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Effect of exogenous enzymes (phytase and xylanase) supplementation on nutrient digestibility and growth performance of Nile tilapia (*Oreochromis niloticus*) fed different quality diets

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ABSTRACT

The use of plant by-products in aqua-feeds contributes to improving the sustainability of aquaculture, but also leads to increased levels of undesirable non-starch polysaccharides (NSP) and phytate. NSP-degrading enzymes (i.e. xylanase) and phytase can be used as a tool to deal with NSP and phytate. A feeding trial was conducted to test whether the effects of phytase and xylanase supplementation on growth performance and nutrient digestibility in Nile tilapia are dependent on diet quality. Two diets were formulated, a control quality (CQ diet; 36% protein and 17% NSP) and a low quality diet (LQ diet; 32% protein and 30% NSP). The difference in diet quality was created by using higher levels of wheat bran, rapeseed meal, sunflower meal, rice bran and wheat dried distiller's grain with solubles in the LQ diet. Both diets had the following enzyme treatments: 1) no enzymes, 2) phytase, and 3) phytase and xylanase. Phytase (*Buttiauxella sp.*) was supplemented at ca. 660 FTU/kg and xylanase at ca. 6596 U/kg. In total, 18 tanks (6 treatments, 3 replicates per treatment) were used with 30 fish each (mean initial body weight 39 g). Fish were restrictively fed at 80% of expected satiation for 42 days, by hand twice daily. Growth was determined by batch weighing of the fish at the start and at the end of the trial. Faeces were collected non-invasively using settling units in order to determine the nutrient digestibility, using yttrium as an inert marker. Fish fed the LQ diet had lower growth (1.35 g/d vs. 1.52 g/d) and nutrient digestibility (except for calcium and ash), compared to the CQ diet ($P < .05$). Phytase improved the digestibility of dry matter, total carbohydrates, NSP, energy, ash, phosphorus and calcium ($P < .01$) Phytase improved growth (g/d) by ca. 7% and phosphorus availability by ca. 29%. The improvement in growth with phytase was comparable between the two diets, improving the FCR from 1.04 to 0.97 and from 1.17 to 1.10 for the CQ and LQ diet, respectively. Xylanase supplementation, on top of phytase, did not enhance growth ($P > .05$). Xylanase improved digestibility of dry matter, energy, total carbohydrates and NSP (from 29.7% to 36.6%) of the LQ diet, but not of the CQ diet (interaction; $P < .05$). In conclusion, the effect of phytase on improved nutrient digestibility and performance was independent of diet quality, whereas the effect of xylanase was dependent on diet quality.

1. Introduction

Aquaculture production is increasing in volume and importance. In 2016, aquaculture accounted for 50% of worldwide fish (including molluscs, crustaceans, seaweeds etc.) production and about 7% of the protein consumed by humans (FAO, 2018). With the expansion of aquaculture, global production of aqua-feeds is also increasing and aqua-feed formulations now include a range of ingredients that were not previously used for feeding fish and other aquatic animals. For example, increasing cost and limited global production of fish-meal and marine fish oils has led to increased use of terrestrial plant ingredients in aqua-feeds (Gatlin III et al., 2007; Hardy, 2010; NRC, 2011; Tacon and Metian, 2015). Coinciding with this change, the levels of phytate and non-starch polysaccharides (NSP) in aqua-feeds have increased, especially when plant by-products are used as feed ingredients. Such products include wheat bran, rice bran, citrus pulp and wheat DDGS (Dried Distiller's Grains with Solubles) (Choct, 1997; Knudsen, 1997; Sinha et al., 2011). These products are of interest as ingredients in aqua-feeds from a sustainability perspective, and also because they are of little value for human consumption (Troell et al., 2014).

Phytate bound phosphorus (P) is unavailable for fish, as fish lack endogenous phytase to hydrolyse inositol-phosphate linkages. Phytate

Abbreviations: NSP, non-starch polysaccharides; ADC, apparent digestibility coefficient; DM, dry matter; CQ, control quality diet; LQ, low quality diet

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has a strong binding affinity to cations, such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}, and K^{+}, to form insoluble salts. This reduces the bioavailability of minerals. Furthermore, phytate can form insoluble complexes with proteins, thereby reducing protein digestibility (Cao et al., 2007; Kumar et al., 2012; Riche and Garling Jr, 2004). NSP is difficult to digest and can induce a wide range of anti-nutritive effects, related to alterations in digesta viscosity, gut physiology, gut morphology, and gut microbiota composition (Sinha et al., 2011). The lowered nutrient availability induced by dietary phytate and NSP can reduce fish growth (Kumar et al., 2012; Maas et al., 2020), thus limiting the potential for inclusion of plant ingredients rich in phytate and NSP in aquafeeds. Furthermore, reduced nutrient utilisation increases waste production, increasing the system waste load in recirculating aquaculture systems (RAS) and eutrophication when waste is discharged to surrounding water bodies (Amirkolaie, 2011; Mainstone and Parr, 2002).

