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Effect of enzymes (phytase and xylanase), probiotics (*B. amyloliquefaciens*) and their combination on growth performance and nutrient utilisation in Nile tilapia

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ABSTRACT

The increased use of plant ingredients in aquafeeds over the last decades, as replacement for fish meal, has led to rising levels of undesired non-starch polysaccharides (NSP) and phytate. Both NSP and phytate degrading enzymes and probiotics have been widely studied. They can be used as a tool to deal with increasing levels of NSP and phytate in aquafeeds. However, studies combining both probiotics and enzymes are scarce in fish. The main objective of the present study was to assess the impact on Nile tilapia (*Oreochromis niloticus*) of enzymes and probiotics, as well as their synergistic effect. Parameters measured were: growth; nutrient digestibility; body composition; and the energy, nitrogen (N), P and Ca balance. Diets were supplemented with, and without, an enzyme mix (phytase at 1000 FTU/kg and xylanase at 6000 U/kg) and with, and without, probiotics (three strains of *B. amyloliquefaciens* at 60 mg/kg feed), according to a 2 × 2 factorial arrangement. This resulted in a control treatment (CON-CON) without enzymes and probiotics, an enzyme treatment (ENZ-CON), a probiotic treatment (CON-PRO) and a treatment with both enzymes and probiotics (ENZ-PRO). In total, 16 tanks (4 replicates/treatment) were used with 35 fish each (mean initial weight 39 g). Fish were restrictively fed equal amounts of dry matter for 42 days. Both enzymes ($P < 0.001$) and probiotics ($P < 0.05$) improved growth (g/d) and FCR when applied individually. The combination of enzymes and probiotics showed an interaction effect ($P < 0.05$) on growth and FCR. Enzymes improved growth to a greater extent than probiotics, whereas the combination of enzymes and probiotics did not further enhance growth. The CON-CON treatment had the highest FCR (1.33), the CON-PRO treatment a slightly lower FCR (1.27); the lowest FCR (1.11) was found for both treatments with enzymes (ENZ-CON and ENZ-PRO). Enzyme supplementation improved the digestibility of all nutrients ($P < 0.01$), whereas probiotics enhanced fat digestibility ($P < 0.01$). Additionally, enzyme supplementation increased retained P (mg/d), retained N (mg/d) and N efficiency ($P < 0.001$). Probiotic supplementation affected the energy requirements for maintenance (kJ/kg$^{0.8}$/d; $P < 0.05$). Dietary supplementation of either enzymes or probiotics had positive effects on the measured parameters, but the combination of enzymes and probiotics did not have a synergistic effect.

1. Introduction

As a finite resource, fishmeal is becoming increasingly unsustainable, from both an environmental and economic point of view. Thus, over recent decades, it has been increasingly replaced in aquafeeds by plant ingredients and the breadth of alternative ingredients is steadily growing (Bendiksen et al., 2011; Oliva-Teles et al., 2015; Tacon and Metian, 2015). High inclusion levels of plant ingredients coincide with antinutritional factors, of which non-starch polysaccharides (NSP) and phytate are probably the most prominent. NSP and phytate can negatively affect fish performance and health in various ways. Moreover, phytate and NSP are largely indigestible by fish due to the absence of the digestive enzymes required to break them down. Therefore, NSP and phytate have a low nutritional value, which limits the inclusion of plant

Abbreviations: NSP, non-starch polysaccharides; CFU, colony-forming unit; MEm, energy requirements for maintenance; DP, digestible phosphorus; RP, retained phosphorus; DCa, digestible calcium; RCa, retained calcium..

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ingredients (rich in NSP or phytate) in fish diets (Francis et al., 2001; Sinha et al., 2011; Kumar et al., 2012).

Tilapia is cultured in more than 100 countries and, in terms of production, it is the second most farmed fish in the world. Outside of Africa, most of the farmed tilapia belongs to the genus *Oreochromis,* of which Nile tilapia (*Oreochromis niloticus*) represents more than 90% (Wang and Lu, 2016). Tilapia are omnivorous and are capable of filter-feeding on phytoplankton, zooplankton and detritus (Garcia-Pérez et al., 2000; Azim et al., 2003). Tilapia is widely used as model species, including for studies on exogenous enzyme supplementation in fish diets. Carbohydrases, like xylanase, β-glucanase and cellulase, target NSP by hydrolysing the long polysaccharide chains into reduced sugars, which can provide energy through fermentation (Bergman, 1990; Bedford, 2000; Choct and Kocher, 2000). As a warm-water species with a long gut, the tilapia is well adapted for feeding on plant ingredients and has a high potential for fermentation in the gut. These factors make it a suitable fish for studies on the effect of exogenous enzyme supplementation (Metzler-Zebeli et al., 2010; Maas et al., 2020). Indeed, many studies have been performed on tilapia, looking at how dietary carbohydrase enzymes can improve NSP digestibility (e.g., Lin et al., 2007; Li et al., 2009; Yigit and Olmez, 2011; Adeoye et al., 2016a; Hlophe-Ginindza et al., 2016; Maas et al., 2018; Hassaan et al., 2019; Maas et al., 2019).

In the last two decades, probiotics have appeared as an eco-friendly feed additive and alternative to antibiotics, in enhancing fish immunity and disease resistance. In addition, probiotics have been shown to improve nutrient utilisation and feed efficiency, resulting in improved growth. Probiotics alter the composition of the gut microbial community, increase the capacity to produce digestive enzymes (amylases, proteases, and lipases, etc.) and raise the supply of nutrients, like short-chain fatty acids (SCFA) and amino acids (Hai, 2015; Banerjee and Ray, 2017; Hoseinifar et al., 2017; Dawood et al., 2019; Kuebutornye et al., 2020). Many studies on probiotics (*Bacillus*) have been performed using tilapia (Aly et al., 2008a; Reda et al., 2016; Liu et al., 2017; Abarike et al., 2018), including a review by Hai (2015).

