplemented with free protease (HF-FP), free carbohydrase (HF-FC), and a mix of free protease and free carbohydrases (HF-MFPFC), distillers dried grains with solubles were used as a source of fiber in high fiber diets. The level in the diet of free protease (FP) and protected (PP) was 175 g per metric ton, the level of free carbohydrase (FC), protected carbohydrase (PC) and the mix of free protease and carbohydrase (MFPFC) was 125 g per metric ton. The diets were offered to sex reversed juvenile tilapia (mean initial weight 9.29 ± 0.11 g) over a 70 days growth trial. Four replicate groups of 20 fish per aquaria were offered the test diets at near satiation levels. At the conclusion of the growth trial, survival was near 100% and weight gain was around 1000%. In general, fish maintained on the high fiber diet performed slightly poorer than those on the lower fiber diet. Concerning enzyme supplements, apparent net energy retention was significantly different (P= 0.0001) in low fiber diets when free and protected proteases were added. However, for low and high fiber diets there were no significant (P > 0.05) differences in final mean weight, percent weight gain, thermal unit

growth coefficients, survival, feed conversion ratio or apparent net protein retention. Overall, there were no clear advantages detected to the protected enzymes. Dry matter and energy digestibility were significantly improved by the addition of free carbohydrase and a mix of free protease and free carbohydrase when supplemented to a low and high fiber diets. Exogenous enzymes have shown to be a promising way to improve digestibility in commercial Nile tilapia diets.

1. Introduction

The development of commercial feeds for aquaculture has been traditionally based on fish meal as the main protein source because of its high protein content and essential amino acid profile (Tacon et al., 1993; Watanabe, 2002; El-Saidy and Gaber, 2004). However, the global fishmeal price has increased more than two fold in recent years (FAO, 2016) due to shortage in the supply. The use of alternative feed ingredients, including plant sources (Salze et al., 2010; Herath et al., 2016; Aydin et al., 2017; da Silva et al., 2017; Martins et al., 2017; Al-Thobaiti et al., 2018; Khalifa et al., 2018), animal sources (Montoya-Mejia et al., 2017; Moutinho et al., 2017; Wang et al., 2017; Devic et al., 2018), algae (Sarker et al., 2018; Simanjuntak et al., 2018; Younis et al., 2018) and restaurant food waste (Nasser et al., 2018) are a viable option for decreasing fish meal use and decreasing formulation cost (Mbahinzireki et al., 2001; Thiessen et al., 2003; Ai and Xie, 2005). However, the presence of anti nutritional factors and low digestibility of diets when some alternative feed ingredients are included can impair their use as fish meal replacements in aquaculture feeds.

Supplementation of diets with exogenous enzymes is considered effective to eliminate the anti-nutritional factors and improve utilization of dietary energy and amino acids, resulting in improved fish performance (Lin et al., 2007) and gut health (Castillo and Gatlin, 2015). Dietary proteases and carbohydrases are used in aquatic animals to improve the digestibility of diets when plant-based ingredients are included in the formulation.

Exogenous protease can compensate for the deficiency of endogenous enzymes especially for young animals and assist in the breakdown of macromolecular proteins, improving their digestibility (Shi et al., 2016). Carbohydrases are used to assist in the breakdown of hemicellulose which are part of the cell wall. As described by Ebringerová (2005), among the hemicelluloses are

the xyloglycans (xylans) and mannoglucans (mannans). Xylans are the most abundant hemicellulose type in the plant kingdom and mannans are part of the non starch polysaccharide (NSP) fraction in plant-based feed ingredients. The enzymes required to digest NSP, such as beta-xylans or beta mannans, are very scarce or even absent among fish species (Kuz'mina, 1996) and the ability to use NSP by the fish depends on the nature of the microbial population residing in the gut (Sinha et al., 2011). The NSP fraction influences digesta viscosity, gut morphology, physiology and mucus layer, affecting the endogenous secretion of water, proteins, electrolytes and lipids. These changes can lead to a reduced nutrient digestibility (Leenhouwers et al., 2006; Sinha et al., 2011).

The addition of exogenous proteases and carbohydrases have been studied in fish. In rainbow trout, addition of protease to canola, pea - based diets resulted in significant improvements in apparent digestibility for crude protein, energy, lipid and dry matter (Drew et al., 2005). Dalsgaard et al. (2012) supplemented protease to soybean meal-containing diets for rainbow trout and reported a significantly increase in the apparent digestibility of protein, lipid, phosphorus and dry matter. Farhangi and Carter (2007) fed juvenile rainbow trout, diets supplemented with protease and carbohydrase alone or in combination to a de-hulled lupin-based feeds. No effects on performance were observed, but the mixed enzyme significantly improved the protein efficiency ratio and the apparent digestibility of dry matter, protein and gross energy. In contrast, Yigit et al. (2014) reported that rainbow trout supplemented with a mix of beta mannanase and alpha galactosidase at two levels (1 g kg^{-1} and 2 g kg^{-1}) to a control diet including soybean meal did not affect growth parameters, feed efficiency and digestibility. Also, rainbow trout (*Oncorhynchus mykiss*) fry diets containing canola and supplemented with cellulase, phytase, pectinase or an

enzyme mix showed no differences in growth parameters, feed conversion ratio, dry matter, protein or lipid digestibility with enzyme supplementation (Yigit and Keser, 2016).

Digestibility of crude protein and crude lipid were significantly improved in juvenile Gibel carp (Liu et al., 2018) fed incremental increases in dietary protease up to 300 mg/kg. Carter et al. (1994) reported a positive effect on performance and feed efficiency in Atlantic salmon smolt (Salmo salar L) when supplementing a combination of proteolytic enzymes and carbohydrases to a diet containing 340 g/kg soybean meal, no increase was observed in the apparent digestibility of nitrogen and carbon.

In tilapia (*Oreochromis niloticus* × *Oreochromis aureus*), the digestibility of dry matter and crude protein increased by the supplementation of protease (Li et al., 2016). Lin et al. (2007) reported that addition of a commercial enzyme complex of neutral protease, beta glucanase and xylanase improved growth performance, but no effect was detected in apparent digestibility of protein, lipid and gross energy in *Oreochromis niloticus* × *Oreochromis aureus*. Adeoye et al. (2016) fed Nile tilapia probiotics, a mix of enzymes (containing phytase, protease and xylanase), and the combination of enzymes and probiotic. Tilapia fed diets supplemented with enzymes plus probiotics performed better than tilapia fed only the probiotic supplemented diets in terms of final body weight, feed conversion ratio and protein efficiency ratio. Hlope-Ginindza et al. (2016) reported that *Oreaochomis mosambicus* fed a kikuyu-based diet supplemented with a multi enzyme complex composed of cellulase, xylanase and phytase had improved growth, lower feed conversion rate values, increased protein efficiency, higher protein digestibility and increased activity of fish enzymes up to 0.5 g per kg addition to the diet. Inclusion of beta mannanase improved growth, feed efficiency and feed conversion ratio and also increased the intestinal enzyme activity in Nile tilapia (Chen et al., 2016). Red hybrid tilapia fed diets containing 40%

palm kernel meal did not improve growth and feed utilization when a combination of protease, cellulase, glucanase, pectinase and pure mannanase were added to the diets (Ng and Chong, 2002). Caspian Salmon fed two multienzyme complexes which consisted of a combination of protease, lipase, phytase, alpha amilase, cellulase, amiloglucosidase, beta glucanase, pentosonase, hemicellulase, xylanase, pectinase, acid phosphatase, acid phytase and endo- beta mannanase, amylase, xylanase, cellulose and alpha galactosidase improved growth and feed utilization when enzymes were included in the diet in a multi enzyme complex at levels of 0.5 g kg^{-1} and 2.5 g kg^{-1} (Zamini et al., 2014). Nile Tilapia can tolerate higher dietary fiber and carbohydrate concentrations than most other cultured fish (Elsayed and Teshima, 1992) and have the ability to feed on wide range of foods. Hence the purpose of this study was to evaluate the efficacy of using commercial protease and carbohydrase enzymes on growth performance, nutrient retention and nutrient digestibility in practical diets for juvenile tilapia *(Oreochromis niloticus*).

2. Materials and Method

2.1. Experimental diets

Ten practical tilapia diets were formulated to contain 32% protein and 6% lipids (Table 1). The test diets were formulated to meet the nutritional requirements of the Nile tilapia (NRC, 2011). Six diets were formulated to contain low levels of fiber (LF) and included a basal diet (LF) and a basal diet supplemented with free protease (LFFP), protected protease (LFPP), free carbohydrase (LFFC), protected carbohydrase (LF-PC), and a mix of free protease and carbohydrase (LF-MFPFC). Additionally, to evaluate the effects in higher fiber diets, a second basal diet (HF) was formulated using 30% distillers dried grains with solubles as a replacement for soybean meal. The HF basal diet was then supplemented with free protease (HF-FP), free carbohydrase (HF-FC), and a mix of free protease and carbohydrase (HF-MFPFC). The level in the diet of free protease (FP)

and protected (PP) was 175 g per metric ton, the level of free carbohydrase (FC), protected carbohydrase (PC) and the mix of free protease and carbohydrase (MFPFC) was 125 g per metric ton. The test diets were prepared at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground dry ingredients and oil were weighted and then mixed using a food mixer (Hobart Corporation, Troy, Ohio, USA) for 15 minutes. Boiling water was then blended into the mixture at ~ 30% in order to attain an appropriate consistency for pelleting. Diets were then extruded through a 3-mm diameter die in a meat grinder, air dried at < 50°C to a moisture content of 8-10 % and stored at room temperature. A sample of 150 g of each feed was collected and analyzed for proximate composition (AOAC 930.15, AOAC 990.03, AOAC 2003.05, Ankom.Tech, AOAC 942.05 were used for moisture, protein, fat, fiber and ash analysis respectively) by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, USA (Table 2).