Supplementation of diets with NSP-degrading enzymes (i.e., xylanase, β-glucanase and cellulase) and phytase can (partly) neutralise the negative effects of phytate and NSP on digestion and thereby increase fish growth (Castillo and Gatlin III, 2015; Kumar et al., 2012; Maas et al., 2018). Numerous studies have been performed with phytase supplementation in different fish species, such as rainbow trout (*Oncorhynchus mykiss*) (Cheng et al., 2004; Sugiura et al., 2001), sea bass (*Dicentrarchus labrax*) (Oliva-Teles et al., 1998), Nile tilapia (*Oreochromis niloticus*) (Liebert and Portz, 2005; Portz and Liebert, 2004) and Asian catfish (*Pangasius pangasius*) (Debnath et al., 2005). Across studies and species, phytase supplementation increases dietary P availability. In addition, several studies show that phytase improves the availability of other minerals and thereby promotes growth (Kumar et al., 2012). Maas et al. (2018) showed that phytase supplementation also improved total carbohydrate digestibility, which was related to an increase in NSP digestibility. The results from studies on NSP-degrading enzymes in fish are less conclusive than those on phytase. Some studies found no or limited effects (Ogunkoya et al., 2006; Yigit and Olmez, 2011), whereas others found improved nutrient digestibility and growth (Ghomi et al., 2012; Lin et al., 2007). Due to the variety in type and dose of enzymes used, individually or in combination, and the large differences in diet composition (e.g. type of NSP), it is difficult to make comparisons across studies with regard to the effect of NSP-degrading enzymes (Adeola and Cowieson, 2011; Castillo and Gatlin III, 2015). In most studies on NSP-degrading enzymes in fish, NSP digestibility has not been measured, although it could help explain the large variations in apparent digestibility coefficients (ADCs) reported for other nutrients. Part of the variability might relate to differences in nutrient and/or ingredient composition of diets, because NSP composition of the diet affects the response to enzyme supplementation in terms of growth and nutrient digestibility (Maas et al., 2019). In broilers, the magnitude of the effect of exogenous enzymes on ileal amino acid digestibility is influenced by the nutritional characteristics of the control diet (Cowieson and Bedford, 2009). It is hypothesized that the effect of enzyme supplementation can be influenced by the quantity of the target substrate, such as phytate or NSP. We expect that when the control diet is of a low quality (i.e. low digestibility) or when anti-nutritional effects are expressed, there is a greater potential for improvement (digestibility and/or improved growth) with enzyme supplementation. Furthermore, the level of the target substrate might influence the optimal dose of the enzyme. Flexibility in dosing of enzyme products is preferred over fixed doses so that the economic viability of the product is optimized (Adeola and Cowieson, 2011; Cowieson and Ravindran, 2008).

Hitherto, there have been no studies carried out to evaluate whether the effect of enzyme supplementation is dependent on the quality of the diet, i.e. the level of dietary NSP and phytate. Therefore, the present study investigated whether the effects of phytase and xylanase supplementation differ when supplemented to diets of different quality. In a feeding trial using a control quality and low quality diet, the effect of phytase, xylanase, and their interactions on nutrient digestibility and growth of Nile tilapia were evaluated.

Table 1
Ingredient composition of the experimental diets.

Control Quality (CQ) diet		Low Quality (LQ) diet
Ingredients (g/kg)		
Maize	70	70
Maize starch	150	–
Soyabean meal	100	100
Wheat	59.3	58.8
Wheat gluten meal	120	80
Wheat bran	60	125
Rapeseed meal	50	120
Sunflower meal	50	120
Rice bran full fat	60	125
Wheat DGGS[†]	50	120
Fish oil	10	10
Rapeseed oil	20	15
Palm oil	20	15
Soy protein concentrate	90	–
Pea protein concentrate	50	–
Vitamin & mineral premix[‡]	10	10
Calcium carbonate ($CaCO_3$)	5	5
Monocalcium phosphate (MCP)	14.5	13
DL Methionine	5.5	4.5
L-Lysine HCl	3.5	6.5
L-Threonine	2.0	2.0
Yttrium oxide	0.2	0.2

[†] DDGS; dried distillers grains with solubles.
[‡] Premix composition. Vitamins (expressed as IU or mg/kg of final diet): vitamin B1, 10; vitamin B2, 10; vitamin B6, 10; vitamin B5, 10; vitamin B3, 20; boitine, 0.2; B-12, 0.015; folic acid, 2; vitamin C, 100; vitamin E, 100 IU; A-vitamin A palmitate, 3000 IU; D-Rovimix D3–500, 2400 IU; K_3 K-menadione sodium bisulphite (51%), 10; inositol, 400; choline, 1500; Antioxidant BHT (E300–321), 100; calcium propionate, 1000. Minerals (expressed as mg/kg of final diet): ferric sulphate, 50; zinc sulphate, 30; cobalt sulphate, 0.1; copper sulphate, 10; Sodium selenite, 0.5; manganese sulphate, 20; magnesium sulphate, 500; chromic chloride, 1; calcium iodate, 2.

2. Materials and methods

2.1. Diets

The impact of enzyme supplementation on nutrient digestibility and growth in tilapia were measured using control quality (CQ) and low quality (LQ) diets. These diets were supplemented with either phytase (CQ + Ph, LQ + Ph), phytase and xylanase (CQ + PhX, LQ + PhX) or not supplemented (CQ + 0, LQ + 0). The CQ diet variants were formulated to have moderate levels of wheat DDGS, wheat bran, rapeseed meal, sunflower meal and rice bran (Table 1). The LQ variants had higher levels of these ingredients and no maize starch or pea and soy protein concentrates. This resulted in a higher NSP and phytate content in the LQ + 0, LQ + Ph and LQ + PhX diets than in the CQ + 0, CQ + Ph and CQ + PhX diets (Table 2 and Supplementary data (S1)). The diets were formulated to have 5.4 g of available P/kg dry matter (DM) using monocalcium phosphate as the P source. The LQ diets had a lower crude protein content than the CQ diets (ca. 32% vs ca. 36%), but the diets were formulated to have similar crude protein to gross energy ratios (S1). Diets supplemented with phytase (*Buttiauxella* sp. phytase, DuPont Nutrition and Bioscience, Leiden, NL) had a phytase activity of ca. 660 FTU/kg DM, and diets with added xylanase (Danisco xylanase, DuPont Nutrition and Bioscience, Leiden, The Netherlands) had xylanase activity that was higher than originally intended (6000 U/kg DM) (Table 2). Yttrium oxide was included as the inert marker for digestibility studies.

