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Effect of ingredient particle sizes and dietary viscosity on digestion and faecal waste of striped catfish (*Pangasianodon hypophthalmus*)

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Abstract

The ingredients' particle size and dietary viscosity may alter digestion, performance and faecal waste management of fish. This study aimed to assess the effect of grinding screen sizes of feed ingredients and dietary viscosity on digestibility, faecal waste and performance of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878). The experiment had a 2 × 3 factorial-design: two feed mesh particle sizes, by grinding ingredient mixtures at two screen sizes (0.8 versus 1.0 mm); and three dietary viscosity levels, created by exchanging carboxymethylcellulose by guar gum (GG) (0, 3 and 6 g of GG/kg of diet). Six diets were assigned to 18 tanks, each connected to three faecal settling tanks. All aquaria were stocked with 20 fish (82 g per fish). After 52 experimental days, dietary viscosity negatively affected both feed digestibility and performance of striped catfish; as a result, the amount of organic matter in the culture system through faeces had increased significantly. The coarse diets significantly increased the digestibility of dry matter and carbohydrate but worsened feed conversion ratio. Increasing dietary viscosity tended to increase the viscosity and moisture content of the faeces, but significantly accelerated the faecal disintegration through the reduction of both faecal recovery and the amount of recovered faeces.

KEYWORDS
digestion, faecal waste, grinding screen size, guar gum level, performance, striped catfish

1 | INTRODUCTION

The importance of sustainability in aquaculture is increasing. One aspect of sustainability is maintaining optimal water quality in combination with minimizing waste discharge to the surrounding environment. This is also the case for striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) culture in Vietnam (De Silva & Phuong, 2011; De Silva et al., 2010; Phan et al., 2009). Management of water quality in the ponds is performed by water exchanged. Shortly after stocking of striped catfish, the frequency of water exchange is daily to weekly and increases to twice daily close to harvesting (Phan et al., 2009). More than 60% of the farms in the Mekong area discharge this water directly

into rivers (Phan et al., 2009). Managing faecal waste is an important tool to control waste discharge. This can be performed by increasing the digestibility of the feed and/or improving the characteristics of the egested faeces. When the digestibility of a feed increases, less faeces is produced. Altering the properties of the egested faeces may enable an easier and more complete removal of the faecal pellet from the water. Earlier studies on digestibility of ingredients in striped catfish (Da, Lundh, & Lindberg, 2012; Hien, Phuong, Le TU, & Glencross, 2010) focused on nutrient uptake and not on faecal waste aspects. Studies in trout (Brinker, 2007, 2009; Brinker, Koppe, & Rösch, 2005) and Nile tilapia (Amirkolaie, El-Shafai, Eding, Schrama, & Verreth, 2005; Amirkolaie, Verreth, & Schrama, 2006; Schneider et al., 2004)

demonstrated that both the characteristics of the faecal pellet (e.g., the stability and removal efficiency from the water) and the amount of faeces produced were affected by the dietary composition.

More specifically, binders that alter the dietary viscosity like soluble non-starch polysaccharides (NSPs) of which guar gum (GG) is an example have been used to improve the removal efficiency of faeces (Sinha, Kumar, Makkar, De Boeck, & Becker, 2011). Brinker et al. (2005) found that the inclusion of GG increased the stability of faeces in trout. However, in Nile tilapia, GG leads to an opposite effect as it decreased the faecal recovery efficiency (Amirkolaie, Leenhouwers, Verreth, & Schrama, 2005). It has also been shown that the (negative) impacts of GG are dose dependent, for example, on growth (Janphirom, Chaiprasert, Thongthieng, Suwannathep, & Songkasiri, 2010); digestibility (Refstie, Svihus, Shearer, & Storebakken, 1999; Storebakken, 1985); nutrients absorption (Smits, Veldman, Verkade, & Beynen, 1998; Van der Klis, Verstegen, & Van Voorst, 1993); gastric emptying (Shiau, Yu, Hwa, Chen, & Hsu, 1988); and the properties of the chyme like viscosity osmolality, pH and the moisture content (van der Klis, Van Voorst, & Van Cruyningen, 1993). Information on the impact of dietary viscosity (e.g., by inclusion of GG) on digestion and faecal waste characteristics in striped catfish is lacking.

Another way to alter the digestibility in fish is to vary the processing conditions of the feed ingredients (e.g., grinding screen size). Regarding particle size after grinding, there is no information on the impact of the characteristics of the faeces in fish generally or in striped catfish specially. We hypothesized that the impact of dietary viscosity (e.g., by GG inclusion) to produce stable faeces is dependent on the particle size of the undigested material in the faecal pellet.

This study assessed the effect of ingredient particle size (by altering screen size at grinding) and the dietary viscosity on feed digestibility, fish performance, intestinal dry matter (DM) content and on viscosity, type of faeces (recovered and non-recovered) and particle sizes of faeces produced by striped catfish.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

The experiment lasted 52 days and was conducted in a 2 × 3 factorial protocol with three replications for each treatment. The experiment aimed to investigate the effect of two screen sizes during grinding of the feed ingredients and of three different dietary viscosity levels. The two screen sizes were 0.8 mm (fine) and 1.0 mm (coarse). The three dietary viscosity levels were created by adding 0, 3 or 6 g/kg GG to a basal diet. This basal diet mimicked a practical striped catfish diet. It contained fishmeal, soybean meal, rice bran, defatted rice bran, cassava and sunflower meal as main ingredients and carboxyl methyl cellulose (CMC) as a pellet binder (Table 1). GG was exchanged by CMC in the basal diet. The chemical composition of the diets is given in Table 2. One batch of the basal ingredient mixture was grinded with a hammer mill (manufactured by Stolz, France, and operated at Vinh Hoan Co. in 2007) using the mesh size 1.0 mm. Half of this grinded mixture was used to make the three "Coarse" treatments: C-GG0, C-GG3 and C-GG6 with, respectively, 0, 3

