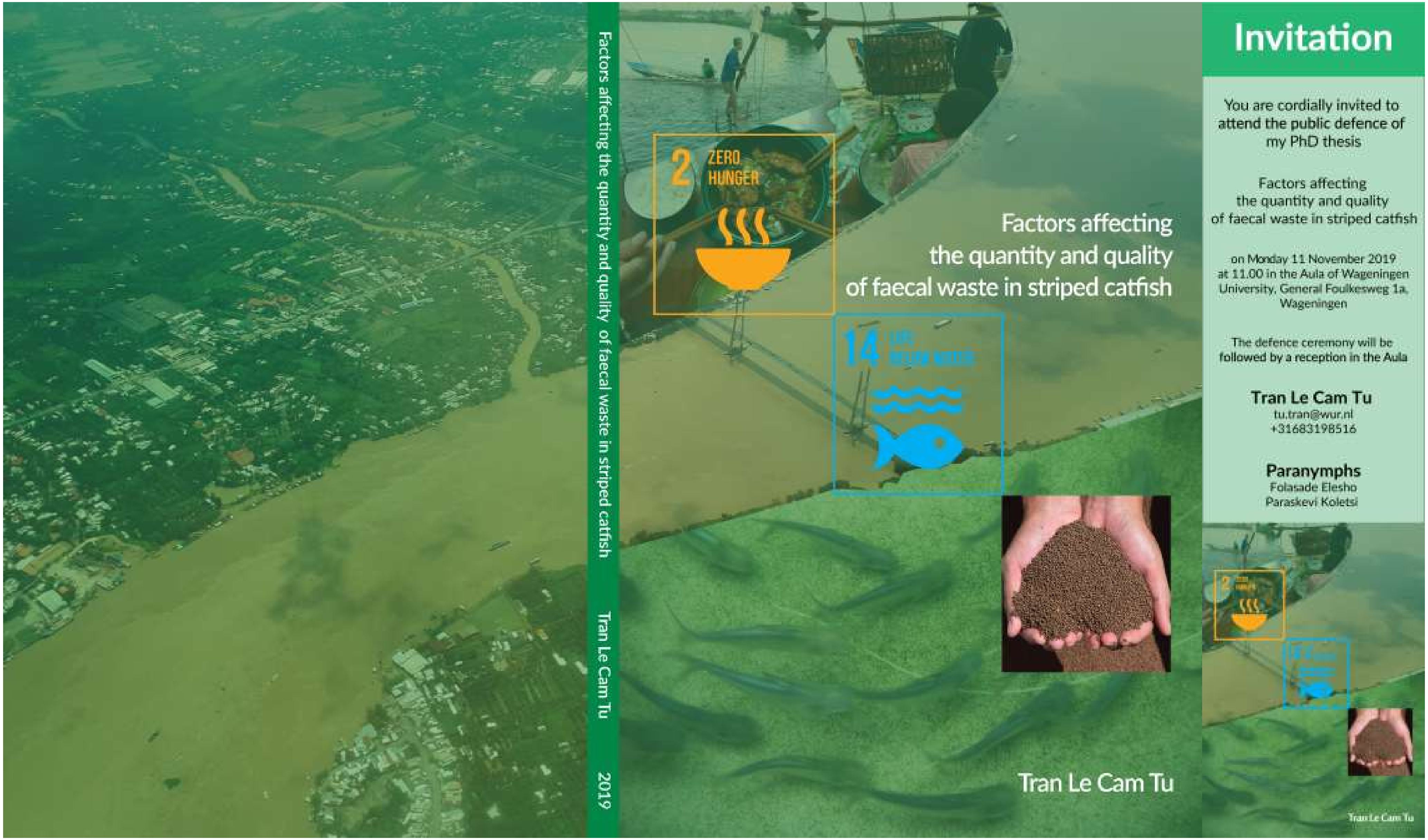
Factors affecting the quantity and quality of faecal waste in striped catfish
Tran Le Cam Tu
2019
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Factors affecting
the quantity and quality
of faecal waste in striped catfish
Tran Le Cam Tu
Invitation
You are cordially invited to attend the public defence of my PhD thesis
Factors affecting the quantity and quality of faecal waste in striped catfish
on Monday 11 November 2019 at 11.00 in the Aula of Wageningen University, General Foulkesweg 1a, Wageningen
The defence ceremony will be followed by a reception in the Aula
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Paranymphs
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Paraskevi Koletsi
Tran Le Cam Tu

Propositions

1. Binders with viscous properties must not be used in striped catfish feeds.
   (This thesis)
2. The ingredient composition of striped catfish feeds is more important than the macronutrient composition to steer the quantity and quality of faecal waste.
   (This thesis)
3. Biotechnology eliminates specific crop growing zones.
4. Managing an ecosystem is more difficult than managing a bank system.
5. Food can be a silent killer.
6. WUR’s PhD regulations regarding international opponents are inconsistent with its ambition to be sustainable.

Propositions belonging to the thesis, entitled

Factors affecting the quantity and quality of faecal waste in striped catfish

Trần Lê Cẩm Tú

Wageningen, 11 November 2019

Factors affecting the quantity and quality of faecal waste in striped catfish

Trần Lê Cẩm Tú

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This research was conducted under the auspices of the Graduate School, Wageningen Institute of Animal Sciences (WIAS).

Factors affecting the quantity and quality of faecal waste in striped catfish

Trần Lê Cẩm Tú

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 11 November 2019
at 11 a.m. in the Aula.

Trần Lê Cẩm Tú
Factors affecting the quantity and quality of faecal waste in striped catfish
116 pages.
PhD thesis, Wageningen University, Wageningen, the Netherlands (2019)
With references, with summary in English.
ISBN: https://doi.org/10.18174/501438
DOI: 978-94-6395-138-8

To my parents

Abstract

Tran-Tu, L.C. (2019). Factors affecting the quantity and quality of faecal waste in striped catfish. PhD thesis.

Wageningen University and Research, The Netherlands.

This study investigated the nutritional interventions to alter/improve faecal pellet quality (which could lead to better and more complete removal of faecal waste) and the enhancement of the stability of egested faeces. In this thesis, striped catfish was taken as a model species, to study the options for aquaculture waste management. Up till now, it is not clear if nutritional interventions targeted to reduce the amount of faecal waste can go along with improving the faecal quality. The main aim of this research was to study the impact of different nutrition interventions on both the quantity and quality of faecal waste produced by striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878). The most ideal nutritional interventions would be those that reduce the quantity and at the same time, improve the quality of faeces. To reach the aim, we conducted four studies. Firstly, we determined the characteristics of waste production and digestibility of dietary ingredients. Fifteen commonly applied ingredients in feed formulation of striped catfish were studied: protein-rich ingredients, ingredients relatively rich in non-starch polysaccharides, and oil and starch-rich ingredients. Secondly, we tested the impacts of the ingredients' particle size and the dietary viscosity on digestion, performance and faecal waste management of this fish. Thirdly, we analysed the dose-response relationship between dietary viscosity and nutrient digestibility and faecal waste characteristics in striped catfish and the long-term impacts of viscosity on the performance of striped catfish. Lastly, we assessed the relationship between the physical and chemical characteristics of chyme and apparent faecal nutrient digestibility. In particular, this study determined the effect of dietary viscosity on chyme characteristics in different segments of the gastrointestinal tract (GIT) of striped catfish, and the progression of nutrient digestion throughout the GIT. In all our studies we have noticed that the ingredient composition of a feed strongly determines the amount of faecal waste produced on a diet. Faecal quantity can be altered by feed technology/feed processing conditions (e.g., grinding screen size). For striped catfish, feed and faecal pellet binders, which increase the dietary viscosity, should not be used in the diet because it reduces the dry matter digestibility even at very low inclusion levels. The negative impact of dietary viscosity on digestion in striped catfish appears very proximal in the gastrointestinal tract (stomach). This negative impact on digestion is not compensated after the evacuation of the chyme from the stomach. The stability of faecal waste is determined by the ingredient composition of the feed consumed by the fish.

Contents

Chapter 1

General Introduction

Aquaculture production in the world is growing rapidly and is expected to reach 140 million tonnes in 2050 (Waite *et al.*, 2014a). This fast growth is the result of a combination of increasing the area for the production and of intensification. The latter means higher inputs in the system which has as a consequence that more waste is released from the production system. Boyd (2015) estimated the amount of total waste produced (kg of dry matter) from fish farming 4.4 to 4.8 per kg of dry matter fish production if the FCR (feed conversion ratio) is between 1.5 to 1.6. Releasing such a large discharge of waste straight into the open water will decrease the quality of the recipient surface water and associated ecosystem (Turcios and Papenbrock, 2014). The waste produced from aquaculture farming is rich in nitrogen and phosphorus (Nhut *et al.*, 2017). Loss of nutrient-rich waste leads to both economic and environmental inefficiency. To enable the continued growth of aquaculture the nutrient efficiency of the production system should be improved. Reuse of this waste as nutrient input for other agricultural practices has been studied. That includes the use of wastewater and sludge in producing rice or vegetables for human food (Trieu and Lu, 2014), or producing duckweed for animal feed (Cheng and Stomp, 2009), or microalgae for functional food or medicines (Muller-Feuga, 2000). In these integrated farming systems, the wasted nutrients are recovered into the new valuable biomass. This is meaningful for material transformation (form waste to useful nutrients), for the environment (purifying the effluents before they are discharged) and for stability. Reusing wasted nutrients is one of the approaches to waste management. The possibility of reusing waste largely depends on the waste removal efficiency which on its turn is linked with the type and the amount of waste produced. To address aquaculture waste management more effectively, understanding the origin of the waste, the types, its composition, and the factors affecting the waste is important.