Extruded diets 3 mm pellets were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands). The oils (fat) and enzymes were added to the pelleted diets by vacuum coating (Vacuüm core coater, Pegasus®-10VC, ¼ H/VV nozzle nr. 6502), at the research facilities of the Animal Science Group, Wageningen University and Research, The Netherlands. The enzymes were diluted 1:50 with demineralized water, and 5 mL of diluted enzymes per kg feed were added to the oils. Diets

Table 2
Analysed chemical composition and enzyme activity of the experimental diets.

	Control Quality (CQ) diet			Low Quality (LQ) diet		
	CQ + 0	CQ + Ph	CQ + PhX	LQ + 0	LQ + Ph	LQ + PhX
Analysed nutrient content (g/kg DM)						
Dry matter (DM, g/kg)	936	931	933	954	953	948
Crude protein	353	357	362	317	317	330
Crude fat	87	87	89	98	87	92
Carbohydrates[†]	500	496	490	513	524	509
Starch	308	306	302	191	195	190
Sugars[‡]	16	16	16	20	20	20
NSP[§]	176	174	172	302	309	300
Gross energy (kJ/g DM)	20.5	20.9	20.8	20.7	20.7	20.6
Ash	59	59	59	72	72	69
Phosphorus	9.9	9.7	9.7	12.2	12.1	11.5
Calcium	7.4	7.3	7.2	7.6	7.6	7.3
Magnesium	3.1	3.1	3.1	4.6	4.6	4.3
DP/DE[¶]	19.4	19.1	19.5	18.8	18.6	18.7
Enzyme acivity						
Phytase (FTU/kg)	b.d.	662	621	b.d.	667	667
Xylanase (U/kg)	n.m.	n.m.	6348	n.m.	n.m.	6844

Notes. +0, no enzymes added; +Ph, phytase added; +PhX, phytase and xylanase added; b.d., below detection; n.m., not measured.
[†] The carbohydrate content (on DM basis) was calculated as: 1000 – (ash + crude protein + crude fat).
[‡] Sugars calculated as: starch and sugars – starch.
[§] Non-starch polysaccharides calculated as: carbohydrates – (starch + sugars).
[¶] DP/DE calculated using the level of protein (P) and energy (E) in the diet and the digestibility (D) of both P and E (Table 4).

without enzymes were coated with 5 mL of demineralized water per kg of feed. Diets were produced ca. 4 weeks prior to the start of the trial and stored in a refrigerator (4 °C) throughout the entire trial.

2.2. Fish, rearing conditions and housing facilities

This experiment was evaluated by the Animal Welfare Body of Wageningen University, The Netherlands. All procedures applied to the animals were in line with Dutch legislation (Act on Animal Experiments) and were classified as not being an animal experiment according to Dutch legislation.

The experiment was conducted in the Aquaculture Research Facility of Wageningen University and Research, The Netherlands. Male Nile tilapia (*Oreochromis niloticus*) of the strain Silver NMT™ were obtained from a commercial fish breeder (Til-Aqua international, Someren, The Netherlands) at ca. 0.2 g individual weight. Sixteen days prior to the start of the trial, fish were held in 120-L tanks (100 fish/tank, 31 g initial weight) in the same room and under the same conditions (water quality, photoperiod, etc.) as the feeding trial. Fish were fed a commercial feed (ME-1.8 MP Presta, containing 55% protein and 15% fat, Skretting, Stavanger, Norway) prior to the start of the trial. Eighteen rectangular (70 × 35 × 40) glass tanks of 70-L (60-L effective volume), placed in three rows of six were used in the trial. Each tank was covered with a lid that prevented light from penetrating from the top. The tanks were connected to the same RAS, which resulted in a common water supply with the same water quality for the inflow of each tank. The RAS consisted of a sump, settling tank and trickling filter. Each tank was connected to a swirl separator (AquaOptima AS, column height 44 cm; diameter 24.5 cm) with a detachable glass bottle to collect waste feed spills and faeces. The water flow through each tank was set at 7 L min^{-1} using a hand held liquid rotameter, and all tanks contained a cylinder shaped air stone to ensure adequate oxygenation. The photoperiod was set to 12 h light: 12 h dark (lights on 7:00, lights off 19:00). The light intensity was increased to the intended level over a period of 30 min (start 06:30) and decreased in the evening over a period of 30 min (start 18:30). The daily water refreshment of the system was 300-L.

Water quality parameters were measured three times per week (Monday-Wednesday-Friday), in the morning before feeding to ensure that the water quality parameters remained within the pre-set ranges. Water samples were collected from the common outflow. The average temperature was 27.5 °C ± 0.2. The pH range was set between 7.0 and 7.8. Sodium bicarbonate was added if the pH tended to drop below 7.0. The pH ranged between 6.8 and 7.8 during the trial. Dissolved oxygen levels of the common outflow stayed above 6.2 mg L^{-1}. Conductivity was 7800 μS/cm at stocking, which was gradually lowered and kept between 3000 and 4000 μS/cm after week one. Total ammonia and nitrite levels of the outflow remained below 0.1 mg L^{-1} and nitrate below 400 mg L^{-1}.

