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Fishmeal hydrolysation and non-protein energy sources affect the kinetics of nutrient digestion in the gastrointestinal tract of African catfish (*Clarias gariepinus*)

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ABSTRACT

The kinetics of nutrients digestion and development of chyme characteristics in African catfish (*Clarias gariepinus*) were assessed in response to fishmeal hydrolysation and non-protein energy (NPE) sources. Four diets were formulated to contain starch or fat as NPE source, and fishmeal or hydrolysed fishmeal as protein source in a 2 × 2 factorial design. Juvenile African catfish (average weight, 63 g) were stocked in glass experimental tanks connected to a common recirculation aquaculture system and were fed restrictively for 3 weeks. Four hours after the consumption of a single meal, fish were dissected to collect chyme from the gastrointestinal tract (GIT). Chyme was collected from stomach, proximal- and distal intestine and analysed for dry matter (DM) content, crude protein (CP) and marker concentration. Postprandial water fluxes to the GIT and stomach evacuation were calculated using yttrium oxide (Y_2O_3) as an inert marker. Faecal DM and CP apparent digestibility coefficient (ADC) were determined by a marker method. Results showed that fishmeal hydrolysation had no effect ($P > 0.1$) on the DM content in all the GIT compartments. However, replacing dietary fat by starch resulted in a higher DM content in the stomach ($P < 0.01$). In the proximal intestine, NPE did not influence the chyme DM content ($P > 0.1$) but in the distal intestine, chyme DM was higher at the “fat diets” ($P < 0.01$). “Starch-diets” had a larger water influx into the stomach compared to “fat diets” ($P < 0.001$), but also a larger water re-absorption in the distal intestine ($P < 0.05$). The inert marker and DM evacuation rate from the stomach was affected by NPE source ($P < 0.05$) and was slower in the starch-fed fish. Hydrolysation of fishmeal increased the digestibility of CP in the stomach, but this effect of hydrolysation was dependent on the energy source, indicated by the interaction effect ($P < 0.05$). The increase in digestibility of CP in the stomach of the diets containing hydrolysed fishmeal was larger at the “fat diets”. In the other GIT compartments, CP digestibility were similar between diets ($P > 0.1$). However, hydrolysation of fishmeal had no effect on faecal ADC of CP. Our results suggest that the hydrolysation of fishmeal can alter the process of digestion along the GIT. In addition, dietary macronutrient composition can alter the postprandial digestion of nutrients in the GIT without being reflected in the faecal digestibility.

1. Introduction

Fish diets are usually formulated based on the faecal nutrient digestibility of ingredients, which only accounts for the total amount of dietary nutrients that was apparently digested and assumed to be absorbed along the gastrointestinal tract (GIT) (Chen, 2017; NRC, 2011). This does not take into consideration the kinetics of nutrients digestion along the GIT. In pig and poultry nutrition, more attention is now directed towards assessing chyme characteristics, nutrient passage rates and the degree of absorption along the GIT after feed ingestion. This is because ileal and faecal digestibility of nutrients differ among feed ingredients (Chen, 2017). Next to nutrient digestibility, it has been shown in poultry that the digestion kinetics of nutrient influences feed utilization (Liu et al., 2013). A poultry study revealed that the starch

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Abbreviations: NPE, non-protein energy; GIT, gastrointestinal tract; ADC, apparent digestibility coefficient; DM, dry matter.

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digestion rate is higher than the protein digestion rate. In the same study, reducing the starch digestion rate by altering dietary composition increased nitrogen retention and reduced FCR without affecting the total starch digestibility. (Liu et al., 2013). It was hypothesised that slowly digestible starch reduced the catabolism of amino acids by enterocytes (Liu and Selle, 2015). Knowledge on the dynamics of nutrients could be applied by nutritionist to formulate balanced diets that can improve nitrogen retention and better FCR.

In fish, only few studies have investigated diet-elicited effects on digestion of nutrients in different compartments of the GIT (i.e., stomach, proximal-, mid- and distal intestine) (Harter et al., 2015; Maas et al., 2021; Tran-Tu et al., 2019). Dietary fat replacement by starch reduced protein disappearance in the stomach (Harter et al., 2015), which coincided with a lower stomach dry matter content and increased water influxes in the stomach of African catfish (*C. gariepinus*) (Harter et al., 2013). Increasing the dietary viscosity (induced by guar gum addition) decreased the digestibility of protein and dry matter in all GIT compartments of striped catfish (*Pangasianodon hypophthalmus*), while chyme dry matter content declined and chyme viscosity increased only in the stomach (Tran-Tu et al., 2019). In Nile tilapia (*Oreochromis niloticus)*, diets supplemented with an enzyme cocktail (phytase and xylanase) had an improved faecal protein digestibility, which occurred from the proximal intestine onward (Maas et al., 2021). In this same study on Nile tilapia, dietary probiotic supplementation increased the disappearance of protein in the stomach while it reduced the faecal protein digestibility, but the differences were small (Maas et al., 2021). Starch is now increasingly used to replace fat as a cheap non-energy protein source due to its availability and low cost (Harter et al., 2015). African catfish and several other omnivorous species can readily handle high inclusions of carbohydrates (Belal, 1999; Bureau et al., 1995; Kirchgessner et al., 1986). However, during starch digestion, a large amount of osmotically active mono- and disaccharides are produced (Harter et al., 2013), but quantitative information on the starch hydrolysis throughout the GIT is lacking. Bucking and Wood (2006) have suggested that these compounds drive the addition of water to the GIT, which is reflected in an increased water influx in the stomach of fish (Harter et al., 2013). Furthermore, starch induces viscosity in the stomach of fish, which often goes together with a lower chyme dry matter content but mainly in the stomach and proximal intestine (Amirkolaie et al., 2006; Harter et al., 2013; Harter et al., 2015; Leenhouwers et al., 2007a). Despite these research efforts in addressing the impact of dietary characteristics on digestion, most studies have mainly focused on dietary non-protein energy sources. They, however, do not provide information on the kinetics of protein digestion as affected by dietary protein sources in the GIT of fish. Such information is important to understand the interaction of dietary protein and energy on nutrient passage dynamics in the GIT, especially now that increasing amounts of less expensive and more sustainable alternative protein ingredients are used to replace fishmeal in aquafeeds.

Fishmeal is in many cases superior regarding attractability, palatability and bioavailability of nutrients compared to alternative protein sources in aquafeeds. Hydrolysation of protein sources can increase the attractability, palatability and/or bioavailability of nutrients in ingredients (Silva et al., 2017). Several studies have demonstrated that hydrolysation of protein sources increases the nutritional values as it enhances growth without adversely affecting protein quality. This positive effect of hydrolysation has been shown for both animal (e.g., fish, shrimp, milk & feather meal) and plant protein sources (e.g., rapeseed, cottonseed, wheat gluten and soybean meal) (Gui et al., 2010; Leal et al., 2010; Muranova et al., 2017; Siddik et al., 2021; Xu et al., 2017; Yuan et al., 2019). Fish protein hydrolysates are products that originated from the conversion of inexpensive and underutilized fish by-products into a commercially valuable protein ingredient by the action of enzymatic hydrolysis. The use of fish protein hydrolysate has gained great attention by fish nutritionists due to its nutritional composition, amino acid profile and antioxidant properties (Chalamaiah et al., 2012; Swanepoel and Goosen, 2018). Partial replacement of fishmeal by fishmeal hydrolysate in the diet of African catfish improved growth and feed efficiency (Swanepoel and Goosen, 2018). In humans, protein hydrolysates resulted in a faster postprandial increase of plasma AAs than their non-hydrolysed equivalents, which suggests a quicker absorption of AA in the GIT (Morifuji et al., 2010). This implies that the passage rate of fishmeal can be altered by the process of hydrolysation thereby influencing chyme characteristics and faecal digestibility.