The free protease complex used in this study is an alkaline serine protease complex produced from bacterial fermentation. The protected protease is a microencapsulated protease complex composed of vegetable fat and bacterial fermentation extract. The enzymatic activity of both products was 18,000 unit/g. One unit of protease is equivalent of the amount of enzyme that releases 1 nmol of 4-nitroaniline per minute from Succ-AAPF-pNA at pH 9.0 and 40°C. The free and microencapsulated carbohydrase complex is a combination of xylanase and beta-mannanase. The activity of xylanase in the products was 270 unit/g defined as the quantity of enzyme that releases one micromole of xylose per minute at pH 4.5 and 30°C. The activity of beta-mannanase in the products was 2,790 unit/g defined as the quantity that liberates one micromole of reducing sugar (mannose equivalents) in one minute from a mannan-containing substrate (locust bean gum) at pH 6.0 and at 50°C. In the free protease-carbohydrase complex, activity of both carbohydrases

was similar to those mentioned above but the protease activity was >5000 unit/g. All the enzymes were supplied by JEFO Nutrition Inc. (Saint-Hyacinthe, Quebec, Canada).

2.2. Culture methods

Juvenile sex- reversed Nile tilapia (mean initial weight 9.29 ± 0.11 g) were randomly stocked into 75-L aquaria which are a component of a 2,500-L indoor recirculation system at 20 fish per aquarium at the E.W. Shell Fisheries Center, Auburn, Alabama. Each diet was randomly assigned to the tanks and offered to fish in four replicate aquaria for the duration of a 70-day growth trial. Samples of fish from the initial stocking were retained for later protein retention analysis.

Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using air stones in each aquarium and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen and water temperature were measured twice a day using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, Ohio) while pH, TAN and Nitrite-N were measured once per week. Photoperiod was set at 14 h light and 10 h dark.

Diets were offered to fish at 3.0-6.0% BW daily, according to fish size and divided into two equal feedings each day. Test diets were applied two times per day (0800 and 1600 h) for a 70-day experimental period. Fish were weighed every week for the first two weeks and every other week thereafter. Daily feed rations were calculated based on % body weight. The ration was adjusted each week based on growth and observation of the feeding response. At the end of the growth trial, fish were counted and group weighed to determine weight gain, survival and feed conversion ratio. At the conclusion of the trial, four fish were randomly collected from every aquarium and frozen at 20 °C for later biochemical analysis. These whole-body fish samples were

homogenized in a food processor and sent to Midwest Laboratories (Omaha, NE, USA) for proximate and mineral analyses as per AOAC procedures (AOAC 930.15, AOAC 990.03, AOAC 954.02, AOAC 942.05 were used for moisture, protein, fat, fiber and ash analysis respectively; AOAC 985.01 was used for mineral analysis).

Growth performance indexes including weight gain, feed conversion ratio (FCR), survival, apparent net protein retention, apparent net energy retention, thermal unit growth coefficient, hepatosomatic index and intraperitoneal fat index were computed using the following calculations:

g) Weight gain (g) = Average final weight (g) – Average initial weight (g)

h) Feed conversion ratio (FCR) = dry feed intake/ wet weight gain.

i) Survival (%) = (Initial fish number – Final fish number)/Initial fish number × 100

j) Apparent net protein retention (ANPR, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.

k) Apparent net energy retention (ANER, %) = (final weight × final energy content) - (initial weight × initial energy content) × 100 / energy intake.

l) Thermal-unit growth coefficient (TGC) = (final weigh$^{1/3}$ - initial weight$^{1/3}$) / (temperature × day) × 100.

m) Hepatosomatic Index (HI) = liver weight/fish weight × 100

n) Intraperitoneal fat Index (IFI)= intraperitoneal fat weight/fish weight × 100

2.3. Digestibility

In order to assess the digestibility of the diets, 1% Chromic Oxide was added to a sub-sample the low fiber diets (Table 1, LF, LF-MFPFC, LF-FC, HF, HF-MFPFC, HF-FC). Digestibility coefficients of test diets were determined using groups of 8 fish (~40 g weight). Fish were allowed to acclimate to the various test diets for four days before starting the collection of feces. Prior to each feeding the tanks and fecal settling chambers (FSC) were cleaned. Fish were

offered two feeding and all feces collected using a settling system. Feces were stored in sealed plastic containers and stored in a freezer until processed. Samples were collected for four days until a suitable quantity was obtained for analyses (~1 g dry weight). Daily samples were pooled by tank and three replicate aquaria (n=3) were utilized for each treatment. Dry matter, crude protein, total energy was determined for the fecal and diet samples according with established procedures. Crude protein content was analyzed using the micro-Kjeldahl method (Ma and Zuazaga, 1942). Total energy content using a micro-calorimetric adiabatic bomb calorimeter using benzoic acid as standard (Model 1425, Parr Instrument Co. Moline, IL, USA). Chromic oxide content testing followed the McGinnis and Kasting (1964) procedures. Apparent digestibility coefficients of the dry matter, protein, and energy for each diet were calculated according to Cho et al. (1982) using the following formulae:

$$\text{ADMD (\%)} = 100 - [100 \times (\%\ Cr^2O^3 \text{ in feed} / \%\ Cr^2O^3 \text{ in feces})]$$

$$\text{APD or AED (\%)} = 100 - [100 \times ((\%\ Cr^2O^3 \text{ in feed\%} / \%\ Cr^2O^3 \text{ in feces}) \times (\%\ \text{nutrient feces} / \%\ \text{nutrient feed}))]$$

2.4. Statistical analyses

Statistical analyses were conducted using SAS system for windows, (V9.4. SAS Institute, Cary, NC). Initial weight, final mean weight, TGC, percent weight gain, feed conversion rate, apparent nutrient retention of protein and energy, dry matter, protein and energy digestibility coefficients were analyzed using a one-way analysis of variance to determine significant ($P < 0.05$) differences among the treatment means followed by Student-Neuman Keuls multiple range test to distinguish significant differences between treatment means. Using the paired subset of diets, two way analysis of variance was used to determine interactions between fiber level and enzyme supplementation and protected and free interactions. Survival was analyzed by logistic (binary) regression.

3. Results

3.1 Water quality

During the experimental period dissolve oxygen, temperature, salinity, pH, total ammonia nitrogen, and nitrite were maintained within acceptable ranges for Nile tilapia at 6.0 ± 0.89 mg L^{-1}, 27.70 ± 0.71 °C, 1.06 ± 0.64 ppt, 7.0 ± 0.79, 0.06 ± 0.03 mg L^{-1}, 0.04 ± 0.02 mg L^{-1} over the 70-d trial period.

3.2Growth performance

Parameters of growth performance of fish offered the low fiber level diets are summarized in Table 3a. No significant (P > 0.05) differences were detected for initial mean weight, final mean weight, percent weight gain, thermal unit growth coefficient (TGC), survival, feed conversion ratio (FCR) as free protease, protected protease, free carbohydrase, protected carbohydrase and the mix of free protease and free carbohydrase were added to the diets. Initial mean weight was not significantly different (P= 0.160) for the dietary treatments (9.30 ± 0.10 g). Final mean weight was unchanged (P = 0.317) by the addition of different enzymes (98.31 – 104.43 g). Percent weight gain was unchanged (P = 0.499) by dietary treatments (954.8 – 1032.9 %). Thermal unit growth coefficient (TGC) was not significantly different (P = 0.398) among treatments (0.129 – 0.135). Percent survival was unchanged (P = 0.701) by dietary treatments (98.75 – 100.00%). Feed conversion ratio was not affected (P = 0.064) by the addition of enzymes (1.15 – 1.26).

Parameters of growth performance for fish fed high fiber level diets are summarized in Table 3b. Initial mean weight, final mean weight, percent weight gain, thermal unit growth coefficient (TGC), survival, feed conversion ratio (FCR) of the fish fed the various diets were not significantly (P>0.05) influenced by the addition of free protease, free carbohydrase, and the mixed of free protease and free carbohydrase which were added to the diets. Initial mean weight (9.26 ±

0.12 g) was not significantly different (P= 0.840) among fish assigned to the various dietary treatments. Final mean weight (95.42 – 98.83 g) was unchanged (P = 0.799) by the addition of different enzymes. Percent weight gain was unchanged (P = 0.734) by dietary treatments (927.8 – 966.8 %). Thermal unit growth coefficient (TGC) was not significantly different (P = 0.762) among treatments (0.109 – 0.113). Survival was 100% across all dietary treatments. Feed conversion ratio was not affected (P = 0.498) by the addition of enzymes (1.13 – 1.18).