1.1 Types of waste and their impacts

In an aquaculture system, there are mainly three types of waste: settleable solids, suspended solids, and dissolved waste. Solid waste is derived from uneaten feed and faeces (Figure 1.1) and can be further divided into settleable solids and suspended solids (Dauda *et al.*, 2018). The solid particles which are large and heavy settle to the bottom of the system. The smaller and lighter solid particles remain suspended in the water column of the system. The ratio between settleable and suspended solids may be further decreased because of the swimming activities of cultured animals and water disturbance from aeration which both may also aggravate the disintegration of the larger particles into smaller ones. The settleable solids accumulate on the pond bottom during the culture period and are known as sludge in the farmed pond. The toxic products, *e.g.* hydrogen sulphide, acetic acid, lactic acid, *etc.* produced by anaerobic activities at the pond bottom may affect the fish health and indirectly also the farmer's profit by lowering the feed efficiency (Wahab *et al.*, 2003). Suspended solids increase the turbidity of water and clogging the fish gills which may impact fish breathing and even lead to death. Moreover, because of the decomposition from bacterial activities in the system, a part of the settleable solids and suspended solids are decayed into dissolved waste. This transformation consumes oxygen in the water thus deplete the available oxygen in the water column. Also, the possibility of the amount of dissolved waste increases because of the leaching and simple dissolving of nutrients from solid waste. Solid waste can be reduced by managing the feeding practice and/or improving the digestibility. The digestibility improvement can be done by diet formulation, dietary additive supplementation (*e.g.* exogenous enzymes), and/or feed technology. The more feed is digested, the fewer faeces are produced leading to less solid waste in the system.

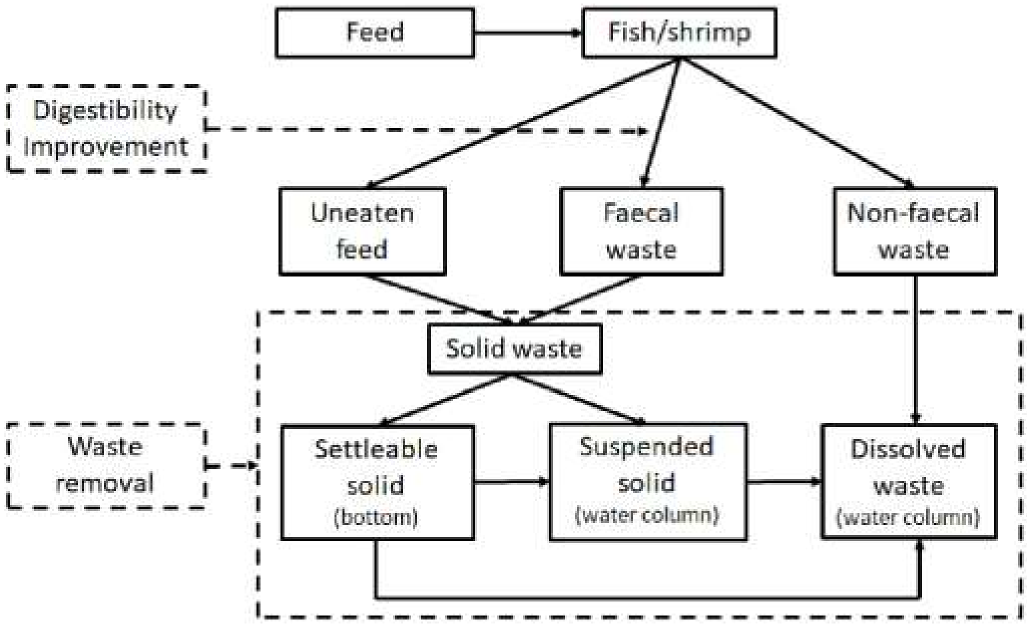

Figure 1.1 Types of waste in an aquaculture system.

Part of the dissolved waste originates from solid waste. The other part of the dissolved waste is related to the metabolic activity of fish, which results in the excretion of metabolites in the form of branchial and urinary (mainly nitrogen) losses and through respiration (mainly carbon dioxide) and in the form of heat losses. The dissolved waste in the water column contains primarily nitrogen, carbon, and phosphorus. The lost nutrients easily lead to eutrophication (Kokou and Fountoulaki, 2018) if many ponds directly discharge wastewater into the river. The approximate amount of nitrogen loss as dissolved waste via the gills and urine is 30 to 60% of the dietary nitrogen (Schneider *et al.*, 2004). So, it means that the nitrogen (protein) contents in the feed are wasted (Kabir *et al.*, 2019). High concentrations of ammonia, a product from dissolved waste, is toxic for fish and harmful to the fish growth (Wahab *et al.*, 2003). The nitrogen loss can be mitigated by improving the dietary amino acids profile (*e.g.* dietary amino acids balance) and the ratio between dietary digestible protein and digestible energy (Hua and Bureau, 2010). The phosphorus loss can be managed by increasing the available dietary digestible phosphorus (for example, by adding digestive enzymes, such as phytase) or increasing bioflocs into the system (Kuhn *et al.*, 2010).

1.2 Factors affecting waste production in aquaculture

The amount and types of waste produced in aquaculture are affected by three main factors, the feed, the species under concern and the production system.

The feed quality is related to the dietary composition and ingredients used to formulate the diet. The macro-nutrients, *i.e.* protein, fat, ash, fibre, non-starch polysaccharides (NSP) in the diet affect the digestibility or the amount of faeces produced and also alter the types of faecal solids (Amirkolaie, 2005; Teuling, 2018). The balance of amino acids in the dietary protein alters the amount of nitrogen in faecal and non-faecal waste produced. Also, the fat levels and fatty acid compositions in the diet impact the amount and types of faecal waste. In addition, and often more important, the type ingredients included into a diet and the interaction between these ingredients affect the produced waste.

The amount and types of faecal waste depend also on the cultured species. The digestion of a diet differs between carnivores, omnivores and herbivorous species. Low-quality plant ingredients are better digested by omnivores (*e.g.* striped catfish) and herbivores (*e.g.* grass carp) compared to carnivores (*e.g.* snakehead). Whereas high-quality protein sources (both plant and animal origins) are highly digestible for all types of fish but the differences in digestibility between them are small. The characteristics of the faeces differ among different species. String faeces are produced by *e.g.*, tilapia, black tiger prawn, white leg shrimp, knife fish, while solid faecal pellets are found in *e.g.*, rainbow trout, barramundi, salmon, lobster and diarrhea-like faeces in *e.g.*, striped catfish, African catfish and snakehead.

The husbandry conditions have an impact on the utilization of feed and thus the waste produced. Moreover, the role of waste is different in different production systems. For example, in cage culture, the waste is entirely released to the host waterbody while in a recirculation aquaculture system (RAS), waste is partly neutralized. In a balanced pond, waste, to a certain extent, is used as fertilizing nutrient. Therefore, the amount of waste at the end of the day also varies between the systems. The other settings of the production system, such as water flow rate in a RAS or raceway, can impact the stability of the faeces and the rate of the transformation of the waste stream from the settleable solid to the suspended solid and then to dissolved waste.

1.3 The quantity and quality of faecal waste and related studies

The quantity of faecal waste is how much waste (or faeces) is produced per unit of fish production or feed consumed. It is determined based on the amount of indigestible feed or indirectly by the FCR. If the digestibility of the diet is improved, fewer faeces are produced.

Digestibility of diets and ingredients were mentioned in many studies, *e.g.* in tilapia (Köprücü and Özdemir, 2005), salmon (Burr *et al.*, 2011), rainbow trout and seabass (Glencross, 2011). However, previous articles concentrated on the digestibility from the perspective of increasing the nutrient utilization efficiency, not from the perspective of waste management. Recently, because of the development of RAS, the awareness on the importance of (solid) waste management has got priority, not only for economics and operation of the systems but also for its impact on fish health and environment. Some studies measured the amount of faecal production in tilapia (Amirkolaie *et al.*, 2005; Amirkolaie *et al.*, 2006). Moreover, the quantity of faecal waste shows the amount of nutrient losses through faeces (Schneider *et al.*, 2004; Maas *et al.*, 2018).

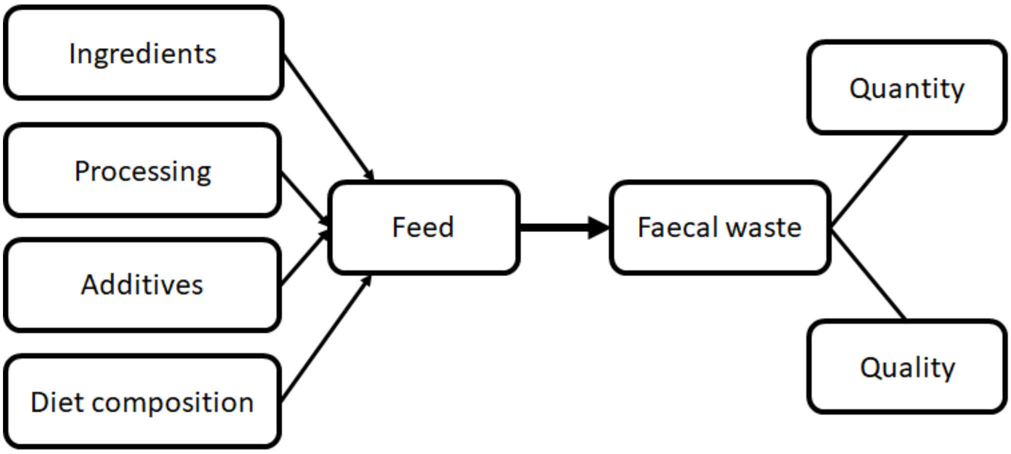

Figure 1.2 Dietary factors affect the quantity and quality of faecal waste.

The quality of faecal waste is related to the characteristics and the removal rate of faeces, *e.g.*, whether the faeces are stable, settleable and easy to remove from the system for treatment. Parameters such as dry matter, viscosity, recovery rate, particle sizes of faecal solids, *etc.* constitute the faecal characteristics. Moist solid faeces are easily and quickly disintegrated. The viscous faeces show a strong connection between molecules and thus these faecal solids will be stable. Concomitantly viscous faeces lead to larger faecal solid particles than less viscous faeces. The quality of faeces is determined by the types of dietary ingredients, processing, and the inclusion of additives in the diet (Figure 1.2). Understanding the faecal quality helps to improve the faeces recovery or to supply the potential information for treating the dissolved waste (non-recovered faeces). The stability of faeces of rainbow trout was improved by increasing the faecal viscosity thus enlarging the particle size of faeces to settle easily (Brinker *et al.*, 2005; Brinker, 2007). A high concentration of volatile fatty acids in the distal intestine was related to a low faecal removal percentage in Nile tilapia (Amirkolaie *et al.*, 2006). Leenhouwers *et al.* (2007b) showed a decrease in faecal dry matter with increasing dietary viscosity as a result of replacing different ingredients in African catfish and that influenced the removal of the faeces. In addition, there are some studies (Leenhouwers *et al.*, 2007a; Harter *et al.*, 2013) which observed that the variation in the characteristics of chyme related to the faecal characteristics.