The overall kinetics of dietary protein digestion is related to the passage rate of digesta along the GIT, which depends on the physicochemical properties (e.g. solubility, viscosity, water binding capacity) of the digesta (Chen, 2017). Therefore, understanding the difference in digestion and absorption rates of nutrients (e.g., energy and protein) along the GIT as affected by ingredient characteristics is important for understanding the differences in faecal ADC and thus for formulating balanced aquafeeds. In relation to the observed effects of fat replacement with starch, we hypothesised that the hydrolysation of dietary protein (fishmeal in this study) increases the protein digestion in the proximal intestine. It was expected that hydrolysation of fishmeal would lead to a more rapid stomach evacuation as compared to the non-hydrolysed fishmeal, which might relate to alteration in chyme characteristics. Furthermore, we proposed that these effects might be affected by the type dietary non-energy protein (i.e., fat vs starch).

To substantiate these assumptions, four diets were formulated to contain two types of energy sources (fat vs starch) and protein sources (fishmeal vs hydrolysed fishmeal). These diets were fed to African catfish with the aim to (1) assess the effect of fishmeal hydrolysation on chyme characteristics in different segments of the GIT, (2) investigate the water balance in the GIT in response to dietary macronutrients, and (3) determine potential interactions between fishmeal hydrolysation and dietary non-protein energy sources on the kinetics of digestion in African catfish.

2. Materials and methods

2.1. Ethics statement and research facility

The study (project number 2018.W.0014.003) was carried out in accordance with the Dutch law on the use of animals (Act on Animal Experiments) for scientific purposes and was approved by the Central Animal Experiments Committee (CCD) of The Netherlands. This experiment was conducted in the research facility of CARUS-ARF at Wageningen University, The Netherlands. Fish were kept and handled in agreement with EU-legislation.

2.2. Experimental diets

This study aimed to examine the effect of fishmeal hydrolyzation and non-protein energy sources on the chyme characteristics and digestion kinetics in African catfish. Therefore, four diets were formulated according to a 2 by 2 factorial design which differed in protein sources (fishmeal or fishmeal hydrolysate, respectively NH-FM versus H-FM)) and type of non-protein energy sources (fat or starch, respectively FD versus SD). The four experimental diets were extruded with a 1.7 die size into 3 to 3.5 mm pellets by Skretting ARC Norway using a twin-screw extruder (Wenger, Sabetha, KS, U.S.A). Meal mixes were preconditioned for 80 s resulting in an outlet dough temperature of 70 °C for all diets. Die and barrel temperature were equal within each diet production run and was 65, 70, 60 and 70 °C for diets “NH-FM + SD”, “NH-FM + FD”, “H-FM + SD” and “H-FM + FD”, respectively. The extrusion resulted in a pellet width of 3.5, 2.1, 3.5 and 2.2 mm, a pellet length of 2.7, 3.0, 2.9 and 3.8 mm and a bulk density (directly after extrusion) of 380, 390, 420 and 420 g/L for the “NH-FM + SD”, “NH-FM + FD”, “H-FM + SD” and “H-FM + FD” diet, respectively. Diets were formulated to be iso-nitrogenous and iso-energetic. The difference between the starch and fat diets was created by replacing 320 g wheat starch by 121.5 g rapeseed oil. These amounts of both ingredients provide the same amount of gross energy (GE). Cellulose was added to the fat diets to compensate for the higher energy content of rapeseed oil. The ingredients and analysed chemical composition of the diets are given in Table 1. Hydrolysis of the fishmeal was performed using a proprietary enzymatic process. After undergoing hydrolysis, the hydrolysate was transferred to a storage tank from where it was continuously pumped into the preconditioner. Enzyme activity was completely stopped once the enzymes were exposed to the high temperatures in the preconditioner. Yttrium oxide (Y_2O_3) was included in all diets as inert marker for measuring water fluxes, DM and protein digestion along the GIT. Feeds were kept in cold storage at 4 °C throughout the experiment and a representative sample was taken for analysis.

2.3. Fish and housing conditions

Juvenile African catfish (*C. gariepinus*) of an average individual body weight of 63 g were obtained from a commercial hatchery (Fleuren & Nooijen BV, Nederweert, The Netherlands). The fish comprised of a mixed-sex population. Two weeks prior to the experiment, fish were fed a commercial diet to adapt to the experimental conditions. At the start of the experiment, fish were randomly stocked (40 fish per tank) into 12 aquaria (200 L) connected to a common recirculation aquaculture system (comprising of a trickling filter, sump and drum filter). Each tank was equipped with air stones and swirl separators (AquaOptima AS, column height 44 cm; diameter 24.5 cm) for the collection of faeces and spilled pellets. The water flow rate was set at 7 L/min, temperature was 28 °C and the photoperiod regime was kept at 12 h light: 12 h dark. Water quality parameters were monitored regularly and maintained at the optimal levels for African catfish: pH, 7.5 ± 0.30; ammonium, 0 ± 0.00 mg/L; nitrite, 0.12 ± 0.075 mg/L; nitrate, 175 ± 82 mg/L; conductivity, 3287 ± 848 μS; and dissolved oxygen concentration, 6.21 ± 0.37 mg/L. Water refreshment was performed based on NO_3 removal from the system to keep NO_3 levels within limits (< 500 mg/L).

Table 1
Formulation and proximate composition of the experimental diets.

%	NH-FM + SD	NH-FM + FD	H-FM + SD	H-FM + FD
Fishmeal	35.17	35.17	17.59	17.59
Fishmeal hydrolysate	0.00	0.00	17.59	17.59
Wheat	15.09	15.10	15.09	15.10
Gelatinized wheat starch	32.00	0.00	32.00	0.00
Rapeseed oil	0.00	12.15	0.00	12.15
Fish oil	3.00	3.00	3.00	3.00
Wheat bran	15.00	15.00	15.00	15.00
Moisture loss[1]	−2.58	0.00	−2.58	0.00
Yttrium oxide	0.10	0.10	0.10	0.10
Mineral & vitamin premix	0.22	0.22	0.22	0.22
Cellulose[2]	2.00	19.26	2.00	19.26
Analysed proximate composition (g/kg dry matter)				
Dry matter	911	927	891	914
Crude protein	325	312	311	306
Crude fat	88	218	85	227
Ash	62	61	61	61
Phosphorus	11.1	10.7	10.7	10.4
Calcium	11.0	11.0	10.8	10.5
Magnesium	1.9	1.8	1.8	1.8
Total carbohydrate	525	409	543	407
Energy (kJ/g)	20.3	23.4	20.0	23.3
Energy excl. cellulose (kJ/g)[3]	20.0	20.1	19.7	20.0

NH-FM, non-hydrolysed fishmeal; H-FM, hydrolysed fishmeal; SD, starch diet; FD, fat diet.

[1] The production of NH-FM + SD and H-FM + SD diets was targeted at a dry matter content of 92%, which resulted in an expected water loss during extrusion of 2.58%.

[2] Qualicel® pc 150 (CFF GmbH & Co. KG, Gehren, Germany).

[3] The calculated energy content excluding the energy from the added cellulose.

2.4. Experimental procedure

The four diets were randomly assigned (in triplicate) to the twelve aquaria. To prevent variability in measurements due to differences in feed intake, fish were hand-fed restrictively in the morning (8:00 h) and afternoon (16:00 h) for 3 weeks. Feeding level was fixed at 19.8 $g/kg^{0.8}/d$ to rule out the effects of feeding level on the chyme characteristics. The daily ration was increased throughout the experimental period by predicting growth using a FCR of 1. During the first week, feeding level was gradually increased from 20% to 100% of the intended ration to allow adaptation to diets. In the case of mortality, the feeding list of the respective tank was adjusted for the remaining number of fish to maintain equal feed intake among treatments. After each meal, the uneaten and spilled pellets were collected and counted for the accurate determination of feed intake. Faeces were collected twice daily from week 2 till the end of the trial, using swirl separators attached to each experimental tank. The faecal collection bottles underneath the swirl separators were suspended in ice to minimize bacterial degradation. Faeces samples were pooled per tank and frozen (−20 °C) until chemical analysis.

At the end of the experiment, thirteen fish were randomly selected from each tank for collecting chyme samples. Sampling was done 4 h after feeding a single meal. These fish were euthanized using an overdose of 2-phenoxy-ethanol (3.0 mL/L). Fish were individually weighed and dissected to collect chyme from the gastrointestinal tract (GIT). For this sampling, the GIT was divided into three compartments: stomach and two equal parts of the gut, representing proximal and distal intestine. Chyme samples were pooled per tank (i.e., 13 fish) and per compartments, weighed (to determine weight wet) and frozen (−20 °C) for further analysis.