Both NSP and phytate degrading enzymes and probiotics have been widely studied and can be used as a tool to deal with increasing levels of NSP, as well as to improve the sustainability of the aquaculture sector. However, studies combining probiotics and enzymes are scarce. The combination of enzymes and probiotics may result in a complementary mode of action. It is hypothesized that enzymes enhance the breakdown of NSP and stimulate the fermentation of NSP in the gut, producing SCFA. Probiotics (e.g. *Bacillus* spp.) may fare better in an SCFA enhanced gut, using the metabolites of NSP fermentation as an energy source. Vice versa, probiotics can alter the gut environment and are known to stimulate the activity of digestive enzymes, potentially favoring the breakdown and fermentation of NSP. In broilers, the synergy between probiotics and enzymes was tested in various studies (Seidavi et al., 2017; Wealleans et al., 2017; Konieczka et al., 2018). Konieczka et al. (2018) showed positive effects of interaction between enzymes (xylanase and β-glucanase) and probiotics (*Bacillus subtilis*) in terms of feed intake, (raised) concentrations of SCFA in the ileum, and (increased) bacterial enzyme activity in the caecal digesta. Wealleans et al. (2017) observed beneficial effects on nutrient digestibility and growth when enzymes (xylanase, amylase, and protease) and probiotics (3 strains of *Bacillus amyloliquefaciens*) were combined, suggesting a synergistic effect between enzymes and probiotics in broilers. To our knowledge, only Adeoye et al. (2016b) and Dai et al. (2019) tested the combined effect of enzymes and probiotics in fish. Adeoye et al. (2016b) focussed on the effects on growth, intestinal histology and microbiome in tilapia; whereas Dai et al. (2019) tested the effects on growth, digestive enzymes and the gut microbiome in snakehead. However, neither study tested for the interaction between enzymes and probiotics and a diet low in NSP was used. Therefore, in the present study we used a diet high in NSP for investigating the synergistic effect between dietary enzymes (phytase and xylanase) and probiotics (three strains of *B. amyloliquefaciens*) on growth, nutrient digestibility, body composition and the energy, nitrogen and P balances in Nile tilapia.

2. Materials and methods

This study was approved by the Central Committee on Animal Experiments (CCD), under the advice of the Animal Experiment Committee (DEC) of The Netherlands (permit no. 2018.W-0010.002). It was also approved by the Ethical Committee for Animal Experiments of Wageningen University, The Netherlands, and carried out according to Dutch law (Act on Animal Experiments).

2.1. Diets

The effect of enzymes and probiotics was tested according to a 2 × 2 factorial arrangement. The first factor was supplementation with and without enzymes, using an enzyme mix of phytase (Axtra® PHY, *Buttiauxella* sp. phytase at 1000 FTU/kg, DuPont Animal Nutrition) and xylanase (Danisco® Xylanase at 6000 U/kg, DuPont Animal Nutrition). The reason for using this enzyme mix is that the combination of phytase and xylanase is shown to be effective in Nile tilapia (Maas et al., 2018). The second factor was the incorporation with and without probiotics (Enviva® PRO 202 GT 60 mg kg feed providing 150,000 CFU/g feed, DuPont Animal Nutrition). Enviva® PRO 202 GT is a probiotic product that contains three strains of *B. amyloliquefaciens* spores, which are heat resistant. This resulted in a control treatment without enzymes and probiotics (CON-CON), a probiotic treatment (CON-PRO), an enzyme treatment (ENZ-CON) and a treatment with both enzymes and probiotics (ENZ-PRO). A basal diet was used for the incorporation of both enzymes and probiotics (Table 1). The basal diet was free of fish meal and formulated according to commonly applied levels of low quality ingredients, such as: wheat bran, rapeseed meal, sunflower meal, rice bran and wheat dried distillers grains with solubles. The choice of these low quality ingredients gave an approximate NSP level of 316 g per kg DM diet. This high NSP level was also used to make it a challenging diet,

Table 1
Ingredient composition of the basal diet (%).

Ingredients (%)	
Maize	7.0
Soya bean meal	10.0
Wheat	6.88
Wheat gluten meal	3.0
Wheat bran	15.0
Rapeseed meal	10.0
Sunflower meal	10.0
Full-fat rice bran	15.0
Wheat DGGS[a]	10.0
Fish oil	1.0
Rapeseed oil	1.5
Palm oil	1.5
Hydrolysed feathermeal	5.0
Premix[b]	1.0
Calcium carbonate ($CaCO_3$)	0.7
Dicalcium phosphate	1.1
DL-Methionine	0.45
L-Lysine	0.65
L-Threonine	0.20
Yttrium oxide	0.02

[a] Dried Distillers Grain with Solubles.

[b] Premix composition (PVO 40/01, Sparos Lda, Olhão, Portugal). Vitamins (expressed as IU or mg/kg of final diet): vitamin A retinol acetate 20,000; vitamin B1, 30; vitamin B2, 30; vitamin B3, 200; vitamin B5, 100; vitamin B6, 20; vitamin B12, 0.1; vitamin C, 1000; vitamine D3, 2000; vitamin E, 100; boitine, 0.3; inositol, 500; betaine, 500; choline chorine. Minerals (expressed as mg/kg of final diet): copper sulphate, 9; ferric sulphate, 6; potassium iodide, 0.5; manganese oxide, 9.6; sodium selenite, 0.1; magnesium hydroxide, 7.5; calcium, 600; chlorine, 250.

which may magnify the possible effect of the applied treatments. Extruded diets (3 mm pellets) were produced by SPAROS Lda. (Portugal). During extrusion, the temperature was kept below 110 °C. A batch of basal diet was extruded with and without probiotics. The enzymes (in liquid form), or placebo solution were mixed with the oils (fish, rapeseed and palm oil) and then vacuum coated at the research facilities of the Animal Science Group, Wageningen University, The Netherlands. The enzymes were diluted 1:50, with demineralised water, to increase the volume and to ensure a more homogenous dispersal of the enzymes during coating. The analysed colony-forming unit (CFU), enzyme recovery and nutrient composition are given in Table 2. Diets with (PRO-CON and PRO-ENZ) and without (CON-CON and CON-ENZ) probiotics had an average *Bacillus* colony-forming unit count of 7.9×10^4 and 3.0×10^3.