1.4 Striped catfish culture in Mekong Delta

In this thesis, the case of striped catfish (*Pangasionodon hypophthalmus*) culture in Vietnam was used as a model to study aquaculture waste management because of several reasons. It is considered as the most intensive fish farming done in ponds and is growing very fast in the Mekong Delta in Vietnam. The sustainability of this farming and its growth will depend on waste management as the Vietnamese government already put a restriction on the direct discharge of waste to the Mekong river and its branches. The pressure of the international trend of replacing fishmeal in the feed by plant-based ingredients might increase the waste produced from current farming practice.

Striped catfish (commonly known as Tra catfish, ca tra or Pangasius) has been commercially produced in Vietnam since the early 2000s. Its farming is mainly situated in 10 provinces of the lower Mekong River Delta (MRD) and in two other provinces (Tay Ninh and Quang Nam) outside the lower MRD region but also in the South of Vietnam. In 2017, the Directorate of Fisheries reported approximately 6100 ha water surface area that was used for farming and a total production of 1.2 million tonnes. These numbers will be expected to increase to respectively 10,000 ha and 1.8 – 2.0 million tonnes in 2020 (Decree No. 1445/QD-TTg, 2013). The fish is farmed intensively in deep (4-6m) ponds and the productivity will range from 400 – 800 tonnes ha^{-1} $crop^{-1}$ (Nguyen, 2013). However, with such intensified farming, about 4 thousands m^3 of water is used per tonne of catfish produced (De Silva and Phuong, 2011).

Striped catfish is known as a diarrhea-like faeces producer. Eighty-two percent of the surveyed striped catfish farms discharged the effluent from the farmed ponds directly into the river or canals (Phan *et al.*, 2009). Nhut *et al.* (2017) reported that per one tonne of dry matter (DM) of fish production, approximately 2.5 tonnes of DM waste were produced, in which there was 45 kg of DM phosphorus and 94 kg of DM nitrogen. From this amount, 1.7 tonnes of DM sludge and 600 kg of DM effluent was discharged. The largest amount of waste in the pond is sludge but the high nutritious effluent might cause negative impacts for not only the environment but also society because mainly inhabitants are living in the MRD. It is obvious that managing the growth of the discharging amount of waste into the Mekong River is crucial for the sustainability of the striped catfish culture.

It is important to look for alternative protein sources for the cultured animal. From 1990 to 2014, the world capture fisheries have remained rather constant at approximately 90 million tonnes yearly (FAO, 2016). Concomitantly, the price of fishmeal climbed yearly. For example, the fishmeal price in Germany and the Netherlands was just about 800 US$ $tonne^{-1}$ in 2005 but had almost doubled in 2015 (FAO, 2016). To reduce the captured pressure from the marine pelagic fish stocks because of the scarcity of fishmeal and fish oil (Boyd, 2015), alternative protein sources for animal feeds, *e.g.* plant-based sources, insects, algae, *etc.* are crucial. This trend is unavoidable for farming practice in the future. However, because of the presence of anti-nutritional factors in plants, plant-based protein ingredients might worsen the digestibility of the feed, leading to more faeces being produced.

1.5 Aim and outline of the research

To manage the faecal waste production in striped catfish, two important approaches are available: 1) reducing the amount of waste, 2) getting the waste out of the farming system quickly and completely (Figure 1.1). In the first approach, the amount of faecal waste can be reduced by improving feed digestibility. So far, few studies are available on the digestibility of feed for striped catfish (Hien *et al.*, 2010; Da *et al.*, 2013) and even few studies address the waste management in striped catfish. Improving feed digestibility may imply that feeds will cost more money (Ngoc, 2016). However, specifically for the case of striped catfish culture, the feed price may hamper farm profitability and the willingness of farmers to use such feed. Despite this, for a balance waste management by reduction of the amount of the faecal waste, more information is required on factor affecting digestibility in striped catfish like ingredients and feed technology. In the second approach, the removal of faecal waste from the water, two ways of intervention can be taken: 1) altering the production systems from traditional ponds toward alternative systems in which solid removal is applied (Nhut, 2016); and 2) nutritional interventions to alter/improve faecal pellet quality (better and more complete removal of faecal waste). The latter targets enhancement of the stability of egested faeces. Up till now, it is not clear if nutritional intervention aimed at reducing the amount of faecal waste can concomitantly go with improving the faecal quality. This thesis aims at studying the impact of nutrition interventions on both the quantity and quality of faecal waste produced by striped catfish (Figure 1.2). The most ideal nutritional interventions would be those that reduce the quantity and increase the quality of faeces.

In **Chapter 2**, the effect of the ingredient composition of feeds on the quantity and quality of faecal waste was addressed. Fifteen commonly applied ingredient in feed formulation of striped catfish were studied regarding their nutrient digestibility, the amount of faeces produced and the removal efficiency by settling. In **Chapter 3**, the addition of a specific additive was assumed to alter the viscosity of the faeces was studied both regarding the quantity and quality of faecal waste. It was hypothesised that increasing the viscosity of faeces would improve the quality of faeces (stability). Additionally, it was assessed if the particle size of the ingredients (*i.e.* a technological treatment) influenced digestibility and faecal stability. Opposite to the expectation, dietary viscosity increased the quantity and decreased the quality of faecal waste. This indicated the importance of dietary viscosity for faecal waste management. It was hypothesised that the level of viscosity applied in this study might have been too high. Therefore, the dose-response of dietary viscosity was tested in the next study regarding the quantity and quality of faecal waste (**Chapter 4**). In **Chapter 5**, the mechanism of how dietary viscosity is influencing the digestion of nutrition throughout the gastrointestinal tract was studied, with a focus on the quantity of faecal wasted. In the final chapter of this thesis (**Chapter 6**) the results, the potential solutions and implications for managing faecal waste of striped catfish are discussed.

Chapter 2

Dietary ingredients affect digestibility and faecal waste of striped catfish (*Pangasionodon hypophthalmus*)

This chapter has been submitted in Aquaculture as:
Tran-Tu, L. C., Thuy-Lam, N. T., Verreth, J. A. J., and Schrama, J.W., 2019. **Dietary ingredients affect digestibility and faecal waste of striped catfish (*Pangasionodon hypophthalmus*)**.

Abstract

The effect of the dietary ingredients on nutrient digestibility and the quantity and quality of the faecal waste produced by striped catfish was assess. This was studied in three experiments. In Exp 1 protein rich ingredients (local fishmeal, defatted soybean meal, meat &bone meal, canola, and full fat soybean meal), Exp 2 ingredients relatively rich in non-starch polysaccharides (defatted rice bran, dried rice bran, wheat bran, palm kernel meal, and dried distillers grains with solubles) and in Exp 3 oil and starch rich ingredients (fish oil, soybean oil, wheat, broken rice, and cassava) were tested. Within experiments, one reference diet and five test diets were tested in triplicate. Nutrient digestibility varied between ingredients. Consequently, the quantity of faecal waste was affected by ingredients composition, ranging from 92 and 249 g kg^{-1} of feed. Also, the recovery rate of faecal waste by settling depended on ingredient composition ranging from 20 to 52%. In conclusion, both the quantity and quality of faecal waste of striped catfish is dependent on the ingredient composition of the diet. The differences in non-recovered faecal waste between ingredients shows that management of faecal waste is possible by diet formulation.

2.1 Introduction

An important quality indicator of an aquafeed is its impact on fish productivity, which is for a large part related to its ingredient composition. To formulate balanced diets, information on nutrient content and digestibility of the used ingredients is essential (Glencross *et al*., 2007). Since nutrient digestibility quantifies the amount of nutrients available for growth, in the past numerous studies have measured apparent digestibility of ingredients, *e.g.* see NRC (1993, 2011). Also, when the potential of an ingredient to serve as an alternative for fishmeal is assessed, its digestibility is usually quantified, like *e.g.*: for soybean meal (Shiau *et al*., 1990), lupines (Glencross *et al*., 2003) and micro algae (Teuling *et al*., 2018; Gong *et al*., 2018). Various factors affect the nutrient digestibility of ingredients and/or aquafeeds like: technological treatments, presence of anti-nutritional factors, environmental conditions (water temperature and oxygen), but also fish species (Francis *et al*., 2001; Glencross *et al*., 2007; Tran-Duy *et al*., 2010; Tran-Tu *et al*., 2018; Teuling *et al*. 2018). Differences among fish species in ingredient digestibility imply that for new cultured species, digestibility studies are again needed for proper diet formulation. Striped catfish *Pangasionodon hypopthalmus* is a recent example of such a newly cultured species. Its commercial farming strongly increased since the early 2000s (De Silva and Phuong, 2011). Yet, relatively few studies on digestibility of ingredients in striped catfish have been reported (Hien *et al*., 2010; Da *et al*., 2013).

Besides providing nutrients for growth and maintenance, aquafeeds are also a main source of waste originating from aquaculture (Cho and Kaushik, 1985; Kokou and Fountoulaki, 2018). The digestibility of a diet directly relates to the amount of faecal waste entering the water (Tran-Tu *et al*., 2018). To keep the water quality optimal for fish while at the same time reducing the impact on surrounding water bodies, a quick and efficient removal of faecal waste is important (Brinker *et al*., 2005). Theoretically, dietary interventions for faecal waste management can aim at steering the quantity (*e.g.*, digestibility) and/or the quality (*e.g.*, stability) of the egested faeces (Kokou and Fountoulaki, 2018). So far, knowledge on dietary factors affecting faecal quality is relatively scarce. Dietary binders, like guar gum, have been shown to alter the removal efficiency of faeces by settling in trout (Brinker, 2007), in tilapia (Amirkolaie *et al*., 2005b) and in striped catfish (Tran-Tu *et al*., 2018). In tilapia (Schneider *et al*., 2004; Amirkolaie *et al*., 2006) and in common carp (Prabu *et al*., submitted) also specific ingredients influence faecal removal efficiency. Information on the effects of specific ingredients on faecal waste quality in other fish species, such as striped catfish, is scarce.

For good faecal waste management, it is important to understand how the dietary ingredients influence the quantity (*e.g.*, digestibility) and quality (*e.g.*, recovery efficiency) of faecal waste. The present study assessed the effect of the dietary ingredient composition on nutrient digestibility and the quantity and quality of the faecal waste produced by striped catfish.