2.3. Experimental procedure

The trial lasted 42 days. At the start, 30 fish were stocked per tank at random (3 rounds of 10 fish), and subsequently batch weighed (per tank) to determine the total biomass. The mean initial body weight at the start of the trial was 39 g. At the end of the trial all fish were batch weighted (per tank) to determine the final weight and to calculate growth parameters. Fish were starved each time for 24 h before batch weighing to allow for the gastro-intestinal tract to empty. The six experimental diets were assigned randomly per set of six tanks. This resulted in 3 replicates per treatment.

The aim within the experiment was to test the effect of the added enzymes and dietary NSP level, on growth and digestibility. Therefore, the fish were fed a restricted equal amount of feed (on DM basis) per tank per day, in order to rule out the effect of feed intake on growth and digestibility. The feeding level was aimed at 16 g $kg^{-0.8}$ body weight (BW)/d, which is about 80% of the expected satiation level. The daily amount of feed was increased throughout the trial by predicting fish growth and weight, using the average start weight of the fish and an expected feed conversion ratio (FCR) of 1.2. At the first feed, the fish were fed 20% of the intended feed level, which was increased in increments over 5 days to 100%. This was done to allow for habituation to the diet and to prevent feed spills. The daily feed ration was divided into two equal portions fed at 09:00 and 15:30 h. The fish were hand fed and portion feeding did not exceed 1 h for all tanks. The fish were fed through a funnel, via a hole in the lid. Fifteen minutes after feeding, the settling bottles (250-mL) connected to the swirl separators were emptied and checked for uneaten pellets. The equivalent amount of uneaten pellets was added to the next feed, to ensure all fish received the same amount of feed (g DM/fish). The feed was sieved to remove small particles and dust, to ensure all feed was consumed and to prevent deterioration of the water quality. Feed was stored in plastic buckets in

a fridge at 4 °C, throughout the trial. Each week a 100 g feed sample of each diet was taken for composition analysis. Each diet sample was added to a specific diet container, which was stored in the fridge at 4 °C, until analysis at the end of the trial.

Faeces were collected for digestibility measurements using a swirl separator, with a detachable set of glass bottles (500-mL). Faeces collection was carried out from the second week onwards. Faeces were collected between the afternoon and morning feeds (between 16:30–08:00 h) and the glass collection bottles were submerged in ice to prevent bacterial degradation of the faeces, and placed in Styrofoam boxes overnight for insulation. Faeces were pooled per week (per tank) and stored in aluminium trays at −20 °C until further analysis.

2.4. Analyses

Feed and faeces were analysed as described by Staessen et al. (2020). Batches of faeces collected per week per tank were dried at 70 °C. The dry weight collected during the last two weeks (pooled) was sufficient to perform all the analyses, hence faeces collected earlier were not used. The faeces were ground using a mixer mill (Retsch Brinkmann, model MM2000) prior to the analysis. Collected faeces and feed were analysed gravimetrically for DM by drying at 103 °C for 4 h until constant weight. Following the DM determination, ash content was determined gravimetrically by incineration in a muffle furnace for 4 h at 550 °C (ISO 5984, 1978). Ashed samples were transferred to volumetric flasks and dissolved in concentrated sulphuric acid solution by autoclaving. Samples were subsequently diluted in water and filtered using a syringe filter (45 μm pores). Finally, Yttrium, P, calcium (Ca) and magnesium (Mg) were analysed using inductively coupled plasma-mass spectrometry (ICP-OES) according to the standard NEN 15510 (2007). The total nitrogen content was measured using the Kjeldahl-method (ISO 5983, 1997), calculating crude protein as N x 6.25 (protein conversion factor). Crude fat was measured gravimetrically using acid hydrolysis followed by petroleum-ether extraction (Soxhlet method; ISO 6492, 1999). Gross energy was measured using a bomb calorimeter (IKA® werke, C7000; IKA analysentechnik, Weitershem, Germany). Starch in feed and faeces was determined enzymatically (NutriControl, The Netherlands). Starch was determined after washing away free sugars with 40% ethanol. The dried residue was digested with Termamyl®, after which starch was hydrolyzed using amyloglucosidase. The glucose units formed were determined using the Luff-Schoorl reagent. Starch + sugars was measured as described above, leaving out the washing with 40% ethanol.

Danisco Animal Nutrition Laboratories (Brabrand, Denmark) analysed the feed samples for phytase activity as described by Yu et al. (2012) and for xylanase activity as described by Romero et al. (2013). For phytase activity, a standard phytase assay method was used (Engelen et al., 2001). Feed samples were reacted with phytic acid at 37 °C and pH 5.5 for 60 min. The reaction was stopped by adding acid vanadate-molybdate reagent, which produces a coloured complex. The optimal density was monitored at 415 nm. One xylanase unit was defined as the amount of enzyme that releases 0.48 μmol of reducing sugar as xylose from wheat arabino xylan per minute at pH 4.2 and 50 °C. Azurine cross linked arabinoxylan isolated from wheat (Megazyme International Ireland Ltd., Bray, Ireland) was used as substrate. Sample extracts (100 μ L^{-1}) were incubated at 50 °C for 60 min, mixed with a vortex and centrifuged at 960 ×*g* for 10 min, after which absorbance of the supernatant was measured with a spectrophotometer at 590 nm against a blank sample and units were calculated in reference to a calibration.

