Received: 4 August 2019 | Revised: 1 November 2019 | Accepted: 4 November 2019
DOI: 10.1111/are.14406

ORIGINAL ARTICLE

Aquaculture Research WILEY

Doses response of dietary viscosity on digestibility and faecal characteristics of striped catfish (*Pangasionodon hypophthalmus*)

L. C. Tu-Tran[1,2] | **Thanh-Cong Nguyen**[2] | **Johan A. J. Verreth**[1] | **Johan W. Schrama**[1]

[1]Aquaculture and Fisheries Group, Wageningen University, Wageningen, The Netherlands

[2]College of Aquaculture and Fisheries, Cantho university, Cantho, Vietnam

Correspondence
Johan W. Schrama, Aquaculture and Fisheries Group, Wageningen University, Wageningen, The Netherlands.
Email: Johan.schrama@wur.nl

Funding information
Wageningen University, Grant/Award Number: SuPa project (WU/AFI/VRF10/WSSD/1)

Abstract

The study analysed the dose–response relationship between dietary viscosity and nutrient digestibility, faecal waste characteristics in striped catfish and the long-term impacts of viscosity on performance of striped catfish. This was done in two experiments: Exp.1 lasted 29 days, in which six dietary viscosity levels were created by including different amount of guar gum (GG; 0, 0.25, 0.5, 1.0, 2.0 or 3.0 g/kg) and Exp.2 lasted 60 days and assessed the long-term effects of three dietary GG levels (0, 0.5 and 3.0 g/kg) were checked. In Exp.1, digestibility of nutrients (except crude fat) decreased linearly with dietary viscosity. With increasing viscosity, removal efficiency of faeces from the water reduced linearly, whereas the total amount of faeces produced and the fraction of big-sized faecal particles (>2 mm). increased linearly. In Exp.1, viscosity did not affect performance. However, in Exp.2, the GG inclusion level of 3.0 g/kg (1.72 cP for the viscosity) affected fish performance. In conclusion, the best strategy for faecal waste management in striped catfish by dietary viscosity is to keep it as low as possible in the diet.

KEYWORDS
digestibility, faecal waste, guar gum, striped catfish

1 | INTRODUCTION

Minimizing the effluent waste of fish farms contributes to a better environmental performance of the sector (Waite et al., 2014). It helps to improve water quality and reduces the waste discharge to the surrounding. This has also been observed in the culture of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) in Vietnam (De Silva & Phuong, 2011; Phan et al., 2009).

Feeds have a major impact on the waste production by aquaculture operations. Aquaculture waste consists of solid waste (e.g. egested faeces) and soluble waste (e.g. orthophosphate and ammonia). The latter directly determines water quality, whereas solid waste can influence both water quality and sediment deposition (Cho & Kaushik, 1990; Hua & Bureau, 2010). The extent of the impact of the solid waste primarily depends on the amount of faeces produced, the stability/disintegration rate of egested faeces (Brinker, 2007) and depending on rearing systems design the removal rate and efficiency of the faeces. Various dietary factors can influence faecal waste production. So far, feed-related interventions to reduce the impact faecal waste (solids) are most often aimed at enhancement of nutrient digestibility (Sales, 2009; Gatlin III et al., 2007). Both in Nile tilapia (Amirkolaie, 2005; Amirkolaie, Leenhouwers, Verreth, & Schrama, 2005b; Schneider et al., 2004) and rainbow trout (Brinker, 2007), it was shown that diet composition can also change the faecal consistency and other physical characteristics of fish faeces. In a recent review, Kokou and Fountoulaki (2018) showed that altering the consistency and properties of fish faeces can affect the nature of the overall environmental impact of aquaculture operations and, depending on the type of rearing system, can also affect the efficacy of effluent management measures. Therefore, another option to manage faecal waste is to improve the faecal stability thereby improving the removal efficiency. This approach was taken in trout (Brinker, 2007) by adding low amounts of dietary binders, which have viscous properties that proved to increase the faecal stability of trout. This concept was also tested in striped catfish (Tran-Tu et al., 2018). However, dietary binders (Brinker, 2007; Brinker, Koppe, & Rösch, 2005; Storebakken, 1985; Storebakken & Austreng, 1987) and bulk agents (Dias, Huelvan, Dinis, & Métailler, 1998), which have viscous properties, can alter the digesta transit time and the macronutrient digestibility. This implies that dietary viscosity is an important factor in faecal waste management by (a) increasing the amount of faeces produced (i.e. reduced digestibility), as was demonstrated in tilapia (Amirkolaie, Leenhouwers, et al., 2005b), African catfish (Leenhouwers, Adjei-Boateng, Verreth, & Schrama, 2006; Leenhouwers, ter Veld, Verreth, & Schrama, 2007b) and striped catfish (Tran-Tu et al., 2018); and (b) by altering the stability/removal efficiency of the faeces, as was demonstrated in trout (Brinker et al., 2005). Studies in fish on the impact of dietary viscosity usually tested a limited amount of treatments and often at relatively high levels of dietary viscosity. However, when dietary viscosity should steer faecal waste by improving the faecal stability, low levels of dietary viscosity should be tested to determine the optimal level of dietary viscosity for faecal stability without affecting fish performance (including nutrient digestibility).

In fish, information about the dose–response relationship between dietary viscosity and nutrient digestibility as well as faecal waste characteristics is scarce. Therefore, a 29-day digestibility experiment (Exp. 1) was done to analyse these relations with dietary viscosity in striped catfish during restrictive feeding. For assessing the impact of dietary viscosity on growth and feed intake, a 60-day growth trial (Exp. 2) was conducted.

2 | MATERIALS AND METHODS

2.1 | Experimental design, fish and diets

This study consisted of two experiments, which were conducted in 2014 at Cantho University in Vietnam. The experiments were carried out in compliance with Vietnamese law. Additionally, the applied experimental procedures were meeting the EU regulations for the care and use of laboratory animals conform to Directive 2010/63/EU.

Experiment 1 (Exp. 1) lasted 29 days and assessed the dose–response relationship of dietary viscosity with nutrient digestibility and faecal waste characteristics. The contrasts in dietary viscosity were created by exchanging carboxyl methyl cellulose (CMC) with guar gum (GG). The dietary GG concentrations were, respectively, 0, 0.25, 0.5, 1.0, 2.0 and 3.0 g/kg. In experiment 2 (Exp. 2) the long-term effect of three dietary viscosity levels (0, 0.5 and 3.0 g of GG kg^{-1} feed) on performance was examined during 60 days. In both experiments, treatments were run in triplicate.