TABLE 1 The amounts (in g/kg) of ingredients used in the basal diet to test three levels of guar gum (x): 0, 3 and 6 g/kg

Ingredients	Amount (as is)
Fish meal[a]	142.9
Soybean meal[b]	168.1
Rice bran[b]	151.8
Defatted rice bran[b]	162.3
Cassava[b]	160.2
Sunflower meal[c]	170.2
Premix vitamin and mineral[d]	10.0
Squid oil[e]	24.5
Chromium oxide	10.0
Guar gum (GG) and carboxyl methyl cellulose (CMC)[f]	10.0

[a]Kien Giang fish meal was produced by Minh Tam Co., Ltd. (Kien Giang province, Vietnam).
[b]Soybean meal, Rice bran, Defatted rice bran and Cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam).
[c]Sunflower meal was imported and supplied by de Heus LLC Co. (Vinh Long province, Vietnam).
[d]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; vitamin 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; $FeSO_4 \cdot H_2O$ 2,000–2,200 mg and other elements such as vitamin B1; acid folic; $CuSO_4 \cdot 5H_2O$; $Ca(IO_3)_2 \cdot H_2O$; Na_2SeO_3; $CoCO_3$; extractant from *Saccharomyces cerevisiae*; mould inhibitor Propionic acid; antioxidants Ethoxyquin and BHT; and fillers $CaCO_3$ and wheat flour (supplied by Provimi Co. Ltd., Vietnam).
[e]Squid oil was produced by Vemedim Co. (Vietnam).
[f]CMC was produced by Xilong Chemical Co., Ltd. (China), and GG was produced by Sigma-Aldrich, Co. (Pakistan). These chemicals were imported by Thanh My Co., Ltd. (Vietnam). Depending the GG inclusion levels (g/kg) in the diet, the ratios between GG and CMC were 0–10, 3–7 and 6–4.

and 6 g/kg GG inclusion. The other half was then grinded using a mesh size of 0.8 mm to make the three "Fine" treatments: F-GG0, F-GG3 and F-GG6. After grinding, ingredient mixture for each treatment was weighed and then vitamin–mineral, marker-Cr_2O_3, GG, CMC and fish oil were added. These mixtures were extruded through the 3-mm die resulting in approximately 4.5-mm-diameter pellets (the extruder was designed and manufactured by the Centre of Technology Research and Application, College of Engineering Technology, Can Tho University in 2010) and thereafter dried at 60°C for 24 hr followed by sieving and storage in a freezer until feeding. The particle size distribution of the "Fine" mixture (0.8 mm screen) was 1.0%, 20.4% and 78.6%, and of the "Coarse" mixture (1.0 mm screen) was 4.2%, 27.1% and 68.7%, for, respectively, the size classes of >0.5, 0.3–0.5 and <0.3 mm. The meal mixtures prior to extrusion and the extruded pellets were sampled and analysed for viscosity.

2.2 | Experimental system and animals

Striped catfish (*Pangasianodon hypophthalmus*) juveniles with an initial body weight of 82 g were bought from a local hatchery. The fish

TABLE 2 Chemical composition of six experimental diets on dry matter basis

	Diet					
	Fine (0.8 mm)			Coarse (1.0 mm)		
Dietary component	GG0	GG3	GG6	GG0	GG3	GG6
Dry matter (g/kg)	882	883	884	889	892	890
Crude protein (g/kg)	318	314	312	313	306	304
Crude fat (g/kg)	57	62	61	65	62	57
Carbohydrate (g/kg)	511	509	510	511	511	526
Crude ash (g/kg)	115	116	117	112	114	114
Chromium oxide (g/kg)	7.5	8.9	9.3	8.1	8.1	8.5
Gross energy (kJ/g)	20.7	20.7	20.8	20.3	20.0	20.0

were a mixed sex population. The juveniles were randomly distributed into 18 plastic digestibility tanks (Allan, Rowland, Parkinson, Stone, & Jantrarotai, 1999) of 170 L; each tank contained 20 fish. The tanks were filled for 80% with water and were aerated with one air stone per tank. The water flow over each tank was set at 3 L/min. Tanks were connected to a semi-recirculation system. During daytime, half of the outflowing water per tank was replaced by de-chlorinated tap water, which had been stored in a 2-m³ tank with continuous aeration.

The digestibility tanks had two main parts: a cylindroconical tank with underneath a settling unit, according to Allan et al. (1999). The first one contained the fish and the second one was for settling of faeces which were collected in a container submerged in ice to prevent bacterial decay of the collected faeces. One difference with the original model was that the 6-mm mesh at the bottom of the holding tank was removed. During feeding, the outlet was closed by a plastic tube (50 mm diameter) to prevent feed from leaving the holding unit in order to give the fish sufficient time to consume the pellets. Thirty minutes after giving the last feed, uneaten floating pellets were collected by nets and the tube was removed.

The photoperiod regime was approximately 12-hr light and 12-hr dark. Water quality was checked daily and maintained for temperature (28–31°C), pH (7.4–7.7), oxygen concentration (5.5–6.4 ppm) and water flow (3 L/min). Total ammonia nitrogen (0.1–0.3 mg/L) and NO_2^--N (0–0.02 ppm) were measured and monitored weekly.