2.2. Fish, rearing conditions and housing facilities

The experiment was performed at the Aquaculture Research Facility (ARF) of Wageningen University, The Netherlands. Male Nile tilapia (*Oreochromis niloticus*; from the strain Silver NMT™) were obtained from a commercial fish breeder (Til-Aqua international, Someren, The Netherlands). Fish were housed in 120 L tanks and fed a commercial diet prior to the start of the experiment. During the experiment a total of 16 rectangular tanks of 60 L (effective volume) were used. The tanks were all connected to the same recirculation system, resulting in a common water supply and ensuring the same water quality for the inflow of each tank. The daily system water refreshment was 300 L. The recirculation system consisted of a sump, settling tank and trickling filter. Every single tank was connected to a swirl separator (AquaOptima AS, column height 44 cm; diameter 24.5 cm), with a detachable glass bottle at the bottom, to count feed spills and collect faeces for each tank separately. The water flow through each tank was set at 7 L/min, using a hand held liquid rotameter. All tanks were provided with a cylinder shaped air stone. The air stone and water flow ensured sufficient dissolved oxygen (DO). The photoperiod was set to 12 h light: 12 h dark (lights on 7:00, lights off 19:00).

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. The average temperature was 27.5 °C (± 0.2). The pH range was set between 7.0 and 7.8. Sodium bicarbonate was added to the system if the pH dropped below 7.0. The pH ranged between 6.8 and 7.9 during the experiment. DO levels of the common outflow stayed above 5.4 mg/L. Conductivity was 8000 μS/cm at stocking, which was gradually lowered and kept around 3000 μS/cm after week one. The total ammonia level was <0.25 mg/L; nitrite <0.15 mg/L; and nitrate <500 mg/L.

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

Treatment	CON		ENZ	
	CON	PRO	CON	PRO
Analysed nutrient content (g/kg DM)				
Dry matter (g/kg)	913	918	906	917
Crude protein	316	313	312	312
Crude fat	84	95	88	97
Total carbohydrates[a]	529	519	528	519
Starch + sugars	208	212	204	207
Non-starch polysaccharides[b]	321	308	323	311
Energy (kJ/g)	20.7	20.7	20.8	20.7
Ash	71	73	72	73
Phosphorus	10.9	11.0	11.0	11.0
Calcium	10.6	11.2	10.6	11.0
Magnesium	3.74	3.72	3.74	3.69
Iron	0.16	0.17	0.16	0.16
Manganese	0.072	0.071	0.072	0.070
Zinc	0.067	0.074	0.066	0.067
Copper	0.021	0.22	0.021	0.022
Yttrium	0.166	0.176	0.168	0.161
Enzyme activity				
Phytase (FTU/kg)	<180	<180	1127	1156
Xylanase (U/kg)	–	386	6372	7620
Probiotics[c]				
Bacillus count (CFU/g)	2.8×10^3	8.5×10^4	3.2×10^3	7.3×10^4

Notes. ENZ: enzyme supplementation; PRO: probiotic supplementation.
[a] The carbohydrate content (on DM basis) was calculated as: 1000 – (ash + crude protein + crude fat).
[b] Non-starch polysaccharides calculated as: carbohydrates – (starch + sugars).
[c] Enviva® PRO 202 GT (Three strains *of B. amyloliquefaciens*) incorporated at 60 mg/kg diet.

2.3. Experimental procedure

The experiment lasted 42 days. The four experimental treatments were assigned randomly to tanks. From a common batch, fish were caught and assigned to one of the 16 tanks at random. Fish were group weighted per tank, while mildly sedated using (0.25 mL/L) 2-phenoxyethanol. Each tank was stocked with 35 fish, having an average initial weight of 39 g. At the end of the experiment all fish were batch weighed per tank and counted, while mildly sedated, to determine the final weight and calculate growth parameters. At the start of the experiment, 20 fish were euthanized using an overdose of 2-phenoxyethanol (3 mL/L), for initial body composition determination. At the end of the experimental period, 10 fish per tank were randomly selected and euthanized for final body composition. Fish samples were stored at −20 °C until further analysis.

The aim of the experiment was to test the effect of the added enzymes and probiotics on growth and nutrient utilisation. Therefore, the fish were fed a restricted equal amount of feed (dry matter (DM)) per tank per day. The feeding level was fixed and 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 experiment 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 to 20% of the intended feeding level, which was increased over 6 days to 100%. The daily feed ration was divided into two equal portions, fed at 9:00 and 15:30 h. The fish were hand fed and the duration of feeding did not exceed 1 h for all tanks.

Feed spills recovered from the settling units were recorded per tank after each feed. The diets were refrigerated (4 °C) throughout the experiment. Once a week, a sample of 100 g was taken from each diet. The feed samples were pooled per treatment and stored (4 °C) until further analysis. Faeces were collected for digestibility studies, using swirl separators for 5 days per week (not the weekends), or until the tray was full for that week. The glass bottles were submerged in ice to prevent bacterial degradation of the faeces. Faeces were collected between the afternoon and morning feed (16:30–8:00 h). Faeces were pooled per week and stored in aluminium trays at −20 °C, until further analysis.

2.4. Analyses

Frozen fish samples (−20 °C) were ground twice, using a meat mincer (Gastromaschinen, GmbH model TW-R 70; Feuma) with a 4.5 mm mesh, and homogenised. Fresh samples were taken for the determination of DM and CP; samples for crude fat and energy were freeze dried prior to analysis. Faeces collected over the previous eight days were oven dried at 70 °C. Feed, faeces and fish samples were analysed according to the same methods. DM content was determined by drying samples for at least 4 h at 103 °C, until constant weight (ISO 6496, 1983). Ash content was determined by incineration, using a muffle furnace for 4 h at 550 °C (ISO 5984, 1978). CP (N x 6.25) was analysed using the Kjeldahl method (ISO 5983, 1979). Crude fat was measured by petroleum-ether extraction (Soxhlet method, ISO 5986). Energy content was measured using a bomb calorimetric, by direct combustion (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 hydrolysed using the Luff-Schoorl reagent. Starch + sugars was measured as described above, but without the washing with 40% ethanol. Yttrium, phosphorus (P), calcium (Ca) and magnesium (Mg) were analysed in the feed and the faeces, using inductively coupled plasma-mass spectrometry (ICP-OES) according to the standard NEN 15510 (2007); iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were analysed in the feed only.

The phytase and xylanase activity in feed samples was analysed by DuPont Innovation Laboratories (Brabrand, Denmark). Phytase was analysed using the methods described by Yu et al. (2012). One phytase unit (FTU) was defined as the amount of enzyme required to release 1 μmol of inorganic P per minute from sodium phytate at pH 5.5 and 37 °C. Xylanase was analysed using the methods described by Romero et al. (2013). One xylanase unit (U) was defined as the amount of enzyme that releases 0.48 μmol of reducing sugar as xylose, from wheat arabinoxylan, per minute at pH 4.2 and 50 °C. The probiotics count (CFU) of spore forming *Bacillus* was enumerated using tryptone soy agar (DuPont in house method, Wilmington, USA).