The basal composition of the experimental diets were identical in both experiments (Table 1). The main ingredients used were fishmeal, soya bean meal, rice bran, defatted rice bran, cassava and sunflower meal (Table 1). These main ingredients were mixed and thereafter grinded using a mesh size of 0.8 mm by a hammer mill (Stolz), which was operated at Vinh Hoan Company. After grinding, this ingredient-mixture was further mixed with the vitamin–mineral premix, squid oil, inert marker/filler (Cr_2O_3 in Exp. 1; SiO_2 in Exp. 2) and the respective experimental dose of GG replacing part of the carboxyl methyl cellulose. The experimental mixtures were produced into approximately 4.5 mm diameter pellets by a single screw extruder with 3 mm die. The extruder was designed and manufactured by the Centre of Technology Research and Application, College of Engineering Technology, Cantho University. After extrusion, pellets were dried at 60°C for 24 hr followed by sieving and storage in a freezer until feeding. Viscosity was

TABLE 1 Ingredient composition (in g/kg) of the experimental diets used in experiment 1 and 2 on as is basis

Ingredients	g/kg
Fish meal[†]	142.9
Soya bean meal[†]	168.1
Rice bran[†]	151.8
Defatted rice bran[†]	162.3
Cassava[†]	160.2
Sunflower meal[†]	170.2
Premix vitamin and mineral[‡]	10.0
Squid oil[†]	24.5
Inert marker[§]	10.0
Guar gum (GG) & carboxyl methyl cellulose (CMC)[¶]	10.0

[†] Kien Giang fish meal was produced by Minh Tam Co., Ltd.; Soya bean meal, rice bran, defatted rice bran and cassava were imported and supplied by Vinh Hoan Co.; Sunflower meal was imported and supplied by de Heus LLC Co.; Squid oil was produced by Vemedim Co.

[‡] Premix vitamin and mineral (UI or mg/kg): vitamin A 800,000 UI; vitamin D 150,000 UI; vitamin E equivalent 10,000 mg; vitamin E 7,500 mg; vitamin C (monophosphate) 7,600 mg; D-Calpan 2,500 mg; Niacin 2,000 mg; viatamin B6 1,500 mg; vitamin B2 1,000 mg; vitamin K3 700 mg; Biotin 10 mg; vitamin B12 2 mg; ZnO: 5,000–5,500 mg; MnO 3,000–3,300 mg; $FeSO_4.H_2O$ 2,000–2,200 mg and other elements such as vitamin B1; acid folic; $CuSO_4.5H_2O$; $Ca(IO_3)_2.H_2O$; Na_2SeO_3; $CoCO_3$; extractant from *Saccharomyces cerevisiae;* mold inhibitor Propionic acid; antioxidants Ethoxyquin and BHT; and fillers $CaCO_3$ and wheat flour (supplied by Provimi Co. Ltd.).

[§] Inert marker being Cr_2O_3 in experiment 1 and SiO_2 in experiment 2.

[¶] CMC was produced by Xilong Chemical Co., Ltd., and GG was produced by Sigma-Aldrich, Co. These chemicals were imported by Thanh My Co., Ltd. Depending on the GG inclusion levels (g/kg) in the diet, the ratios between GG and CMC were 0–10, 0.25 to 9.75, 0.5 to 9.5, 1–9, 2–8 and 3–7.

measured both in the mixture prior to extrusion and in the extruded pellets (after being grinded) according to the method described by Leenhouwers et al. (2006). One gram of the sample was mixed with 4 ml of demi water, incubated for 30 min at 28°C and centrifuged at 12,000g for 10 min, and thereafter supernatant viscosity was immediately measured by Brookfield LVDV-II + cone/plate viscometer. The measured viscosity and analysed chemical composition of the experimental diets are given in Table 2 (Exp. 1) and Table 3 (Exp. 2). Inclusion of guar gum was applied to alter dietary viscosity, because of its high viscose properties without altering the dietary protein and fat content. Consequently, small changes in the diet (<3.0 g/kg) resulted in a dietary viscosity in pellets ranging from 1.58 to 2.16 cP (Table 2).

Striped catfish (*Pangasionodon hypophthalmus*) were bought from a local hatchery. Fish were a mixed-sex population and weighed 95 g in Exp.1 and 100 g Exp. 2 at start of the experiment. In Exp. 1, fish were randomly distributed into 170 L digestibility tanks (for details on tanks see Tran-Tu et al., 2018) and in Exp. 2, into 500 L tanks with a composite cone bottom. In both experiments, each tank contained 20 fish. Tanks were filled for 80% with water and were constantly aerated by one air stone per tank. The water flow through each tank was set at 3 L/min. Within each experiment, all tanks were connected to a semi-recirculation system which was fully running only at night-time. During daytime, half of the amount of outflowing water per tank was replaced by de-chlorinated tap water. This renewal water had been stored in a 2 m^3 tank with continuous aeration prior to being added into filtration tank of the system.

The photoperiod regime was approximately 12 hr light and 12 hr dark. Water quality was checked daily for the temperature and ranged between 28 and 31°C for both experiments. pH ranged between 7.3–7.8 and 7.4–7.7, oxygen concentration between 5.6–6.5 and 5.1–6.4 ppm and water flow was 3.0 and 0.7 L/min for Exp. 1 and 2 respectively. Total ammonia nitrogen TAN (0.1–0.3 mg/L) and NO_2^{-}-N (0–0.03 ppm) for both experiments were measured weekly.

2.2 | Experimental procedure and sample analysis

In both experiments, fish were fed a mixture of the experimental diets for 2 weeks prior to the start of the experiment (before stocking) to allow adaptation to the dietary ingredients. In Exp. 1, fish were fed restrictively in order to prevent contamination of the collected faeces with feed spillage/waste. Fish were fed daily 20 g per tank of one of the six experimental diets for 29 days at 09:00 am. Faeces collection started from week 3 onwards and was collected daily. Faeces collection for 22 hr in total, started at 10:00 a.m. (1 hr after feeding) and lasted till 1 hr prior to the next feeding (8:00 a.m.). During this collection period, the 250-mL collection container was submerged in ice water to prevent bacterial degradation. After collection, the faeces were pooled per tank into an aluminium tray and stored at −20°C (Tran-Tu et al., 2018). In Exp. 2, the fish were fed until apparent satiation twice daily at 09:00 am and 04:00 pm for assessing the impact of dietary viscosity on feed intake. In Exp. 2, no faeces were collected. In both experiments, the uneaten feed was collected, dried at 60°C for 24 hr and weighed.