2.2 Materials and methods

2.2.1 Experimental diets

This study comprised three experiments and was conducted at Can Tho University, Vietnam. The three experiments lasted 61, 53 and 49 days, respectively. Per experiment, five ingredients were studied (15 in total). Within each experiment, one reference (Ref) diet and five test diets were tested in triplicate. In the test diets, 30% of the test ingredient was included into the reference diet, with exception of the test diets for the oil-ingredients in which 10% of the tested oils were included into the reference diet, according to Foster for measuring ingredient digestibility (Forster, 1999).

Table 2.1 The ingredient composition (in g kg^{-1}) of the reference diet, which was used to test the impact of fifteen ingredients on nutrient digestibility and faecal waste characteristics

Ingredients	Amount (g kg^{-1})
Fishmeal [1]	380
Wheat flour [2]	540
Fish oil [1]	30
Carboxyl methyl cellulose [3]	20
Vitamin and mineral premix [4]	10
SiO_2 [3]	10
Cr_2O_3 [5]	10

[1] Fish meal and fish oil was imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam).
[2] Wheat flour was imported and retailed by Hong Ha Co., Ltd (Can Tho city, Vietnam).
[3] Carboxyl methyl cellulose and SiO_2 were produced by Xilong Chemical Co., Ltd. (China) and imported by Thanh My Co. Ltd (Vietnam).
[4] Vitamin and mineral premix (UI or mg kg^{-1}): 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., Vietnam).
[5] Cr_2O_3 was produced by the Merck Group (Germany) and imported by Thanh My Co. Ltd (Vietnam).

The major ingredients of the reference diet (Ref) were imported fish meal, wheat flour and fish oil (Table 2.1). For measuring digestibility coefficients (ADC), chromium oxide (Cr_2O_3) was added to the reference diet at the concentration of 1% as an inert marker. In the test diets, the ingredients were included at 30% (except for the oils, 10%) and the rest was the reference diet. The exact inclusion of each experimental ingredient was based on the measured dry matter content of the test ingredients. The analysed nutrient compositions of the tested ingredients and diets are given in Table 2.2. In experiment 1 (Exp 1), protein rich ingredients (fishmeal, soybean meal, meat and bone meal, rapeseed meal, and toasted full fat soybean meal), in experiment 2 (Exp 2) ingredients relatively rich in non-starch polysaccharides (defatted rice bran, rice bran, wheat bran, palm kernel meal, and dried distillers grains with solubles) and in experiment 3 (Exp 3) oil and starch rich ingredients (fish oil, soybean oil, wheat, broken rice, and cassava) were tested.

Prior to the mixing of all test diets, one basal mixture batch of fishmeal and wheat flour were mixed and grinded by a hammer mill at a mesh size of 1.0 mm. All test ingredients, were similarly but separately grinded at a mesh size of 1.0 mm. For all test diets and the reference diets, all grinded ingredients and non-grinded ingredients (oils, marker, carboxyl-methyl-cellulose and premix) were mixed on dry matter basis according to the amounts given in Table 2.2. The amounts of these mixtures were calculated for all diet treatments.

Subsequently, the mixtures were extruded through a 3 mm-die resulting in approximately 4.5 mm diameter pellets. The extruded pellets were dried at 60°C for 24 hours followed by sieving. The fish oil in the Ref diet (Table 2.1) was added to the mixture prior to extrusion. Regarding the tested oils (fish and soya oil), 5% of these oils were added to the mixture prior to extrusion and the remaining 5% was coated onto the pellets after being dried. After production, pellets were stored in a freezer until feeding. The experimental diets (including the Ref diets) were produced 3 times because for each experiment (one batch) new ingredients were purchased. The hammer mill and the extruder, which were used for pellet production, were designed and manufactured by the Centre of Technology Research and Application, College of Engineering Technology, Can Tho University in 2010. The ingredients and the dietary pellets were sampled and analysed for chemical composition and viscosity (Table 2.2).

2.2.2 Experimental system and animals

Every experiment was conducted using 18 composite tanks (Tran-Tu *et al.*, 2018) of 170 L each. The tanks were filled with 80% water and were aerated with one air stone per tank. 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 the filtration tank of the system. During night-time, the system was run as a closed recirculating system without water renewal. The water flow through the experimental tanks was 3 L min^{-1}. Striped catfish (*Pangasionodon hypophthalmus*) juveniles with an average initial body weight of 130, 98 and 67 g were bought from a local hatchery for Exp 1, 2 and 3, respectively (Supplement Table 1). The fish

Table 2.2 The dietary formulation, chemical compositions of experimental diets and ingredients (on dry matter basis, DM)

	Exp 1						Exp 2						Exp 3					
	Ref1	FM	SBM	MBM	RM	FFSB	Ref2	DFRB	RB	WB	PKM	DDGS	Ref3	FO	SO	WHE	BR	CAS
Formulation																		
Reference diet (%)	100	70	70	70	70	70	100	70	70	70	70	70	100	90	90	70	70	70
Tested ingredient (%)	0	30	30	30	30	30	0	30	30	30	30	30	0	10	10	30	30	30
Diets																		
Dry matter (g kg^{-1})	913	912	917	909	919	943	888	881	875	891	898	882	896	894	904	892	880	888
Crude protein (g kg^{-1})	314	393	393	363	357	353	351	300	268	288	292	350	317	287	286	251	225	232
Crude fat (g kg^{-1})	35	32	23	56	22	74	55	47	69	45	70	51	27	126	121	34	23	22
Crude ash (g kg^{-1})	108	126	105	113	132	109	108	108	91	92	92	98	65	66	65	54	50	52
Carbohydrate (g kg^{-1})	543	448	499	469	490	464	486	546	572	575	546	501	591	522	528	662	703	695
Gross energy (KJ g^{-1})	18.1	18.3	18.3	18.8	17.7	19.2	18.8	18.3	18.9	18.5	19.1	18.9	18.7	20.7	20.6	18.6	18.3	18.3
viscosity (cP)	4.1	5.0	2.5	5.3	1.7	2.3	7.4	1.4	2.1	1.7	5.4	1.7	7.4	6.7	5.7	2.4	4.8	8.7
Ingredients																		
Dry matter (g kg^{-1})	-	875	879	907	887	878	-	947	953	962	875	863	-	-	-	960	890	886
Crude protein (g kg^{-1})	-	628	485	580	387	362	-	167	111	177	185	361	-	-	-	105	79.0	26.3
Crude fat (g kg^{-1})	-	53	17	124	26	181	-	29	105	23	113	42	-	1000	1000	47	11	6.2
Crude ash (g kg^{-1})	-	217	91	160	85	57	-	103	41	51	50	68	-	-	-	16	3.6	27
Carbohydrate (g kg^{-1})	-	102	407	136	502	400	-	701	743	750	652	529	-	-	-	832	907	940
Gross energy (KJ g^{-1})	-	18.7	19.1	20.9	18.8	22.6	-	17.2	19.5	18.0	20.0	19.3	-	39.5	39.5	18.7	17.9	17.0
viscosity (cP)	-	1.9	1.7	1.9	-	1.3	-	1.3	1.2	1.6	1.5	1.4	-	-	-	1.8	0.9	1.0

Exp = experiment; Ref1 = reference diet of the experiment 1; Ref2 = reference diet of the experiment 2; Ref3 = reference diet of the experiment 3. FM = local fishmeal was produced by Minh Tam Co. Ltd (Kien Giang province, Vietnam). SBM = defatted soybean meal; RM = rapeseed meal; WHE = the whole grain of wheat; WB = wheat bran; and CAS = cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam). RB = rice bran and DFRB = defatted rice bran were produced by CALOFIC Co. (Soc Trang province, Vietnam) and supplied by Vinh Hoan Co. MBM = meat and born meal; FFSB = toasted full fat soybean; PKM = palm kernel meal; and DDGS = dried distillers grains with soluble were imported and supplied by De Heus Co. (Vinh Long province, Vietnam). FO = catfish by-product oil was retailed by Thanh My Co., Ltd (Can Tho city, Vitenam). SO = soybean oil was produced by CALOFIC Co. with the label's name Simply.

were mixed sex populations. Per tank 24 fish were stocked and were randomly assigned to tanks. The tank was randomly assigned to one of the 7 experimental diets (3 replicates per diet).

The photoperiod regime was approximately 12 h light and 12 h 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). Total ammonia nitrogen TAN (0.1 – 0.3 mg L^{-1}) and NO_2–N (0 – 0.02 ppm) were measured and monitored weekly.

2.2.3 Experimental procedure and sample analysis

Prior to the start of each experiment, the fish had been acclimatized to the experimental diets for two weeks. Individual fish weights were measured at the beginning and end of the experiment. Fish were fed once daily at 09:00 am. The feeding level was approximately 2% of their body weight. Feed intake per tank was recorded by collecting and weighing spilled and refused feed by the fish per tank 30 minutes after feeding. The spilled feed was dried at 60°C for 24 hours and weighed. Sampling faeces started from the third week (21 days) after stocking onwards and the collection was done daily from 1 hour after feeding till 1 hour before the next feeding. Each faeces sample was collected into an aluminium tray and stored in the freezer until analysis.

During pelleting, ingredient samples were taken and during the experiment about 10 g of each experimental diet was sampled weekly. The faeces samples were dried at 60°C for 24 hours. All samples were ground by a coffee blender and stored in the freezer until analysis. The chemical analyses were done in triplicate. According to standard laboratory methods (AOAC, 2000), the dry matter (DM) content was determined by drying in the oven at 105°C until constant weight; crude protein (N × 6.25) content was measured following the Kjeldahl method; mineral (ash) content by placing in the furnace at 560°C for 4 hours; and crude fat content by solvent (diethyl ether) extraction. The carbohydrate (CHO) content in the sample on dry matter basis was calculated as 1000 – (crude protein + crude ash + crude fat). The gross energy (kJ g^{-1}) was calculated as [(crude protein × 23.6) + (crude fat × 39.5) + (CHO × 17.2)] / 1000. The chromic oxide content of feed and faeces was measured by a spectrophotometer at the wave length 350 nm after digestion of the samples by nitric acid and then oxidized with per-chloric acid (Furukawa and Tsukahara, 1966). The viscosity in grinded pellets and ingredients was determined according to the study of Leenhouwers *et al*. (2006). One gram of these samples was mixed with 4 mL of demi water, incubated for 30 minutes at 28°C and thereafter centrifuged at 12000g for 10 minutes. After centrifuging, the supernatant of each sample was immediately used for measuring the absolute viscosity (cP) by a Brookfield LVDV-II+ cone/plate viscometer.