3.2 Nutrient retention and body composition

In low fiber level diets, apparent net energy retention was significantly different (P = 0.0001) as free and protected proteases were added to the diets (32.37, 38.71, 37.79, 33.79, 31.39, 31.07 % for LF, LF-FP, LF-PP, LF-FC, LF-PC, LF-MFPFC; respectively). Apparent net protein retention (37.58 – 44.60 %) was unchanged (P = 0.399) by the inclusion of proteases and carbohydrases (table 4a) to the basal diet. With regards to high fiber diets, apparent net protein retention (40.12 – 42.24%) and apparent net energy retention (29.24 – 32.567%) were unchanged (P = 0.803; P = 0.429, respectively) by the inclusion of protease and carbohydrases (table 4b). Hepatosomatic index and intraperitoneal fat index was unchanged by addition of enzymes for low and high fiber diets (table 4a and 4b).

Whole body fish composition is summarized in table 5a and 5b. No differences were observed in crude protein (P = 0.786; 14.30 - 15.43%), dry matter (P= 0.547; 24.78 – 26.98%), fat (P = 0.627; 6.38 – 7.36%) and ash (P =0.323; 3.02 – 4.98%) content of whole body fish samples among fish fed free, protected or a mix of protease and carbohydrase in low fiber diets. In diets containing high fiber level, no differences were observed in crude protein (P=0.876; 14.68 - 15.23), dry matter (P= 0.368; 24.85 – 27.3%), fat (P= 0.059; 5.90 – 7.61 %) and ash (P=0.165; 3.50 –

5.08%) content of whole body fish samples among fish fed free or a mix of protease and carbohydrase.

3.2 Digestibility

Digestibility values for low fiber level diets are summarized in table 6a. Dry matter digestibility was significantly different (P= 0.0001) in diets containing free carbohydrase and a mix of free protease and free carbohydrase (52.39, 53.34, 59.38% for LF, LF-FC, LF-MFPFC, respectively). Energy digestibility was significantly improved (P = 0.0003) by the addition of enzymes to the diet (57.17, 63.14, 65.49%, for LF, LF-FC, LF-MFPFC, respectively). Protein digestibility was significantly different (P= 0.0002; 77.11, 83.74, 82.30 for LF, LF-FC, LF-MFPFC, respectively).

Digestibility values for high fiber level diets are summarized in table 6b. Dry matter digestibility was significantly different (P= 0.0011) in diets containing free carbohydrase and a mix of free protease and free carbohydrase (52.22, 56.81, 54.47 8% for HF, HF-FC, HF-MFPFC, respectively). Energy digestibility was significantly different (P = 0.042) by the addition of enzymes to the diet (58.92, 62.56, 60.84%, for HF, HF-FC, HF-MFPFC, respectively). Protein digestibility was significantly different (P= 0.004; 85.45, 87.02, 89.07 for HF, HF-FC, HF-MFPFC, respectively).

In low fiber diets, higher digestibility values were observed when the mix of free protease and free carbohydrase was added to the diets. In contrast, high fiber level diets resulted in higher digestibility values when free carbohydrase was added to the diet.

4. Discussion

The use of exogenous enzymes can be a tool to incorporate different feed ingredients without affecting fish performance by increasing the quality of aquaculture diets. In the present study, diets without enzyme addition presented lower dry matter, energy and protein digestibility coefficients compared to diets containing carbohydrases and the mix of protease and carbohydrases, indicating that enzymes can improve digestibility in tilapia diets, thus increasing the protein and carbohydrate uptake by the fish. These results agree with the findings reported by Li et al. (2016) in *Oreochromis niloticus* × *Oreochromis aureus*, increased digestibility of dry matter and crude protein was observed by the supplementation of protease. These improvements in digestibility can be due by the increase of free amino acids in the diets by the enzyme becoming active with the help of moisture and temperature during processing (Li et al., 2016). In rainbow trout, Farhangi and Carter (2007) indicated that apparent digestibility coefficients of dry matter, crude protein and energy were improved by a multi enzyme protease and carbohydrase due to increases in nutrient digestibility by the stimulation of the release of bile acids, improving emulsification of non starch polysaccharides (De Keyser et al., 2016). Dalsgaard et al. (2012) reported that supplementing protease to soy-containing diets for rainbow trout significantly increased the apparent digestibility of protein, lipid, phosphorus and dry matter, the authors explained an improved nutrient uptake in fish fed soybean meal containing diets by targeting proteinaceous anti-nutrients or hydrolyzing antigenic proteins. Carter et al. (1994) reported no effects of dietary supplementation with combinations of enzymes on the apparent digestibility of nitrogen in Atlantic salmon, however the specific growth rate and feed efficiency were significantly improved. In contrast, Lin et al. (2007) reported that the addition of a commercial enzyme complex of neutral protease, beta glucanase and xylanase had no detectable effect in

apparent digestibility of protein, lipid and gross energy in *Oreochromis niloticus* × *Oreochromis aureus*. The variability of these results may be due to the differences in the enzymes and diet formulations used in these studies. Ogunkoya et al. (2006) found no effect on growth and feed efficiency with the addition of graded levels of enzyme cocktail to rainbow trout offered a soybean meal based diet. The effect of the enzyme inclusion is not predictable at all times due to the non-specific action of the enzymes on the target substrate (Farhangi and Carter, 2007).

Fish final mean weight, feed conversion ratio, percent weight gain, thermal unit growth coefficient and percent survival were not affected by the inclusion of protease and carbohydrase to the diets. This is most likely the result of high nutrient levels which satisfied the nutrition requirement of the tilapia. Hence, supplementation with enzymes did not show positive effects on fish growth. Similar results were observed in rainbow trout fed lupin plus enzyme supplements (Farhangi and Carter, 2007). Ng and Chong (2002) reported that growth performance was not affected in red hybrid tilapia by diets containing 10 to 40% palm kernel meal and beta mannanase at inclusions of 0.01%, 0.05% or 0.1%. In contrast, tilapia supplemented with proteases in low fish meal diets showed improvements in gain and decreased feed conversion ratio with enzyme addition to the diet (Li et al., 2016). Inclusion of beta mannanase improved growth, feed efficiency and feed conversion ratio and also increased the intestinal enzyme activity in Nile tilapia (Chen et al., 2016). Also, similar results are reported by Zamini et al. (2014), the addition of 0.5 g kg^{-1} and 2.5 g kg^{-1} of two multienzyme complexes and the combination containing protease, lipase, phytase, alpha amilase, cellulase, amiloglucosidase, beta glucanase, pentosonase, hemicellulase, xylanase, pectinase, acid phosphatase, acid phytase and endo- beta mannanase, amylase, xylanase, cellulose and alpha galactosidase improved growth and feed utilization in Salmo *Trutta caspius*,

In our study, net energy retention was significantly improved in low fiber diets with the addition of proteases. Dalsgaard et al. (2012) observed no improvement in net energy retention when proteases were added to soybean meal containing diets for rainbow trout. Research on fish suggest that the type of enzymes and its concentration (relative to body weight) affects fish response to enzyme supplementation (Lin et al., 2007).

5. Conclusion

Results demonstrate that the inclusion of protease and carbohydrase improved digestibility of practical tilapia diets and did not alter production performance, nutrient retention or body tissue composition. The use of enzymes in diet formulation for Nile tilapia is an opportunity to increase digestibility while decreasing cost formulation without affecting performance.

Table 1. Ingredient composition (g 100 g^{-1} as-is) of test diets formulated to contain 32% protein and 6% lipid.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
Ingredient	Low Fiber						High Fiber			
MFM [1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
MBM [2]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
SBM [3]	48.00	48.00	48.00	48.00	48.00	48.00	36.80	36.80	36.80	36.80
DDGS [4]							30.00	30.00	30.00	30.00
Fish oil [5]	3.30	3.30	3.30	3.30	3.30	3.30	1.55	1.55	1.55	1.55
Lecithin [6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Corn Starch [7]	5.60	5.582	5.582	5.587	5.587	5.587	2.850	2.832	2.837	2.837
Corn[8]	30.50	30.50	30.50	30.50	30.50	30.50	15.70	15.70	15.70	15.70
Mineral premix[9]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[10]	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride[7]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35% active[11]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[7]	2.50	2.50	2.50	2.50	2.50	2.50	2.80	2.80	2.80	2.80
Lysine HCl[12]							0.20	0.20	0.20	0.20
Enzyme FP[13]		**0.018**						**0.018**		
Enzyme PP[13]			**0.018**							
Enzyme FC[13]				**0.013**					**0.013**	
Enzyme PC[13]					**0.013**					
Enzyme MFPFC[13]						**0.013**				**0.013**

[1] Menhaden Fishmeal, Omega Protein Inc., Houston, TX, USA.
[2] Meat & Bone Meal, Midsouth milling Co., Memphis TN, USA.
[3] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[4] Distillers dried grains with solubles (DDGS) Flint Hills Resources, LLC, Pelham, GA, USA.
[5] Menhaden Fish Oil, Omega Protein Inc., Reedville Houston, VA, USA.
[6] Enhanced D-97, The Solae Company, St. louis, MO, USA.
[7] MP Biomedicals Inc., Solon, Ohio, USA
[8] Faithway Feed Co., LLC., Guntersville, AL, USA

[9] Trace mineral (g/100g Premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.25; Ferrous sulfate, 4.0; Magnesium sulfate anhydrous, 13.86; Manganous sulfate monohydrate, 0.65; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate hepahydrate, 13.19; cellulose, 67.96.