At the start and end of the experiments, fish were batch weighed. In Exp. 2, 20 fish were sampled at the start of the experiment and 10 fish per tank at the end of the experiment for body composition analysis. These fish were killed by putting them in ice water (2:1) after being sedated in a benzocaine solution (0.1 g/L). Per diet, about 10 g of feed was weekly sampled. Faeces and fish body samples were dried at 60°C for 24 hr. Feed, faeces and fish samples were ground by a coffee blender and stored at −20°C. Chemical analyses were done in triplicate. According to standard laboratory methods (AOAC, 2000), the moisture content was

TABLE 2 Analysed chemical composition and viscosity of six experimental diets with different inclusion levels of guar gum (GG) used in experiment 1 (on dry matter basis)

Dietary component	Diet code†					
	GG0	GG0.25	GG0.5	GG1.0	GG2.0	GG3.0
Dry matter (g/kg)	889	902	877	889	898	889
Crude protein (g/kg)	291	287	302	291	289	289
Crude fat (g/kg)	59	58	58	57	57	58
Carbohydrate (g/kg)	528	534	519	530	532	531
Crude ash (g/kg)	113	112	112	112	113	113
Chromium oxide (g/kg)	8.9	8.8	8.9	8.6	9.4	9.4
Dietary viscosity (cP):						
Mixture prior to extrusion	1.80	1.86	1.90	2.07	2.25	2.49
Pellets	1.58	1.63	1.72	1.81	1.96	2.16

†Diet code GG0, GG0.25, GG0.5, GG1.0, GG2.0 and GG3.0, refer to diets with a inclusion level of 0, 0.25, 0.5, 1.0, 2.0 and 3.0 g guar gum/kg feed.

determined by drying in the oven at 105°C until constant weight; crude protein (N × 6.25) content following the Kjeldahl method; crude ash content by placing in the furnace at 560°C for 4 hr; and crude fat content by solvent extraction. The carbohydrate content was calculated as dry matter minus crude protein crude ash minus crude fat. The chromic oxide content was measured by a spectrophotometer at the wavelength 350 nm after digestion of the samples by nitric acid and then oxidation with perchloric acid (Furukawa & Tsukahara, 1966).

TABLE 3 Analysed chemical composition the three experimental diets with different inclusion levels of guar gum (GG) used in experiment 2 (on dry matter basis)

	Diet code[†]		
Dietary component	**GG0**	**GG0.5**	**GG3.0**
Dry matter (g/kg)	920	926	918
Crude protein (g/kg)	296	295	296
Crude fat (g/kg)	34	41	43
Carbohydrate (g/kg)	555	552	548
Crude ash (g/kg)	115	112	113
Feed price (thousand VND kg^{-1})[‡]	13.9	14.0	14.4

[†] Diet code GG0, GG0.5 and GG3.0 refer to diets with an inclusion level of 0, 0.5 and 3.0 g guar gum/kg feed.

[‡] VND is Vietnamese Dong

2.3 | Calculations and statistical analysis

In Exp. 1, the apparent digestibility coefficients of nutrients (Nutrient ADC) were calculated from the concentrations of the inert marker and the respective nutrient (dry matter, crude protein, crude fat, carbohydrate or crude ash) in feed and faeces (g/kg) by the following equation (Cho & Kaushik, 1985):

$$\text{Nutrient ADC} = 1 - \left(\frac{\text{Marker}_{\text{diet}} \times \text{Nutrient}_{\text{faeces}}}{\text{Marker}_{\text{faeces}} \times \text{Nutrient}_{\text{diet}}} \right)$$

The percentage of faeces recovery was computed from the total amount of marker collected with faeces (collected amount of faecal DM times marker concentration) and the total amount of marker consumed with feed (according to Amirkolaie, Leenhouwers, et al., 2005b). The faecal waste consists of the recovered faeces and the

TABLE 4 The estimated relationships[†] between dietary viscosity measured on the pellets of the pelleted experimental diets (X in cP) and digestibility of nutrients, performance traits and faecal characteristics in striped catfish

Parameter[a]	**Equation (Y = intercept (±SE) + β (±SE) × X or Y = intercept (±SE) + β (±SE) × X + α (±SE) × X^2)**	***R*^{2}**	***p* value (linear component)**	***p* value (quadratic component)**
Digestibility				
Dry matter (%)	Y = 89.0 (±2.5) − 6.6 (±1.4) × X	.59	<.001	–
Crude protein (%)	Y = 95.1 (±1.3) − 4.0 (±0.73) × X	.65	<.001	–
Crude fat (%)	Y = 93.2 (±1.5) + 1.5 (±0.81) × X	.19	.073	–
Crude ash (%)	Y = 66.8 (±6.8 − 17.9 (±3.7) × X	.59	<.001	–
Carbohydrate (%)	Y = 89.4 (±3.0) − 6.3 (±1.6) × X	.48	.001	–
Performance				
FMW (g)	111 (±0.73)	–	.188	–
DWG (g d^{-1})	0.57 (±0.03)	–	.181	–
FCR	1.1 (±0.05)	–	.197	–
Survival rate (%)	98.6 (±0.54)	–	.883	–
Faecal waste characteristics				
Faeces recovery (%)	Y = 85.6 (±5.8) − 26.4 (±3.2) × X	.81	<.001	–
Faeces recovery (%)	Y = 197 (±61) − 147 (±65) × X + 32 (±18) × X^2	.85	–	.084
TFP (g/kg feed DM)	Y = 110 (±25) + 65.8 (±14) × X	.59	<.001	–
RF (g/kg feed DM)	Y = 153 (±14) − 36.9 (±7.9) × X	.58	<.001	–
NRF (g/kg feed DM)	Y = −42.8 (±23) + 103 (±12) × X	.81	<.001	–
Faecal particles >2 mm (%)	Y = −0.80 (±13) + 21.0 (±6.9) × X	.37	.008	–
Faecal particles >2 mm (%)	Y = −263 (±129) + 306 (±140) × X − 76.2 (±37) × X^2	.51	–	.059

[a] FMW, Final mean weight; DWG, Daily weight gain; FCR, Feed conversion ratio; TFP, total amount of faeces produced; RF, the amount of recovered faeces; and NRF, the amount of non-recovered faeces.