2.5. Performance

Growth performance was calculated, as described in Saravanan et al. (2012). The growth (in g/d) was calculated as the difference between the average individual initial (W_i) and final (W_f) body weight per tank, divided by the duration (t) of the trial in days (d). The specific growth rate (SGR in % BW /d) was calculated as (ln (W_f) - ln (W_i)) x 100 / t. The daily absolute feed intake (in g DM/d) was calculated as FI_{tot} / (n × t), where n is the number of fish per tank and FI_{tot} the total feed intake (in g DM), corrected for mortality and feed spills. The FCR was calculated as daily absolute feed intake / growth. The feed intake expressed as percentage of BW (%/d) was calculated as (FI_{tot} / W_G) x (100 / t), where W_G is the geometric mean body weight (in g) calculated as √(W_i x W_f). The survival of fish per tank was calculated as (N_f / N_i) x 100, where N_f is the final number of fish and N_i the initial number. The protein efficiency ratio (PER) was calculated as growth / protein intake, where the protein intake (in g/d) was calculated as FI_{tot} x (% protein diet / 100).

2.6. Digestibility

Yttrium oxide was used as an inert marker to calculate the apparent digestibility coefficient (ADC in %) of dry matter, crude protein, crude fat, total carbohydrate, starch, NSP, gross energy, ash, P, Ca and Mg for each tank. The ADC was calculated as (Cheng and Hardy, 2002):

$$ADC\ (\%) = (1-((Y_{diet}/Y_{faeces}) \times (N_{faeces}/N_{diet}))) \times 100$$

where Y is the concentration of Yttrium in the diet and faeces and N is the quantity of nutrient or energy content in the diet and faeces. The total amount of carbohydrates in feed and faeces was calculated on a DM basis as: 1000 – (crude protein + crude fat + ash). The NSP fraction was calculated as: total carbohydrates – (starch + sugars). As the total carbohydrates and NSP fraction were calculated (as above), the means incorporated the accumulated errors in all the other analyses, leading to increased error (SEM). Therefore, this data must be interpreted with care.

2.7. Statistical analyses

The main effect of diet (quality) was tested according to a 2 by 3 factorial design using a General Linear Model (GLM), where the first factor is the diet (CQ vs LQ) and the second factor the enzyme treatment; 1) no enzymes, 2) phytase, and 3) phytase and xylanase. This means that when the CQ or LQ diet is mentioned without enzyme treatment, reference is made to the average of diets based on CQ or LQ, respectively. This 2 by 3 factorial design was also used to calculate the standard error of means (SEM) presented in Tables 3 and 4. The effect of phytase and the interaction between diet and phytase was tested according to a two-way ANOVA on part of the data set, using both diets with no enzymes and with only phytase supplementation, thus excluding the diets with the combined phytase and xylanase supplementation. Likewise, the effect of xylanase and the interaction between diet and xylanase was tested according to a two-way ANOVA on part of the data set, using both diets supplemented with phytase and the combined supplementation with phytase and xylanase, thus excluding the diets with no enzymes. When an interaction was found ($P < .05$) between diet and phytase and/or xylanase, a Tukey HSD (honest significant difference) with multiple comparison with 95% level of significance was used to compare treatment means. All data were expressed as the mean per treatment of three replicates. All statistical analyses were carried out using the IBM Statistical Package for the Social Sciences (SPSS) program (v 25.0, New York, United states).

3. Results

3.1. Performance

The average initial weight was 39.3 g and statistically identical for all treatments ($P > .1$). The overall survival rate within the trial was high at 99.4% and unaffected by dietary treatment (Table 3). As intended, fish on different dietary treatments received equal amounts of feed (1.50 g DM/d). There was a dietary effect on all performance parameters ($P < .05$). The lower quality of the LQ diet lowered the

Table 3
Growth performance of Nile tilapia over 42 days. Values are means (3 replicates) and standard error of the mean (SEM).

	Common Quality (CQ) diet[†]			Low Quality (LQ) diet				P – value				
	CQ + 0	CQ + Ph	CQ + PhX	LQ + 0	LQ + Ph	LQ + PhX	SEM	Diet	Phytase[‡]	Xylanase[§]	Diet x Phy[‡]	Diet x Xyl[§]
No. tanks	3	3	3	3	3	3	-	-	-	-	-	-
No. fish/tank	30	30	30	30	30	30	-	-	-	-	-	-
Survival (%)	100	100	97.8	100	98.9	100	1.04	ns	ns	ns	ns	ns
Tank biomass start (g)	1164	1167	1179	1182	1183	1178	14.4	ns	ns	ns	ns	ns
Tank biomass end (g)	2987	3130	3050	2795	2874	2955	33.7	***	**	ns	ns	#
Initial body weight (g)	39.6	39.3	39.1	39.4	39.2	39.3	0.48	ns	ns	ns	ns	ns
Final body weight (g)	99.6	104.3	104.0	93.2	96.9	98.5	0.56	***	***	ns	ns	ns
Feed intake (FI)												
Total FI (g DM/tank)	1895	1898	1877	1891	1877	1904	7.8	ns	ns	ns	ns	*
FI (g DM/d)	1.50	1.50	1.50	1.5	1.50	1.51	0.000	-	-	-	-	-
FI (% BW/d)	2.59	2.54	2.52	2.6	2.55	2.56	0.018	ns	ns	ns	ns	ns
Growth												
Growth (g/d)	1.45	1.56	1.54	1.28	1.37	1.41	0.018	***	***	ns	ns	ns
SGR (%/d)	2.24	2.35	2.32	2.05	2.14	2.19	0.031	***	**	ns	ns	ns
FCR	1.04	0.97	0.98	1.17	1.10	1.07	0.012	***	***	ns	ns	ns
PER	2.72	2.89	2.83	2.69	2.87	2.83	0.047	ns	***	*	ns	ns

Notes. +0, no enzymes added; +Ph, phytase added; +PhX, phytase and xylanase added; DM, dry matter; BW, body weight; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; ns, not significant $P > .1$; **, $P < .01$; ***, $P < .001$.