[10] Vitamin (g/kg Premix): Thiamin HCl, 0.44; Riboflavin, 0.63; Pyridoxine HCl, 0.91; D-pantothenic acid, 1.72; Nicotinic acid, 4.58; Biotin, 0.21; Folic acid, 0.55; Inositol, 21.05; Menadione sodium bisulfite, 0.89; Vitamin A acetate (500,000 IU g-1), 0.68; Vitamin D3 (400,000 IU g-1), 0.12; DL-alpha-tocoperol acetate (250 IU g-1), 12.63; cellulose 955.59.

[11] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[12] Ajinomoto Heartland Inc., Chicago, IL, USA.

[13] Jefo Nutrition Inc, Saint-Hyacinthe, QC, CA

Table 2. Proximate composition and amino acid profile of test diets (as is basis).

	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
	Low fiber						High fiber			
Crude protein*	32.00	29.14	27.09	30.30	30.13	29.78	31.41	31.23	32.30	31.87
Moisture	9.17	13.28	15.15	5.91	6.70	8.31	6.95	6.23	5.88	8.10
Crude Fat	7.35	5.33	4.70	5.19	4.54	4.82	7.07	6.50	6.77	6.45
Crude Fiber	4.25	3.59	3.48	7.00	4.50	3.60	7.45	6.46	5.11	6.14
Ash	8.03	7.40	7.21	7.70	7.65	7.60	8.47	8.46	8.57	8.43
Alanine	1.54	1.41	1.38	1.52	1.48	1.49	1.68	1.66	1.75	1.70
Arginine	2.14	1.96	1.86	2.08	2.01	2.04	1.89	1.91	2.00	1.96
Aspartic Acid	3.20	2.92	2.77	3.08	3.01	3.01	2.76	2.79	2.86	2.86
Cysteine	0.40	0.37	0.37	0.40	0.39	0.39	0.45	0.44	0.44	0.45
Glutamic Acid	5.27	4.85	4.67	5.14	5.02	5.01	4.79	4.80	4.90	4.84
Glycine	1.63	1.48	1.46	1.65	1.55	1.62	1.60	1.56	1.64	1.59
Histidine	0.81	0.74	0.69	0.78	0.77	0.76	0.80	0.80	0.83	0.81
Isoleucine	1.36	1.25	1.17	1.33	1.30	1.29	1.30	1.31	1.36	1.33
Leucine	2.45	2.28	2.16	2.40	2.35	2.32	2.65	2.62	2.75	2.69
Lysine	2.03	1.82	1.71	1.91	1.88	1.88	1.88	1.89	2.01	1.96
Methionine	0.52	0.46	0.42	0.47	0.47	0.47	0.49	0.49	0.52	0.52
Ornithine	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03
Phenylalanine	1.50	1.42	1.30	1.45	1.42	1.41	1.49	1.48	1.55	1.53
Proline	1.67	1.56	1.43	1.64	1.59	1.60	1.79	1.78	1.83	1.81
Serine	1.27	1.17	1.19	1.18	1.16	1.17	1.23	1.23	1.28	1.29
Taurine	0.15	0.15	0.14	0.15	0.16	0.15	0.12	0.12	0.13	0.11
Threonine	1.18	1.07	1.04	1.12	1.09	1.10	1.13	1.14	1.18	1.18
Tryptophan	0.44	0.42	0.39	0.41	0.42	0.42	0.42	0.43	0.43	0.40
Tyrosine	0.98	0.93	0.86	0.94	0.93	0.93	0.97	0.99	1.04	1.01
Valine	1.56	1.42	1.35	1.53	1.50	1.49	1.56	1.55	1.62	1.57

*Crude Protein = %N x 6.35

Table 3a. Growth response of juvenile tilapia (9.30 ± 0.10 g) fed for 70 d on six low fiber diets with inclusion of proteases and carbohydrases [+].

Diet	Treatment[+]	Final mean Weight (g)	Weight Gain (%)	Thermal-unit growth coefficient	Survival (%)	Feed conversion ratio	ANPR %	ANER %	HI	IFI
1	LF	104.4	1014.9	0.134	100.00	1.08	37.58	32.37[b]	1.73	2.03
2	LF-FP	105.6	1032.9	0.135	100.00	1.06	41.64	38.71[a]	1.79	2.06
3	LF-PP	102.2	999.3	0.133	98.75	1.07	43.65	37.79[a]	1.38	1.85
4	LF-FC	98.3	970.9	0.129	98.75	1.08	43.70	33.79[b]	1.62	1.67
5	LF-PC	98.7	954.9	0.130	100.00	1.09	44.60	31.39[b]	1.68	1.75
6	LF-MFPFC	102.2	997.4	0.132	98.75	1.07	42.58	31.07[b]	1.81	1.60
PSE		2.60	29.9	0.002	0.88	0.03	2.41	0.87	0.23	0.41
P value		0.317	0.499	0.398	0.701*	0.064	0.399	0.001	0.276	0.736

PSE= Pooled Standard Error, n=4.* Analyzed by binary regression. ANPR= Apparent net protein Retention. ANER= Apparent Net Energy Retention. HI=Hepatosomatic index. IFI= Intraperitoneal Fat Index. Significance ($P<0.05$) based on analysis of variance followed by Student Newman Keuls grouping. Superscripts represent significant differences. [+] Jefo Nutrition Inc, Saint-Hyacinthe, QC, Canada.

Table 3b. Growth responses of juvenile tilapia (9.26 ± 0.12 g) fed for 70-days on four high fiber diets with inclusion of proteases and carbohydrases[+].

Diet	Treatment [+]	Final mean Weight (g)	Weight Gain (%)	Thermal-unit growth coefficient	Survival (%)	Feed conversion ratio	ANPR %	ANER %	HI	IFI
7	HF	95.42	927.8	0.113	100.0	1.06	41.32	30.71	1.70	1.43
8	HF-FP	96.31	935.6	0.113	100.0	1.07	42.25	29.24	1.63	1.03
9	HF-FC	95.46	934.6	0.109	100.0	1.12	40.12	30.94	1.31	1.22
10	HF-MFPFC	98.84	966.8	0.111	100.0	1.07	40.95	32.56	1.76	1.33
PSE		2.82	26.4	0.002		0.03	1.53	1.36	0.25	0.41
P value		0.799	0.734	0.762		0.498	0.803	0.429	0.0785	0.552

PSE= Pooled Standard Error, n=4.* Analyzed by binary regression. ANPR= Apparent net protein Retention. ANER= Apparent Net Energy Retention. HI=Hepatosomatic index. IFI= Intraperitoneal Fat Index.Significance ($P<0.05$) based on analysis of variance followed by Student Newman Keuls grouping. [+] Jefo Nutrition Inc, Saint-Hyacinthe, QC, Canada.

Table 4a. Proximate composition (g kg^{-1}, *as is*) of whole body tilapia fed six low fiber diets supplemented with proteases and carbohydrases.

As is basis	Diet 1 LF	Diet 2 LF-FP	Diet 3 LF-PP	Diet 4 LF-FC	Diet 5 LF-PC	Diet 6 LF-FPFC	PSE	P-value
Dry matter	25.23	26.40	24.93	26.98	25.53	24.78	0.87	0.547
Protein	14.30	14.80	14.88	14.98	15.43	14.60	0.54	0.786
Fat	6.52	7.36	6.88	6.77	7.05	6.38	0.42	0.627
Energy	1333	1331	1357	1371	1358	1330	44.94	0.977
Ash	3.61	3.77	3.02	4.98	3.25	3.18	0.63	0.323
Calcium %	1.02	1.08	1.05	1.24	1.34	1.17	0.21	0.880
Copper ppm	1.63	1.68	1.65	1.48	1.45	1.68	0.10	0.829
Iron ppm	15.95	14.83	22.15	13.85	15.58	15.78	2.32	0.209
Magnesium %	0.033	0.033	0.035	0.038	0.035	0.035	0.003	0.783
Phosphorus %	0.608	0.653	0.643	0.720	0.770	0.688	0.100	0.881
Potassium %	0.258	0.253	0.258	0.253	0.253	0.258	0.007	0.972
Sodium %	0.113	0.103	0.108	0.110	0.113	0.113	0.004	0.539
Sulfur %	0.158	0.160	0.163	0.155	0.158	0.158	0.004	0.786
Zinc ppm	19.95	19.25	20.83	19.50	20.30	20.18	1.40	0.972

Table 4b. Proximate composition (g kg-1, as is) of whole body tilapia fed four high fiber diets supplemented with proteases and carbohydrases.

As is basis	Diet 7 HF	Diet 8 HF-FP	Diet 9 HF-FC	Diet 10 HF-FPFC	PSE	P-value
Dry matter	24.85	26.75	27.30	26.35	0.98	0.368
Protein	14.68	14.90	15.23	15.00	0.48	0.876
Fat	6.15	5.90	7.61	7.51	0.24	0.059
Energy	1303	1177	1266	1317	44.62	0.171
Ash	3.50	5.08	4.09	3.87	0.47	0.165
Calcium %	1.00	0.85	1.11	0.97	0.14	0.611
Copper ppm	1.23	1.37	1.70	1.43	0.12	0.161
Iron ppm	13.65	18.35	18.80	16.05	2.34	0.416
Magnesium %	0.030	0.030	0.035	0.033	0.003	0.552
Phosphorus %	0.605	0.520	0.668	0.570	0.068	0.498
Potassium %	0.243	0.253	0.248	0.253	0.011	0.894
Sodium %	0.103	0.103	0.103	0.105	0.006	0.985
Sulfur %	0.153	0.158	0.165	0.158	0.003	0.116
Zinc ppm	19.53	18.43	20.70	18.25	1.15	0.434

Table 5a. Digestibility values of dry matter, energy and protein in a low fiber diet with inclusion of protease and carbohydrase

		Digestibility		
	Enzymes[+]	Dry matter	Energy	Protein
Diet 1	LF	52.39 ± 0.54^{c}	57.17 ± 3.71^{b}	77.11 ± 6.07^{b}
Diet 4	LF-FC	53.34 ± 0.32^{b}	63.14 ± 1.46^{a}	83.74 ± 1.00^{a}
Diet 6	LF-MFPFC	59.38 ± 0.19^{a}	65.49 ± 0.58^{a}	82.30 ± 2.91^{a}
PSE		0.22	1.34	0.84
P-value		0.0001	0.012	0.0002

Significance (P<0.05) determined by one way ANOVA followed by Student Newman Keuls grouping. PSE=Pooled Standard Error. Superscripts represent significant differences.
[+] Jefo Nutrition Inc, Saint-Hyacinthe, QC, Canada.