2.3 | Experimental procedure

Prior to the experiment and before stocking in the digestibility tanks (Figure 1), the fish had been fed a mixture of the experimental diets for 2 weeks to allow adaptation to the dietary ingredients. Fish were individually weighed at the start and end of the experimental period. During the 52-day experimental period, the fish were fed one of the six experimental diets at 2% of their body weight. Striped catfish are very stress sensitive and react quickly to noise and human interference. To minimize disturbance, fish were fed once daily at 09:00 a.m. Faeces collection started from week 3 (i.e., after 21 days of rearing) onwards. Faeces were collected daily from 1 hr after feeding until 1 hr prior to the next feeding. The 250-ml container was pooled per tank into an aluminium tray and stored in the freezer until analysed. Thirty

minutes after ending the feeding, spilled and refused feed by the fish were collected from the container under each tank. This uneaten feed was dried at 60°C for 24 hr and weighed.

On the final sampling day, 8 hr after feeding, the fish were caught by net from each tank and then anaesthetized by immersion in a solution of benzocaine (1 g) in 3 ml of acetone then diluted with fresh
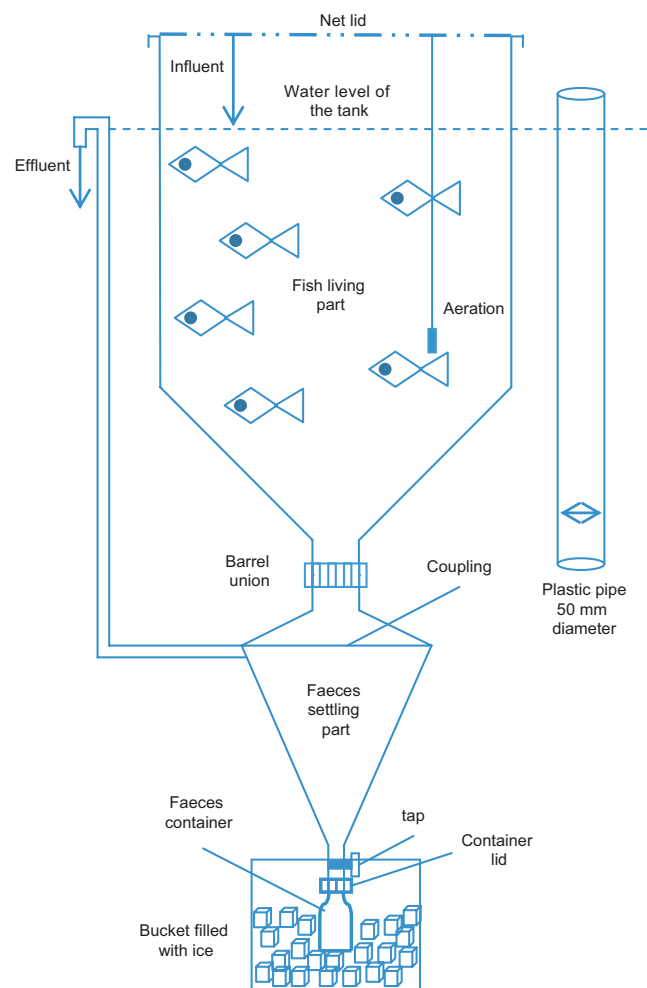
FIGURE 1 Schematic overview of the fish tank with the faecal collection unit as described by Allan et al. (1999) with some modification made

water up to 10 L. The temperature of the solution was maintained at approximately 20°C by adding ice to the larger container in which we placed the immersion bucket. After 10 min of immersion, all fish were individually weighed. Eighteen sedated fish from each tank were put in ice water (2:1) until dead for dissection and collection of the digesta in the gastrointestinal tract (GIT). Before and after dissection, every fish was weighed. Each GIT was divided into four segments: stomach and three equal parts representing for proximal, mid and distal gut. One part of the pooled digesta samples were centrifuged at 12,000 g for 10 min. Viscosity in pellets after grinding and diet mixtures (prior to extrusion) was performed according to Leenhouwers, Adjei-Boateng, Verreth, and Schrama (2006). One gram of these samples was mixed with 4 ml of demi water, incubated for 30 min at 28°C and thereafter centrifuged at 12,000 g for 10 min. Afterwards, we immediately measured the viscosity of the supernatant of each sample by Brookfield LVDV-II + cone/plate viscometer (Leenhouwers et al., 2006). Absolute viscosity was expressed in cP. The other part of the pooled digesta samples was dried and determined the percentage of DM. Here only data on DM and viscosity in the distal part are presented.

2.4 | Sample analysis

About 10 g of each feed was sampled weekly during the experimental period. The faeces samples were dried at 60°C for 24 hr. Both kind of samples were then ground by a coffee blender and stored in the freezer until analysis. The chemical analyses were carried out in triplicate, except for gross energy that was determined in duplicate. According to standard laboratory methods (AOAC, 2000), the moisture content was determined by drying in the oven at 105°C until constant weight; crude protein (N × 6.25) content following the Kjeldahl method; mineral (ash) content by placing in the furnace at 560°C for 4 hr; and crude fat content by solvent extraction. The carbohydrate content in the sample was calculated by the equation 100 – (crude protein + crude ash + crude fat). The gross energy was directly analysed by a bomb calorimetry meter (Parr C6100, USA). The chromic oxide content was measured by a spectrophotometer at the wave length 350 nm after digestion of the samples by nitric acid and then oxidation with per-chloric acid (Furukawa & Tsukahara, 1966).