[†] Only relationships (both linear and quadratic), which were significant ($p < .05$) or tended to be significant ($p < .10$) are given.

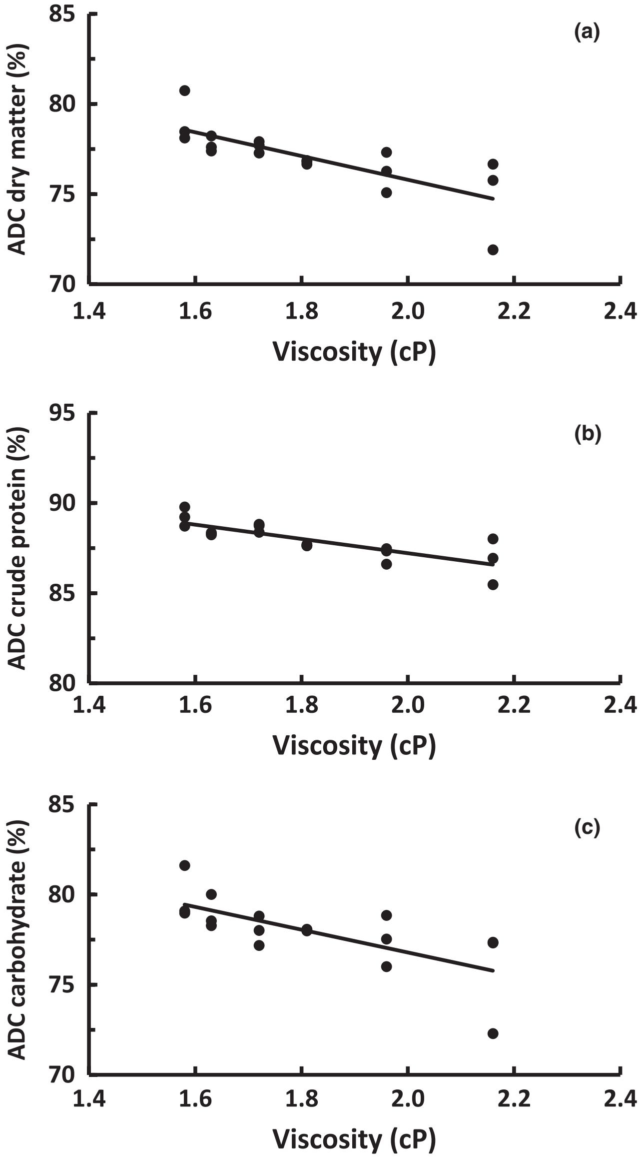

FIGURE 1 The linear relationships between dietary viscosity (cP) measured on the pellets of the experimental diets and nutrient digestibility (%) of: dry matter (a), crude protein (b) and carbohydrates (c). Solid lines indicate a significant relationship ($p < .05$), and the estimated equations are presented in Table 4

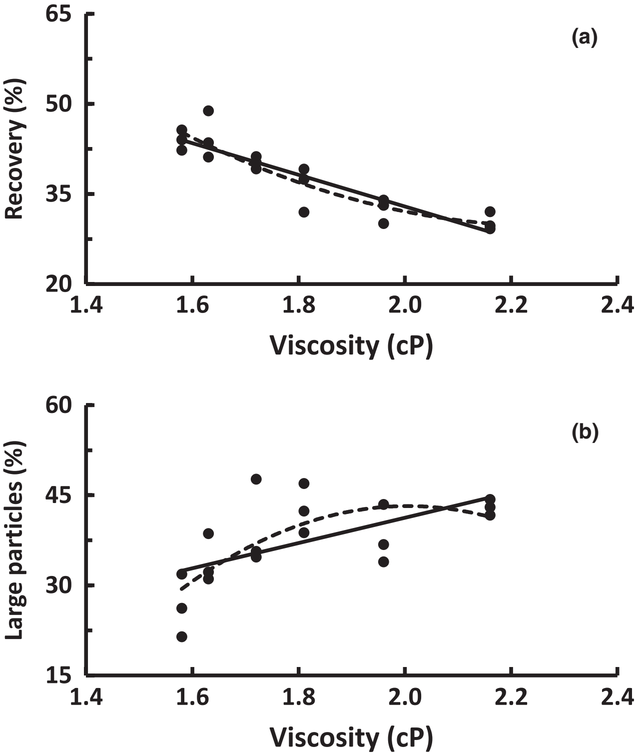

FIGURE 2 The relationships (both linear and quadratic) between dietary viscosity (cP) measured on the pellets of the experimental diets and: the recovery of the faeces from the water by settling (in %) (a); and the percentage of faecal particles being larger than 2 mm measured in faeces of striped catfish collected by settling (b). Solid lines indicate a significant relationship ($p < .05$) and broken lines indicate a tendency ($p < .10$). The estimated equations are presented in Table 4

non-recovered faeces. The total amount of faeces produced was computed from the dry matter digestibility. The amount of non-recovered faeces is the difference between the faeces recovered from the settling tanks and the calculated amount of total faeces produced (expressed in g/kg feed). The faeces collected during the last 3 days of Exp. 1 were used to determine the particle size of faeces. The faeces sample was sieved through a 2 mm mesh while being submerged in water. After sieving both fractions were oven dried at 60°C and weighed. The percentage of particles in the faeces that was bigger or smaller than 2 mm was calculated on DM basis.

Daily weight gain (DWG) was calculated by dividing the difference between the final and initial mean weight by the number of experimental days. Feed conversion ratio (FCR) was calculated as mean weight (as is) gain per fish divided by the mean amount of feed consumed per individual expressed in g DM. Survival rate (SR) was the number of fish harvested divided by number of fish stocked. In Exp. 2, the daily satiation feed intake per fish was computed from the average amount of feed fed minus the uneaten feed (on DM basis). The net protein utilization (NPU) and fat retention (FR) were calculated from initial and final fish body composition dividing by the respective nutrient intake. The cost of feed kg^{-1} of fish gain was determined by multiplying FCR and the feed price.