2.2.4 Calculations and statistical analysis

The apparent digestibility coefficients (ADC) of nutrients (*i.e.* dry matter, crude protein, crude fat, carbohydrate, crude ash and gross energy) in the diet was measured by an indirect method using chromic oxide as inert marker (Cho and Kaushik, 1985):

$ADC = 100 – [(Marker_{diet} \times Nutrient_{faeces}) / (Marker_{faeces} \times Nutrient_{diet})] \times 100$

Where: the $Marker_{diet}$ and $Marker_{faeces}$ are the concentrations (g kg^{-1} DM) of chromium oxide in the diet and faeces, respectively. The $Nutrient_{diet}$ and $Nutrient_{faeces}$ are the concentrations (g kg^{-1} DM) of the nutrient in the diet and faeces, respectively.

The apparent digestibility coefficients of nutrient in the tested ingredient ($ADC_{test\ ingredient}$) was calculated as follows (Bureau and Hua, 2006):

$ADC_{test\ ingredient} = ADC_{test\ diet} + [(ADC_{test\ diet} - ADC_{ref\ diet}) \times (b \times D_{ref\ diet} / a \times D_{test\ ingredient})]$

where: a = inclusion level of the test ingredient in the test diet; b = inclusion level of the reference diet into the test diet; (a + b) = 1; $ADC_{test\ diet}$ = nutrient digestibly of the test diet; $ADC_{ref\ diet}$ = nutrient digestibly of the reference diet; $D_{ref\ diet}$ = nutrient content in the reference diet; and $D_{test\ ingredient}$ = nutrient content in the test ingredient.

The initial and final mean weights (IMW & FMW) of the fish were determined by weighing the total biomass and counting the total number of fish in each tank. Daily weight gain (DWG) was calculated by dividing the difference between the final and initial mean weight (mean weight gain) by the number of experimental days. The feed intake (FI) was calculated by the mean feed intake (on DM basis) of each tank divided by experimental days. Feed conversion ratio (FCR) was based on the mean weight gain per fish and on the mean amount of consumed feed per individual in g DM. Survival rate (SR) was the final number of fish as the percentage of the number of stocked fish.

The total amount of faecal DM collected by the settling unit was recorded. From this amount and the chromic oxide content of the faeces, the total amount of chromic oxide collected was calculated. The faecal recovery percentage (FR%) was computed as the total amount of chromic oxide collected with the faeces divided by the total amount of chromic oxide consumed with the feed during the period of faeces collection (Amirkolaie *et al*., 2005b). From the FR% and ADC of DM, the total amount of faeces produced (TFP), the amount of non-recovered faeces (NRF) and amount of faeces removed from the water (RF) using the settling tanks. TFP, NRF and RF were expressed in DM per kg of feed DM.

The waste production characteristics (FR%, TFP, RF or NRF) of each ingredient was calculated analogue to the calculation of ingredient ADC as follows:

$WPC_{test\ ingredient} = WPC_{test\ diet} + [(WPC_{test\ diet} - WPC_{ref\ diet}) \times (b \times D_{ref\ diet} / a \times D_{test\ ingredient})]$

where: a = inclusion level of the test ingredient in the test diet; b = inclusion level of the reference diet into the test diet; (a + b) = 1; $WPC_{test\ diet}$ = waste production characteristic of the test diet; $WPC_{ref\ diet}$ = waste production characteristic of the reference diet; $D_{ref\ diet}$ = dry matter content in the reference diet; and $D_{test\ ingredient}$ = dry matter content in the test ingredient.

Data expressed as percentage were transformed by arcsinus transformation prior to statistical analysis. 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. Performance, nutrient digestibility and faecal waste characteristics measured at diet level were tested for the effect of diet and experiment by two-way ANOVA without the interaction effect. Nutrient digestibility and faecal waste characteristics at ingredient level were tested for the effect of diet by one-way ANOVA. The IBM SPSS software package version 23 was used for statistical analysis.

2.3 Results

The type of ingredient in the di*et al*tered the faecal waste in striped catfish, *e.g.*, waste production varied between experimental diets and ingredients (Table 2.3). The reference (Ref) diet was produced newly for each experiment. The removal efficiency of faeces from the water by settling (FR%) for the reference diets was similar between the 3 experiments ($P>0.10$), being 42%. However, within experiments, FR% varied between the test diets. This was reflected by the significant difference in FR% between ingredients ($P<0.001$).

Specifically, fish fed the test diets that contained cassava, broken rice, fishmeal and full fat soybean had a low FR%, ranging between 20 and 27% at diet level. This was also reflected in the calculated FR% of these ingredients, which appeared to have negative FR% values at ingredient level. This implies that these ingredients also reduced the FR% of the reference diet (basal mixture) within these test diets. The highest FR% was found in palm kernel meal, both at diet level and ingredient level (73 and 52%, respectively). In general, ingredients with a relatively high fibre content (tested in Exp 2) improved FR%, while the "protein rich" (Exp 1), "fat" and "starch rich" ingredients (Exp 3) reduced the FR%.

In contrast to FR%, the total amount of faeces produced (TFP) at the reference diets was strongly different between experiments ($P<0.001$; 160, 92 and 127 g DM kg^{-1} of DM feed fed, respectively). At diet level as well as ingredient level, TFP was strongly different between ingredients ($P<0.001$), which paralleled the findings of ADC of DM (Table 2.4). At ingredient level, soybean oil induced the striped catfish to excrete the largest amount of faecal waste

Table 2.3 Effect of diet/ingredient on faecal waste characteristics in striped catfish

	Exp 1						Exp 2						Exp 3							P values	
	Ref 1	FM	SBM	MBM	RM	FFSB	Ref 2	DFRB	RB	WB	PKM	DDGS	Ref 3	FO	SO	WHE	BR	CAS	SEM	Exp	Diet
	Waste production of diets																				
FR%	42	26	34	35	30	27	42	49	44	40	52	43	42	34	34	34	24	20	7.2	0.995	0.317
TFP	160	158	165	206	207	209	92	159	135	195	218	189	127	164	196	196	182	249	6.7	<0.001	<0.001
RF	67	39	55	73	62	57	39	78	59	79	113	82	53	55	67	66	43	49	12	0.146	0.005
NRF	93	119	110	133	145	152	54	81	76	116	105	107	74	109	129	130	139	200	13	0.029	<0.001
	Waste production of ingredients																				
FR%	-	-12	15	20	1.6	-6.1	-	64	48	35	73	45	-	14	16	15	-18	-31	18	-	0.008
TFP	-	156	177	314	318	326	-	316	235	434	512	415	-	493	812	357	309	532	20	-	<0.001
RF	-	-24	30	90	52	36	-	169	108	172	285	183	-	71	190	97	21	40	39	-	<0.001
NRF	-	179	148	225	266	289	-	146	127	262	227	231	-	422	621	260	289	492	43	-	<0.001

Presented values are means (n=3) per diet/ingredient within each experiment. Exp = experiment; Ref1 = reference diet of the experiment 1; Ref2 = reference diet of the experiment 2; Ref3 = reference diet of the experiment 3. FM = local fishmeal was produced by Minh Tam Co. Ltd (Kien Giang province, Vietnam). SBM = defatted soybean meal; RM = rapeseed meal; WHE = the whole grain of wheat; WB = wheat bran; and CAS = cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam). RB = rice bran and DFRB = defatted rice bran were produced by CALOFIC Co. (Soc Trang province, Vietnam) and supplied by Vinh Hoan Co. MBM = meat and born meal; FFSB = toasted full fat soybean; PKM = palm kernel meal; and DDGS = dried distillers grains with soluble were imported and supplied by De Heus Co (Vinh Long province, Vietnam). FO = catfish by-product oil was retailed by Thanh My Co., Ltd (Can Tho city, Vitenam). SO = soybean oil was produced by CALOFIC Co. with the label's name Simply. FR% = faeces recovery (%); TFP = the amount of faeces produced (g DM kg^{-1} of DM feed); RF = recovered faeces (g DM kg^{-1} of DM feed); NRF = non recovered faeces (g DM kg^{-1} of DM feed).

Table 2.4 Effect of diet/ingredient on nutrient digestibility in striped catfish (in %)

	Exp 1						Exp 2						Exp 3							P values	
	Ref 1	FM	SBM	MBM	RM	FFSB	Ref 2	DFRB	RB	WB	PKM	DDGS	Ref 3	FO	SO	WHE	BR	CAS	SEM	Exp	Diet
ADC diets																					
Dry matter	84.0	84.2	83.5	79.4	79.3	79.1	90.8	84.1	86.5	80.5	78.2	81.1	87.3	83.6	80.4	80.4	81.8	75.1	0.67	<0.001	<0.001
Crude protein	86.3	90.2	88.9	88.4	86.9	85.5	96.0	92.5	91.2	91.4	87.6	91.5	93.3	90.7	88.3	90.1	91.2	91.6	0.73	<0.001	<0.001
Crude fat	87.9	90.8	81.4	96.4	55.8	85.6	94.7	86.1	80.7	83.8	91.0	89.1	96.8	95.9	97.0	91.9	87.4	94.1	1.86	<0.001	<0.001
Crude ash	32.8	37.0	43.1	35.3	28.7	29.3	49.4	36.7	29.4	21.8	30.1	42.1	22.9	10.6	-6.99	11.5	8.19	-34.1	4.48	<0.001	<0.001
Carbohydrate	92.7	91.7	88.0	81.1	88.4	84.9	95.8	88.6	94.1	84.2	79.6	80.7	90.6	86.0	83.2	81.7	83.8	77.2	0.87	<0.001	<0.001
Gross energy	89.7	90.9	88.1	86.2	86.1	85.2	95.8	89.9	91.2	86.8	84.1	86.3	92.1	89.9	88.0	85.1	86.1	82.3	0.54	<0.001	<0.001
ADC ingredients																					
Dry matter	-	84.4	82.3	68.6	68.2	67.4	-	68.4	76.5	56.6	48.8	58.5	-	50.7	18.8	64.3	69.1	46.8	2.04	-	<0.001
Crude protein	-	94.5	93.1	90.7	88.3	83.7	-	75.0	57.7	71.0	51.9	81.5	-	-	-	67.5	73.5	(43.4)	2.68	-	<0.001
Crude fat	-	93.6	59.6	101	-1.25	84.7	-	48.9	63.9	22.4	87.0	72.4	-	95.7	97.0	85.1	30.7	(65.4)	5.28	-	<0.001
Crude ash	-	41.0	72.6	38.8	11.5	10.3	-	4.88	-97.3	-117	-68.3	13.9	-	-	-	(-104)	(-658)	-337	16.3	-	<0.001
Carbohydrate	-	(77.3)	73.6	(-41.1)	78.7	62.4	-	77.2	91.4	66.2	50.5	48.0	-	-	-	66.9	72.9	57.4	2.21	-	<0.001
Gross energy	-	93.5	84.7	79.1	78.5	77.0	-	90.2	94.5	79.8	72.1	78.6	-	90.0	86.8	74.3	77.5	63.2	1.51	-	<0.001