2.5 | Calculations and statistical analysis

The apparent digestibility coefficient of nutrient (Nutrient.ADC), such as DM, crude protein, crude fat, carbohydrate, crude ash and gross energy, was revealed by an indirect method using chromic oxide as inert marker (Cho & Kaushik, 1985):

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

where the marker$_{\text{diet}}$ is concentration (g/100 g dry weight) of chromium oxide in the diet. The marker$_{\text{faeces}}$ is concentration (g/100 g dry weight) of chromium oxide in the faeces. The nutrient$_{\text{diet}}$ is concentration (g/100 g dry weight) of nutrient in the diet. And nutrient$_{\text{faeces}}$ is concentration (g/100 g dry weight) of nutrient in the faeces.

The initial and final mean weights of the fish were determined by weighing and counting the total number of fish in each tank. Daily weight gain was calculated by dividing the difference between the final and initial mean weight (mean weight gain) by the number of experimental days. Feed conversion ratio was based on the mean weight gain per fish and on the mean amount of consumed feed per individual in g DM. Survival rate was the percentage of the final number of fish to that of the initial number of stocked fish.

The percentage of faeces recovery was computed using the total amount of Chromic oxide in excreted faeces and the total amount of Chromic oxide in the consumed feed (Amirkolaie, Leenhouwers, et al., 2005), where the total amount of Chromic oxide in excreted faeces is the amount in DM of faeces which was collected multiplied by the Chromic oxide concentration in the faeces. The total amount of Chromic oxide of consumed feed is the total amount of consumed feed in DM multiplied by the chromium oxide concentration in the feed.

Particle size of faecal waste determines the potential of suspended solid formation and also the degree of nutrient leaching from the waste. Therefore, the percentage of particles larger than 2 mm was determined on the faecal waste collected over a period of 22 hr. The measured particle size distribution can be a combination of the disintegration of faecal pellets after egestion as well as the potential aggregation of faecal waste during collection. To prevent clogging of the mesh, the sieving was carried out with the sieve being submerged in water. The daily collected faecal waste with water on top in the collecting containers was gently poured onto a 2-mm mesh sieve while being already submerged in water. After sieving, both fractions were dried and weighed. The percentages of the amount (in DM) of particles in the faeces that were bigger or smaller than 2 mm was calculated.

The faecal waste consists of the recovered faeces plus the non-recovered faeces. The total amount of faeces produced was computed based on the DM 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.

All results were introduced in a database (MS-Excel®), and mean and standard deviations of each treatment were calculated. The data available as percentage were transformed into ASIN results. After that, all data were checked for normal distribution using the One-Sample Kolmogorov–Smirnov test and for homogeneity of variances using Levene's test. Differences between the six treatment diets and the interactions between the two factors were determined by a two-way factorial ANOVA using SPSS 16.0®.

3 | RESULTS

The viscosity of the diets was measured in samples taken before and after extrusion (Figure 2). In the diets, part of the CMC was exchanged by increasing levels of GG. Increasing the dietary GG content resulted in an increased viscosity. This response differed between the grinding size treatments. The increase in viscosity was larger in the "Coarse" diets
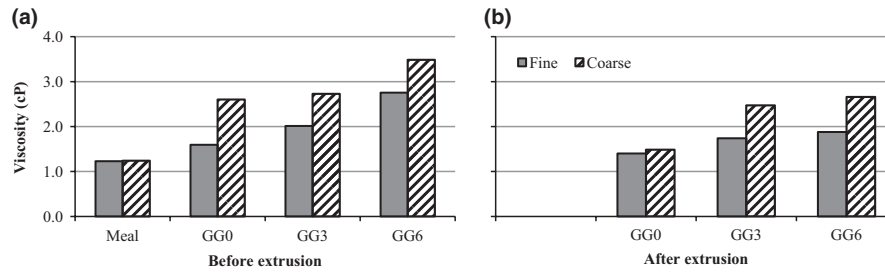
FIGURE 2 Effect of guar gum (GG) inclusion level and grinding scenes size ("Fine" versus "Coarse") on dietary viscosity of ingredient mixtures prior to extrusion (panel a) and pellets after extrusion (panel b). GG0, GG3 and GG6 are diets containing 0, 3 and 6 g GG/kg of diet; GG is exchanged for carboxyl methyl cellulose (CMC) in the diets. "Meal" in panel a revers to ingredient mixtures without GG and CMC

TABLE 3 The effect of grinding screen size (i.e., particle size of DM feed mass) and guar gum levels on the apparent digestibility coefficients of nutrients (mean ± SD) of striped catfish fed the experimental diets

Grinding screen sizes	Guar gum levels (g/kg)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Carbohydrate (%)	Crude ash (%)	Gross energy (%)
Fine (0.8 mm)	0	77.7 ± 1.3	91.8 ± 0.1	95.2 ± 1.4	74.5 ± 2.8	47.8 ± 0.4	84.0 ± 1.1
	3	74.8 ± 0.7	90.2 ± 1.3	95.0 ± 0.5	71.5 ± 0.9	40.0 ± 2.6	81.7 ± 0.4
	6	75.1 ± 0.6	90.6 ± 0.9	94.6 ± 0.1	72.0 ± 1.3	40.4 ± 1.8	81.3 ± 0.4
Coarse (1.0 mm)	0	79.1 ± 0.8	92.6 ± 0.5	96.8 ± 0.6	75.8 ± 1.9	49.6 ± 1.9	85.6 ± 0.7
	3	75.7 ± 0.6	90.0 ± 0.5	94.8 ± 1.7	73.4 ± 1.1	40.4 ± 1.2	81.8 ± 1.0
	6	76.2 ± 1.9	89.5 ± 1.7	95.3 ± 0.3	76.0 ± 2.6	34.8 ± 1.5	81.8 ± 1.6
p Values of the factors							
Grinding screen sizes		.049	.783	.131	.020	.183	.143
Guar gum levels		.001	.004	.127	.087	<.001	<.001
Interaction		.925	.307	.308	.474	.005	.363

than in the "Fine" diets. Moreover, the viscosity in the "Coarse" and "Fine" treatment without CMC and GG addition was equal. Extrusion reduced the viscosity compared to the meal mixtures, but the trends and differences between the dietary treatments remained present.