2.5. Sample analysis

Analyses were performed on the diets, chyme and faeces samples. Feed pellets were grinded. Faecal and chyme samples were freeze-dried, then manually pulverized through a 1 mm screen sieve. The chemical analysis of the feed and faeces were performed in triplicate using the same methods while chyme was analysed in duplicate. The dry matter (DM) was determined by drying at 103 °C in the oven (ISO 6496, 1983) while ash was determined after furnacing for 4 h at 550 °C (ISO 5984, 1978). Crude protein of feed and faeces was quantified according to the Kjeldahl method (ISO 5983, 1979), while the crude protein content of chyme was quantified using the DUMAS method due to the limited amount of chyme samples. Crude fat was determined by differences following extraction with petroleum ether at 40 °C – 60 °C in a Soxhlet apparatus (ISO 6492, 1999) and energy content was quantified using an adiabatic bomb-calorimeter (C7000 IKA®, IKA analysentechnik, Weitershem, Germany; ISO 9831, 1998). Yttrium, phosphorus, calcium and magnesium were detected by using inducted coupled plasma mass spectrometry according to the standard NEN 15510 (ICP-MS, 2007).

2.6. Calculations

The apparent digestibility coefficient (ADC) of nutrients in faeces were calculated according to the formula described by Cheng and Hardy (2002) using yttrium oxide as inert marker; ADC (%) = 100 × [1 − (yttrium concentration in the feed × nutrient concentration in the faeces)/(yttrium concentration in the faeces × nutrient concentration in feed)]. Nutrient ADC per compartment was calculated as follows; ADC (%) = 100 × [1 − (yttrium concentration in the feed × nutrient concentration in the chyme)/(yttrium concentration in the chyme × nutrient concentration in feed)]. The concentrations of yttrium and nutrients were expressed on DM basis. Total carbohydrate was calculated as dry matter minus crude protein minus crude fat minus ash content.

Stomach evacuation rates (g DM/h) were calculated as the amount of dry matter of the ingested feed minus the chyme content (DM) of the stomach divided by the time since the last feeding. The chyme dry matter (g DM mg/yttrium) was calculated as ingested DM on the sampling day divided by the yttrium content (mg) of the ingested feed. Water flux was calculated according the formula described by Harter et al. (2013). The relative water fluxes (mL/g of ingested DM) were calculated from the relative water content of the chyme in one compartment minus the relative water content in the previous compartment divided by the relative ingested DM. Where, relative water content of chyme (g) was expressed in relation to its marker content (mg). This was calculated from the wet weight and DM content of chyme and then converted into a volumetric measure (mL) (assuming that 1 g of water had a volume of 1 mL under the tested conditions).

2.7. Statistical analysis

Data analyses were performed by using SPSS Statistics, version 23.0 for Windows (IBM Corp., Armonk, NY, USA). Nutrient ADCs, chyme characteristics within compartments and stomach evacuation were analysed by two-way ANOVA. Following a significant ANOVA result, means were compared by a multiple comparisons test using Tukey's honest significant difference (HSD).

3. Results

Results for the dry matter (DM) content of chyme in each GIT compartment are depicted in Fig. 1. The average DM content of "starch-diets" was highest in the stomach (0.18 kg/kg); decreased towards the proximal intestine (0.11 kg/kg) and slightly increased in the distal intestine (0.14 kg/kg). Likewise, the DM content of "fat-diets" was highest in the stomach (0.22 kg/kg); decreased towards the proximal intestine (0.12 kg/kg) and slightly increased in the distal intestine (0.16 kg/kg). The stomach chyme DM differed between diets having different non-protein energy sources (NPE) ($P < 0.01$). Fish fed "fat-diets" had higher chyme DM in the stomach compared to those fed "starch-diets". However, NPE did not influence the chyme DM content ($P > 0.1$) in the proximal intestine. In the distal intestine, the DM content was higher ($P < 0.05$) in the fish fed "fat-diets" compared to "starch-diets". The hydrolysation of fishmeal had no effect ($P > 0.1$) on the DM content in all the GIT compartments. Overall, there was no significant interaction between fishmeal hydrolysation and NPE on DM contents of both diets in all the compartments.

Results for the relative water fluxes (RWF) in the GIT are illustrated in Fig. 2. Water fluxes differ between compartments. Within each compartment, no significant interaction between fishmeal hydrolysation and NPE was detected. Water fluxes to the stomach was influenced by the NPE ($P < 0.001$), while a tendency ($P < 0.1$) of the effect of fishmeal hydrolysation was observed. Relative water additions to the stomach were higher ($P < 0.001$) in fish fed "starch-diets" compared to "fat-diets", having 4.07 and 3.15 mL of water added per g of ingested DM, respectively. Both dietary treatments did not influence the water fluxes ($P > 0.1$) in the proximal intestine. When comparing water fluxes in the stomach with proximal intestine, results suggested that the water addition decreased as the chyme progressed distally. The sum of relative water influx to the proximal intestine was not significantly different between diets but numerically higher for "starch-diets" (3.01 mL/g of ingested DM) compared to "fat-diets" (2.24 mL/g of ingested DM). NPE affected ($P < 0.05$) the water fluxes in the distal intestine. A higher water re-absorption in the distal part of the intestine was observed in fish fed "starch-diets" compared to "fat-diets", which was reflected by the relative water flux of −4.64 and − 3.05 mL/g of ingested DM, respectively.

The results for stomach evacuation rate are presented in Table 2. During the first 4 h postprandial, the rate of yttrium evacuation from the stomach was higher ($P < 0.05$) for "fat-diets" (15.3% Y/h) compared to "starch-diets" (12.6% Y/h). Crude protein (CP) evacuation rate from the stomach, was affected by the fishmeal hydrolysation ($P < 0.05$), but not by the type of NPE ($P > 0.1$). The evacuation rate of CP was higher for the hydrolysed fishmeal diets (23.5% CP/h) than for the non-hydrolysed fishmeal (23.0% CP/h). CP left the stomach at a faster rate compared to yttrium (average over all diets, 23.3 CP/h vs. 14.0% Y/h). Similarly, a slightly higher evacuation rate was recorded for DM (average over all diets, 16% DM/h) compared to yttrium. Furthermore, NPE had an effect ($P < 0.05$) on the DM evacuation from the stomach, with "fat-diets" having a higher evacuation rate than "starch-diets". The total yttrium loss (% feed excreted) was calculated (Supplementary Table C). Averaged over diets, 71.40% of the yttrium consumed was found in all compartment after 4 h postprandial. This implies that 4 h after feeding, 28.59% of the consumed yttrium was already excreted via the faeces. However, the amount of faecal yttrium loss was not affected by fishmeal hydrolysation but differed between NPE sources ($P < 0.05$), which was higher for "fat-diets" (37.00% feed excreted) compared to "starch-diets" (20.18% feed excreted).

Apparent digestibility coefficients (ADC) of DM and CP along the GIT are visualised in Fig. 3 & 4 respectively. Hydrolysation of fishmeal increased the digestibility of CP in the stomach and this was dependent on the energy source, as indicated by the interaction between NPE and