All results were introduced in a data-base (MS-Excel®), and mean and standard deviation of each treatment were calculated. Data of Exp. 1 were tested for the effect of dietary viscosity by regression analyses for linear and quadratic relationships. The measured viscosity of the pelleted experimental diets was used as X-variable in these regression analysis. Data of Exp. 2 were tested for the effect of diet by one-way ANOVA. In the case of a significance, post hoc comparison of means was done by Tukey's test. The residuals of all data were checked for normal distribution using the one-sample Kolmogorov–Smirnov test. All the tests were done by using SPSS 25.0®.

3 | RESULTS

3.1 | Experiment 1

Exp. 1 focused on the effect (i.e. dose response) of dietary viscosity on nutrient digestibility and faecal waste parameters. Increasing dietary GG levels increased dietary viscosity of the pellets ($r = .996$; $p < .001$). Mean values per experimental diets for digestibility, performance and faecal waste characteristics are given in supplementary Table S1. Regression analysis showed that dietary viscosity did not affect fish performance during the 29-d experiment period in Exp. 1 ($p > .1$; Table 4). Except for fat, all nutrient digestibility's were negatively affected by dietary viscosity (Figure 1). For all these nutrients, the digestibility was linearly related to dietary viscosity (i.e. the quadratic component was not significant; Table 4). With increasing dietary viscosity, the digestibility of dry matter declined with 6.6% cP^{-1} (Figure 1a), the digestibility of crude protein with 4.0% cP^{-1} (Figure 1b) and the digestibility of carbohydrate with 6.3% cP^{-1} (Figure 1c). However, in contrast with this general pattern, the digestibility of fat tended to increase slightly with increasing dietary viscosity ($p = .073$), although the relation was not significant.

Between the experimental diets, the faeces recovery ranged from 29.2% to 48.9% (Figure 2a). Faeces recovery declined linearly with increasing dietary viscosity ($p < .001$). However, with increasing levels of dietary viscosity (i.e. higher GG inclusion levels), the decline in faeces recovery diminished (Figure 2a). This observation was corroborated by a trend for a quadratic relation between faeces recovery and dietary viscosity ($p = .084$; Table 4). The reduction in dry matter digestibility with increasing dietary viscosity resulted in a linear increase in the total amount of faeces produced per kg of feed (TFP; Table 4). The amount of faeces that was recovered by settling (RF) also declined with dietary viscosity. The increase in TFP and decline in RF resulted in an enhanced amount of non-recovered faeces when the dietary viscosity increased (NRF, Table 4). TFP, RF and NRF were all linearly related to dietary viscosity. Even small increases in dietary viscosity (low inclusion level of GG) resulted in a negative impact on all faecal waste parameters. The particle size distribution of collected faeces by settling, was also linearly related to viscosity ($p < .01$). Increasing the dietary viscosity increased the percentage of particles larger than 2 mm, but this relationship with particle size tended to be quadratic ($p = .059$; Figure 2b). With increasing dietary viscosity levels, the improvement of particle size became smaller (i.e. curved off).

3.2 | Experiment 2

In Exp. 2, fish were fed to satiation. Dietary viscosity (i.e. GG inclusion) did not affect feed intake. In contrast to Exp. 1, growth of the fish over the 60-d experimental period in Exp. 2 was affected by GG inclusion (Table 5). Growth was similar at the diets with a GG inclusion of 0 and 0.5 g/kg, but reduced at the diet with 3.0 g/kg ($p < .05$). Consequently, also FCR was higher at the highest GG inclusion level compared with the other two diets (Table 5). The same pattern between diets was also observed for the net protein utilization efficiency. Fat retention efficiency differed between all diets and declined with increasing GG inclusion level. Due to the increase in FCR (Table 5) together with the increased cost of the experimental diets (Table 3) with increasing GG inclusion levels, the feed cost per unit of growth was higher at the GG level of 3.0 g/kg compared with the other two experimental diets.

TABLE 5 Effect of dietary guar gum level on performance, nutrient retention and feed costs per unit of weight gain in striped catfish during a 60-d growth experiment (Exp. 2)

	Dietary code[†]		
Parameter	**GG0**	**GG0.5**	**GG3.0**
Initial mean weight (g)	100 ± 1.5	100 ± 1.3	100 ± 1.7
Final mean weight (g)	239 ± 15.3^{b}	247 ± 17.2^{b}	177 ± 8.9^{a}
Daily weigh gain (g d^{-1})	2.3 ± 0.26^{b}	2.4 ± 0.27^{b}	1.3 ± 0.16^{a}
Feed intake (g DM d^{-1})	2.6 ± 0.46	3.0 ± 0.48	2.3 ± 0.13
Feed conversion ratio	1.11 ± 0.09^{a}	1.24 ± 0.17^{a}	1.84 ± 0.17^{b}
Survival rate (%)	87 ± 7.6	93 ± 11.5	98 ± 2.9
Dry matter content in final fish body (%)	31.2 ± 2.5	30.8 ± 2.1	28.1 ± 1.1
Net protein utilization	0.48 ± 0.05^{b}	0.42 ± 0.10^{b}	0.23 ± 0.05^{a}
Fat retention	4.1 ± 0.70^{c}	3.0 ± 0.16^{b}	2.1 ± 0.30^{a}
Feed cost per unit weight gain (thousand VND kg^{-1})[‡]	15.5 ± 1.2^{a}	17.4 ± 2.4^{a}	26.6 ± 2.4^{b}

[abc] Values (mean ± *SD*) within rows lacking a common superscript differ significantly ($p < .05$).

[†] Diet code GG0, GG0.5 and GG3.0 refer to diets with an inclusion level of 0, 0.5, and 3.0 g guar gum/kg feed.

[‡] VND is Vietnamese Dong.