The apparent digestibility coefficient (ADC) of crude protein, crude ash and gross energy differed ($p < .05$) among GG levels and was not affected by grinding treatment (Table 3). However, the differences in digestibility between animals receiving diets with and without GG were significant. The interaction between grinding treatment and GG levels was significant ($p < .05$) for the ADC of crude ash. The screen size affected the ADC of DM and carbohydrate ($p < .05$). In general, the "Coarse" diets had a higher ADC than "Fine" diets.

The interaction between grinding treatment and dietary GG levels was present for final mean weight, weight gain and feed conversion ratio ($p < .05$; Table 4). Inclusion of GG negatively affected the performance. However, the interaction effect demonstrated that this impact of GG was dependent on the grinding treatment. At the "Fine" grinding treatment, the negative impact of GG was less strong (Table 4).

The DM level of digesta from the distal gut was significantly affected ($p < .05$) by the GG inclusion level (Table 5). GG inclusion in the diet decreased the DM content. Although not significantly, the viscosity of the distal gut content increased numerically with increasing GG levels.

The GG levels significantly affected faecal recovery (Figure 3a), total faeces produced and non-recovered faeces (Figure 3b,d). The percentage of faecal recovery decreased with the increase in GG levels ($p = .021$). The trend in the amount of total faeces produced and non-recovered faeces ($p = .001$) was similar. However, the inclusion of GG tended to improve the particle size of faeces, but the effect was not significant (Figure 4). The grinding treatment did not affect any of the faecal waste characteristics.

4 | DISCUSSION

4.1 | Feed digestibility, fish performance and amount of faecal waste

Replacement of CMC by GG in the basal diet increased the dietary viscosity (Figure 2). The results show that ADC of nutrients in striped catfish was influenced by dietary viscosity (i.e., GG inclusion). The highest digestibility values are found in the diets without GG. Concomitant with the digestibility results, also the performance was negatively affected with increasing dietary viscosity. This corroborates with data from literature. In a study on rainbow trout, Storebakken (1985) showed that the ADC of protein and fat started

TABLE 4 The effect of grinding screen sizes and guar gum levels on the growth, survival rate and feed conversion ratio (mean ± SD) of striped catfish fed the experimental diets

| Grinding screen sizes	Guar gum levels (g/kg)	Initial mean weight (g per fish)	Final mean weight (g per fish)	Daily weight gain (g per fish per day)	Feed conversion ratio	Survival rate (%)
Fine (0.8 mm)	0	82 ± 0.1	131 ± 11.4	0.95 ± 0.22	1.80 ± 0.26	97 ± 2.9
	3	82 ± 0.2	127 ± 10.0	0.87 ± 0.19	1.99 ± 0.49	98 ± 2.9
	6	82 ± 0.2	127 ± 5.8	0.87 ± 0.11	2.07 ± 0.29	97 ± 5.8
Coarse (1.0 mm)	0	82 ± 0.1	128 ± 9.9	0.89 ± 0.19	1.80 ± 0.35	100 ± 0.0
	3	82 ± 0.2	123 ± 10.2	0.80 ± 0.19	2.18 ± 0.47	97 ± 5.8
	6	82 ± 0.1	118 ± 10.2	0.70 ± 0.20	2.18 ± 0.32	98 ± 2.9
p Values of the factors						
Grinding screen sizes		.583	.100	.097	.069	.463
Guar gum levels		.820	.118	.128	.110	.977
Interaction		.956	.027	.027	.001	.541
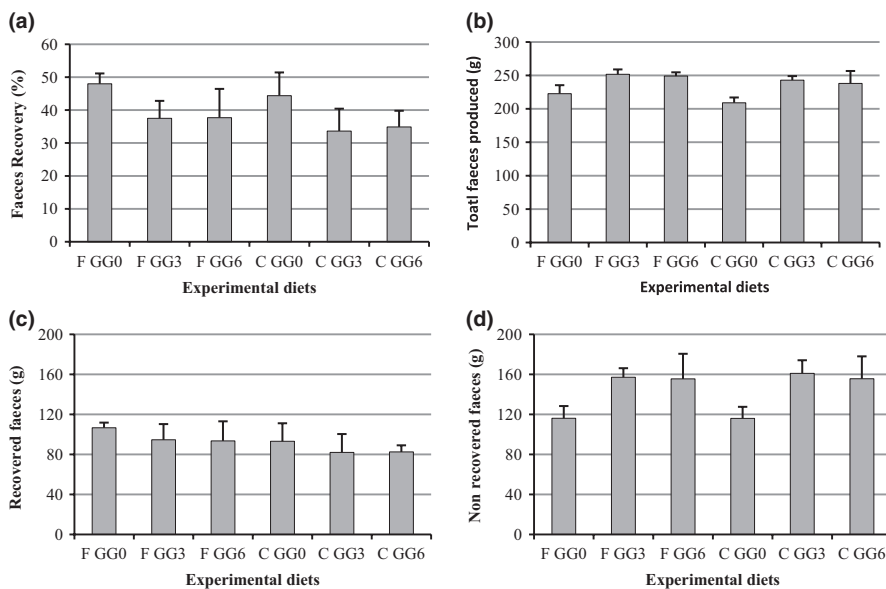
FIGURE 3 (a) Faeces recovery (%), (b) the amount (g DM/kg DM feed) of total faeces produced (TFP), (c) recovered faeces (RF) and (d) non-recovered faeces (NRF) of the six experimental diets refer to dietary GG inclusion and grinding screen sizes for the experimental period. *p* Values of grinding screen sizes, GG levels and interaction of (a) are .252, .021, .992; (b) .052, .001, .922; (c) .105, .313, .990; and (d) .846, .001, .969, respectively