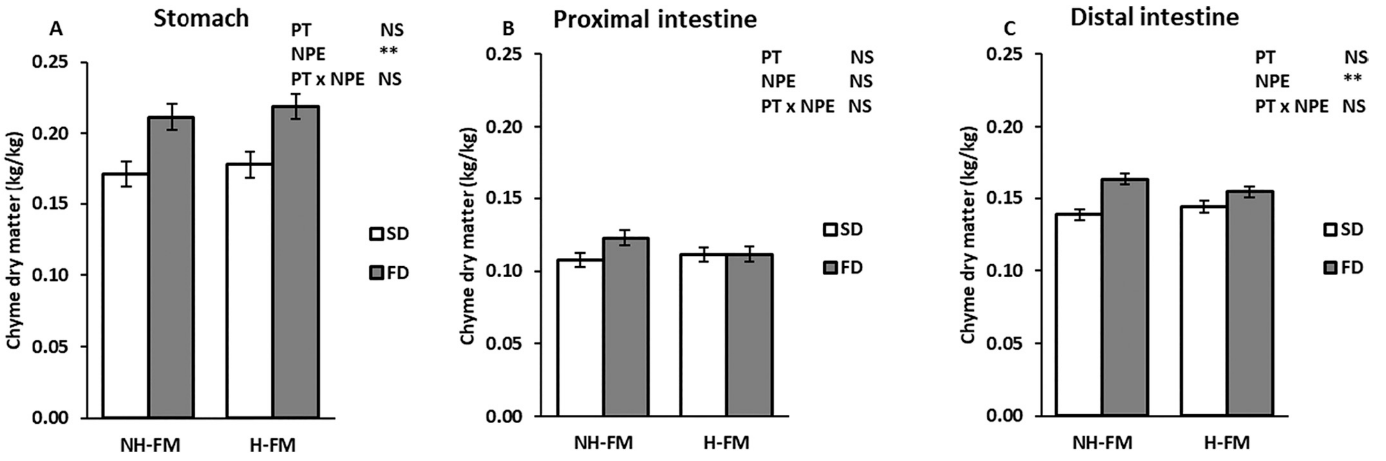

Fig. 1. Dry matter (DM) content of chyme (kg/kg), measured in the stomach, proximal intestine and distal intestine of African catfish. NH-FM, control fishmeal; H-FM, hydrolysed fishmeal; SD, starch diet; FD, fat diet. Presented values are means ($n = 3$) per dietary treatment within each compartment, except for the NH-FM + FD diet in the proximal and distal intestine ($n = 2$). Error bars indicate standard error of means; PT, protein type (non-hydrolysed fishmeal and hydrolysed fishmeal); NPE, non-protein energy source (starch and fat); NS, not significant; **, $P < 0.01$.

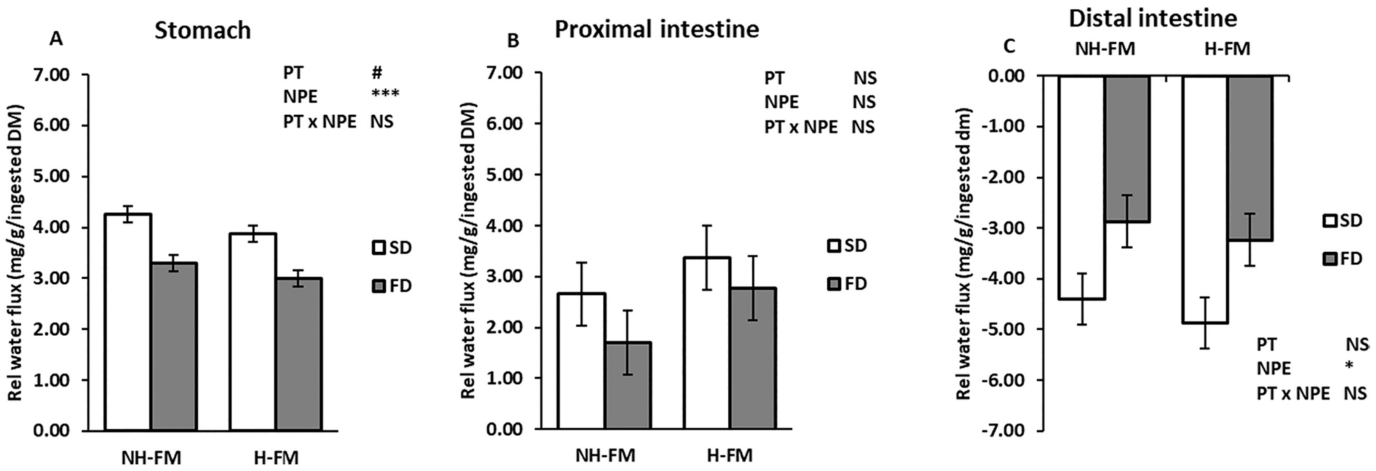

Fig. 2. Relative water flux (mL/g ingested DM), measured in the stomach, proximal intestine and distal intestine of African catfish; NH-FM, control fishmeal; H-FM, hydrolysed fishmeal; SD, starch diet; FD, fat diet. Presented values are means ($n = 3$) per dietary treatment within each compartment, except for the NH-FM + FD diet in the proximal and distal intestine ($n = 2$). Error bars indicate standard error of means; PT, protein type (non-hydrolysed fishmeal and hydrolysed fishmeal); NPE, non-protein energy source (starch and fat); NS, not significant; #, $P < 0.1$; *, $P < 0.05$; ***, $P < 0.001$.

Table 2
Stomach evacuation rate of African catfish after 4 h of feeding diets containing different protein and energy sources.

	Diet							
	Control fishmeal		Hydrolysed fishmeal			*P*-value		
Evacuation	SD	FD	SD	FD	SEM	PT	NPE	PT x NPE
DM (% DM/h)	14.35	16.70	15.47	17.39	0.88	NS	*	NS
CP (% CP/h)	23.17	22.85	23.55	23.49	0.21	*	NS	NS
Yttrium (% Y/h)	12.16	15.10	13.03	15.52	1.08	NS	*	NS

DM. dry matter; Y, yttrium; h, hour; SD, starch diet; FD, fat diet; PT, protein type (non-hydrolysed fishmeal and hydrolysed fishmeal); NPE, non-protein energy source (starch and fat); SEM, standard error of means; NS, not significant; *, $P < 0.05$.

fishmeal hydrolysation ($P < 0.05$). Whereas, both dietary treatments had no significant effect on ADC of CP in the proximal and distal intestine ($P > 0.1$). The fish fed “starch-diets” had higher (38.99%) CP digestibility in the stomach compared to the “fat-diets” (26.76%). Regarding DM digestibility, no interaction effect ($P > 0.1$) of fishmeal hydrolysation and NPE was detected in the stomach. However, NPE sources independently showed a significant effect on ADC of DM in the proximal ($P < 0.05$) and distal intestine ($P < 0.01$). In the proximal intestine, a significantly lower DM digestibility was observed for “starch-diets” (12.18%) compared to the “fat-diets” (26.32%). However, the reverse was the case in the distal intestine with “starch-diets” showing higher values (58.10%) than “fat-diets” (51.25%). Kinetics of digestion of crude ash, phosphorous, calcium and magnesium are provided in Supplementary Table A. The ADC of P increased from stomach towards the distal intestine, but the major part of the digested P already disappeared in the stomach. ADC of P was not different between diets in any gut segments. ADC of Ca averaged over diets was 47, 12 and 25% in stomach, proximal- and distal intestine, respectively. ADC of Ca in the stomach was higher for “starch-diets” than for “fat diets” ($P < 0.01$), while in the proximal intestine, the ADC of Ca was highest at the “fat diets” ($P < 0.05$). In the distal intestine, no differences in ADC of Ca between diets were present. The ADC of Mg in the different gut segments fully paralleled the pattern in ADC of Ca (Supplementary Table A). ADC of P, Ca, and Mg was unaffected by fishmeal hydrolysation in all gut segments ($P > 0.1$).

The result of faecal digestibility showed some similarities with the outcome of the proximal and distal intestine, with no effect ($P > 0.1$) of fishmeal hydrolysation on both ADC of DM and CP (Fig. 3D and 4D). However, the ADC of DM was significantly affected by the dietary energy sources where “fat diets” yielded a lower DM faecal ADC ($P < 0.01$). In contrast, fish fed “fat-diets” showed a higher faecal ADC of CP ($P < 0.001$) but the difference was minor (86.86% for starch and 88.89% for fat diets) (Fig. 4D). There was no interaction effect of fishmeal hydrolysation and energy type on faecal ADC of DM and CP ($P > 0.1$). ADC data of fat, crude ash, energy, calcium, phosphorous and magnesium are given in Supplementary Table B.