4 | DISCUSSION

4.1 | Digestibility and performance

The present study proves that striped catfish is sensitive to minor changes in dietary viscosity. This is reflected by the linear negative relationships between dietary viscosity and macronutrient digestibilities ($p < .001$, except for fat). Similar negative effects on the nutrient digestibility of striped catfish were found between diets with a contrast in dietary viscosity (low vs. high), which was created by inclusion of different amounts of binders (i.e. GG) (Tran-Tu et al., 2018). Similar effects of dietary viscosity were observed in Nile tilapia (Amirkolaie, Leenhouwers, et al., 2005b; Fagbenro & Jauncey, 1995), rainbow trout (Storebakken, 1985), African catfish (Leenhouwers et al., 2006; Leenhouwers, Veld, et al., 2007b), Atlantic salmon (Kraugerud et al., 2007), and also in broiler chickens (Van der Klis, Verstegen, & Van Voorst, 1993). The reduced digestibility of nutrients when binders (e.g. GG or CMC) and/or plant ingredients are included in a diet is assumed to relate to the presences of highly viscous compounds that increase the viscosity of chyme causing malabsorption of nutrients (e.g. minerals, amino acids, etc.) in the intestine (Mudgil, Barak, & Khatkar, 2014). These effects of dietary viscosity are often explained by: (a) the viscous non-starch polysaccharides (NSP), which often increase viscosity, are indigestible and the required enzymes for digestion are absent in fish, and therefore affect all parts of the gastrointestinal tract; (b) viscous NSP absorb water and alter the chyme into a gel, which hamper the mixing of endogenous enzymes and thereby reduce hydrolysis of nutrients; (c) the gelling of chyme reduces diffusion of nutrients thereby reducing their absorption and increasing faecal nutrient losses, such as bile acids, amino acids; (d) the increased viscosity of the chyme may alter/damage the gut mucosa, thereby decreasing the absorption of hydrolysed nutrients by the enterocytes (Sinha, Kumar, Makkar, De Broeck, & Becker, 2011).

Based on the above potential mechanisms, one would expect that all nutrients are affected similarly. However, in the current study, only fat digestion was not hampered by dietary viscosity. Similar observations of a smaller/no impact of dietary viscosity on fat digestion while other nutrients were affected have been reported for Nile tilapia (Amirkolaie, Leenhouwers, et al., 2005b), Atlantic salmon (Kraugerud et al., 2007), and also earlier for striped catfish (Tran-Tu et al., 2018). In contrast, in broilers, the digestibility of fat was significantly reduced by dietary viscosity (Maisonnier, Gomez, & Carré, 2001; Van der Klis et al., 1993). It might be that the location of fat absorption in fish (more distal than for amino acids) plays a role. However in Nile tilapia (Amirkolaie, Verreth, & Schrama, 2006), African catfish (Harter, Verreth, Heinsbroek, & Schrama, 2013) and also in striped catfish (Tran-Tu, Bosma, Verstegen, & Schrama, 2019), it has been shown that the viscosity of the chyme increase as it passages towards the distal intestine. This would support the hypothesis that fat digestion would be even more affected than the digestion of other nutrients because it is absorbed more distally. Why the digestion of the different nutrients (fat, protein, carbohydrates etc.) responds differently to changes in dietary viscosity in striped catfish and other fish species requires further research.

Apart from digestibility, various studies showed that dietary viscosity (by inclusion of specific binders or plant ingredients with viscous NSPs), also reduced the fish performance (i.e. decreased growth and feed intake, increased FCR) of, for example, Nile tilapia (Leenhouwers, Ortega, Verreth, & Schrama, 2007a; Shiau, Yu, Hwa, Chen, & Hsu, 1988) or snakehead (Janphirom, Chaiprasert, Thongthieng, Suwannathep, & Songkasiri, 2010). However, there are also various studies where the effects of Exp. 1 in the current study (Table 4) are not observed like in: African catfish (Harter, Heinsbroek, & Schrama, 2015; Leenhouwers et al., 2006), Nile tilapia (Leenhouwers, Ortega, et al., 2007a) and striped catfish (Tran-Tu et al., 2018). The absence of significant effects on performance in these studies might be due to (among others) differences in duration of the studies and in different levels of dietary viscosity between treatments. In Exp. 2 of our study, which lasted 60 days (Table 5), a reduced growth, protein utilization, fat retention and an increased FCR ($p < .05$) was found but only at the diet with highest viscosity. Next to differences in experimental duration (60 d in Exp. 2 vs. 29 d in Exp. 1), also the feeding level may have caused the absence of effects in Exp. 1 (restricted feeding vs. *ad. libitum* feeding in Exp. 2).

Regarding the cost of feed per kg of fish gain, Exp. 2 (Table 5) indicated that the dietary viscosity (by GG inclusion) increased the feed cost because of the extra cost of GG in combination with the increase in FCR. Thus, increasing dietary viscosity increased feed cost per kg of weight gain in striped catfish.

We believe that the effect of dietary viscosity (induced by binders or by dietary viscous NSP sources) on various response parameters is dependent on species, animal sizes and types of binder or NSP. For instance, increasing the dietary viscosity by adding 80 g/kg GG did not affect the growth of African catfish (Leenhouwers et al., 2006). Similar observations were found in rainbow trout with 3 g/kg GG inclusion (Brinker, 2007, 2009) and in striped catfish with 0.5 g/kg GG inclusion (this study Exp. 2). In contrast, in snakehead dietary, GG inclusions of 0.1 g/kg decreased fish growth (Janphirom et al., 2010) and in Nile tilapia, a dietary level of 3 g/kg GG declined the digestibility of nutrients (Fagbenro & Jauncey, 1995). Also in broiler chickens, GG inclusion of 1.0 g/kg declined the digestibility of nutrients (Maisonnier et al., 2001). Moreover, Storebakken (1985) found that larger fish were able to alleviate the negative effects of dietary viscosity induced by GG inclusion. In rainbow trout, Brinker (2005) reported that no negative effects on nutrient digestion were found in the diets with 3 g/kg GG but a decreased digestibility was shown in the diet with 10 g/kg alginate. The linear effects of dietary viscosity on nutrient digestion, which were found in the current study (Exp. 1), imply that the impact of ingredients/binders depends on their relative viscous property and their inclusion levels into the diet. Even small increases in inclusion level may already reduce the digestibility of nutrients and thus the biological value of a diet.

4.2 | Faecal waste

In the present study, and in line with the effect of reduced digestibility, the total amount of faeces increased with increasing dietary viscosity. Similar observations were reported in Nile tilapia (Amirkolaie, Leenhouwers, et al., 2005b) and earlier in striped catfish (Tran-Tu et al., 2018). In other words, a higher dietary viscosity results in more faeces being produced by fish. Also, in line with the observed effect on dry matter digestibility, the amount of faeces was linearly related to dietary viscosity. Thus in striped catfish, increasing dietary viscosity gives more faecal waste.