Presented values are means (n=3) per diet/ingredient within each experiment. The values in the brackets were excluded from statistical analysis because the contribution of the nutrient of the ingredient in the test diet was less than 10%. Exp = experiment; Ref1 = reference diet of the experiment 1; Ref2 = reference diet of the experiment 2; Ref3 = reference diet of the experiment 3; FM = local fishmeal was produced by Minh Tam Co. Ltd (Kien Giang province, Vietnam). SBM = defatted soybean meal; RM = rapeseed meal; WHE = the whole grain of wheat; WB = wheat bran; and CAS = cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam). RB = rice bran and DFRB = defatted rice bran were produced by CALOFIC Co. (Soc Trang province, Vietnam) and supplied by Vinh Hoan Co. MBM = meat and born meal; FFSB = toasted full fat soybean; PKM = palm kernel meal; and DDGS = dried distillers grains with soluble were imported and supplied by De Heus Co. (Vinh Long province, Vietnam). FO = catfish by-product oil was retailed by Thanh My Co., Ltd (Can Tho city, Vitenam). SO = soybean oil was produced by CALOFIC Co. with the label's name Simply.

Supplemental Table 2.1 Effect of diet/ingredient on performance of striped catfish

	Exp 1						Exp 2						Exp 3							P values	
	Ref 1	FM	SBM	MBM	RM	FFSB	Ref 2	DFRB	RB	WB	PKM	DDGS	Ref 3	FO	SO	WHE	BR	CAS	SEM	Exp	Diet
IMW (g fish^{-1})	130	130	130	130	130	130	98	98	98	98	98	98	67	67	67	67	67	67	0.2	<0.001	0.904
FMW (g fish^{-1})	222	218	209	207	219	212	114	111	116	116	114	116	104	101	102	96.2	94.8	110	6.0	<0.001	0.456
DWG (g d^{-1})	1.51	1.44	1.29	1.27	1.46	1.34	0.31	0.25	0.36	0.34	0.30	0.33	0.76	0.71	0.71	0.60	0.57	0.89	0.11	<0.001	0.473
FI (g tank^{-1} d^{-1})	55.9	46.4	47.8	47.9	51.2	48.9	30.8	31.0	31.2	29.1	29.0	29.4	27.2	27.8	28.1	27.9	25.3	28.0	1.87	<0.001	0.035
FCR	1.32	1.15	1.32	1.34	1.25	1.30	2.27	3.07	1.95	2.17	2.43	2.45	1.14	1.33	1.58	1.44	1.62	0.99	0.37	0.024	0.670
SR (%)	100	100	100	100	98.6	98.6	98.6	94.4	98.6	97.2	98.6	98.6	98.6	93.1	90.3	97.2	87.5	95.8	3.7	0.929	0.638

Presented values are means (n=3) per diet within each experiment. Exp = experiment; Ref1 = reference diet of the experiment 1; Ref2 = reference diet of the experiment 2; Ref3 = reference diet of the experiment 3; FM = local fishmeal was produced by Minh Tam Co. Ltd (Kien Giang province, Vietnam). SBM = defatted soybean meal; RM = grape seed meal; WHE = the whole grain of wheat; WB = wheat bran; and CAS = cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam). RB = rice bran and DFRB = defatted rice bran were produced by CALOFIC Co. (Soc Trang province, Vietnam) and supplied by Vinh Hoan Co. MBM = meat and born meal; FFSB = toasted full fat soybean; PKM = palm kernel meal; and DDGS = dried distillers grains with soluble were imported and supplied by De Heus Co. (Vinh Long province, Vietnam). FO = catfish by-product oil was retailed by Thanh My Co., Ltd (Can Tho city, Vitenam). SO = soybean oil was produced by CALOFIC Co. with the label's name Simply. IMW = initial mean body weight; FMW = final mean body weight; DWG = daily weight gain; FI = feed intake; FCR = feed conversion ratio; and SR = survival rate.

(812 g DM kg^{-1} of DM feed fed) compared to the other ingredients. Also, cassava and palm kernel meal showed very high amounts of total faeces produced, above 500 g DM kg^{-1} of DM feed fed.

The non-recovered faeces (NRF) is the outcome of the amount of faeces produced (TFP) and the recovery rate (RF). Ingredients yielded very different NRF ($P<0.001$). The highest values for NRF were measured in fish fed the diets with the inclusion of either cassava, fish oil or soybean oil, which had an NRF at ingredient level of 492, 422 and 621 g DM kg^{-1} of DM feed fed, respectively. Defatted rice bran, rice bran and soybean meal had the lowest values of NRF, being 127, 146 and 148 g DM kg^{-1} of DM feed fed, respectively.

Nutrient digestibilities of diets and of ingredients are given in Table 2.4. The ADC values of each nutrient was different between the ingredients ($P<0.001$). However, the reference diet showed also large differences between experiments ($P<0.001$). *E.g.*, the ADC of DM during Exp 1, 2 and 3, was 84.0, 90.8 to 87.3%, respectively. At ingredient level, also large differences in nutrient ADC were present. Cassava and palm kernel meal had a digestibility of DM below 50%. The two tested oil sources (fish- and soybean oil) had a high digestibility of crude fat and gross energy, but a low dry matter digestibility. Performance was measured, but as it was not the aim of the experiment, these data are therefore reported in Supplemental Table 2.1. Growth and FCR differed between the experimental diets, whereas mortality was on average 97% and not affected by diet or experiment.

2.4 Discussion

This study assessed the impact of dietary ingredients on the quantity and quality of faecal waste produced by striped catfish. The quantity of faecal waste was strongly dependent on the dietary ingredient composition. The amount of faecal waste produced per kg of feed ranged between 92 and 249 g DM for the two most extreme diets. This reflects the difference in DM digestibility between the different diets ranging between 75 and 91%. For striped catfish, only a limited number of studies report ADC in ingredients (*e.g.* Hien *et al*., 2010; Da *et al*., 2013). The obtained ADC values for the different ingredients in striped catfish seem to fit generally with trends seen in other fish species like *e.g.* tilapia (Köprücü and Özdemir, 2005; Maina *et al*., 2002), hybrid tilapia (Dong *et al*., 2010; Zhou and Yue, 2012), channel catfish (Li *et al*., 2013), striped surubim (Silva *et al*., 2012), snakehead (Yu *et al*., 2013), grouper (Eusebio *et al*., 2004) and loach (Chu *et al*., 2015). Overall, ingredients having higher fibre content had lower nutrient ADCs, and thus gave more faecal waste. Protein sources like soybean meal, rapeseed meal, and meat and bone meal, often used as alternative for fishmeal, had similar protein ADC but increased slightly the faecal waste production. Strikingly, inclusion of oils seemed to negatively affect the digestibility of other nutrients, thereby increasing the faecal waste output. This parallels the finding in tilapia, that exchanging gelatinized maize starch by oils reduced the protein digestibility (Amirkolaie *et al*., 2006; Schrama *et al*., 2012). However, such a negative interference of oil (fat) on other nutrient digestions is not consistent between fish species. *E.g.* in African catfish, it was found that exchanging gelatinized starch (30%) by oils (12.5%) improved protein ADC (Harter *et al*., 2014).

In this study, the impact on ingredients on the quality of faecal waste was quantified by measuring the faecal recovery by settling. Average over all 18 diets, the recovery rate was 36%. This is well in line with our earlier observations in striped catfish (Tran-Tu *et al.* 2018). The recovery rate of faecal waste in striped catfish is much lower than values found for Nile tilapia (~70%; Amirkolaie *et al*., 2005b), common carp (~75%; Prabhu *et al*., submitted) and rainbow trout (~80%; Meriac *et al*., 2014). The low recovery rate of faecal waste confirms the visual subject observations that striped catfish produces diarrhoea-like faeces and that very few faecal pellets are present without a mucus layer. In contrast, tilapia and common carp faeces is mostly encapsulated in a mucus envelope. Thus, the efficacy to remove solid faecal waste by settling strongly differs between fish species. Consequently, the type of solid waste removal (settling *vs.* drum filters) and system design (amount of mechanical strain on faecal pellets), which is most optimal might differ between fish species. The importance of the collections method of faeces is reflected by the very low faeces recovery (<12%) in Nile tilapia when using Choubert faeces collectors (Schneider *et al*., 2004). In contrast Nile tilapia studies using settling columns behind the fish tank had all faeces recoveries well above 40% (Amirkolaie, 2005; Amirkolaie *et al*., 2005a,b).

The present study clearly shows that in striped catfish the dietary ingredient composition affects the faecal removal efficiency by settling, which ranged between 20 to 52%. The impact of dietary ingredient composition on faecal recovery was also found in Nile tilapia (Amirkolaie *et al*., 2005), common carp (Prabhu *et al*., submitted) and rainbow trout (Meriac *et al*., 2015), all using settling as collection method, but not in Nile tilapia (Schneider *et al*., 2004) when using Choubert collectors. The latter is mostly due to the very low recovery rates (<12%) when using Choubert collectors.