4. Discussion

DM content of the chyme was highest in the stomach, decreased in the proximal intestine and slightly increased in the distal intestine. In the same light, most water was added to the stomach and less to the proximal intestine while in the distal intestine, water was re-absorbed. Harter et al. (2013) also reported similar results for African catfish as well as Bucking and Wood (2006) for rainbow trout (*Oncorhynchus mykiss*). The study of Bucking and Wood (2006) on water fluxes in the GIT of rainbow trout revealed a large addition of water to the stomach over the first 12 h after feeding a single meal. Addition of large amounts of water to the stomach is due to the physiological demands resulting from the consumption of dry diets by fish (Bucking and Wood, 2006). This water influx may be of exogenous (postprandial drinking) or endogenous (addition of digestive juices) origin. Although, freshwater (FW) fish are known to be hyperosmotic to their environment and to drink less water than marine fish (Perrott et al., 1992), water uptake in their intestine can be controlled in the same way as marine species do. In addition, FW fish are able to regulate the influx of interstitial water to the stomach based on their nutritional demands (Harter et al., 2013). The lower addition of water to the proximal intestine can be explained by the previous water influx to the stomach. In a study investigating water fluxes in African catfish (Harter et al., 2013), 59% of water in the proximal intestine originated from the stomach, which led to a decreased water addition in this compartment to compensate for the

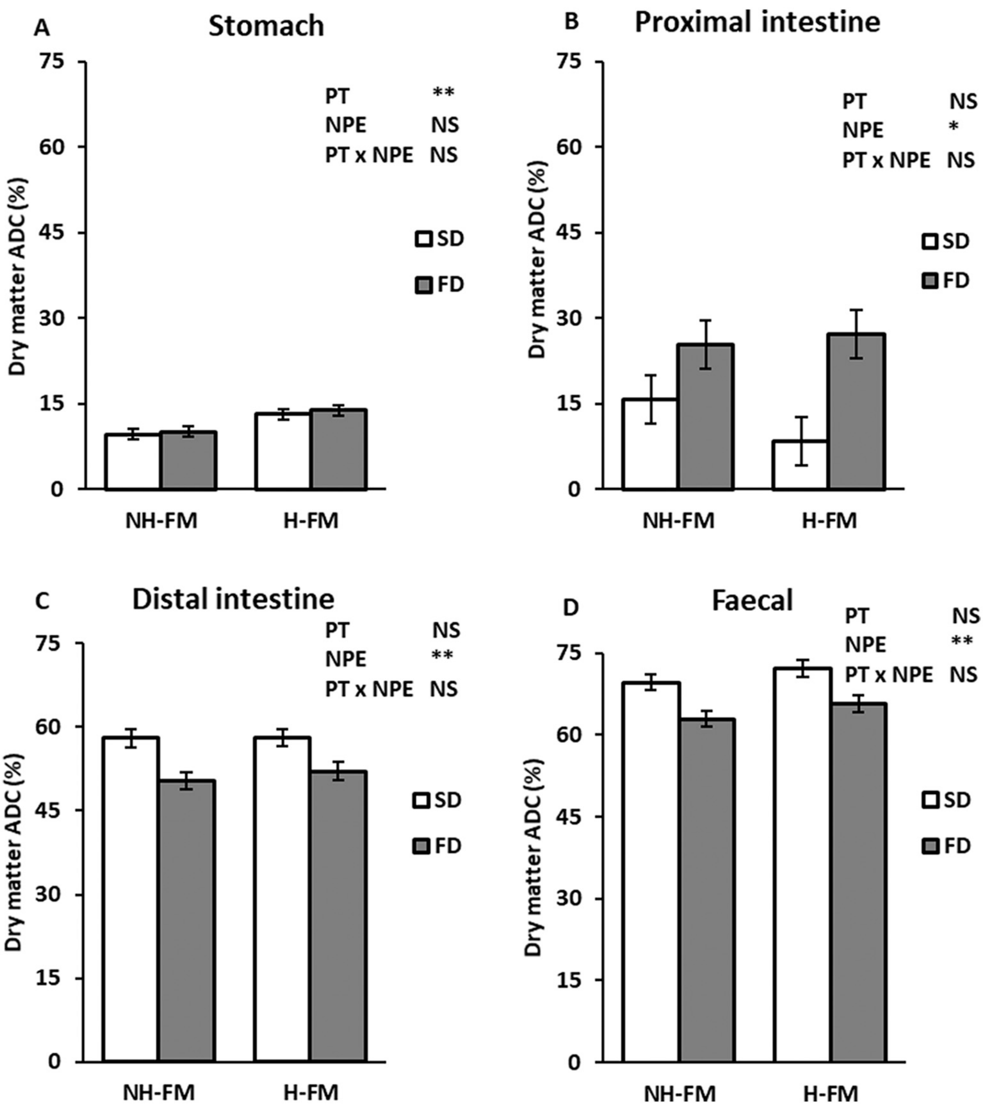

Fig. 3. Apparent digestibility coefficient (ADC) of dry matter in the stomach, proximal intestine, distal intestine and feces of African catfish; NH-FM, control fishmeal; H-FM, hydrolysed fishmeal; SD, starch diet; FD, fat diet. Presented values are means ($n = 3$) per dietary treatment within each compartment, except for the NH-FM + FD diet in the proximal intestine ($n = 2$). Error bars indicate standard error of means; PT, protein type (non-hydrolysed fishmeal and hydrolysed fishmeal); NPE, non-protein energy source (starch and fat); NS, not significant; *, $P < 0.05$; **, $P < 0.01$.

initial surplus influx. Another part of the water in the proximal intestine originates from water that is secreted together with bicarbonate. The latter being secreted to neutralize the acidic stomach chyme entering the proximal intestine. It is very likely that this also occurs in African catfish as this is supported by the current observation of a drop in ADC of Ca from 47% in the stomach to 17% in the proximal intestine, which indicates that there is an influx of Ca into the proximal intestine. Also, intestinal fluids such as, bile and pancreatic enzymes released to this compartment after feeding contributes to the influx of water in the proximal intestine (Grosell et al., 2000). In the current study, the flux of water was negative in the distal part of the intestine, which was also observed in rainbow trout (Bucking and Wood, 2006). According to Bucking and Wood (2006), the negative result could be due to the net reabsorption of water that was added in the previous compartments. However, a more logical explanation might be the net reabsorption of digestive fluids gained from the previous compartments. There is a possibility of passive absorption of water with the fluids at the distal end of the gut. However, the mechanism that surrounds the interstitial reabsorption of digestive fluids and water in the GIT of fish requires further investigation.

The observed higher stomach DM content for fish fed “fat-diets” than for “starch-diets” is in line with the study of Harter et al. (2015) on African catfish. Harter et al. (2015) suggested that the hydrophobic properties in a high fat diet would interfere with water mixing with chyme. This is also a likely explanation for the observed differences in the water balance results observed in this study, in which more water was added to “starch-diets” than “fat-diets”. Hydrolysis of starch produces large amounts of osmotically active mono- and disaccharides, which are thought to result in the addition of water to the GIT (Harter et al., 2013), but also the water binding capacity of starch itself might contribute to the lower DM content in the stomach. The higher ADC of Ca and Mg in the stomach at the “starch-diets” compared to the “fat-diets” might suggest that there is a difference in pH in the stomach resulting in an increased dissolving of Ca and Mg. As the drop in ADC of Ca between stomach and proximal intestine was larger at the “starch-diets” than at the “fat-diets”, this implies that the influx of Ca in the proximal intestine was higher at the “starch-diets” compared to the “fat-diets”. These observations about the ADC of Ca might be an indication that the increased acid-secretion in the stomach requires more bicarbonate secretion in the proximal intestine. However, this hypothesis requires further testing. The present study showed also a large reabsorption of water at the distal intestine when fish were fed “starch-diets”. Regarding the protein source, non-hydrolysed fishmeal diets sparked a tendency of more water addition to the stomach of African catfish compared to the hydrolysed fishmeal diets. The hydrolysis process required by fishmeal diets might have increased the need for both endo- and exogenous water addition. Nevertheless, water addition and re-absorption was not significantly different among the protein diets in