However, the impact of dietary viscosity on faecal waste characteristics may differ between fish species. In a series of studies in rainbow trout, it was proven that dietary inclusion of GG (increasing dietary viscosity) can be effective to improve faecal waste characteristics, especially through the formation of larger faecal particles (Brinker, 2007, 2009; Brinker & Friedrich, 2012; Brinker et al., 2005). These larger particles could reduce the solid load and potential leachable material within aquaculture operations. Opposite to these observations in rainbow trout, in Nile tilapia, dietary GG inclusion increased the amount of organic load by reducing the faeces recovery (Amirkolaie, El-Shafai, Eding, Schrama, & Verreth, 2005a). The current study demonstrated that also in striped catfish dietary GG inclusion could increase the faecal particle size, similar as in rainbow trout. However, despite this increased particle size, and in contrast to the studies on rainbow trout, dietary viscosity reduced the faeces recovery efficiency similar as was found in tilapia. Currently, there is not a clear explanation for this observation that faecal recovery efficiency declines and that the percentage of large faecal particles increases with dietary viscosity in striped catfish. However, it should be realized that the faecal particle size is measured only in the recovered faecal waste and not in the total amount of egested faeces.

These apparent contrasting results are all based on studies applying GG supplementation. Structurally, galactomannans are the main component in GG and bean gum and are comprised of β-(1 → 4)-linking mannan chains with α-(1 → 6)-linked galactosyl side groups (McCleary, Clark, Dea, & Rees, 1985). In the process of forming a gelling solution, galactomannans combine with water molecules to build the stable intermolecular junction zones between chain sequences. The GG solutions are rheologically stable, because of the continuous re-entanglement of the interpenetrating network, which could explain the formation of larger faecal particles (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). However, higher inclusion levels of GG could also decrease the stability of the faeces (Storebakken, 1985) because of increased fermentation. The gelling property can be disrupted by gas, which can be produced between the inter-strands and cause the faecal pellet to break into smaller pieces. The gas follows from the fermentation process by bacterial activities (Sinha, Kumar, Makkar, De Boeck, & Becker, 2011). The difference in culture temperature between trout versus tilapia and striped catfish will affect the bacterial activity within the faeces. In tilapia, it has been shown that native starch vs. gelatinized starch reduced starch digestibility but also increased the concentration of volatile fatty acids in the chyme of the distal intestine (Amirkolaie et al., 2006), which suggested increased fermentation inside the fish. This increased fermentation coincided with a strongly reduced faecal recovery. Also in an earlier study with striped catfish (Tran-Tu et al., 2019), it was found that with higher GG inclusion levels, the pH in the mid intestine was slightly reduced, which might be an indication for higher fermentation activity inside the intestine. Obviously, the role of the gut microflora on faecal waste management in fish requires more attention in future research.

5 | CONCLUSION

In striped catfish, digestion of macronutrients, with exception for fat, was negatively affected by dietary viscosity. Within the studied range of viscosity levels, digestibility of these macronutrients, the faecal removal efficiency and the total amount of faecal waste produced were all linearly related to dietary viscosity. Increasing dietary viscosity (e.g. by using dietary binder) is not suitable for faecal waste management in striped catfish, since it increases the amount of faeces produced, worsens the removal efficiency by settling and negatively affects the nutritional value of the diets. Only the particle size of collected faeces improved with dietary viscosity. The best strategy for faecal waste management in striped catfish by dietary viscosity is to keep it as low as possible in the diet.

ACKNOWLEDGMENTS

We thank Tran Thanh Tan, Nguyen Hong Quyet Thang, Pham Minh Tam and Tran Thi Phuong Hang for their assistance in feed preparation, the experiment and the laboratory work in Vietnam, Leon Heinsbroek for developing the measurement of faecal particle distribution, Ronald Booms for the laboratory work in NL, Ep Eding for his advice to improve the experimental system management, Martin Verstegen and Kabir Kazi for revising the English, Tran Thi Thanh Hien, Nguyen Thanh Phuong and Do Thi Thanh Huong for their proper advice. This study was funded by the SuPa project (WU/AFI/VRF10/WSSD/1), in full 'Waste management for Pangasius culture in the Mekong Delta of Vietnam'.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

L. C. Tu-Tran https://orcid.org/0000-0001-6140-498X
Johan W. Schrama https://orcid.org/0000-0001-7156-8806

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

REFERENCES

Amirkolaie, A. K. (2005). *Dietary carbohydrate and faecal waste in the Nile tilapia* (*Oreochromis niloticus* L.) (p. 143). PhD thesis, Wageningen University and Research, Wageningen.

Amirkolaie, A. K., El-Shafai, S. A., Eding, E. H., Schrama, J. W., & Verreth, J. A. J. (2005a). Comparison of faecal collection method with high- and low-quality diets regarding digestibility and faeces characteristics measurements in Nile tilapia. *Aquaculture Research*, *36*, 578–585. https://doi.org/10.1111/j.1365-2109.2005.01257.x

Amirkolaie, A. K., Leenhouwers, J. I., Verreth, J. A. J., & Schrama, J. W. (2005b). Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus L.*). *Aquaculture Research*, *36*, 1157–1166. https://doi.org/10.1111/j.1365-2109.2005.01330.x

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. https://doi.org/10.1016/j.aquaculture.2006.06.039

Brinker, A. (2007). Guar gum in rainbow trout (*Oncorhynchus mykiss*) feed: The influence of quality and dose on stabilisation of faecal solids. *Aquaculture*, *267*, 315–327. https://doi.org/10.1016/j.aquaculture.2007.02.037

Brinker, A. (2009). Improving the mechanical characteristics of faecal waste in rainbow trout: The influence of fish size and treatment with a non-starch polysaccharide (guar gum). *Aquaculture Nutrition*, *15*, 229–240. https://doi.org/10.1111/j.1365-2095.2008.00587.x

Brinker, A., & Friedrich, C. (2012). Fish meal replacement by plant protein substitution and guar gum addition in trout feed. Part II: Effects on faeces stability and rheology. *Biorheology*, *49*, 27–48.

Brinker, A., Koppe, W., & Rösch, R. (2005). Optimised effluent treatment by stabilised trout faeces. *Aquaculture*, *249*, 125–144. https://doi.org/10.1016/j.aquaculture.2004.12.029

Cho, C. Y., & Kaushik, S. J. (1985). Effect of protein intake on metabolizable and net energy values of fish diets. In C. B. Cowey, A. M. Mackie & J. G. Bell (Eds.), *Nutrition and feeding in fish*, (pp. 95–117). Londres, UK, GBR.

Cho, C. Y., & Kaushik, S. J. (1990). Nutritional energetics in fish: Energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Review of Nutrition and Dietetics*, *61*, 132–172.