2.5. Calculations

The growth (in g/d) was calculated as the difference between the average individual initial weight (W_i) and the final (W_f) body weight, per tank, divided by the duration (t) of the experiment in days (d). The specific growth rate (SGR in % body weight /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} is the total feed intake (in g DM), corrected for dead fish and feed spills. The geometric mean body weight (W_G in g) was calculated as $\sqrt{(W_f \times W_i)}$ and the mean metabolic body weight (MBW in $kg^{0.8}$) as $(W_G/1000)^{0.8}$.

The FCR was calculated as daily absolute feed intake / growth. The survival of fish per tank was calculated as (Nf / Ni) x 100, where Nf is the final number of fish and Ni the initial number.

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):

$$\text{ADC } (\%) = \left(1-\left(\left(Y_{diet}/Y_{faeces}\right) \text{ x } \left(N_{faeces}/N_{diet}\right)\right)\right) \text{ x } 100$$

Y is the concentration of Yttrium in the diet and faeces (in g/kg DM) and N is the quantity of nutrients (in g/kg DM), or energy content (in kJ/g DM), in the diet and faeces. The total amount of carbohydrates (g/kg) 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).

The energy (E kJ/d), nitrogen (N mg/d), P and Ca (mg/d) balance parameters were calculated per tank and expressed on a per fish basis). The parameters were calculated as described by Saravanan et al. (2012). Generally, balance parameters are expressed in relation to metabolic body weight. However in the present study, fish were fed an equal amount of DM in diets of identical nutrient composition, and thus with similar amounts of nitrogen, energy, P and Ca. The final weights of the fish were affected by enzyme and probiotic supplementation (see result), therefore using metabolic body weight would already create differences in balance parameters, due to the differences in final body weight.

For the N balance, N intake was calculated as: the product of feed intake and dietary N content; digestible N intake as N intake times the digestibility coefficient of N; retained N as the difference between final and initial N body mass; branchial urinary N (BUN) losses as the digestible N intake minus retained N. The N efficiency was calculated as retained N (RN) divided by digestible N (DN). P, Ca and Mg balances were calculated according to the same principle as the N balance. For the energy balance, energy intake (GE) was calculated as: the product of feed intake and dietary energy content; digestible energy (DE) intake as GE times the energy digestibility coefficient (GE x (ADC E / 100); branchial and urinary E (BUE) losses as BUN losses times the energy content of NH_3-N (BUN x 24.9 kJ/g N), assuming that all N was excreted as NH_3-N; metabolisable E intake (ME) as DE minus BUE (ME = DE – BUE); Retained E (RE) as the difference between final and initial body energy content; heat production (HE) as metabolisable E minus RE (HE = ME - RE); RE as protein (REp) as the product of retained protein (RN x 6.25) and 23.7, where 23.7 is the energy content of 1 g protein; and RE as fat (REf) as the difference between RE and REp (REf = RE – REp), assuming RE only in the form of fat and protein. The energy requirements for maintenance (MEm) were calculated from the metabolisable E (ME) and the energy retained as protein (REp) and fat (REf). The following formula was used to calculate the MEm = ME – ((REp / 0.5) + (REf / 0.9)). In this calculation, an energetic utilisation efficiency of metabolisable E for protein gain of 50% was assumed; similarly an energetic utilisation efficiency of metabolisable E for fat gain of 90% was assumed (Lupatsch et al., 2003).

2.6. Statistics

For all statistical analysis, the tank was considered as the experimental unit. A two-way ANOVA was used to test for significance of the effects of enzyme and probiotic supplementation (and their combined effect), for all data. When an interaction effect was detected ($P < 0.05$), a Tukey HSD (honest significant difference) test was conducted (with multiple comparisons and 95% level of significance), to compare treatment means. All data were expressed as mean per treatment of four replicates. All statistical analyses were carried out using the IBM Statistical Package for the Social Sciences (SPSS)(v 25.0; New York, NY, USA).

3. Results

3.1. Performance

Survival was high (>99.3%) and unaffected by the treatment (Table 3). The initial weight (39 g) was similar between treatments ($P > 0.1$). Conforming to the experimental design, absolute feed intake was identical among treatments (1.69 g DM/d). Enzyme supplementation (phytase and xylanase) increased growth rate (g/d), specific growth rate (SGR %/d) and decreased FCR ($P < 0.001$; Table 3). Addition of probiotics increased the growth rate and decreased FCR ($P < 0.05$), and tended to improve SGR ($P < 0.10$). For all performance traits (growth, SGR and FCR), there was an interaction effect between enzyme and probiotics ($P < 0.05$). Enzymes improved the growth rate to a greater extent than probiotics, whereas the combination of enzymes and probiotics did not further enhance growth. The CON-CON treatment had the highest FCR (1.33), followed by the CON-PRO treatment, which had a significantly lower FCR (1.25). The lowest FCR (1.11) was found for those treatments containing either enzymes or a combination of enzymes and probiotics.

3.2. Digestibility

The apparent digestibility coefficients (ADC) of all nutrients, were enhanced for diets supplemented with enzymes (Table 4). Enzyme supplementation had a large impact on ash digestibility and mineral availability; ADC of Ash, P, Ca and Mg were increased by 48, 51, 135 and 35%, respectively. Probiotic supplementation reduced CP digestibility ($P < 0.05$) and tended to reduce NSP ADC ($P = 0.084$).Diets supplemented with enzymes (ENZ-CON and ENZ-PRO) had a higher NSP ADC, compared to diets without enzymes (CON-CON and CON-PRO) (36.1%

Table 3
Effect of enzyme and probiotic supplementation on the performance of Nile tilapia over 42 days.