TABLE 5 The effect of grinding screen sizes and guar gum levels on the chyme characteristics (mean ± SD) of the dissected distal gut of striped catfish fed the experimental diets

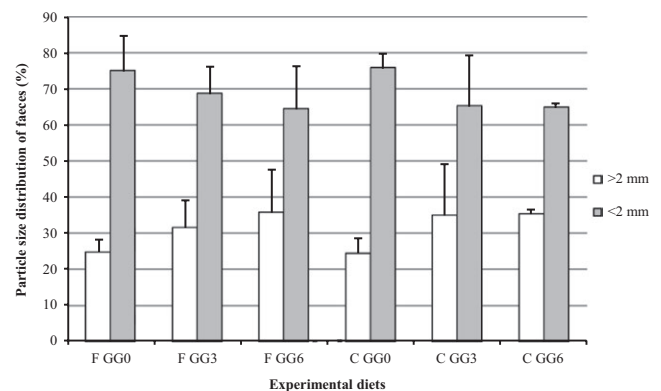| Grinding screen sizes	Guar gum levels (g kg^{-1})	Dry matter (g kg^{-1})	Viscosity (cP)
Fine (0.8 mm)	0	150 ± 1.3	2.09 ± 0.39
	3	130 ± 2.0	2.60 ± 0.89
	6	122 ± 3.4	2.67 ± 0.76
Coarse (1.0 mm)	0	147 ± 4.4	2.50 ± 0.27
	3	126 ± 3.4	2.56 ± 0.76
	6	120 ± 7.3	3.86 ± 1.17
p Values of the factors			
Grinding screen sizes		.133	.178
Guar gum levels		<.001	.123
Interaction		.914	.403

FIGURE 4 The effect of grinding screen sizes and guar gum levels on particle size distribution of faeces. *p* Values of grinding screen sizes, GG levels and interaction are .825, .111, .893 for the particle bigger than 2 mm and .922, .241, .789 for another particle, respectively

to reduce already at 20 g/kg GG in the diet, while fish final weight and feed efficiency was decreased at 100 g/kg GG in the diet. Negative effects of GG on performance were also found in African catfish (Leenhouwers et al., 2006) and in Nile tilapia (Amirkolaie, Leenhouwers, et al., 2005).

Comparison of literature shows that the effects of dietary GG levels on digestibility and performance differ among fish species. Just 0.1 g of GG/kg diet, for instance, already caused a lower growth in snakehead (Janphirom et al., 2010). On the other hand, African catfish did not show any difference in performance when GG was added up to 80 g GG/kg diet (Leenhouwers et al., 2008). In the case of rainbow trout, small fish (e.g., 43 g of body weight) were more severely affected by GG than large fish (e.g., 642 g of body weight) (Brinker, 2009). Negative effects of GG are also documented in broiler chickens fed maize diets which included 0 to 3 g/kg GG (Maisonnier, Gomez, & Carré, 2001).

Overall, the variability between fish species in the response to dietary GG inclusion is large. Part of this variability is most likely due to experimental design factors such as age of fish, dietary inclusion level and basal diet composition. For example, the current study demonstrated that the impact of dietary GG inclusion of on dietary viscosity dependents on grinding procedure. In a coarsely grinded mixture, the increase in viscosity was larger with inclusion level of GG compared to a finely grinded mixture (Figure 2a). Moreover, also the process technique and conditions might play a role. The current study shows that extrusion decreased the viscosity compared to the mixtures prior to extrusion. Similarly, it can be hypothesized that studies on dietary viscosity might have different outcomes between, for example, steam pelleting (Amirkolaie et al., 2006) versus extrusion in (Brinker, 2009 or current study). But for sure part of the variance will be due to species differences. Factors that might have an impact are the pH in the stomach, being very low in, for example, Nile tilapia (Saravanan et al., 2013); microbial activity in the intestine as hypothesized by Amirkolaie (2005) especially in warm water fish species; and anatomical/morphological difference of the gastrointestinal tract (e.g., stomachless fish). This topic deserves more research on understanding the species differences.

Guar gum, a soluble NSP, is hardly digested by aquatic species. In general, NSP is indigestible because of the absence of intestinal enzymes for degrading NSPs such as β-glucanases and β-xylanases (Kuz'mina, 1996). This inability of NSP digestion often goes together with a decrease in digestion and absorption of other nutrients like protein, lipid (Smits et al., 1998) and minerals (Sinha et al., 2011). It is suggested that undigested NSP can hinder the effectiveness of endogenous enzymes by binding or reduced mixing of chyme because of increased viscosity. Therefore, the supplementation with NSP-degrading enzymes may improve the digestibility, not only of the NSP, but also of the other nutrients (Stone, Allan, & Anderson, 2003) and thereby also performance (Ai et al., 2007).