De Silva, S. S., & Phuong, N. T. (2011). Striped catfish farming in the Mekong Delta, Vietnam: A tumultuous path to a global success. *Reviews in Aquaculture*, *3*, 45–73. https://doi.org/10.1111/j.1753-5131.2011.01046.x

Dias, J., Huelvan, C., Dinis, M. T., & Métailler, R. (1998). Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass *(Dicentrarchus labrax)* juveniles. *Aquatic Living Resources*, *11*, 219–226.

Fagbenro, O., & Jauncey, K. (1995). Water stability, nutrient leaching and nutritional properties of moist fermented fish silage diets. *Aquacultural Engineering*, *14*, 143–153. https://doi.org/10.1016/0144-8609(94)P4432-B

Furukawa, A., & Tsukahara, H. (1966). On the acid digestion method for the determination of chromic oxide as an index subsatnce in the study of digestibility of fish feed. *Nippon Suisan Gakkaishi*, *32*, 502–506.

Gatlin, D. M. III, Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., ...Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research*, *38*, 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

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

Harter, T. S., Verreth, J. A. J., Heinsbroek, L. T. N., & 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. https://doi.org/10.1371/journal.pone.0055245

Hua, K., & Bureau, D. P. (2010). Quantification of differences in digestibility of phosphorus among cyprinids, cichlids, and salmonids through a mathematical modelling approach. *Aquaculture*, *308*, 152–158. https://doi.org/10.1016/j.aquaculture.2010.07.040

Janphirom, T., Chaiprasert, P., Thongthieng, T., Suwannathep, S., & Songkasiri, W. (2010). Increasing fish feed stability using guar gum: Case study with *Channa striata*. *Asian Journal of Food and Agro-Industry*, *3*, 8.

Kokou, F., & Fountoulaki, E. (2018). Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. *Aquaculture*, *495*, 295–310. https://doi.org/10.1016/j.aquaculture.2018.06.003

Kraugerud, O. F., Penn, M., Storebakken, T., Refstie, S., Krogdahl, Ã. S., & Svihus, B. (2007). Nutrient digestibilities and gut function in Atlantic salmon *(Salmo salar)* fed diets with cellulose or non-starch polysaccharides from soy. *Aquaculture*, *273*, 96–107. https://doi.org/10.1016/j.aquaculture.2007.09.013

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. *Aquaculture Nutrition*, *12*, 111–116. https://doi.org/10.1111/j.1365-2095.2006.00389.x

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. https://doi.org/10.1016/j.aquaculture.2007.10.044

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. https://doi.org/10.1016/j.aquaculture.2007.01.003

Maisonnier, S., Gomez, J., & Carré, B. (2001). Nutrient digestibility and intestinal viscosities in broiler chickens fed on wheat diets, as compared to maize diets with added guar gum. *British Poultry Science*, *42*, 102–110. https://doi.org/10.1080/00071660020035154

McCleary, B. V., Clark, A. H., Dea, I. C. M., & Rees, D. A. (1985). The fine structures of carob and guar galactomannans. *Carbohydrate Research*, *139*, 237–260. https://doi.org/10.1016/0008-6215(85)85089-8

Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., & Price, J. (1981). Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydrate Polymers*, *1*, 5–21. https://doi.org/10.1016/0144-8617(81)90011-4

Mudgil, D., Barak, S., & Khatkar, B. S. (2014). Guar gum: Processing, properties and food applications—a review. *Journal of Food Science and Technology*, *51*, 409–418. https://doi.org/10.1007/s13197-011-0522-x

Phan, L. T., Bui, T. M., Nguyen, T. T. T., Gooley, G. J., Ingram, B. A., Nguyen, H. V., ...De Silva, S. S. (2009). Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. *Aquaculture*, *296*, 227–236. https://doi.org/10.1016/j.aquaculture.2009.08.017

Sales, J. (2009). Linear models to predict the digestible lipid content of fish diets. *Aquaculture Nutrition*, *15*, 537–549. https://doi.org/10.1111/j.1365-2095.2008.00620.x

Schneider, O., Amirkolaie, A. K., Vera-Cartas, J., Eding, E. H., Schrama, J. W., & Verreth, J. A. J. (2004). Digestibility, faeces recovery, and related carbon, nitrogen and phosphorus balances of five feed ingredients evaluated as fishmeal alternatives in Nile tilapia, *Oreochromis niloticus* L. *Aquaculture Research*, *35*, 1370–1379. https://doi.org/10.1111/j.1365-2109.2004.01179.x

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*, 345–354. https://doi.org/10.1016/0044-8486(88)90118-4

Sinha, A. K., Kumar, V., Makkar, H. P. S., De Boeck, G., & Becker, K. (2011). Non-starch polysaccharides and their role in fish nutrition - a review. *Food Chemistry*, *127*, 1409–1426. https://doi.org/10.1016/j.foodchem.2011.02.042

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. https://doi.org/10.1016/0044-8486(85)90004-3

Storebakken, T., & Austreng, E. (1987). Binders in fish feeds: II. Effect of different alginates on the digestibility of macronutrients in rainbow trout. *Aquaculture*, *60*, 121–131. https://doi.org/10.1016/0044-8486(87)90304-8

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. https://doi.org/10.1016/j.aquaculture.2019.01.047

Tran-Tu, L. C., Hien, T. T. T., Bosma, R. H., Heinsbroek, L. T. N., Verreth, J. A. J., & Schrama, J. W. (2018). Effect of ingredient particle sizes and dietary viscosity on digestion and faecal waste of striped catfish *(Pangasianodon hypophthalmus)*. *Aquaculture Nutrition*, *24*, 961–969.

Van der Klis, J. D., Verstegen, M. W. A., & Van Voorst, A. (1993). Effect of a soluble polysaccharide (carboxy methyl cellulose) on the absorption of minerals from the gastrointestinal tract of broilers. *British Poultry Science*, *34*, 985–997. https://doi.org/10.1080/00071669308417658

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S., …& Phillips, M. (2014). *Improving productivity and environmental performance of aquaculture. In Working paper, Installment 5. In Creating a Sustainable Food Future*. Washington, DC: World Resources Institute.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Tu-Tran LC, Nguyen T-C, Verreth JAJ, Schrama JW. Doses response of dietary viscosity on digestibility and faecal characteristics of striped catfish (*Pangasionodon hypophthalmus*). *Aquac Res*. 2020;51:595–604. https://doi.org/10.1111/are.14406