Table 5b. Digestibility values of dry matter, energy and protein in a high fiber diet with inclusion of protease and carbohydrase

		Digestibility		
	Enzymes*	Dry matter	Energy	Protein
Diet 7	Control HF	52.22 ± 0.91^{c}	58.92 ± 1.63^{b}	85.45 ± 0.20^{b}
Diet 9	HF-FC	56.81 ± 0.91^{a}	62.56 ± 0.77^{a}	87.02 ± 0.92^{a}
Diet 10	HF-MFPFC	54.47 ± 0.38^{b}	60.84 ± 1.43^{ab}	89.07 ± 4.14^{a}
PSE		0.45	0.77	0.84
P-value		0.001	0.042	0.0003

Significance (P<0.05) determined by one way ANOVA followed by Student Newman Keuls grouping. PSE= Pooled Standard Error. Superscripts represent significant differences.
* Jefo Nutrition Inc. (Saint-Hyacinthe, QC, CA)

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Chapter IV

NILE TILAPIA GROWTH PERFORMANCE USING XYLANASE AND BETA GLUCANASE

Abstract

The purpose of this study was to evaluate the production performance of Nile tilapia *Oreaochromis niloticus* when supplemented with a commercial beta xylanase and beta glucanase enzymes. Two practical basal tilapia diets were formulated to contain 32% protein and 6% lipids. One basal diet formulated to contain a low level of fiber (LF) based on soybean meal, was modified by top coating liquid enzyme to produce five levels of enzyme inclusion (0.00, 0.015, 0.030, 0.045, and 0.060 g/100g). A second basal diet was formulated to contain a high level of fiber (HF). To increase the fiber content, 30% distillers dried grains with solubles were used as a replacement for soybean meal. This basal diet was modified by top coating liquid enzyme to produce five levels of enzyme inclusion (0.00, 0.015, 0.030,0.045, and 0.060 g/100g). The test diets were offered to sex reversed juvenile tilapia (mean initial weight 10.31 ± 0.31 g) over a 70 - day growth trial. Four replicate groups of 20 fish per aquaria were offered the test diets at near satiation levels. At the conclusion of the growth trial, survival was near 100% and weight gain was around 600%. The inclusion of beta xylanase and beta glucanase resulted in significant differences in final mean weight ($P = 0.0029$), percent weight gain ($P = 0.0128$), thermal unit growth coefficients ($P = 0.0046$) with no change ($P > 0.05$) in feed conversion ratio ($P= 0.2153$), apparent net protein retention, apparent net energy retention, hepatosomatic index and intraperitoneal fat index. In general, fish maintained on the high fiber diet performed better to the addition of the enzyme. The use of beta xylanase and beta glucanase showed clear advantages, improving growth performance of Nile tilapia.

1. Introduction

In the search for cost effective protein sources for aquaculture feeds, the option of including plant-based protein sources as feed ingredients has received considerable attention (Salze et al., 2010; Herath et al., 2016; Aydin et al., 2017; da Silva et al., 2017; Martins et al., 2017; Al-Thobaiti et al., 2018; Khalifa et al., 2018). Plant-based proteins can be used to decrease the cost of feed formulations while serving as a fish meal alternative. Fish meal is high in protein content and has balanced essential amino acid profile (Tacon et al., 1993; Watanabe, 2002; El-Saidy and Gaber, 2004). One disadvantage of plant-based ingredients is possible antinutritional factors and indigestible carbohydrates. As described by Ebringerová (2005), plants have hemicelluloses which are carbohydrates that are the building component of the cell walls and are associated with proteins. The hemicelluloses are very diverse, including the xyloglycans (xylans) and beta glucans. Xylans are the most abundant hemicellulose type in plant the kingdom and beta glucans are part of the non starch polysaccharide (NSP) fraction in plant ingredients. Non starch polysaccharides (NSP) are difficult to digest because the enzymes are very scarce or even absent among fish species (Kuz'mina, 1996). Therefore, the ability of the fish to digest NSP depends on its gut microflora (Sinha et al., 2011). Processing treatments, such as extrusion, have little efficiency altering NSP content of plant feedstuffs to improve its nutritional value (Welker et al., 2014). The NSP fraction influences digesta viscosity, gut morphology, physiology and the mucus layer, affecting the endogenous secretion of water, proteins, electrolytes and lipids. These changes can lead to a reduced nutrient digestibility (Leenhouwers et al., 2006; Sinha et al., 2011). The addition of enzymes to the diet to break down the non starch polysaccharide fraction has been an effective strategy to improve the utilization of fiber content in plant ingredients by releasing dietary energy and amino acids, resulting in improved fish performance (Lin et al., 2007) and health of the

intestine (Castillo and Gatlin, 2015). Due to the fact that non structural polysaccharides comprise many compounds, different types of carbohydrases exist, however the market is dominated by xylanase and glucanase (Adeola and Cowieson, 2011).

The performance responses of carbohydrase addition is the current subject of study in different species of fish. Some studies have not showed effects to carbohydrase supplementation. Dalsgaard et al. (2012) found no differences in feed conversion ratio or fish performance in rainbow trout when supplementing beta-glucanase, xylanase and protease alone or in combination to diets with high inclusion levels of soybean meal, sunflower meal or rapeseed meal. Farhangi and Carter (2007) fed juvenile rainbow trout de-hulled lupin-based diets supplemented with protease and carbohydrases alone or in combination. No effects on performance were observed among the lupin containing diets, but the mixed enzyme de hulled lupin – based diet had significantly higher protein efficiency ratio. In rainbow trout, average weight gain and thermal growth coefficient was unaffected by diets containing soybean meal up to 20% incorporating an enzyme complex reported to have xylanase, amylase, cellulase, protease and beta glucanase activity to the level of 1 g kg-1 or 2.5 g kg-1 (Ogunkoya et al., 2006). Rainbow trout (Oncorhynchus mykiss) fry diets containing canola meal and supplemented with cellulase, phytase, pectinase or an enzyme mix showed no difference in weight gain and feed conversion ratio with enzyme supplementation (Yigit and Keser, 2016).

In contrast, several studies have shown positive effects on growth performance. Diets with the addition of 400mg of xylanase and 800 mg of glucanase and the combination had a significant increase ($P = 0.003$) in specific growth rate of Japanese seabass *Lateolabrax japonicus* (Ai et al., 2007). Atlantic salmon smolt (Salmo salar L) fed a combination of proteolytic enzymes and carbohydrases in a diet containing 340 g/kg soybean meal reported a positive effect on final mean

weight and feed conversion ratio (Carter et al., 1994). Also in Atlantic salmon, Jacobsen et al. (2018) indicated that diets containing soybean meal and a combination of xylanase and phytase had significantly higher final weight and thermal unit growth coefficient than diets without enzyme addition. Caspian Salmon fed trout diets containing two multienzyme complexes and a combination containing protease, lipase, phytase, alpha amylase, cellulase, amiloglucosidase, beta glucanase, pentosonase, hemicellulase, xylanase, pectinase, acid phosphatase, acid phytase and endo- beta mannanase, amylase, xylanase, cellulose and alpha galactosidase improved growth and feed utilization when enzymes were included in the diet in a multi enzyme complex at levels of 0.5 g kg-1 and 2.5 g kg -1 (Zamini et al., 2014). *Labeo rohita* diets were supplemented with xylanase and phytase in non-fermented and fermented rice bran based diets, the authors reported percent weight gain, protein and energy retention in the fish were significantly improved and feed conversion ratio was significantly lower (P=0.0001) in the diet containing exogenous enzymes (Ranjan et al., 2018). Lin et al. (2007) reported that supplementation of a commercial enzyme complex of neutral protease, beta glucanase and xylanase improved final mean weight as dietary enzyme increased from 0.0 to 1.0 g kg-1 ($P < 0.05$) in *Oreochromis niloticus* × *Oreochromis aureus*. Nile tilapia were fed a combination of probiotic and a mix of enzymes (containing xylanase, protease and phytase). Final mean weight, feed conversion rate and protein efficiency ratio were significantly improved ($P < 0.05$) by enzyme with probiotics treatment diet (Adeoye et al., 2016). *Oreaochomis mosambicus* fed a multi enzyme complex composed of cellulase, xylanase and phytase was added to a kikuyu based diet at a rate of 0, 0.25, 0.5, 0.75 and 1 g kg^{-1}. Fish fed the level of 0.5 g kg inclusion of enzyme to the diet showed better fish growth, lower FCR values, and had higher protein efficiency (Hlophe-Ginindza et al., 2016). Nile tilapia (*Oreochromis niloticus*) were fed an enzyme complex containing beta glucosidase, pectinase, xylanase,

endoglucanase, amylase, protease and phytase, fish fed with enzyme on top post extrusion at a level of 800 ppm showed significantly ($P < 0.05$) higher final mean weigh and weigh gain (Martins et al., 2018). The purpose of this study was to evaluate the efficacy of using a commercial carbohydrase enzyme composed of beta xylanase and beta glucanase on growth performance and nutrient retention in practical diets for juvenile tilapia (*Oreochromis niloticus*).