In the current study, the higher values of faeces recovery (approximately 40 – 52%) were obtained with the diets containing the fibre-source ingredients, *i.e.* defatted rice bran, dried rice bran, wheat bran, palm kernel meal and dried distillers grains with solubles, representing the group with high NSP content. Meriac *et al*. (2015) working with rainbow trout, found a similar impact of NSP content. However, in common carp (Prabhu *et al*., submitted) such an impact of NSP was less clear. The difference in response to NSP might relate the type of NSP, viscous versus non-viscous NSP. Amirkolaei *et al*. (2005b) showed in tilapia that viscous NSP (guar gum) strongly reduced the faecal recovery compared to non-viscous NSP (cellulose). In striped catfish it was also observed that increasing dietary viscosity by guar gum inclusion negatively affected the faecal recovery (Tran-Tu *et al.,* 2018). However, in the current study dietary viscosity did not correlate with faecal recovery (data not shown) nor with dry matter ADC. The absence of such a relationship in the current study suggests that in striped catfish other factors than dietary viscosity (*e.g.* types of ingredients, variety of ingredients over time, processing conditions) determine the quantity and quality of the faecal waste.

Strikingly the faecal recovery rate of the reference diets used in all three experiments were fully identical (Table 3), despite the fact that for every experiment the diets were newly produced and a new batch of basal ingredients was purchased. This demonstrates that faecal recovery is consistent over time (between experiments). In contrast, digestibility of the reference diets differed between the experiments. This might suggest that process conditions have more impact on the amount of faeces produced and less on the quality of faecal waste.

In fish farming, the amount of non-recovered faecal waste per kg of feed is important for 1) water quality management within ponds; and 2) the waste output of the farm discharged via the water to surrounding water bodies. In the 18 diets tested in this study, 77% of the variation in non-recovered faecal waste was related to variability in dry matter ADC and 62% to variability in faecal recovery rate.

This study shows that the selection of ingredients within a diet (diet formulation) can steer the non-recovered faecal waste. *E.g.* in this study on striped catfish, fishmeal showed the highest digestibility but a very low recovery rate of faeces. A similar observation was found in rainbow trout (Brinker and Friedrich, 2012). Despite the high dry matter ADC, the amount of non-recovered faeces was intermediate at the fishmeal diet due to the poor recovery rate. On the other hand, rice bran had the lowest amount of non-recovered faecal waste due its high recovery rate and a reasonable dry matter ADC. This shows that combining dry matter ADC data with recovery rate data will make management/reduction of non-recovered faecal waste through diet composition more effective.

2.5 Conclusion

This study showed that both the quantity and quality of faecal waste of striped catfish is dependent on the ingredient composition of the diet. Ingredients with a high fibre content (like palm kernel) increased the faecal quality (recovery percentage) but also increased the quantity of the produced faeces. The oils, protein rich ingredients and starch rich ingredients all had a negative effect on the faecal quality. Protein rich feedstuffs, reduced the amount of faecal waste because of its high digestibility. In contrast, oils and starch rich ingredients (like cassava) negatively affected nutrient digestibility and thereby increased the amount of faecal waste. The observed differences in non-recovered faecal waste between ingredients in the current study implies that management of faecal waste is possible by dietary ingredient composition, but is most effective if the impact of ingredient on quantity and quality is combined.

Chapter 3

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

This chapter has been published as:

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 (*Pangasionodon hypophthalmus*).** *Aquaculture Nutrition* 24 (3), 961-969. https://onlinelibrary.wiley.com/doi/full/10.1111/anu.12632

Abstract
The ingredients' particle size and the dietary viscosity may alter digestion, performance and faecal waste management of fish. This study aimed to assess the effect of grinding screen sizes of the feed ingredients and dietary viscosity on digestibility, faecal waste and performance of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878). The experiment had a 2x3 factorial design: two feed mash particle sizes, by grinding the ingredient mixture at two screen sizes (0.8 versus 1.0 mm); and three dietary viscosity levels, which were created by exchanging carboxyl methyl cellulose by guar gum (GG) (0, 3, and 6 g of GG kg^{-1} of diet). The six diets were assigned to 18 tanks, each connected to three faecal settling tanks. All aquaria were stocked with 20 fish of 82 g $fish^{-1}$ on average. 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.

3.1 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 (Phan *et al.*, 2009, De Silva *et al.*, 2010, De Silva and Phuong, 2011). Management of water quality in the ponds is done 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 done 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 (Hien *et al.*, 2010, Da *et al.*, 2012), focused on nutrient uptake and not on faecal waste aspects. Studies in trout (Brinker *et al.*, 2005, Brinker, 2007, Brinker, 2009) and Nile tilapia (Schneider *et al.*, 2004, Amirkolaie *et al.*, 2005a, Amirkolaie *et al.*, 2006) 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 NSPs (non–starch polysaccharides) of which guar gum (GG) is an example, have been used to improve the removal efficiency of faeces (Sinha *et al.*, 2011). Brinker *et al.* (2005) found that the inclusion of GG increased the stability of faeces in trout. However, in Nile tilapia GG lead to an opposite effect as it decreased the faecal recovery efficiency (Amirkolaie *et al.*, 2005b). It has also been shown that the (negative) impacts of GG are dose dependent *e.g.* on growth (Janphirom *et al.*, 2010); digestibility (Storebakken, 1985, Refstie *et al.*, 1999); nutrients absorption (Van der Klis *et al.*, 1993b, Smits *et al.*, 1998); gastric emptying (Shiau *et al.*, 1988) and the properties of the chyme like viscosity osmolality, pH and the moisture content (Van der Klis *et al.*, 1993a). 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 hypothesised 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 content, and on viscosity, type of faeces (recovered and non-recovered), and particle sizes of faeces produced by striped catfish.

3.2 Materials and methods

3.2.1 Experimental diets

The experiment lasted 52 days and was conducted in a 2 x 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 3 dietary viscosity levels were created by adding 0, 3, or 6 g kg^{-1} guar gum (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 3.1). 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 and 6 g kg^{-1} 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 hours 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 mm, 0.3 to 0.5 mm and <0.3 mm. The meal mixtures prior to extrusion and the extruded pellets were sampled and analysed for viscosity.

3.2.2 Experimental system and animals

Striped catfish (*Pangasionodon hypophthalmus*) juveniles with an initial body weight of 82 g were bought from a local hatchery. The fish were a mixed sex population. The juveniles were randomly distributed into eighteen plastic digestibility tanks (Allan *et al.*, 1999) of 170 L (Figure 3.1); 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^{-1}. 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 m^3 tank with continuous aeration.

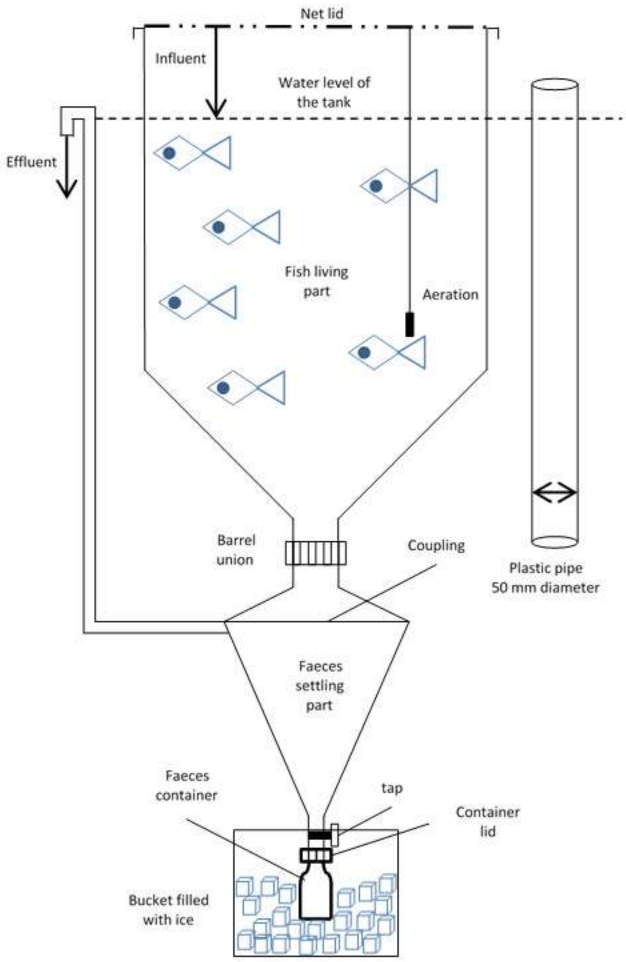

Figure 3.1 Schematic overview of the fish tank with the faecal collection unit as described by Allan *et al*. (1999) with some modification made.

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 h light and 12 h 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^{-1}). Total ammonia nitrogen TAN (0.1 – 0.3 mg L^{-1}) and NO_2^--N (0 – 0.02 ppm) were measured and monitored weekly.

Table 3.1 The amounts (in g kg^{-1}) of ingredients used in the basal diet to test three levels of guar gum (x): 0, 3 and 6 g kg^{-1}

Ingredients	Amount (as is)
Fish meal [1]	142.9
Soybean meal [2]	168.1
Rice bran [2]	151.8
Defatted rice bran [2]	162.3
Cassava [2]	160.2
Sunflower meal [3]	170.2
Premix vitamin and mineral [4]	10.0
Squid oil [5]	24.5
Chromium oxide	10.0
Guar gum (GG) & Carboxyl methyl cellulose (CMC) [6]	10.0

[1] Kien Giang fish meal was produced by Minh Tam Co., Ltd. (Kien Giang province, Vietnam).

[2] Soybean meal, Rice bran, Defatted rice bran and Cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam).

[3] Sunflower meal was imported and supplied by de Heus LLC Co. (Vinh Long province, Vietnam).

[4] Premix vitamin and mineral (UI or mg kg^{-1}): 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., Vietnam).

[5] Squid oil was produced by Vemedim Co. (Vietnam).

[6] 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^{-1}) in the diet, the ratios between GG and CMC were 0 to 10, 3 to 7, and 6 to 4.