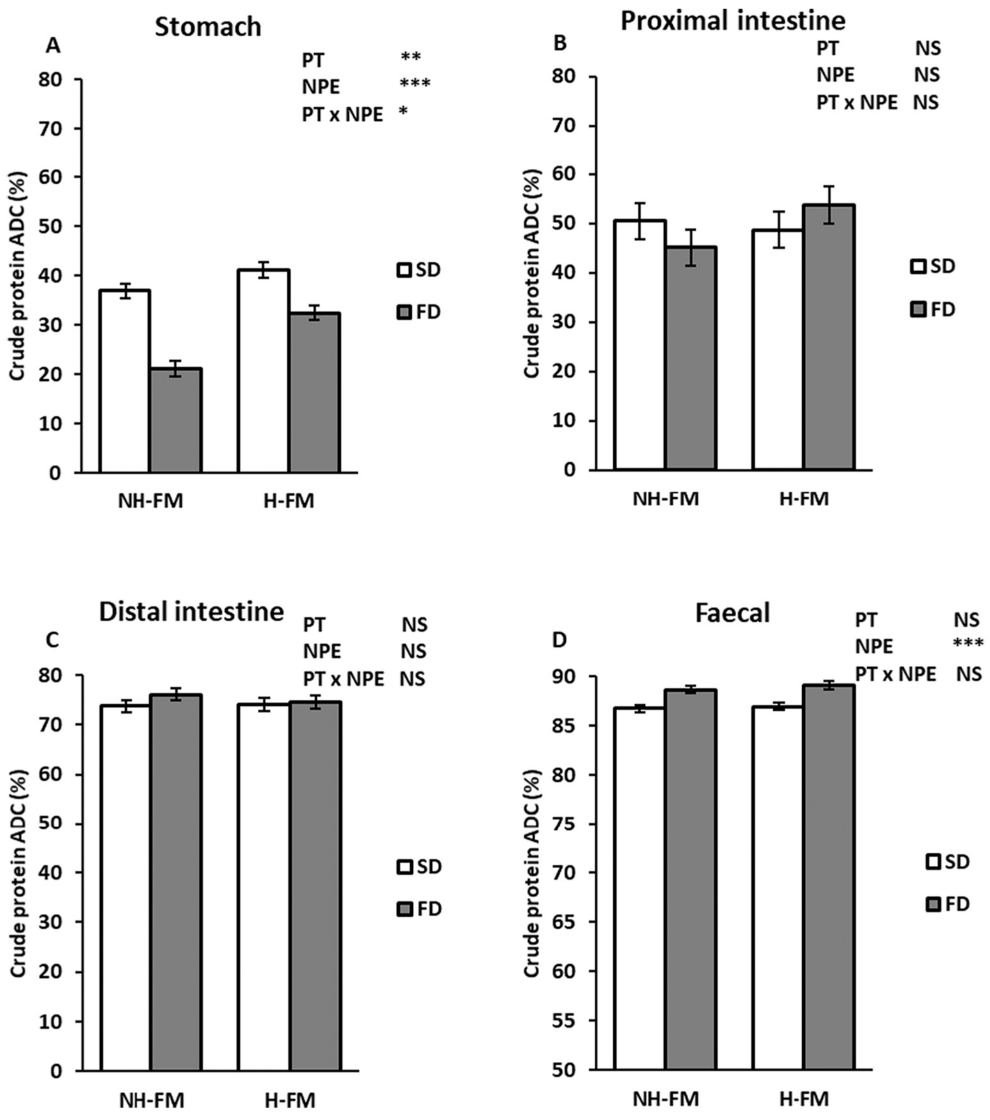

Fig. 4. Apparent digestibility coefficient (ADC) of crude protein (CP) in the stomach, proximal intestine, distal intestine and feces of African catfish; NH-FM, control fishmeal; H-FM, hydrolysed fishmeal; SD, starch diet; FD, fat diet. Presented values are means ($n = 3$) per dietary treatment within each compartment, except for the NH-FM + FD diet in the proximal intestine ($n = 2$). Error bars indicate standard error of means; PT, protein type (non-hydrolysed fishmeal and hydrolysed fishmeal); NPE, non-protein energy source (starch and fat); NS, not significant; *, $P < 0.05$; *, $P < 0.01$; ***, $P < 0.001$.

subsequent compartments. However, a good explanation for our observation of the water fluxes along the GIT for both type of fishmeal diets is lacking.

In the current study, the DM evacuation rate of “fat-diets” was higher in the stomach of African catfish than “starch-diets”. This observation does not substantiate that a higher chyme DM content of “fat-diets” would prolong its retention time in the stomach thereby delaying evacuation time, as suggested by Harter et al. (2015). Chyme with a higher DM is expected to remain in the stomach until appropriate liquefaction is achieved, until then will evacuation occur. However, the improper mixture of water and ‘fat-diet’ due to its high lipid content may have led to the quicker evacuation of the chyme, especially the liquid portion. On the other hand, it appears that the high viscous nature of starch upon reaction with water in the stomach may be associated with a longer passage rate (Amirkolaie et al., 2006). Literature shows that the kinetics of DM along the GIT is negatively related to the viscosity of the diet (Leenhouwers et al., 2006; Leenhouwers et al., 2007a; Leenhouwers et al., 2007b). Starch has been shown to increase chyme viscosity in the stomach of African catfish, which can slow down the passage rate (Harter et al., 2015). Amirkolaie et al. (2006) reported that gelatinized starch as used in the present study increased chyme viscosity in the stomach more than in other segments in Nile tilapia. Furthermore, the longer retention time of the starch diets in the stomach may also be related to the fish needing to achieve a more natural degree of liquefaction of the diet, in an attempt to reach the water content of natural prey (Bucking and Wood, 2006). This may also explain why more water addition was required by the “starch-diets”. The passage rate of CP through the stomach in fish fed the non-hydrolysed fishmeal diets was lower than for hydrolysed fishmeal diets. This can be explained by the need to undergo further hydrolysis by the action of stomach acid and enzymes prior to absorption in contrast to the fishmeal hydrolysate that had been partially broken down. Consequently, it is expected that fishmeal hydrolysate will display higher solubility and to some extent, the liquid phase with solved protein will evacuate from the stomach at a faster rate.

The hydrolysation of fishmeal resulted in higher DM and CP digestibility in the stomach, although, absorption of nutrient is not expected to take place in this compartment, rather compounds are broken down by the action of enzymes. This observation is striking and may be explained by differences in evacuation times between the protein and inert marker. The CP in the stomach had a higher evacuation rate (23.52%) compared to the inert marker (14.27%), which could have led to inaccurate calculations. Calculation errors may occur when dissociation between chyme and marker happens as they proceed along the GIT (Bucking and Wood, 2006). When proteins are hydrolysed in the stomach, peptides can/will move to the next segment of the GIT.

However, if the dissolved peptides exit the stomach before the marker (here: yttrium) as suggested by results from the current study, the ADC may be overestimated (Harter et al., 2015). This is because the peptides are no longer present in the stomach and neither absorbed in this compartment. Since fish stomachs are unable to absorb larger molecules such as peptides (Uys and Hecht, 1987), the high ADC of CP in the stomach indicates that a fraction of the peptides had already moved into the next section of the GIT. It has been reported that the liquid and solid fraction of chyme may not always move at the same pace. Bucking and Wood (2006) used ballotini beads and polyethylene PEG to investigate the movement of substances in rainbow trout. They observed a continuous association of marker and chyme as they transit along the GIT in their study. However, the liquid portion of the chyme was reported to slightly move faster than the solid part from the stomach.

There is an overall indication of improved nutrients digestibility due to fishmeal hydrolysation in the current study (Supplementary table B). The positive effect of using protein hydrolysate on fish growth has been discussed in many studies (Cahu et al., 1999; Chalamaiah et al., 2012). The growth of crucian carp (*Carassius auratus gibelio*) was significantly higher in fish fed cottonseed hydrolysate diet compared to a diet containing unprocessed cottonseed meal (Gui et al., 2010). In another study on Nile tilapia, shrimp protein hydrolysate was shown to be a good protein source with no adverse effects on growth and nutrient utilization (Leal et al., 2010). In a study with humans, it was demonstrated that protein hydrolysates show a faster and greater postprandial increase of plasma AAs than their non-hydrolysed equivalents (Morifuji et al., 2010). Pre-digested fish protein was seen to be absorbed quicker by Atlantic salmon (*Salmo salar*) than intact fish protein, which resulted in a faster and higher postprandial peak of essential amino acids in the plasma (Espe and Lied, 1994; Espe et al., 1993). Hydrolysed fishmeal contains protein with short peptides, which can easily dissolve in water, are highly digestible (Chalamaiah et al., 2012) and well utilised for growth (Khieokhajonkhet and Surapon, 2020; Refstie et al., 2004) and thus can be regarded as fast digestible protein.