Apart from affecting endogenous enzyme efficiency, the viscosity or gelling property of a diet (by inclusion, e.g., GG or other soluble NSPs) can also accelerate the release and losses of endogenous products such as bile acids, enzymes and mucus and may even cause the shedding of the mucosa layer thus increasing the excretion of these products. Secondly, the viscosity can also limit the distribution of digestive enzymes throughout the substrates. This leads to increased losses of nutrients through the faeces. Third, viscosity can also alter gut morphology, for example thicken the unstirred water layer adjacent to the mucosa, and thus reduce the absorption of hydrolysed nutrients (de Lange, 2000). Lastly, an interaction occurs between various components of NSP and minerals in the chyme, so that it reduces the absorption of the minerals (Sinha et al., 2011). This effect was reported in studies on broiler chickens fed CMC which is known as a soluble NSP (van der Klis, Verstegen, et al., 1993). Rye, which has a high NSP content, prevented the absorption of Ca, Mg, Na and P in African catfish (Leenhouwers, ter Veld, Verreth, & Schrama, 2007). In Nile tilapia, it decreased only Na absorption (Leenhouwers, Ortega, Verreth, & Schrama, 2007). Soy products caused the loss of extra Na in the faeces of Atlantic salmon (Storebakken et al., 1998). The current study confirms the latter, because GG addition (i.e., NSP supplementation) affected the ADC of non-NSP nutrients, protein and ash, in the diet.

Regarding the effect of the grinding size of the ingredients, this study indicated that a coarse grinding increased the ADC of DM and carbohydrate. However, regarding FCR the impact of grinding size depended on the dietary viscosity. At the GG0 treatment, there was no difference in FCR paralleling the equal measured dietary viscosity (Figure 2). While at the other GG treatments, coarse grinding increased the FCR. Results of earlier studies on how particle size of ingredients affects animal digestibility are conflicting. For example, fine grinding (3-mm hammer-mill screen) elevated ADC of gross energy in pigs compared to coarse grinding with 6-mm screen (Callan, Garry, & O'Doherty, 2007). Another pig study demonstrated higher ADCs when using a 3.0-mm compared to a 2.5-mm screen mesh with grinding (Moreira et al., 2009). The ADC of crude protein increased when the grinding screen size increased from 2.0 to 3.0 mm, but it was reduced at a screen size of 3.5 mm (Moreira et al., 2009). The improvement of digestibility may result from the increase in the uniformity and the surface area of ingredients (Callan et al., 2007). However, within the range of grinding sizes which are appropriate for an animal at a certain growth stage, the coarse diets enlarged the intestinal crypts (both in height and volume) more than fine diets did. This caused not only a better protection against intestinal infections (Brunsgaard, 1998) but also a better nutrient absorption. Moreover, Callan et al. (2007) reported that fine diets improved feed efficiency because of a decrease in feed wastage.

Due to the digestibility effects, there was a difference in the amount of faecal waste produced. The more nutrients were digested, the less amount of faeces was produced. In the current study, increasing dietary viscosity induced striped catfish to produce more faeces. This is in accordance with research on Nile Tilapia where 80 g GG/kg of diet increased the amount of faecal waste (Amirkolaie, Leenhouwers, et al., 2005).

4.2 | Faecal waste

This study revealed that increasing dietary viscosity significantly decreased the DM content of faeces, the percentage of faeces recovery

and the amount of recovered faeces but increased the amount of total faeces produced. In addition, inclining dietary viscosities also seemed to elevate the viscosity of digesta in the distal intestine and particle size of the faecal waste in this catfish, but these results did not show any significant difference between treatments. This tendency of an increased viscosity in the proximal intestine by dietary inclusion of GG was also found in the research on tilapia (Amirkolaie, Leenhouwers, et al., 2005).

GG is a galactomannan which contains linear chains of β–(1→4) mannan. It is water-soluble and able to absorb water (Sinha et al., 2011); this may have resulted in the increased water content in the faeces of striped catfish. In addition, GG is highly viscose and has been shown to increase digesta viscosity in the proximal intestine (Amirkolaie, Leenhouwers, et al., 2005; Leenhouwers et al., 2006). However, in the current study, despite the reduced DM content of the digesta in the distal intestine of striped catfish, no effect on viscosity was found. The absence of a difference in viscosity in the current study might be due to stimulation of fermentation with increasing GG levels. The fermentation of carbohydrates occurs which is indicated by increased levels of volatile fatty acids in the distal part of the intestine in broiler (van der Klis, Van Voorst, et al., 1993) or in fish like Nile tilapia (Leenhouwers, ter Veld, et al., 2007) and African catfish (van der Klis, Van Voorst, et al., 1993; Leenhouwers, ter Veld, et al., 2007; Sinha et al., 2011). The enhanced fermentation may have neutralized the viscose properties of GG and thereby its positive impact on faecal stability through binding the faecal pellet together.

Before starting the experiment, it was expected that increasing faecal stability would be reflected in larger faecal pellets and that these larger faecal pellets would be more efficiently removed from the water. The current study showed that increasing dietary viscosity by GG inclusion in the diet reduced the faecal removal efficiency in the experimental units. However, diets did not affect the size of faecal pellets. Numerically, there was a tendency for an increased faecal pellet size with increasing dietary viscosity levels. This observation might be due to the fact that in the current study, the contrasts in dietary viscosity levels were not large enough because GG was placing CMC in the diet. The absence of a relation between faecal pellet size and recovery efficiency might also be due to the fact that particle size was determined on faeces collected by settling. This may have led to a non-representative sampling of faecal pellets. The collection method may have selected on the stable part of the faecal pellets, that is, thereby standardizing the faecal pellet size already.