[†] Exact measured values of enzyme activity are given in Table 2.

[‡] The effect of phytase and the interaction between diet and phytase (Phy) were tested according to a 2 by 2 factorial design using a two way ANOVA, using only the diets without xylanase supplementation (+0 and + Ph).

[§] The effect of xylanase and the interaction between diet and xylanase (Xyl) were tested according to a 2 by 2 factorial design using a two way ANOVA, using only the diets with phytase supplementation (+Ph and + PhX).

growth rate and SGR and increased the FCR. Phytase supplementation improved the growth rate and lowered the FCR (Table 3; $P < .001$). The improvement in growth with phytase was comparable between the two diets, improving the FCR from 1.04 to 0.97 and from 1.17 to 1.10 for the CQ and LQ diet, respectively. In contrast to the effect of phytase, xylanase did not affect any of the parameters ($P > .1$). Numerically, a slightly better FCR, SGR, growth rate (g/d) and final body weight was observed for the LQ diet with xylanase, while those parameters were highly comparable for the CQ diet. The PER averaged 2.81 for all treatments, and was not influenced by diet quality ($P > .1$). Phytase supplementation improved the PER ($P < .001$), whereas xylanase supplementation decreased the PER ($P < .05$). No interactions between the diet quality and phytase or xylanase were present ($P > .1$).

3.2. Digestibility

The low quality of the LQ diet lowered the digestibility of all nutrients ($P < .05$) except Ca and ash (Table 4). The DM digestibility was on average 79.6% for the CQ diet versus 70.4% for the LQ diet. As expected, phytase supplementation improvement the digestibility of the ash fraction and the minerals P, Ca and Mg ($P < .001$). The P digestibility improved from 51.4% to 65.3% for the CQ diet and from 45.0% to 58.8% for the LQ diet. In addition, phytase supplementation resulted in a higher digestibility of DM, carbohydrates and energy ($P < .01$). There was an effect of xylanase supplementation as well as an interaction effect between xylanase and the diet for DM, carbohydrate and energy digestibility ($P < .05$). For DM, carbohydrate and

Table 4
Apparent digestibility coefficient (ADC) of Nile Tilapia fed the experimental diets for 42 days. Values are means (3 replicates) and the standard error of the mean (SEM).

	Common Quality (CQ) diet[†]			Low Quality (LQ) diet				*P* - value				
	CQ + 0	CQ + Ph	CQ + PhX	LQ + 0	LQ + Ph	LQ + PhX	SEM	Diet	Phytase[‡]	Xylanase[§]	Diet x Phy[‡]	Diet x Xyl[§]
ADC (%)												
Dry matter	78.6	80.3^{x}	79.8^{x}	68.2	69.9^{z}	73.0^{y}	0.38	***	**	**	ns	**
Crude protein	93.1	93.4	93.3	89.8	89.9	90.6	0.19	***	ns	ns	ns	ns
Crude fat	92.4^{a}	93.3^{a}	93.6	89.7^{b}	88.6^{b}	90.5	0.47	***	ns	#	*	ns
Carbohydrates	70.6	72.4^{x}	71.5^{x}	55.2	57.9^{z}	61.9^{y}	0.54	***	**	*	ns	**
Starch	99.1	99.3	99.1	97.9	98.1	98.0	0.09	***	ns	ns	ns	ns
NSP	17.9	22.5^{x}	20.4^{x}	25.3	29.7^{z}	36.6^{y}	1.26	***	**	#	ns	**
Energy	82.7	83.7^{x}	83.5^{x}	73.3	74.2^{z}	76.8^{y}	0.25	***	**	**	ns	**
Ash	38.0	49.1	45.5	36.4	46.2	47.7	1.97	ns	***	ns	ns	ns
Phosphorus	51.4	65.3	64.2	45.0	58.8	59.1	0.63	#	***	ns	ns	ns
Calcium	4.4	31.2	20.7	5.2	19.4	24.8	5.98	ns	***	ns	#	ns
Magnesium	55.3^{c}	69.4^{a}	68.3	49.3^{d}	59.9^{b}	60.4	0.73	*	***	ns	*	ns

Notes. +0, no enzymes added; +Ph, phytase added; +PhX, phytase and xylanase added; NSP, non-starch polysaccharides; ns, not significant $P > .1$; # tendency, $P < .1$; *, $P < .05$; **, $P < .01$; ***, $P < .001$; Means within the same row not sharing a common letter are significantly different ($P < .05$), using "abcd" for interactions between diet and phytase and "xyz" for interactions between diet and xylanase.

[†] Exact measured values of enzyme activity are given in Table 2.

[‡] The effect of phytase and the interaction between diet and phytase (Phy) were tested according to a 2 by 2 factorial design using a two way ANOVA, using only the diets without xylanase supplementation (+0 and + Ph).