In the current study, the ADC of CP in the stomach was higher for the fish fed the "starch-diets". This is in contrast to the study of Harter et al. (2015) in which the fat diet had a higher ADC of CP in the stomach than the starch diet. This contrast between both studies, might relate to various aspects. Firstly, digestion is a dynamic process that relates with the time after consuming a meal. In other words, sampling time after feeding will be important especially for the conditions occurring in the stomach; like the postprandial decline in stomach chyme DM (Bucking and Wood, 2006; Harter et al., 2013), in stomach chyme pH (Saravanan et al., 2013) and in stomach chyme osmolality (Bucking and Wood, 2006), all these observation concerned freshwater fish. In addition, time related differences in digestive enzyme activity might be involved. Furthermore, a hampered mixing of stomach chyme with gastric fluids containing enzymes at the"fat-diets" may be an explanation for the reduced digestibility in the stomach in current study. This hampered mixing at the "fat-diets" might be related to a high lipophilic characteristic but also to the supplementation of cellulose. Cellulose was added to the "fat-diets" in order to have an equal stomach filing directly after given an equal meal (equal protein and energy consumption), as decreasing stomach pH and DM content is dependent on meal size. In contrast to the current study, Harter et al. (2013, 2015) did not add a dietary filler to their fat-diet. The difference between the "starch-diets" and "fat-diets" regarding DM content and water influx in the stomach were fully comparable. Still though, the difference in stomach ADC of CP between these studies might be related to the addition of a filler in the current study. However, this seems not to be a plausible explanation as non-viscous dietary carbohydrate fillers increase stomach emptying rate of DM, like carboxymethylcellulose in tilapia (Shiau et al., 1988) and wheat bran in rainbow trout (Hilton and Slinger, 1983). However, the addition of indigestible cellulose to the "fat-diets" in the current study is the major reason for the lower ADC of DM in the distal intestine and faeces. As the chyme progresses to the proximal intestine, bile and other pancreatic digestive fluids are released for further digestion, especially for fat. As such, the hydrophobic problem will be solved and the proper interaction between digestive enzymes and nutrients present in the diet is enabled. This is supported by the increased ADC of CP of "fat-diets" in the subsequent compartments as well as the faecal digestibility. This observation is in line with the study of Harter et al. (2015), whereby CP in the fat diet was better digested at the distal part of the GIT of African catfish. However, it is noteworthy that "fat-diets" had the highest overall faecal ADC for CP, fat and ash, a reflection of what occurred at the posterior end of the GIT, which did not previously occur in the anterior part. This implies that absorption took place throughout the whole intestine of African catfish in accordance with previous studies (Bucking and Wood, 2006; Harter et al., 2013), but differences in faecal ADC can be created in the distal part of the intestine.

The effect of fishmeal hydrolysation on CP digestibility observed in the stomach did not directly reflect in the CP digestibility in other GIT compartments and faecal ADC of CP. Considering this outcome, it can be speculated that the effect of dietary composition on the digestion rate of CP in the stomach could be compensated in other compartments of the GIT as the chyme passes through the gut. Thus, lack of differences among the ADC of CP of the different protein diets in the proximal and distal intestine (also faecal ADC) indicate that a prolonged retention time of the non-hydrolysed fishmeal in the stomach resulted in the slow release of AAs and di- and tri-peptides. This resulted in high digestibility (similar to hydrolysed fishmeal) up to the end of the GIT (Chen, 2017). These results indicate that the mechanism of hydrolysis and absorption of proteins was rather similar among both diets in these compartments compared to the stomach.

Currently, fish diets are mostly formulated based of the faecal digestibility of nutrients, which only accounts for the total quantity of dietary nutrients that was apparently absorbed in the GIT (NRC, 2011). This does not take into account the kinetics of protein/nutrient digestion along the gut. As such, it provides less information (compared to kinetics of protein digestion study) on the timing of release and absorption of AAs and nutrients along the GIT after meal ingestion (Liu and Selle, 2015). Information on the kinetics of protein and energy digestion in fish is scarce. Results of the current study showed that the hydrolysation of fishmeal increased the crude protein digestibility in the stomach of African catfish, but this was dependent on the dietary non-protein energy. However, this effect did not translate into other compartments in the gastrointestinal tract and overall faecal digestibility. Replacement of dietary fat by starch increased water addition to the stomach but reduced the passage rate of chyme from the stomach. In African catfish (Harter et al., 2015) and Nile tilapia (Amirkolaie et al., 2006), replacing dietary fat by starch increased stomach chyme viscosity. Dietary supplementation with viscous non-starch polysaccharides (NSP) reduced the stomach evacuation rate of DM (Storebakken, 1985; Nikolopoulou et al., 2011). In contrast, non-viscous NSP, like cellulose, stimulate gastric emptying (Hilton and Slinger, 1983; Shiau et al., 1988). Exchanging fat by starch in African catfish without cellulose being used as diet filler, strongly increased stomach chyme viscosity coinciding with a reduced increased gastric emptying rate of DM at 2 h postprandial (Harter et al., 2015). Therefore, the viscous nature of starch after water solubility together with the inclusion of cellulose in the "fat-diets" likely caused the lower the stomach evacuation rate at the "starch-diets" compared to the "fat-diets", while the "starch-diets" had lower dry matter content compared to the "fat-diets".

High water fluxes inside the GIT are often considered as being not ideal. This might relate to the fact of an association with "diarrheal" like faeces. E.g., Enteritis induced by soybean meal in the distal intestine of salmon often coincides with reduced faecal DM content (Refstie et al., 1999). However, for a proper function of the digestive tract in fish fed dry pellets with a DM content >90%, a proper influx of fluid is essential. Bucking and Wood (2006) clearly showed that in rainbow trout, stomach emptying only starts if stomach chyme DM content is smaller than ~45%. Thus, a proper influx of water is needed to start gut emptying as well as to enable proper mixing of chyme with digestive enzymes, bile etc. From the current study, it was observed that dietary macro-nutrient composition influences the water influx in the stomach. Therefore, it can be concluded that water fluxes, digesta passage rate and the kinetics of digestion along the GIT are dependent on dietary macronutrient composition. Next to dietary composition, it can further be hypothesised that physical pellet characteristics, like water solubility and pellet hydration time, can influence water fluxed in the stomach and also digestive kinetics. As extrusion process conditions determine such pellet characteristics (e.g., Wang et al., 2021), it is worthwhile to assess the relationship between feed process conditions (i.e., physical pellet characteristics) and digestion kinetics.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.737425.

References

Amirkolaie, A.K., Verreth, J.A.J., Schrama, J.W., 2006. Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (*Oreochromis niloticus* (L.)). Aquaculture. 260, 194–205.

Belal, I., 1999. Replacing dietary corn with barley seeds in Nile tilapia, *Oreochromis niloticus* (L.), feed. Aquac. Res. 30, 265–269.

Bucking, C., Wood, C.M., 2006. Water dynamics in the digestive tract of the freshwater rainbow trout during the processing of a single meal. J. Exp. Biol. 209, 1883–1893.

Bureau, D., De La Nouee, J., Jaruratjamorn, P., 1995. Effect of dietary incorporation of crop residues on growth, mortality and feed conversion ratio of the African catfish, Clarias gariepinus (Burchell). Aquac. Res. 26, 351–360.

Cahu, C., Infante, J.Z., Quazuguel, P., Le Gall, M., 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture. 171, 109–119.

Chalamaiah, M., Dinesh Kumar, B., Hemalatha, R., Jyothirmayi, T., 2012. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. Food Chem. 135, 3020–3038.

Chen, H., 2017. Protein digestion kinetics in pigs and poultry. PhD thesis. Wageningen University, The Netherlands, pp. 9–76.

Cheng, Z.J.J., Hardy, R.W., 2002. Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (Oncorhynchus mykiss). Aquaculture. 212, 361–372.

Espe, M., Lied, E., 1994. Do Atlantic salmon (Salmo salar) utilize mixtures of free amino acids to the same extent as intact protein sources for muscle protein synthesis? Comp. Biochem. Physiol. A Physiol. 107, 249–254.