Treatments	CON		ENZ			*P*-values		
	CON	PRO	CON	PRO	SEM	ENZ	PRO	ENZ*PRO
Survival (%)	99.3	97.9	100.0	100.0	1.19	ns	ns	ns
Initial body weight (g)	38.9	39.1	38.9	39.2	0.26	ns	ns	ns
Final body weight (g)	92.1[a]	95.5[b]	102.8[c]	103.2[c]	0.78	***	*	#
Feed intake (g DM/d)	1.69	1.69	1.69	1.69	0.00	–	–	–
Growth								
Growth (g/d)	1.27[a]	1.34[b]	1.52[c]	1.52[c]	0.015	***	*	*
SGR (%/d)	2.05[a]	2.12[b]	2.31[c]	2.31[c]	0.015	***	#	*
FCR	1.33[a]	1.25[b]	1.11[c]	1.11[c]	0.014	***	*	*

Notes. ENZ, enzyme (effect) supplementation; PRO, probiotic (effect) supplementation; ENZ*PRO, interaction effect; SGR, specific growth rate; FCR, feed conversion ratio. Values are means and standard error of the mean (SEM). Means within the same row not sharing a common letter are significantly different ($P < 0.05$). ns, not significant, $P > 0.1$; #, tendency $P < 0.10$; * $P < 0.05$; *** $P < 0.001$.

Table 4
Effect of enzyme and probiotic supplementation on nutrient digestibility (ADC, %) of Nile tilapia over 42 days.

Treatment	CON		ENZ			*P*-values		
	CON	PRO	CON	PRO	SEM	ENZ	PRO	ENZ*PRO
Dry matter	67.5	68.2	72.3	71.3	0.88	***	ns	ns
Crude protein	89.1	89.0	89.7	89.4	0.11	***	*	#
Crude fat	87.1[a]	89.8[b]	91.0[bc]	91.6[c]	0.57	***	**	*
Total carbohydrates	55.9	56.1	61.7	59.5	1.39	**	ns	ns
Starch	98.2	98.1	98.5	98.3	0.14	**	ns	ns
NSP	28.6	27.3	38.4	33.6	2.22	***	#	ns
Energy	72.4	72.9	75.9	74.9	0.74	***	ns	ns
Ash	34.0	36.5	52.1	51.1	1.49	***	ns	ns
Phosphorus	41.2	44.4	64.8	64.2	1.51	***	ns	ns
Calcium	14.4[a]	20.9[a]	42.5[b]	40.4[b]	2.66	***	ns	*
Magnesium	46.0	48.4	63.8	63.4	1.52	***	ns	ns

Notes. ENZ, enzyme (effect) supplementation; PRO, probiotic (effect) supplementation; ENZ*PRO, interaction effect; NSP, non-starch polysaccharides. Values are means and standard error of the mean (SEM). Means within the same row not sharing a common letter are significantly different ($P < 0.05$). ns, not significant, $P > 0.1$; #, tendency $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

versus 27.9%; $P < 0.001$). Probiotic supplementation improved fat ADC ($P < 0.05$), but this effect was influenced by enzyme supplementation (interaction; $P < 0.05$). The improvement in fat ADC by probiotics was stronger in the absence of enzymes. The ADC of fat improved from 87.1%, for the CON-CON treatment, to 91.6%, for the ENZ-PRO treatment. Ca ADC was also affected by an interaction between enzymes and probiotics ($P < 0.05$). Probiotic supplementation increased Ca ADC for treatments without enzyme supplementation, but decreased Ca ADC in treatments with enzyme supplementation (Table 4).

3.3. Body composition

Body protein content (g/kg) was unaffected by the dietary treatments ($P > 0.1$; Table 5). Enzyme supplementation reduced the body fat content by 11 g/kg fresh weight ($P < 0.001$). This corresponds to a lower energy content ($P < 0.01$), and a tendency towards a lower DM content, for fish fed diets containing enzymes. Enzyme supplementation had a large impact on the body mineral content. Ash, P, Ca and Mg levels were higher in fish fed diets with enzymes ($P < 0.001$). Compared to the initial body composition, ash and mineral contents were higher in fish fed diets with enzymes, whereas ash and mineral content was lower in fish fed diets without enzymes. Probiotic supplementation resulted in a higher body ash content ($P < 0.05$), although numerically the difference was small. Probiotic supplementation tended to increase body P content ($P = 0.055$). There was no interaction effect between enzymes and probiotics on body composition parameters ($P > 0.1$; Table 5).

3.4. Balances

The nitrogen (N), energy (E) phosphorus (P) and Calcium (Ca) balances, expressed on an individual fish basis as mg/d or kJ/d, are displayed in Table 6. Parallel to the protein digestibility, probiotic

Table 5
Effect of enzymes and probiotic supplementation on body composition (on fresh weight basis, g/kg) of Nile tilapia over 42 days.

Treatment	Initial	CON		ENZ			*P*-values		
		CON	PRO	CON	PRO	SEM	ENZ	PRO	ENZ*PRO
Dry matter	273	284	285	280	279	2.5	#	ns	ns
Crude protein	152	152	152	153	151	1.6	ns	ns	ns
Crude fat	85	99	98	89	86	2.1	***	ns	ns
Energy (kJ/g)	6.9	7.5	7.6	7.1	7.0	0.12	**	ns	ns
Ash	32.7	28.2	28.8	34.5	35.6	0.35	***	*	ns
Phosphorus	5.4	4.6	4.7	5.8	6.0	0.07	***	#	ns
Calcium	8.6	7.1	7.4	9.6	9.7	0.15	***	ns	ns
Magnesium	0.30	0.27	0.27	0.31	0.32	0.005	***	ns	ns

Notes. Initial, initial body composition; ENZ, enzyme (effect) supplementation; PRO, probiotic (effect) supplementation; ENZ*PRO, interaction effect. Values are means and standard error of the mean (SEM). ns, not significant, P > 0.1; #, tendency P < 0.10; * P < 0.05; ** $P < 0.01$; *** $P < 0.001$.

Table 6
Effect of enzyme and probiotic supplementation on nitrogen, energy, phosphorous, calcium and magnesium balances of Nile tilapia over 42 days.