2. Material and Method

2.1 Experimental diets

Two practical basal tilapia diets were formulated to contain 32% protein and 6% lipids (Table 1). The test diets were formulated to meet the nutritional requirements of the Nile tilapia (NRC, 2011). One basal diet formulated to contain low level of fiber (LF) based on soybean meal was modified by top coating of liquid enzyme to produce five levels of enzyme inclusion (0.00, 0.015, 0.030, 0.045 and 0.060 g/100g). Additionally, a second basal diet was formulated to contain a high level of fiber (HF). In order to increase the fiber content 30% distillers dried grains with solubles were used as a replacement for soybean meal, this basal diet was modified by application of liquid enzyme to produce five levels of enzyme inclusion (0.00, 0.015, 0,030, 0.045, and 0.060 $g100g^{-1}$). The test diets were prepared at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground dry ingredients and oil were weighed and then mixed using a food mixer (Hobart Corporation, Troy, Ohio, USA) for 15 minutes. Diets were then extruded through a 3-mm diameter die in an extruder (Ex 30, Exteec, Brazil). After cooling, the liquid enzyme was applied post pellet to obtained ten diets, a basal low fiber and high fiber diet with inclusion of enzyme to obtain 5 levels of enzyme, respectively. Diets were air dried at < 50°C to a moisture content of 8-10 %, and stored at room temperature. A sample of 150 g of each feed was collected and analyzed for

proximate composition (AOAC 930.15, AOAC 990.03, AOAC 2003.05, Ankom. Tech, AOAC 942.05 were used for moisture, protein, fat, fiber and ash analysis respectively) by Midwest Laboratories, Omaha, NE, USA (Table 2).

The enzyme used in this study was Natugrain TS L (BASF Corporation, Ludwigshafen, Germany) an enzyme complex of endo 1- 4 β- xylanase and endo 1- 4 β- glucanase produced by *Aspergillus niger*. One thermostable endo-xylanase unit (5600 TXU/g) is defined as the amount of enzyme which liberates 5 micromole reducing sugars, measured as xylanase equivalents per minute from a buffer solution containing 1 g arabinoxylan per 100 ml at pH 3.5 and 40 °C. One thermoestable endo-glucanase unit (2500 TGU/g) is defined as the amount of enzyme which liberates 1 micromole reducing sugars, measured as glucose equivalents per minute from a buffer solution containing 0.714 g beta-glucan per 100 ml at pH 3.5 at 40 °C.

2.2. Culture methods

Juvenile sex reverse Nile tilapia (mean initial weight 10.34 ± 0.31g) were randomly stocked into 75-L aquaria which are a component of a 2,500-L indoor recirculation system at 20 fish per aquarium at the E.W. Shell Fisheries Center, Auburn, Alabama. Each diet was randomly assigned to the tanks and offered to fish in four replicate aquaria for the duration of a 70-day growth trial. Samples of fish from the initial stocking were retained for later protein analysis.

Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using air stones in each aquarium and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen and water temperature were measured twice a day using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, Ohio) while pH, TAN and Nitrite-N were measured once per week. Photoperiod was set at 14 h light and 10 h dark.

Diets were offered to fish at 3.0-6.0% BW daily, according to fish size and divided into two equal feedings each day. Test diets were applied two times per day (0800 and 1600 h), for a 70-day experimental period. Fish were weighed every week for the first two weeks and every other week thereafter. Daily feed rations were calculated based on % body weight. The ration was adjusted each week based on growth and observation of the feeding response. At the end of the growth trial, fish were counted and group weighed to determine weight gain, survival and feed conversion ratio. At the conclusion of the trial, four fish were randomly collected from every aquarium and frozen at 20 °C for later biochemical analysis and hepatosomatic and intraperitoneal index. These whole-body fish samples were homogenized in a food processor and sent to Midwest Laboratories (Omaha, NE, USA) for proximate and mineral analyses as per AOAC procedures (AOAC 930.15, AOAC 990.03, AOAC 954.02, AOAC 942.05 were used for moisture, protein, fat, fiber and ash analysis respectively; AOAC 985.01 was used for mineral analysis).

Growth performance indexes including weight gain, feed conversion ratio (FCR), survival, apparent net protein retention, apparent net energy retention, thermal unit growth coefficient, hepatosomatic index and intraperitoneal fat index were computed using the following calculations:

o) Weight gain (g) = Average final weight (g) – Average initial weight (g)

p) Feed conversion ratio (FCR) = dry feed intake/ wet weight gain.

q) Survival (%) = (Initial fish number – Final fish number)/Initial fish number × 100

r) Apparent net protein retention (ANPR, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.

s) Apparent net energy retention (ANER, %) = (final weight × final energy content) - (initial weight × initial energy content) × 100 / energy intake.

t) Thermal-unit growth coefficient (TGC) = (final weigh$^{1/3}$ - initial weight$^{1/3}$) / (temperature × day) × 100.

u) Hepatosomatic Index (HI) = liver weight/fish weight × 100

v) Intraperitoneal fat Index (IFI)= intraperitoneal fat weight/fish weight × 100

2.3. Statistical Analysis

Statistical analyses were conducted using SAS system for windows, (V9.4. SAS Institute, Cary, NC). All data was subjected to a two way analysis of variance to determine interactions between fiber level and enzyme level. Initial weight, final mean weight, thermal unit growth coefficient, percent weight gain, feed conversion rate, dry matter, apparent nutrient retention of protein and energy, hepatosomatic index and intraperitoneal fat index were analyzed using a one-way analysis of variance to determine significant ($P < 0.05$) differences among the treatment means followed by Student-Neuman Keuls multiple range test to distinguish significant differences between treatment means.

3.Results

3.1 Water quality

During the experimental period dissolve oxygen, temperature, salinity, pH, total ammonia-nitrogen (TAN), and nitrite-nitrogen were maintained within acceptable ranges for Nile tilapia at 6.0 ± 0.76 mg L^{-1}, 28.34 ± 0.24 °C, 2.97 ± 0.93 ppt, 7.0 ± 0.59, 0.06 ± 0.03 mg L^{-1}, 0.04 ± 0.02 mg L^{-1} over the 70-d trial period.

3.2Growth performance

Parameters of growth performance from two-way analysis of variance are summarized in Table 3. No significant interactions were observed among the fiber content and the level of enzyme for final mean weight, percent weight gain, thermal unit growth coefficient (TGC), survival, feed conversion ratio (FCR), apparent net protein retention, apparent net energy retention,

hepatosomatic index and intraperitoneal fat index. As there were no significant effects of diet type pooled means according to the enzyme level were determined (Table 3). Initial mean weight (10.34 ± 0.31 g) was not significantly different (P= 0.2553) among fish assigned to the various dietary treatments. Final mean weight (65.1 – 79.1 g) was significantly different (P = 0.0029) by the addition of different enzymes levels. Percent weight gain was significantly influenced (P = 0.0128) by dietary treatments (541.8 – 652.8 %). Thermal unit growth coefficient (TGC) was significantly different (P = 0.046) among enzyme levels (0.095 – 0.107). Survival was unchanged (P = 0.6965) across all dietary treatments (98.33 – 100%). Feed conversion ratio was not affected (P = 0.2153) by the level of enzyme (1.30 – 1.40).

As there are biological differences between diet, the data were also analyzed by diet type and one-way ANOVA which is summarized in table 4a for low fiber diets and 4b for high fiber diets. In low fiber diets, final mean weight, percentage weight gain, thermal unit growth coefficient, survival, feed conversion ratio, apparent net protein retention, apparent net energy retention, hepatosomatic index and intraperitoneal fat were not affected by the addition of the enzyme (table 4a). In contrast, when high fiber diets were supplemented with beta xylanase and beta glucanase final mean weight was significantly increased (P=0.0006; 61.83-79.56), percentage weight gain was significantly improved (P =0.0005; 514.62-650.63%), thermal unit growth coefficient was improved (P = 0.0002; 0.0919-0.1076). Survival was unchanged (P= 0.4380; 96.66 - 100.00%) across dietary treatments, feed conversion ratio was significantly different (P = 0.0092) among dietary treatments (1.19 – 1.33), apparent net protein retention, apparent net energy retention, hepatosomatic index and intraperitoneal fat index were not affected by the inclusion of the enzyme in high fiber diets (P > 0.05) (table 4b).

3.2 Nutrient retention and body composition

Apparent net protein retention (P = 0.6743; 35.35 - 37.63%) apparent net energy retention (P = 0.2382; 23.17 – 26.047%) hepatosomatic index (P=0.1784; 2.24 – 2.63%) and intraperitoneal fat (P = 0.6116; 0.87-1.07%) were unchanged by the inclusion of endo beta xylanase and beta glucanase (table 3).