Espe, M., Lied, E., Torrissen, K.R., 1993. Changes in plasma and muscle free amino acids in Atlantic salmon (Salmo salar) during absorption of diets containing different amounts of hydrolysed cod muscle protein. Comp. Biochem. Physiol. A Physiol. 105, 555–562.

Grosell, M., O'donnell, M., Wood, C., 2000. Hepatic versus gallbladder bile composition: in vivo transport physiology of the gallbladder in rainbow trout. Am. J. Phys. Regul. Integr. Comp. Phys. 278, R1674–R1684.

Gui, D., Liu, W., Shao, X., Xu, W., 2010. Effects of different dietary levels of cottonseed meal protein hydrolysate on growth, digestibility, body composition and serum biochemical indices in crucian carp (*Carassius auratus gibelio*). Anim. Feed Sci. Technol. 156, 112–120.

Harter, T.S., Verreth, J.A., Heinsbroek, L.T., Schrama, J.W., 2013. Isoenergetic replacement of fat by starch in diets for African catfish (*Clarias gariepinus*): effect on water fluxes in the gastro intestinal tract. PLoS One 8, e55245.

Harter, T., Heinsbroek, L., Schrama, J., 2015. The source of dietary non-protein energy affects in vivo protein digestion in African catfish (*Clarias gariepinus*). Aquac. Nutr. 21, 569–577.

Hilton, J.W., Slinger, S.J., 1983. Effect of wheat bran replacement of wheat middlings in extrusion processed (floating) diets on the growth of juvenile rainbow trout (Salmo gairdneri). Aquaculture. 35, 201–210.

Khieokhajonkhet, A., Surapon, K., 2020. Effects of fish protein hydrolysate on the growth performance, feed and protein utilization of Nile tilapia (Oreochromis niloticus). Int. J. Agric. Technol. 16, 641–654.

Kirchgessner, M., Kürzinger, H., Schwarz, F., 1986. Digestibility of crude nutrients in different feeds and estimation of their energy content for carp (*Cyprinus carpio* L.). Aquaculture. 58, 185–194.

Leal, A.L.G., de Castro, P.F., de Lima, J.P.V., de Souza Correia, E., de Souza Bezerra, R., 2010. Use of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus*, L.) feeds. Aquac. Int. 18, 635–646.

Leenhouwers, J.I., Adjei-Boateng, D., Verreth, J.A.J., Schrama, J.W., 2006. Digesta viscosity, nutrient digestibility and organ weights in African catfish (*Clarias gariepinus*) fed diets supplemented with different levels of a soluble non-starch polysaccharide. Aquac. Nutr. 12, 111–116.

Leenhouwers, J.I., Ortega, R.C., Verreth, J.A.J., Schrama, J.W., 2007a. Digesta characteristics in relation to nutrient digestibility and mineral absorption in Nile tilapia (*Oreochromis niloticus* L.) fed cereal grains of increasing viscosity. Aquaculture. 273, 556–565.

Leenhouwers, J.I., ter Veld, M., Verreth, J.A.J., Schrama, J.W., 2007b. Digesta characteristiscs and performance of African catfish (*Clarias gariepinus*) fed cereal grains that differ in viscosity. Aquaculture. 264, 330–341.

Liu, S., Selle, P., 2015. A consideration of starch and protein digestive dynamics in chicken-meat production. World's Poultry Sci. J. 71, 297–310.

Liu, S., Selle, P., Cowieson, A., 2013. The kinetics of starch and nitrogen digestion regulate growth performance and nutrient utilisation of broilers fed coarsely ground, sorghum-based diets. Anim. Prod. Sci. 53, 1033–1040.

Maas, R.M., Deng, Y., Dersjant-Li, Y., Petit, J., Verdegem, M.C.J., Schrama, J.W., Kokou, F., 2021. Exogenous enzymes and probiotics alter digestion kinetics, volitile fatty acid content and microbial interactions in the gut of Nile tilapia. Sci. Rep. 11, 8221.

Morifuji, M., Ishizaka, M., Baba, S., Fukuda, K., Matsumoto, H., Koga, J., Higuchi, M., 2010. Comparison of different sources and degrees of hydrolysis of dietary protein: effect on plasma amino acids, dipeptides, and insulin responses in human subjects. J. Agric. Food Chem. 58, 8788–8797.

Muranova, T., Zinchenko, D., Kononova, S., Belova, N., Miroshnikov, A., 2017. Plant protein hydrolysates as fish fry feed in aquaculture. Hydrolysis of rapeseed proteins by an enzyme complex from king crab hepatopancreas. Appl. Biochem. Microbiol. 53, 680–687.

Nikolopoulou, D., Moutou, K.A., Fountoulaki, E., Venou, B., Adamidou, S., Alexis, M.M., 2011. Patterns of gastric evacuation, digesta characteristics and pH changes along the gastrointestinal tract of gilthead seabream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). Comparative Biochemstry and Physiology. A. 158, 406–414.

NRC, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.

Perrott, M., Grierson, C., Hazon, N., Balment, R., 1992. Drinking behaviour in sea water and fresh water teleosts, the role of the renin-angiotensin system. Fish Physiol. Biochem. 10, 161–168.

Refstie, S., Svihus, B., Shearer, K.D., Storebakken, T., 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean product. Anim. Feed Sci. Technol. 79, 331–345.

Refstie, S., Olli, J.J., Standal, H., 2004. Feed intake, growth, and protein utilisation by post-smolt Atlantic salmon (Salmo salar) in response to graded levels of fish protein hydrolysate in the diet. Aquaculture. 239, 331–349.

Saravanan, S., Geurden, I., Orozco, Z.G.A., Kaushik, S.J., Verreth, J.A.J., Schrama, J.W., 2013. Dietary electrolyte balance affects the nutrient digestibility and maintenance energy expenditure of Nile tilapia. Br. J. Nutr. 111, 1948–1957.

Shiau, S.Y., Yu, H.L., Hwa, S., Chen, S.Y., Hsu, S.I., 1988. The influence of carboxymethylcellulose on growth, digestion, gastric emptying time and body composition of Tilapia. Aquaculture 70, 345354.

Siddik, M.A., Howieson, J., Fotedar, R., Partridge, G.J., 2021. Enzymatic fish protein hydrolysates in finfish aquaculture: a review. Rev. Aquac. 13, 406–430.

Silva, T.C.d., Rocha, J.D., Moreira, P., Signor, A., Boscolo, W.R., 2017. Fish protein hydrolysate in diets for Nile tilapia post-larvae. Pesq. Agrop. Brasileira 52, 485–492.

Storebakken, T., 1985. Binders in fish feeds I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. Aquaculture. 47, 11–26.

Swanepoel, J.C., Goosen, N.J., 2018. Evaluation of fish protein hydrolysates in juvenile African catfish (*Clarias gariepinus*) diets. Aquaculture. 496, 262–269.

Tran-Tu, L.C., Bosma, R.H., Verstegen, M.W.A., Schrama, J.W., 2019. Effect of dietary viscosity on digesta characteristics and progression of digestion in different segments of the gastrointestinal tract of striped catfish (Pangasionodon hypophthalmus). Aquaculture. 504, 114–120.

Uys, W., Hecht, T., 1987. Assays on the digestive enzymes of sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae). Aquaculture. 63, 301–313.

Wang, H., Ma, S., Yang, J., Qin, Y., Cheng, H., Xue, M., Li, J., 2021. Optimization of the process parameters for extruded commercial sinking fish feed wiht mixed plant protein sources. J. Food Process Eng. 44, e13599.

Xu, H., Mu, Y., Liang, M., Zheng, K., Wei, Y., 2017. Application of different types of protein hydrolysate in high plant protein diets for juvenile turbot (Scophthalmus maximus). Aquac. Res. 48, 2945–2953.

Yuan, X., Jiang, G., Cheng, H., Cao, X., Shi, H., Liu, W., 2019. An evaluation of replacing fish meal with cottonseed meal protein hydrolysate in diet for juvenile blunt snout bream (Megalobrama amblycephala): Growth, antioxidant, innate immunity and disease resistance. Aquac. Nutr. 25, 1334–1344.