Treatments	CON		ENZ			*P*-values		
	CON	PRO	CON	PRO	SEM	ENZ	PRO	ENZ*PRO
Nitrogen (N) balance (mg/d)								
Gross N intake	85.3	84.7	84.5	84.1	0.000	–	–	–
Digestible N intake (DN)	76.0	75.4	75.9	75.1	0.068	**	***	ns
Branchial and urinary N loss	45.0	42.5	38.3	38.3	0.74	***	ns	ns
Retained (RN)	30.9	32.9	37.5	36.7	0.75	***	ns	#
N efficiency (RN/DN)	40.7	43.6	49.5	48.9	0.98	***	ns	ns
Energy (E) Balance (kJ/d)								
Gross E intake	35.0	35.0	35.2	34.9	0.013	–	–	–
Digestible E intake (DE)	25.3	25.5	26.7	26.1	0.18	***	ns	#
Branchial and urinary E loss	1.1	1.1	1.0	1.0	0.023	**	ns	ns
Metabolisable E	24.2	24.4	25.7	25.2	0.19	***	ns	#
Heat E	14.2	13.6	14.8	14.3	0.32	#	ns	ns
Retained E (RE)	10.0	10.8	10.9	10.9	0.26	#	ns	ns
Retained E as protein	4.6	4.8	5.5	5.4	0.11	***	ns	ns
Retained E as fat	5.4	5.9	5.4	5.4	0.26	ns	ns	ns
E maintenance (MEm)	9.1	8.1	8.7	8.3	0.34	ns	#	ns
E maintenance (kJ/kg$^{0.8}$/d)	86.3	76.0	78.9	75.0	2.90	ns	*	ns
E efficiency (RE/DE, %)	39.4	42.3	41.0	41.6	1.02	ns	ns	ns
Phosphorus (P) balance (mg/d)								
Gross P intake	18.4	18.6	18.6	18.5	0.013	–	–	–
Digestible P intake	7.6	8.3	12.0	11.9	0.20	***	ns	#
Branchial and urinary P loss	2.5	2.5	2.8	2.3	0.24	ns	ns	ns
Retained P	5.1	5.8	9.3	9.6	0.16	***	**	ns
P efficiency (RP/DP, %)	67.5	70.3	76.8	80.9	1.93	***	ns	ns
Calcium (Ca) balance (mg/d)								
Gross Ca intake	18.4	18.6	18.6	18.5	0.013	–	–	–
Digestible Ca intake (DCa)	2.7^{a}	3.9^{a}	7.9^{b}	7.5^{b}	0.35	***	ns	*
Branchial and urinary Ca loss	−5.1	−5.0	−7.6	−8.3	0.44	***	ns	ns
Retained Ca (RCa)	7.7	8.9	15.5	15.8	0.34	***	#	ns
Ca efficiency (RCa/DCa, %)	310	234	197	212	25.1	*	ns	#
Magnesium (Mg) balance (mg/d)								
Gross Mg intake	6.3	6.3	6.3	6.2	0.001	–	–	–
Digestible Mg intake (DMg)	2.9	3.0	4.0	3.9	0.067	***	ns	ns
Branchial and urinary Mg loss	2.6	2.7	3.6	3.5	0.066	***	ns	ns
Retained Mg (RMg)	0.31	0.34	0.48	0.50	0.011	***	#	ns
Ca efficiency (RMg/DMg, %)	10.8	11.2	12.0	12.6	0.35	**	ns	ns

Notes. ENZ, enzyme (effect) supplementation; PRO, probiotic (effect) supplementation; ENZ*PRO, interaction effect; RN, retained nitrogen; DN, digestible nitrogen; RE, retained energy; DE, digestible energy; RP, retained phosphorous; DP, digestible phosporous. Values are means and standard error of the mean (SEM). ns, not significant, $P < 0.1$; #, tendency $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

supplementation affected the DN intake negatively ($P < 0.001$). Besides that, probiotic supplementation did not affect the N balance ($P > 0.1$). Enzyme supplementation reduced branchial and urinary N losses ($P > 0.001$) and increased RN ($P > 0.001$). This pattern of reduced branchial and urinary N loses and increased RN, was reflected by significantly higher (17%) N efficiency in diets containing enzymes ($P < 0.001$). N efficiency was comparable for the ENZ-CON and ENZ-PRO treatments (49.5% and 48.9%); the CON-PRO treatments had a lower N efficiency (43.6%); and the treatments without enzymes or probiotics (CON-CON) had the lowest N efficiency (40.7%). There was a tendency for an interaction effect of enzymes and probiotics on the RN ($P = 0.099$). It was observed that probiotic supplementation increased RN only in treatments without enzyme supplementation (30.9 vs. 32.9 mg/d), not in treatments with enzymes (37.5 vs. 36.7 mg/d).

Enzyme supplementation reduced branchial and urinary E loss ($P < 0.01$) and increased metabolisable E intake ($P < 0.001$). In line with a higher metabolisable E intake, there was a tendency for higher heat E ($P = 0.076$) and RE ($P = 0.061$) with enzyme supplementation. Similar to RN, E retained as protein (RN multiplied by 23.7 kJ/g) was higher for fish fed diets with enzymes, compared to fish fed diets without enzymes. In contrast, E retained as fat was unaffected by any of the dietary treatments ($P > 0.1$). In diets supplemented with enzymes, increased E retained as protein, and unchanged E retained as fat, resulted in leaner fish. Expressed in kJ/kg$^{0.8}$/d, MEm was reduced with probiotic supplementation ($P < 0.05$). Averaged over probiotic treatments, MEm for treatments with and without probiotics was 75.5 and 82.6 kJ/kg$^{0.8}$/d, respectively (Table 6). The energy efficiency (RE/DE) was unaffected by the dietary treatments ($P > 0.1$), ranging between 39.4 and 42.3%.

Parallel to P digestibility, the dietary P intake (DP) increased ($P < 0.05$) by 51% with enzyme supplementation. Despite the higher DP intake, branchial and urinary P loss was unaffected by the dietary treatments ($P > 0.1$). Both enzyme and probiotic supplementation enhanced retained P (RP) ($P < 0.01$); the largest improvement was demonstrated with enzyme supplementation. Enzyme supplementation enhanced the RP (averaged over the control treatments) from 5.5 mg/d (CON-CON and CON-PRO treatments) to 9.4 mg/d (ENZ-CON and ENZ-PRO treatments); whereas probiotic supplementation enhanced the RP (averaged over the enzyme treatments) from 7.2 mg/d (CON-CON and ENZ-CON treatments) to 7.7 mg/d (CON-PRO and ENZ-PRO treatments). The P efficiency (RP/DP) was affected by enzyme supplementation ($P < 0.001$). The lowest P efficiency was found with the CON-CON treatment (67.5%), followed by the CON-PRO (70.3%), ENZ-CON (76.8%) and ENZ-PRO (80.9%) treatments. P balance traits were not affected by an interaction effect between enzymes and probiotics ($P > 0.05$).