Whole body fish composition is summarized is summarized in table 5, no differences were observed in crude protein (P=0.2826; 14.05 - 15.05%), dry matter (P= 0.4428; 24.62– 26.31%), fat (P= 0.2311; 6.00 – 6.95%) and ash (P=0.7322; 3.74 – 4.14%) content of whole body fish samples among fish fed various levels of beta xylanase and beta glucanase.

4. Discussion

The use of xylanase and beta glucanase can improve performance and alleviate the negative effects of non starch polysaccharides in Nile Tilapia plant-based diets. Nile tilapia can tolerate higher dietary fiber and carbohydrate concentrations than most other cultured fish (Elsayed and Teshima, 1992). Our study indicated that final mean weight, percent weight gain and thermal unit growth coefficient were improved by enzyme addition to the diets. Compared to control diets the level of 0.03% and 0.06% inclusion was significantly higher. These results are in agreements with the findings presented by Adeoye et al. (2016) with Nile tilapia fed probiotic and enzyme addition improving final mean weight. Also, in Japanese seabass *Lateolabrax japonicus* (Ai et al., 2007). Atlantic salmon (Carter et al., 1994; Jacobsen et al., 2018), Caspian Salmon (Zamini et al., 2014), *Labeo rohita* (Ranjan et al., 2018) growth performance was improved by the addition of carbohydrases. This positive response of growth might be explained because enzyme addition can increase nutrient availability and digestibility by the stimulation of the release of bile acids improving emulsification of non-starch polysaccharides (De Keyser et al., 2016), and also

exogenosus enzyme supplementation can promote the secretion of endogenous enzymes by the fish (Magalhaes et al., 2016).

Hepatosomatic index and intraperitoneal fat index were not different among xylanase and beta glucanase levels, similar results were found by Adeoye et al. (2016) in *Oreochromis niloticus* fed probiotics and a complex of enzymes. Ranjan et al. (2018) reported no differences in hepatosomatic index in *Labeo rohita* supplemented exogenous enzymes to fermented and non fermented de oiled rice bran. In contrast, some studies have shown no effect to carbohydrase supplementation on growth performance. Dalsgaard et al. (2012) found in rainbow trout no differences in feed conversion ratio or fish performance when supplementing beta-glucanase, xylanase and protease to diets with high inclusion levels of soybean meal, sunflower meal or rapeseed meal. Farhangi and Carter (2007) fed juvenile rainbow trout a de-hulled lupin-based diet, with protease and carbohydrases alone or in combination and no effects on performance were observed. Rainbow trout fry diets containing canola meal and supplemented with cellulase, phytase, pectinase or an enzyme mix showed no difference in weight gain and feed conversion ratio with enzyme supplementation (Yigit and Keser, 2016). The response to exogenous enzymes supplemented to the diet differ according to fish species, ingredient matrix and also to the different enzyme activity or enzyme complex that is used.

5. Conclusion

Results demonstrate that the inclusion of endo 1-4 beta xylanase and 1-4 beta glucanase improved growth performance of practical tilapia diets and did not alter feed conversion ratio, nutrient retention or body tissue composition, the use of enzymes is an opportunity to increase the use of plant based ingredients in formulation for Nile tilapia

Table 1. Ingredient composition (g 100g $^{-1}$as is) of basal diet formulated to contain 32% protein and 6% lipid.

	Low fiber					High fiber				
Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
MFM [1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
PBM [2]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
SBM [3]	49.10	49.10	49.10	49.100	49.10	34.00	34.00	34.00	34.00	34.00
DDGS [4]						30.00	30.00	30.00	30.00	30.00
Fish oil [5]	3.23	3.23	3.23	3.23	3.23	2.10	2.10	2.10	2.10	2.10
Lecithin [6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Corn Starch [7]	0.03	0.03	0.03	0.03	0.03	2.00	2.00	2.00	2.00	2.00
Corn[8]	32.50	32.50	32.50	32.50	32.50	16.50	16.50	16.50	16.50	16.50
Mineral and Vitamin premix[9]	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Choline chloride[7]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 35% active[11]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[7]	3.04	3.04	3.04	3.04	3.04	3.04	3.04	3.04	3.04	3.04
Lysine HCl[12]						0.26	0.26	0.26	0.26	0.26
β xylanase - β glucanase [13]	**0.00**	**0.01**	**0.02**	**0.03**	**0.04**	**0.00**	**0.01**	**0.02**	**0.03**	**0.04**

[1] Menhaden Fishmeal, Omega Protein Inc., Houston, TX, USA.
[2] Poultry Byproduct Meal, Midsouth milling Co., Memphis TN, USA.
[3] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[4] Distillers dried grains with solubles (DDGS) Flint Hills Resources, LLC, Wichita, KS, USA.
[5] Menhaden Fish Oil, Omega Protein Inc., Reedville, VA, USA.
[6] Enhanced D-97, The Solae Company, St. louis, MO, USA.
[7] MP Biomedicals Inc., Solon, Ohio, USA
[8] Faithway Feed Co., LLC., Guntersville, AL, USA
[9] Trace mineral (g/100g Premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.25; Ferrous sulfate, 4.0; Magnesium sulfate anhydrous, 13.86; Manganous sulfate monohydrate, 0.65; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate hepahydrate, 13.19; cellulose, 67.96. Vitamin (g/kg Premix): Thiamin HCl, 0.44; Riboflavin, 0.63; Pyridoxine HCl, 0.91; D-pantothenic acid,

1.72; Nicotinic acid, 4.58; Biotin, 0.21; Folic acid, 0.55; Inositol, 21.05; Menadione sodium bisulfite, 0.89; Vitamin A acetate (500,000 IU g-1), 0.68; Vitamin D3 (400,000 IU g-1), 0.12; DL-alpha-tocoperol acetate (250 IU g-1), 12.63; cellulose 955.59.

[11] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[12] Ajinomoto Heartland Inc., Chicago, IL, USA.

[13] Natugrain TS L, BASF Corporation, Ludwigshafen, Germany.

Table 2. Proximate composition of test diets (as is basis).

	Low fiber					High fiber				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
(%) β xylanase - β glucanase [2]	0.00	0.01	0.02	0.03	0.04	0.00	0.01	0.02	.03	0.04
Crude protein*	32.40	32.20	32.30	32.90	33.10	33.60	33.70	33.40	33.20	33.90
Moisture	7.33	8.85	8.94	9.01	8.98	6.69	8.76	9.09	9.04	8.85
Crude Fat	6.68	6.84	6.70	6.55	6.54	6.89	6.91	6.82	6.72	6.74
Crude Fiber	2.04	1.88	1.96	2.23	1.85	2.39	2.66	2.82	2.78	2.98
Ash	7.84	7.67	7.41	7.24	7.70	8.46	8.14	7.74	7.94	7.73
β xylanase (TXU) –		720	1590	1860	3020		620	1380	1920	3100
β glucanase (TGU) activity unit/kg		350	780	920	1270		300	630	810	1180

*Crude Protein = %N x 6.35

[2] Natugrain TS L (BASF Corporation, Ludwigshafen, Germany)

Table 3. Growth response of juvenile tilapia (10.31 ± 0.31 g) fed for 70 d a low fiber diet with inclusion of four level of β xylanase - β glucanase.

	Enzyme level (%)[+]	Final mean Weight (g)	Weight Gain (%)	Thermal-unit growth coefficient	Survival (%)	Feed conversion ratio	ANPR %	ANER %	HI	IFI
Two way anova										
Model P value		0.0186	0.0454	0.0208	0.6171	0.2532	0.6777	0.5193	0.2140	0.0826
Fiber		0.4137	0.2576	0.3323	0.7412	0.8640	0.1777	0.8691	0.1057	0.7038
Enzyme level		0.0229	0.0128	0.0046	0.6965	0.2153	0.6743	0.2382	0.1784	0.6116
Fiber * Enzyme		0.4933	0.4515	0.3878	0.322	0.2295	0.6791	0.6605	0.5302	0.0143
Pooled by diet type										
	0.000	65.1[b]	541.8[b]	0.095[b]	100.00[a]	1.35[a]	36.65[a]	23.17[a]	2.40[a]	0.94[a]
	0.015	71.9[ab]	594.6[ab]	0.101[ab]	98.33[a]	1.40[a]	37.63[a]	23.77[a]	2.24[a]	0.98[a]
	0.030	79.1[a]	652.8[a]	0.107[a]	100.00[a]	1.30[a]	37.58[a]	26.04[a]	2.51[a]	1.07[a]
	0.045	71.9[ab]	598.2[ab]	0.101[ab]	98.33[a]	1.38[a]	35.35[a]	24.08[a]	2.57[a]	0.87[a]
	0.060	74.3[a]	612.8[ab]	0.103[a]	99.17[a]	1.34[a]	36.32[a]	24.26[a]	2.63[a]	1.08[a]
	PSE	14.3	28.91	0.0029	3.53	0.002	4.35	2.22	0.12	0.05

PSE= Pooled Standard Error, n=8. ANPR= Apparent net protein Retention. ANER= Apparent Net Energy Retention. HI=Hepatosomatic index. IPI= Intraperitoneal Fat Index.Significance ($P<0.05$) based on two way analysis of variance followed by Student Newman Keuls grouping. [+] Natugrain TS L, BASF Corporation, Ludwigshafen, Germany

Table 4a. Growth response of juvenile tilapia (10.31 ± 0.31 g) fed for 70 d a low fiber diet with inclusion of four level of β xylanase - β glucanase.