[§] The effect of xylanase and the interaction between diet and xylanase (Xyl) were tested according to a 2 by 2 factorial design using a two way ANOVA, using only the diets with phytase supplementation (+Ph and + PhX).

energy, the digestibility was significantly higher for the LQ diet with xylanase, compared to the LQ diet without xylanase, while the CQ diet did not benefit from the supplementation of xylanase. Without enzyme supplementation, 17.9% and 25.3% of the NSP was digested for the CQ and LQ diet, respectively. Phytase improved the NSP ADC from 17.9% to 22.5% for the CQ diet and from 25.3% to 29.7% for the LQ diet ($P < .01$). The improvement in NSP digestibility with xylanase supplementation depended on the diet as shown by the interaction effect ($P < .01$). Supplementing xylanase did not lead to a further increase in NSP ADC for the CQ diet, while the NSP ADC was significantly higher with xylanase supplementation (29.7% vs. 36.6%) for the LQ diet.

4. Discussion

4.1. Diet quality

The experiment investigated whether the effect of phytase and xylanase supplementation on nutrient digestibility and growth depends on the quality of the diet. Two diets were used, a control quality (CQ) diet and a low quality diet (LQ), with a NSP content of 174 and 304 g/kg DM respectively. The difference in diet quality is a combination of factors, besides the difference in NSP content, the LQ diet had higher phytate levels (S1), lower protein levels (Table 2) and a different protein source composition (Table 1). The lower quality of the LQ diet reduced all growth performance parameters, which is in line with previous studies with Nile tilapia using diets with a contrast in NSP level (Haidar et al., 2016; Maas et al., 2019). As a consequence of the lower quality (NSP level), the digestibility of the carbohydrate fraction was reduced from an ADC of 70.6% for the CQ diet to 55.2% for the LQ diet without enzymes. This study shows that, besides these direct effects of lower diet quality (increased NSP level), the protein, fat, ash, P and Mg digestibility was reduced as well. NSP can have multiple anti-nutritional factors, such as the deconjugation of bile acids (which are important for fat digestion), affecting the gut morphology (i.e., shortening of villi length) and affecting the digesta viscosity which can reduce the mixing of digestive enzymes. All these factors can contribute to reduced nutrient digestibility (Choct, 1997; Choct and Kocher, 2000; Sinha et al., 2011). Furthermore, soy and pea protein concentrates were used (14% in total) in the CQ diet, whereas all the protein in the LQ diet originated from low quality ingredients, which elevated the level of, amongst others, dietary NSP. Likewise, the percentage of ash and minerals that originated from the mineral premix was higher for the CQ diet. The choice of ingredients is a factor that influences the quality of the diet, which in turn is known to influence the overall nutrient ADC, irrespective of the nutrient composition (Alonso et al., 2000; Cheng and Hardy, 2003).

NSP was shown not to be inert for endogenous digestion, which is in line with several studies using Nile tilapia (Amirkolaie et al., 2005; Haidar et al., 2016; Leenhouwers et al., 2007; Maas et al., 2019). In the present study, an endogenous (without enzyme supplementation) NSP digestibility of 17.9% and 25.3% was found for the CQ and LQ diet, respectively. The ADC values are comparable to the average calculated NSP digestibility of 24.3% for tilapia across studies in a recent review by Maas et al. (2020). The NSP source and composition is a known factor influencing the endogenous NSP digestibility (Leenhouwers et al., 2007; Maas et al., 2019). However, in the present study, the theoretical NSP composition was kept comparable, by incorporating the NSP rich ingredients (wheat bran, rapeseed meal, sunflower meal, rice bran, and wheat DGGS) in a similar ratio into the diets. With the used NSP levels in the diets (Table 2) and the NSP ADCs (Table 4), it was calculated that approximately 31 g versus 77 g of NSP was digested endogenously per kg DM feed fed, for the CQ and LQ diets, respectively. On the contrary, Haider et al. (2016) showed that the NSP digestibility of the same diet drastically decreased with an increase in feeding level, which suggest a threshold in the amount of NSP that can be digested. It is unclear what caused the higher NSP ADC, and a more than two-fold increase in absolute NSP digestion, for the LQ diet compared to the CQ diet.

4.2. Phytase

Supplementation of microbial phytase in the diet improved P availability and overall growth performance, while reducing P excretion for several fish species (Cao et al., 2007; Cheng et al., 2004; Kumar et al., 2012; Oliva-Teles et al., 1998). Hereby, the induced anti-nutritional effect of phytate was partly compensated, reducing the need for inorganic P (Cao et al., 2007). In line with other studies on Nile tilapia (Liebert and Portz, 2005; Maas et al., 2018; Portz and Liebert, 2004), the growth performance was improved for both diets with phytase supplementation. The available P level (in g/kg DM feed) was 5.1 for the CQ diet and 5.4 for the LQ diet, well above the stated P requirement for Nile tilapia for growth of 4.5 g/kg DM feed (Prabhu et al., 2013). In line with a comparable improvement in growth, the improvement in P digestibility was comparable between the CQ and LQ diets. The large and comparable impact phytase has on both growth performance and P digestibility for the CQ and LQ diets suggests that P availability was limited for growth, which suggests that the stated available P level of 4.5 (g/kg DM feed) is too low to support optimal growth. In the present study, an enzyme dose of 660 ± 24 FTU/kg diet was measured (aimed for 1000 FTU/kg diet). The enzyme dose used was below the optimal range reported for tilapia by Liebert and Portz (2005) of 750–1000 FTU/kg. The increase in P digestibility is highly comparable between both diets, while the level of P in the diet is approximately 23% higher for the LQ diet. From the P level in the diet (Table 2) and the P ADC (Table 4), it was calculated that 1.36 and 1.67 g P per kg DM feed administered, was made available with phytase, for the CQ and LQ diets, respectively. This suggest that the dose of phytase (660 ± 24 FTU/kg) was not limiting for increasing the P availability of both diets (see section "Xylanase" below). The slightly higher P digestibility for the CQ diet compared to the LQ diet, both with and without enzymes, can be attributed to the higher percentage of highly digestible inorganic P (monocalcium phosphate) in the total P content in the CQ diet. The effect phytate and phytase have on, in particular, the bioavailability of minerals and the amino acid digestibility, is well described (Cao et al., 2007; Francis et al., 2001; Nolan et al., 1987). To our knowledge, the effect of phytase on improving NSP digestibility has not been reported before. Maas et al. (2018) showed a strong increase in carbohydrate digestibility with phytase supplementation, and suggested that this was due to improved NSP digestibility. NSP are structural components of plants which primarily can be found in cell walls (Sinha et al., 2011). Where phytate, the main storage form of P in plants, is found within the plant largely depends on the type of plant. In cereals for example, phytate is mainly concentrated in the aleurone layer, whereas in legumes, phytate is distributed throughout the whole seed (Skoglund et al., 2009). It can be hypothesized that when, for example, the phytate in the aleurone layer is broken down through phytase supplementation, the NSP, located in the aleurone layer becomes more accessible for endogenous digestion, with increased NSP digestibility as result.