5 | CONCLUSION

Increasing dietary viscosity by inclusion of GG had no positive impact on faecal waste management in striped catfish culture, because it negatively affected nutrient digestibility and also reduced the removal efficiency of faeces from the water by settling. Faecal pellet stability was not enhanced with increasing dietary viscosity indicated by the absence of impacts on faecal viscosity and faecal pellet size. Grinding the ingredients on different screen size had no impact on the characteristics of the egested faeces. However, due to impacts on digestibility, the grinding procedure altered the amount of faecal waste produced. Grinding at a larger screen size (1 mm versus 0.8 mm) increased the digestibility of DM and carbohydrate but the elevating impact on the feed conversion ratio dependent on the dietary viscosity.

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REFERENCES

Ai, Q., Mai, K., Zhang, W., Xu, W., Tan, B., Zhang, C., & Li, H. (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147*, 502–508.

Allan, G. L., Rowland, S. J., Parkinson, S., Stone, D. A. J., & Jantrarotai, W. (1999). Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: Development of methods. *Aquaculture, 170*, 131–145.

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

Amirkolaie, A. K., El-Shafai, S. A., Eding, E. H., Schrama, J. W., & Verreth, J. A. J. (2005). 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.

Amirkolaie, A. K., Leenhouwers, J. I., Verreth, J. A. J., & Schrama, J. W. (2005). 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.

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.

AOAC (2000). *Official methods of analysis of the Association of Official Analytical Chemists*. Washington, DC: AOAC.

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.

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.

Brinker, A., Koppe, W., & Rösch, R. (2005). Optimised effluent treatment by stabilised trout faeces. *Aquaculture, 249*, 125–144.

Brunsgaard, G. (1998). Effects of cereal type and feed particle size on morphological characteristics, epithelial cell proliferation, and lectin binding patterns in the large intestine of pigs. *Journal of Animal Science, 76*, 2787–2798.

Callan, J. J., Garry, B. P., & O'Doherty, J. V. (2007). The effect of expander processing and screen size on nutrient digestibility, growth

performance, selected faecal microbial populations and faecal volatile fatty acid concentrations in grower–finisher pigs. *Animal Feed Science and Technology*, *134*, 223–234.

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, GBR: Academic Press.

Da, C. T., Lundh, T. R., & Lindberg, J. E. (2012). Evaluation of local feed resources as alternatives to fish meal in terms of growth performance, feed utilisation and biological indices of striped catfish (*Pangasianodon hypophthalmus*) fingerlings. *Aquaculture*, *364-365*, 150–156.

De Silva, S. S., Ingram, B. A., Nguyen, P. T., Bui, T. M., Gooley, G. J., & Turchini, G. M. (2010). Estimation of nitrogen and phosphorus in effluent from the striped catfish farming sector in the Mekong Delta. *Vietnam. Ambio*, *39*, 504–514.

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.

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

Hien, T. T. T., Phuong, N. T., Le Tu, T. C., & Glencross, B. (2010). Assessment of methods for the determination of digestibilities of feed ingredients for Tra catfish, *Pangasinodon hypothalamus*. *Aquaculture Nutrition*, *16*, 351–358.

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.

Kuz'mina, V. V. (1996). Influence of age on digestive enzyme activity in some freshwater teleosts. *Aquaculture*, *148*, 25–37.

de Lange, C. F. M. (2000) *Characterisation of the non-starch polysaccharides* (pp. 77–92). Wageningen, The Netherlands: Wageningen Pers.

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.

Leenhouwers, J. I., Ortega, R. C., Verreth, J. A. J., & Schrama, J. W. (2007). 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., Pellikaan, W. F., Huizing, H. F. A., Coolen, R. O. M., Verreth, J. A. J., & Schrama, J. W. (2008). Fermentability of carbohydrates in an in vitro batch culture method using inocula from Nile tilapia (*Oreochromis niloticus*) and European sea bass (*Dicentrarchus labrax*). *Aquaculture Nutrition*, *14*, 523–532.

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

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.

Moreira, I., Kutschenko, M., Paiano, D., Scapinelo, C., Murakami, A. E., & de Quadros, A. R. B. (2009). Effects of different grinding levels (particle size) of soybean hull on starting pigs performance and digestibility. *Brazilian Archives of Biology and Technology*, *52*, 1243–1252.

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.

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 products. *Animal Feed Science and Technology*, *79*, 331–345.

Saravanan, S., Geurden, I., Orozco, Z. G., Kaushik, S. J., Verreth, J. A., & Schrama, J. W. (2013). Dietary electrolyte balance affects the nutrient digestibility and maintenance energy expenditure of Nile tilapia. *British Journal of Nutrition*, *110*, 1948–1957.

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.

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.

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.

Smits, C., Veldman, A., Verkade, H., & Beynen, A. (1998). The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine. *Poultry Science*, *77*, 1534–1539.

Stone, D. A. J., Allan, G. L., & Anderson, A. J. (2003). Carbohydrate utilization by juvenile silver perch, *Bidyanus bidyanus* (Mitchell). IV. Can dietary enzymes increase digestible energy from wheat starch, wheat and dehulled lupin? *Aquaculture Research*, *34*, 135–147.

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.

Storebakken, T., Kvien, I. S., Shearer, K. D., Grisdale-Helland, B., Helland, S. J., & Berge, G. M. (1998). The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal fed to Atlantic salmon (*Salmo salar*): Evaluation of different faecal collection methods. *Aquaculture*, *169*, 195–210.

van der Klis, J. D., Van Voorst, A., & Van Cruyningen, C. (1993). Effect of a soluble polysaccharide (carboxy methyl cellulose) on the physicochemical conditions in the gastrointestinal tract of broilers. *British Poultry Science*, *34*, 971–983.

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.