Diet	(%) β xylanase - β glucanase [+]	Final mean Weight (g)	Weight Gain (%)	Thermal-unit growth coefficient	Survival (%)	Feed conversion ratio	ANPR %	ANER %	HI	IFI
1	0.000	68.2	569.07	0.0981	100.00	1.27	37.35	23.52	2.45	1.21
2	0.015	74.9	628.79	0.1044	100.00	1.26	40.01	24.45	2.10	0.99
3	0.030	78.6	655.07	0.1072	100.00	1.21	38.13	25.12	2.26	1.02
4	0.045	70.6	583.63	0.0996	96.66	1.33	35.63	23.36	2.52	0.61
5	0.060	74.1	616.60	0.1033	98.33	1.28	36.26	24.50	2.58	1.21
	PSE	3.94	37.20	0.0037	1.66	0.05	2.21	1.38	0.30	0.31
	P value	0.4142	0.5065	0.4458	0.5437*	0.6252	0.6658	0.8795	0.2887	0.1184

PSE= Pooled Standard Error, n=4.* Analyzed by binary regression. ANPR= Apparent net protein Retention. ANER= Apparent Net Energy Retention. HI=Hepatosomatic index. IPI= Intraperitoneal Fat Index. Significance (P<0.05) based on analysis of variance followed by Student Newman Keuls grouping. [+] Natugrain TS L BASF Corporation, Ludwigshafen, Germany.

Table 4b. Growth response of juvenile tilapia (10.38 ± 0.35 g) fed for 70 d a high fiber diet with inclusion of four levels of β xylanase - β glucanase.

Diet	Enzyme level (%)[+]	Final mean Weight (g)	Weight Gain (%)	Thermal-unit growth coefficient	Survival (%)	Feed conversion ratio	ANPR %	ANER %	HI	IFI
6	0.000	61.827[c]	514.62[c]	0.0919[c]	100.00	1.33[ab]	35.95	22.81	2.37	0.67
7	0.015	69.009[b]	560.58[bc]	0.0980[b]	96.67	1.35[a]	35.25	23.10	2.37	0.97
8	0.030	79.565[a]	650.63[a]	0.1076[a]	100.00	1.19[b]	37.05	26.91	2.75	1.12
9	0.045	73.315[ab]	612.82[ab]	0.1028[ab]	100.00	1.24[ab]	35.07	24.80	2.62	1.13
10	0.060	74.415[ab]	609.00[ab]	0.1032[ab]	100.00	1.23[ab]	36.39	24.02	2.68	0.96
	PSE	2.1836	16.965	0.0018	1.4907	0.0318	1.13	1.11	0.29	0.22
	P value	0.0006	0.0005	0.0002	0.4380*	0.0092	0.7297	0.1202	0.3586	0.1008

PSE= Pooled Standard Error, n=4.* Analyzed by binary regression. ANPR= Apparent net protein Retention. ANER= Apparent Net Energy Retention. HI=Hepatosomatic index. IFI= Intraperitoneal Fat Index.Significance ($P<0.05$) based on analysis of variance followed by Student Newman Keuls grouping. [+] Natugrain TS L. BASF Corporation, Ludwigshafen, Germany.

Table 5. Proximate composition (g kg^{-1}, *as is*) of whole body tilapia of juvenile tilapia fed for 70 d with inclusion of four level of β xylanase - β glucanase.

	Enzyme level (%)[+]	Dry matter	Protein (%)	Fat (%)	Ash (%)
Model P value		0.4132	0.6368	0.5632	0.4545
Fiber		0.7500	0.5348	0.5983	0.8403
Enzyme level		0.4428	0.2826	0.2311	0.7322
Fiber*Enzyme		0.2523	0.8590	0.8118	0.1645
Pooled enzyme level					
	0.000	25.71	14.31	6.18	3.72
	0.015	26.31	15.05	6.95	4.14
	0.030	25.62	14.12	6.78	3.88
	0.045	24.62	14.05	6.00	3.91
	0.060	25.58	14.41	6.97	3.74
	PSE	1.091	0.3337	0.3912	0.1602

PSE= Pooled Standard Error, n=8. Significance ($P<0.05$) based on two way analysis of variance followed by Student Newman Keuls grouping. [+] Natugrain TS L, BASF Corporation, Ludwigshafen, Germany.

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Chapter V

SUMMARY AND CONCLUSIONS

The use of plant based ingredients in tilapia diets is a common practice in the aquaculture industry, this due to decrease availability of fish meal worldwide. However, the replacement of fish meal by plant-based ingredients can impair productivity, due to anti nutritional factors present. Among this antinutrients are the non starch polysaccharides, which are part of the structural component of plant based feedstuffs. In the digestive system of fish, the enzymes to digest non starch polysaccharides are scarce or even absent. This can reduce the ability of fish to obtain the nutrients from plant-based diets. In order to increase the availability of nutrients by fish, the addition of exogenous enzymes that can break down non starch polysaccharides as a solution to increase the digestibility of practical diets and improved performance in Nile tilapia *Oreochromis niloticus*.

The first study described in this dissertation was conducted to evaluate the production performance and digestibility of Nile tilapia *Oreochromis niloticus*, when supplemented with a commercial beta mannanase enzyme. A basal diet designed to contain 32% protein and 6% lipid using primarily plant-based protein sources allowing high levels of "low" digestibility ingredients. The basal diet was modified to produce four levels (0.00, 0.05, 0.10, 0.20%) of enzyme supplementation. At the conclusion of the growth trial, neither performance parameters, apparent energy nor net protein retention improved by beta mannanase addition. The inclusion of beta mannanase to the diet resulted in a linear increase in dry matter digestibility (P= 0.0004; R2 =0.75), energy digestibility (P=0.0003; R2 =0.74) and protein digestibility (P=0.0247; R2 =0.41). As compared to the basal diet, the enzyme supplementation of 0.1 and 0.2% of the diet significantly

improved digestion above that of the basal diet. Results demonstrate that the inclusion of beta mannanase improved digestibility of practical tilapia diets but did not alter production performance, nutrient retention or body tissue composition.

The second study was designed to evaluate the production performance and digestibility of Nile tilapia *Oreochromis niloticus* when supplemented with commercial protease and carbohydrases. Ten practical tilapia diets were formulated to contain 32% protein and 6% lipids. Six diets were formulated to contain a low level of fiber (LF) and included free protease (LF-FP), protected protease (LF-PP), free carbohydrase (LF-FC), protected carbohydrase (LF-PC), and a mix of free protease and carbohydrases (LF-MFPFC). Four diets were formulated to contained high levels of fiber (HF) and included a basal diet (HF) and a basal diet supplemented with free protease (HF-FP), free carbohydrase (HF-FC), and a mix of free protease and free carbohydrases (HF-MFPFC). Dried distillers grains was used as a source of fiber in high fiber diets. The level in the diet of free protease (FP) and protected (PP) was 175 g per metric ton, the level of free carbohydrase (FC), protected carbohydrase (PC) and the mix of free protease and carbohydrase (MFPFC) was 125 g per metric ton. Under the conditions of this study, fish maintained on the high fiber diet performed slightly poorer than those on the lower fiber diet. Concerning enzyme supplements, apparent net energy retention was significantly different ($P= 0.0001$) in low fiber diets when free and protected proteases were added. However, for low and high fiber diets there were no significant differences ($P > 0.05$) in animal performance and apparent net protein retention. Overall, there were no clear advantages detected to the protected enzymes. When enzymes were supplemented in low and high fiber diets, dry matter and energy digestibility were significantly improved by the addition of free carbohydrase and a mix of free protease and free

carbohydrase. Based on these results, exogenous protease and carbohydrase enzymes have shown to be a promising way to improve digestibility in commercial tilapia diets.

The third study investigated the production performance of Nile tilapia *Oreaochromis niloticus* when supplemented with commercial beta xylanase and beta glucanase enzymes. Two practical basal tilapia diets were formulated to contain low levels of fiber (LF) based on soybean meal was modified by top coating of liquid enzyme to produce five levels of enzyme inclusion (0.00, 0.015, 0.030,0.045, and 0.060 g/100g). A second basal diet was formulated to contain a high level of fiber (HF). To increase the fiber content 30%, dried distillers grains were used as a replacement for soybean meal, this basal diet was modified by top coating liquid enzymes to produce five levels of enzyme inclusion (0.00, 0.015, 0.030, 0.045, and 0.060 $g100g^{-1}$). The inclusion of beta xylanase and beta glucanase resulted in significantly improved growth parameters, final mean weight ($P = 0.0029$), percent weight gain ($P = 0.0128$), thermal unit growth coefficients ($P = 0.0046$) with no change ($P > 0.05$) in feed conversion ratio ($P= 0.2153$), apparent net protein retention, apparent net energy retention, hepatosomatic index and intraperitoneal fat index. In general, fish maintained on the high fiber diet performed better with the addition of the enzyme. The use of beta xylanase and beta glucanase showed clear advantages improving growth performance on Nile tilapia.

Based on the results of these studies, exogenous enzymes such as beta mannase, protease, xylanase and betaglucanase can be used to increase digestibility of practical tilapia diets and improved performance of Nile tilapia when plant-based ingredients are the major components of the diets. However, the increase in performance is not always consistent and depends on the type of the enzyme and substrate make up of the diet.

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