Enzyme supplementation affected all Ca balance traits ($P < 0.05$; Table 6). Retained Ca (RCa) for all treatments was higher than the digestible Ca intake (DCa), indicating water-borne Ca uptake, as shown by the negative branchial and urinary Ca losses. Enzyme supplementation enhanced the RCa from 8.3 mg/d (averaged over the control

treatments: CON-CON and CON-PRO) to 15.7 mg/d (averaged over the enzyme treatments: ENZ-CON and ENZ-PRO); whereas probiotic supplementation had a tendency ($P = 0.057$) to increase the RCa. An increase in Ca efficiency with decreasing levels of DCa can be observed. This suggests that for the RCa, the relative contribution of Ca uptake from the water increased with decreasing levels of DCa.

Enzyme supplementation increased all Mg balance traits ($P < 0.05$; Table 6). Enzyme supplementation enhanced the retained Mg (RMg) from 0.31 mg/d (averaged over the control treatments: CON-CON and CON-PRO) to 0.49 mg/d (averaged over the enzyme treatments: ENZ-CON and ENZ-PRO). Probiotics only tended to increase the RMg ($P = 0.082$). The Mg efficiency was low with values ranging between 10.8% (CON-CON) and 12.6% (ENZ-PRO). Mg balance traits were unaffected by an interaction effect between enzymes and probiotics ($P > 0.05$).

4. Discussion

Dietary supplementation of enzymes (phytase and xylanase), as well as probiotics (three strains of *B. amyloliquefaciens*), enhanced growth performance of Nile tilapia. Previous studies, using the same enzymes (xylanase and phytase; but including other dietary enzymes) (Lin et al., 2007; Adeoye et al., 2016a; Maas et al., 2018; Maas et al., 2019) and various probiotics (Aly et al., 2008a; Aly et al., 2008b; Wang et al., 2008; Ayyat et al., 2014; Reda and Selim, 2015; Saputra et al., 2016; Abarike et al., 2018) also observed improvements in growth performance of Nile tilapia. In the present study, the FCR of the CON-CON treatment (without enzymes and probiotics) was high (1.33); fish of a comparable size normally have a FCR of around 0.90 (Amirkolaie et al., 2006; Saravanan et al., 2012; Adeoye et al., 2016b; Maas et al., 2018). This high FCR with the CON-CON treatment can be attributed to the higher dietary NSP level (i.e. lower diet quality), which resulted in a lower dry matter digestibility (67.5%) and thus lower growth rate (Sinha et al., 2011; Maas et al., 2020).

One of the objectives of this study was to explore the synergy between enzyme and probiotic supplementation. The positive effect of both enzymes and probiotics did not result in a synergistic effect on growth. In the present study, the improvement in growth was greater with enzyme supplementation (FCR with ENZ-CON treatment: 1.11) than with probiotics (FCR with CON-PRO treatment: 1.25), compared to the control treatment (FCR with CON-CON treatment: 1.33). The combination of enzymes and probiotics (ENZ-PRO) did not further enhance growth, compared to the enzyme supplemented treatment (ENZ-CON), but maintained the same values for the growth parameters. It was expected that the combination could show an additive or synergistic effect, however this was not observed in this study. Compared to the control treatment (CON-CON), the enzymes alone (ENZ-CON) enhanced the growth rate (g/d) by approximately 20%, when feeding the same diet at the same feeding level (g/d). This effect the enzymes (ENZ-CON) had on the growth rate could have resulted in realizing the maximum growth potential of the diet. A study by Adeoye et al. (2016b) also tested the combined effect of enzymes and probiotics; they found the improvement on growth to be minimal. This could partly be due to the use of a commercial diet, which alone (without enzymes and probiotics) already had a good FCR (0.94). A high nutritional value of the control diet, as in the study of Adeoye et al. (2016b), or a large improvement in nutritional value through enzyme supplementation (present study), may limit any further improvement in diet quality through additional additives, such as other enzymes and probiotics (Cowieson and Bedford, 2009; Maas et al., 2019). In addition, many studies on broilers showed that the effect of probiotics is more effective under challenging conditions (Cao et al., 2013; Zhang et al., 2016). It could be expected that under increasingly challenging conditions (i.e. a commercial setting), the combination could lead to better growth performance. Although the probiotics did not further enhance growth performance in the presence of enzymes, the postulated effects probiotics have, like improvements in immune response, disease resistance and feed intake (Newaj-Fyzul et al., 2014; Hai, 2015; Kuebutornye et al., 2020), remain plausible, as this was not investigated in the present study. In the study of Adeoye et al. (2016b), the effect of probiotics on the intestinal morphology was maintained in the presence of enzymes. Therefore it cannot be concluded, from the present data, whether or not the use of probiotics, in the presence of enzymes, can have positive impacts, such as improvements in gut health.

Like the growth parameters, there were no synergistic effects between enzyme and probiotic supplementation on digestibility. The tendency of probiotics to reduce NSP digestibility could have contributed to lowered nutrient ADCs. The combination of enzymes and probiotics (ENZ-PRO) resulted in the highest fat digestibility (91.6%), although not significantly higher compared to the treatment with only enzymes (ENZ-CON; 91.0%). In this study, fish fed the control treatment (CON-CON) displayed a NSP digestibility of 28.6%. That tilapia can endogenously digest NSP, is in line with recent meta-analysis across studies, where an average NSP digestibility for tilapia of 24.3% was reported (Maas et al., 2020). Supplementation with phytase and xylanase significantly increased the NSP digestibility by approximately 29%. Likewise, Maas et al. (2018) and Maas et al. (2019) showed that the combination of phytase and xylanase can increase NSP digestibility. Contrary to the effect of the enzymes, the probiotics had a tendency to reduce NSP digestibility, from 33.5% (average CON-CON and ENZ-CON) to 30.5% (average PRO-CON and PRO-ENZ). To the best of our knowledge, effects of probiotics on NSP digestibility have not previously been reported in fish. Probiotics (*B. amyloliquefaciens*) are known to affect the gut in various ways (physiology, gut microbiota, production of metabolites, pH, etc.), altering the gut environment (Hai, 2015; Dai et al., 2019; Dawood et al., 2019). Changes in the gut environment may have led to less favourable conditions for the supplemented enzymes (phytase and xylanase) and endogenous digestive enzymes, thereby reducing NSP digestibility. It has been shown that the NSP level can affect the digestibility of nutrients other than NSP (Haidar et al., 2016; Maas et al., 2018; Maas et al., 2020). In addition, enzymes had a strong effect on nutrient digestibility and retention of N, energy and P, which were all numerically higher for the enzyme treatment (ENZ-CON), compared to the probiotic treatment (CON-PRO). If enzymes and probiotics compete for the same substrate, this might explain the lack of additivity.