4.3. Xylanase

The effect of xylanase was tested in diets that were all supplemented with phytase. In our study, xylanase did not further enhance growth performance. Studies that have tested the sole effect of xylanase in fish are scarce. A few studies have tested the main effect of xylanase and showed an improvement in growth performance in Nile tilapia (Hassaan et al., 2019), rainbow trout (Dalsgaard et al., 2012), Jian carp (*Cyprinus carpio* var. Jian) (Jiang et al., 2014) and Japanese seabass (*Lateolabrax japonicus*) (Ai et al., 2007). All these studies were done without phytase supplementation to the diet. In poultry it is shown that the magnitude of the effect of enzyme supplementation on ileal amino acid digestibility is largely dependent on the nutritional value of the control diet. It is suggested that the effect of carbohydrases (like xylanase) is less when the diet already contains phytase, because phytase can improve the diet's nutritional value compared to a control diet without enzymes (Cowieson and Bedford, 2009). Likewise, in tilapia it is suggested that a control diet

low in NSP and phytate, which results in good growth performance, has little to gain from the supplementation of enzymes (Maas et al., 2019). Similarly, the supplementation of phytase in the present study, could have maximized the growth potential of the diet, muting the effect of xylanase. On the contrary, Maas et al. (2018) showed that in Nile tilapia, the effect of xylanase on growth performance was enhanced in the presence of phytase. Although not improving growth rate, xylanase improved the digestibility of DM, total carbohydrates and energy. An interaction effect was present for DM, total carbohydrates, NSP and energy; the digestibility was significantly higher for the LQ diet with xylanase supplementation, while the CQ diet did not benefit (Table 4). Although not statistically significant, both growth rate (g/d) and FCR were improved by approximately 3% when xylanase was supplemented to the LQ diet. Although the NSP digestibility with only phytase supplementation to the LQ diet was already higher compared to the CQ diet (22.5% vs. 29.7%), the digestibility increased from to 29.7% to 36.6%, whereas the NSP digestibility of CQ diet did not improve. As mentioned before, because the composition of the NSP fraction of the diets is theoretically comparable, a comparable response was expected. If the enzyme dose was limiting for NSP degradation, a lower response for the LQ diet would be expected, because for each 1% increase (absolute) in NSP ADC, 75% more NSP was digested in the LQ diet than in the CQ diet. The NSP digestibility of the LQ diet is higher compared to the CQ diet when supplemented with both phytase and xylanase (20.4% vs 36.6%), which resulted in three times more NSP digested per kg diet fed, than for the CQ diet (35 vs. 110 g/kg diet). Why a larger NSP fraction remained undigested in the CQ diet remains unclear. The effect of xylanase on NSP digestibility might have been partially muted by the endogenous NSP digestibility as well as by the effect of phytase on NSP digestibility, considering that both work on the same NSP fraction. For xylanase it is evident that it cleaves the β 1,4 backbone of xylan, which is part of the hemicellulose fraction (Collins et al., 2005). It is generally accepted that the NSP fractions pectin and hemicellulose are less inert to digestion compared to cellulose and lignin (Amirkolaie et al., 2005; Maas et al., 2019; Sinha et al., 2011). Which part of the NSP fraction is digested endogenously and which part is digested as a result of phytase, remains unclear. With the improvement of DM digestibility, the amount of fecal waste (100% - DM ADC) decreased by approximately 10%, reducing the pressure on RAS purification systems or decreasing the nutrient load in the effluent when discharged to surface waters.

5. Conclusion

Our study confirmed the negative effect of poor quality diet (high NSP levels, etc.), formulated using low quality ingredients, on growth performance and on digestibility of DM, total carbohydrates, NSP, energy, ash and the minerals P, Ca and Mg in Nile tilapia. Phytase improved the growth performance and nutrient digestibility of DM, energy, total carbohydrates, ash, P and Ca independently of the quality of the diet. Xylanase supplementation on top of phytase did not enhance growth performance of neither the control quality nor the low quality diet. The effect of xylanase on nutrient digestibility depended on the diet quality; xylanase enhanced the digestibility of DM, energy, total carbohydrates and NSP of the low quality diet, while the control quality diet remained unaffected.

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Credit authorship contribution statement

Roel M. Maas: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – original draft. **Marc C.J. Verdegem:** Conceptualization, Writing - Review & Editing. **Theodor** L. **Stevens:** Investigation, Data Curation. **Johan W. Schrama:** Conceptualization, Methodology, Writing – Review & Editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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