Although probiotics did not further enhance the growth rate in the presence of enzymes, when supplemented on their own (CON-PRO), probiotics increased the growth rate by approximately 5.5%, compared to the control treatment (CON-CON). The basal diets already contained large amounts of spore forming *Bacillus*, however the inclusion of *B. amyloliquefaciens* still improved growth performance. The improvement in growth rate for the probiotic treatment (CON-PRO) is best explained by looking at the N and energy balances. Probiotics are known to produce digestive enzymes and to stimulate enzyme activity (amylases, proteases, lipases, etc.), which can result in enhanced nutrient digestibility (Chen et al., 2016; Dawood et al., 2019; Makled et al., 2019; Kuebutornye et al., 2020). In the present study, it is notable that probiotic supplementation resulted in a strong increase in fat digestibility, whilst digestibility of other nutrients did not increase. Protein digestibility was actually negatively affected by the presence of probiotics. This suggests that a factor other than the production of digestive enzymes/stimulation of enzyme activity, is responsible for the increase in fat digestibility, for instance, changes in fat emulsification or chyme characteristics. The average energy requirement for maintenance (MEm) was 79.1 ± 4.4 kJ/kg$^{0.8}$/d, which is considerably higher than estimated by some other studies (Lupatsch et al., 2010; Schrama et al., 2012; Maas et al., 2018), where the MEm ranged between 54.7 and 64.1 kJ/kg$^{0.8}$/d. Saravanan et al. (2013) showed that the diet can have a strong effect on the MEm, when the dietary electrolyte balance disturbs the acid-base homeostasis, resulting in an increase in MEm from 57 to 88 kJ/kg$^{0.8}$/d. In the study by Haidar et al. (2016), the average estimated MEm was high (103 kJ/kg$^{0.8}$/d); here an increase in dietary NSP level increased the MEm by 14%, suggesting that the energy cost of digestion increases with higher levels of NSP (and its subsequent effect

on the intestinal microbial balance).

Probiotics can affect the gut microbiota, as well as the gut barrier function (Balcázar et al., 2007; Nayak, 2010; Jutfelt, 2011; Ige, 2013; Zhou et al., 2013), which might have affected the energy requirements for maintenance (i.e. through reduced cost of digestion). What caused the effect of probiotics on the MEm in the present study remains unclear and needs further investigation. The probiotic treatment (CON-PRO) retained about 8% more energy, compared to fish on the control treatment (CON-CON), however this was not statistically significant. The retained E (kJ/d) was highly comparable between the CON-PRO (10.8), ENZ-CON (10.9) and ENZ-PRO (10.9) treatments. Although the digestible N intake was numerically lower for the probiotic treatment (CON-PRO), compared to the control treatment (CON-CON), the retained N, and thus the N efficiency, was higher (numerically) for the probiotic treatment (43.6%) compared to the control treatment (40.7%). Likewise, both El-Haroun et al. (2006) and Makled et al. (2019) showed an improved protein efficiency ratio (PER) in Nile tilapia, when feeding diets supplemented with probiotics.

Besides the significant effect of enzymes on nutrient digestibility, both the N retention and N efficiency were increased, by approximately 20%, for the treatments containing enzymes (ENZ-CON and ENZ-PRO), compared to the control treatment (CON-CON). This significant effect of enzymes on the N balance is in accordance with the substantial improvement (+ 20%) in growth rate. As N retention comes simultaneously with water gain, the effect of increasing N retention is large. This explains the 13% higher growth rate with enzyme supplemented diets, compared to diets supplemented with probiotics (CON-PRO), despite the comparable energy retention. An increase in N retention and efficiency, with the use of enzymes (phytase and xylanase), was previously observed in Maas et al. (2018), however the cause of this increased N efficiency is unclear.

It is uncertain from the results, whether the available dietary P levels were sufficient to sustain maximal body P content. The amount of P in the diets was 11 g/kg DM feed, which is considerably higher than the recommended level of 4.0 g/kg (according to the NRC, 2011). The diets were formulated to have an available P level of 4.7 g/kg DM feed. This was to meet the P requirements for growth and for attaining maximal whole-body P concentration, in accordance with meta-analysis across fish species (excluding Rainbow trout) by Prabhu et al. (2013). In this study, the calculated available P (g/kg DM) (using Tables 2 and 5) for the different treatments was: 4.5 (CON-CON), 4.9 (CON-PRO), 7.1 (ENZ-CON) and 7.1 (ENZ-PRO). The P availability with the enzyme supplemented treatments was higher, subsequently both the P retention (absolute, mg/d; Table 6), as well as the body P content (g/kg; Table 5), increased. This indicates that the available P levels in the diets without enzymes were too low to sustain maximal whole body P concentrations. The relationship between available dietary P levels (g/kg DM) and whole-body P content (g/kg live weight) was extrapolated according to a linear broken line model, by Prabhu et al. (2013). Prabhu et al. (2013) suggest that, above the level required to sustain maximal whole P concentrations, P will be excreted (branchial and urinary P loss) thereby reducing the P efficiency (retained P/digestible P). However, in the present study, fish retained relatively more P with increasing available P levels, resulting in a higher P efficiency. The average P efficiency for the enzyme treatments was 78.9%, in comparison to 68.9% for the treatments without enzymes (Table 6). It cannot be speculated whether the available P levels, in the enzyme treatments, were above the level required to sustain maximal body P content. Maas et al. (2018) found that the P efficiency stayed rather constant with increasing available P levels (using phytase), showing a high average value of 92%.

To summarise, both enzymes and probiotics enhanced growth performance, with the largest improvement due to enzymes. The enzymes improved nutrient digestibility of all nutrients, whereas probiotics enhanced fat digestibility. Besides affecting nutrient digestibility, enzymes stimulated a significant increase in retained N and P, and N efficiency. Probiotics reduced the energy requirements for maintenance, thereby increasing retained energy (numerically). Individually, both enzymes and probiotics had positive effects on the measured parameters, however a synergistic or complementary mode of action between enzymes and probiotics was not observed in this study.

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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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