Table 3.2 Chemical composition of six experimental diets on dry matter basis

Dietary component	Diet					
	Fine (0.8 mm)			Coarse (1.0 mm)		
	GG0	GG3	GG6	GG0	GG3	GG6
Dry matter (g kg^{-1})	882	883	884	889	892	890
Crude protein (g kg^{-1})	318	314	312	313	306	304
Crude fat (g kg^{-1})	57	62	61	65	62	57
Carbohydrate (g kg^{-1})	511	509	510	511	511	526
Crude ash (g kg^{-1})	115	116	117	112	114	114
Chromium oxide (g kg^{-1})	7.5	8.9	9.3	8.1	8.1	8.5
Gross energy (kJ g^{-1})	20.7	20.7	20.8	20.3	20.0	20.0

3.2.3 Experimental procedure

Prior to the experiment and before stocking in the digestibility tanks, the fish had been fed a mixture of the experimental diets for two 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. In order to minimize disturbance, fish were fed once daily at 09:00 am. Faeces collection started from week 3 (i.e. after 21 days of rearing) onwards. Faeces were collected daily from 1 hour after feeding until 1 hour 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 hours and weighed.

On the final sampling day, eight hours 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 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 minutes 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 gastro-intestinal 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 12000*g* for 10 minutes. Viscosity in pellets after grinding and diet mixtures (prior to extrusion) was done according to Leenhouwers *et al.* (2006). One gram of these samples was mixed with 4 mL of demi water, incubated for 30 minutes at 28°C and thereafter centrifuged at 12000*g* for 10 minutes. 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 dry matter (DM). Here only data on DM and viscosity in the distal part are presented.

3.2.4 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 hours. Both kind of samples were then ground by a coffee blender and stored in the freezer until analysis. The chemical analyses were done in triplicate, except for gross energy that was done 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 x 6.25) content following the Kjeldahl method; mineral (ash) content by placing in the furnace at 560°C for 4 hours; 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 analyzed 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 and Tsukahara, 1966).

3.2.5 Calculations and statistical analysis

The Apparent Digestibility Coefficients of nutrient (Nutrient.ADC) such as dry matter, crude protein, crude fat, carbohydrate, crude ash and gross energy was revealed by an indirect method using chromic oxide as inert marker (Cho and Kaushik, 1985):

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

Where: the marker $_{diet}$ is concentration (g 100 g^{-1} dry weight) of Chromium oxide in the diet. The marker $_{faeces}$ is concentration (g 100 g^{-1} dry weight) of Chromium oxide in the faeces. The nutrient $_{diet}$ is concentration (g 100 g^{-1} dry weight) of nutrient in the diet. And nutrient $_{faeces}$ is concentration (g 100 g^{-1} 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 *et al.*, 2005b), 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 2mm was determined on the faecal waste collected over a period of 22 hours. 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. In order to prevent clogging of the mesh, the sieving was done 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 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.
All results were introduced in a data-base (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®.

3.2 Results

The viscosity of the diets was measured in samples taken before and after extrusion (Figure 3.2). In the diets, part of the CMC was exchanged by increasing levels of guar gum (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 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.

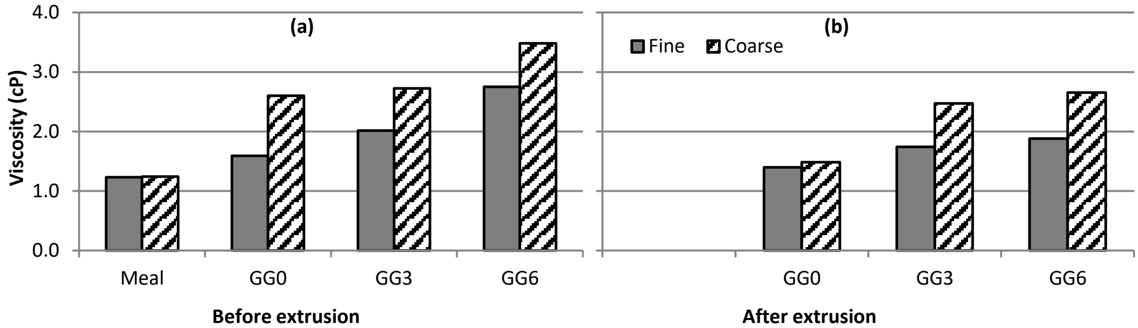

Figure 3.2 Effect of guar gum (GG) inclusion level and grinding scenes size ("Fine" versus "Coarse") on dietary viscosity of ingredient mixtures prior to extrusion (panel a) and pellets after extrusion (panel b). GG0, GG3 and GG6 are diets containing 0, 3 and 6 g GG kg^{-1} of diet; GG is exchanged for carboxyl methyl cellulose (CMC) in the diets. "Meal" in panel a revers to ingredient mixtures without GG and CMC.

The apparent digestibility (ADC) of crude protein, crude ash and gross energy differed (P<0.05) among GG levels, and were not affected by grinding treatment (Table 3.3). 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<0.05) for the ADC of crude ash. The screen size affected the ADC of dry matter and carbohydrate (P<0.05). 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<0.05; Table 3.4). 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 3.4).

Table 3.3 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^{-1})	Dry matter (%)	Crude protein (%)	Crude fat (%)	Carbohy-drate (%)	Crude ash (%)	Gross energy (%)
Fine	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
(0.8 mm)	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	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
(1.0 mm)	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		0.049	0.783	0.131	0.020	0.183	0.143
Guar gum levels		0.001	0.004	0.127	0.087	0.000	0.000
Interaction		0.925	0.307	0.308	0.474	0.005	0.363

Table 3.4 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^{-1})	Initial mean weight (g $fish^{-1}$)	Final mean weight (g $fish^{-1}$)	Daily weight gain (g $fish^{-1}$ day^{-1})	Feed conversion ratio	Survival rate (%)
Fine	0	82±0.1	131±11.4	0.95±0.22	1.80±0.26	97±2.9
(0.8 mm)	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	0	82±0.1	128±9.9	0.89±0.19	1.80±0.35	100±0.0
(1.0 mm)	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		0.583	0.100	0.097	0.069	0.463
Guar gum levels		0.820	0.118	0.128	0.110	0.977
Interaction		0.956	0.027	0.027	0.001	0.541

The dry matter level of digesta from the distal gut was significantly affected (P<0.05) by the GG inclusion level (Table 3.5). 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 fecal recovery (Fig 3.3a), total faeces produced and non-recovered faeces (Fig 3.3b, 3.3d). The percentage of fecal recovery decreased with the increase of GG levels ($P = 0.021$). The trend in the amount of total faeces produced and non-recovered faeces ($P = 0.001$) was similar. However, the inclusion of GG tended to improve the particle size of faeces, but the effect was not significant (Fig 3.4). The grinding treatment did not affect any of the fecal waste characteristics.

3.3 Discussion

3.3.1 Feed digestibility, fish performance and amount of faecal waste

Replacement of CMC by GG in the basal diet increased the dietary viscosity (Figure 3.2). The results show that apparent digestibility 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 to reduce already at 20 g kg^{-1} GG in the diet, while fish final weight and feed efficiency was decreased at 100 g kg^{-1} 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 *et al.*, 2005b).

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^{-1} 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^{-1} 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^{-1} GG (Maisonnier *et al.*, 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. *E.g.* 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 increased in viscosity was larger with inclusion level of GG compared to a finely grinded mixture (Figure 3.2a). 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 *e.g.* 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 *e.g.* 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.

Table 3.5 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 (mPa·s)
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		0.133	0.178
Guar gum levels		0.000	0.123
Interaction		0.914	0.403

Guar gum, a soluble NSP, is hardly digested by aquatic species. In general NSP are 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 of 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 *et al.*, 2003) and thereby also performance (Ai *et al.*, 2007).

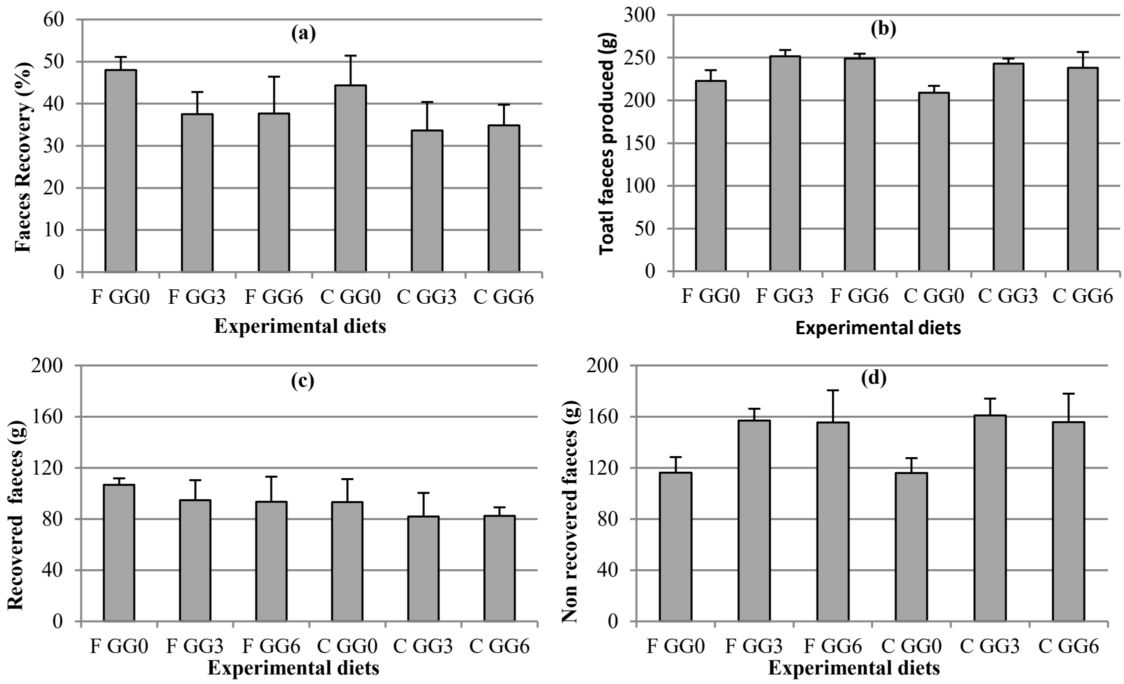

Figure 3.3 (a) Faeces recovery (%), (b) the amount (g DM kg^{-1} DM feed) of total faeces produced (TFP), (c) recovered faeces (RF) and (d) non-recovered faeces (NRF) of the six experimental diets refer to dietary GG inclusion and grinding screen sizes for the experimental period. P values of grinding screen sizes, GG levels and interaction of (a) are 0.252, 0.021, 0.992; (b) 0.052, 0.001, 0.922; (c) 0.105, 0.313, 0.990; and (d) 0.846, 0.001, 0.969, respectively.

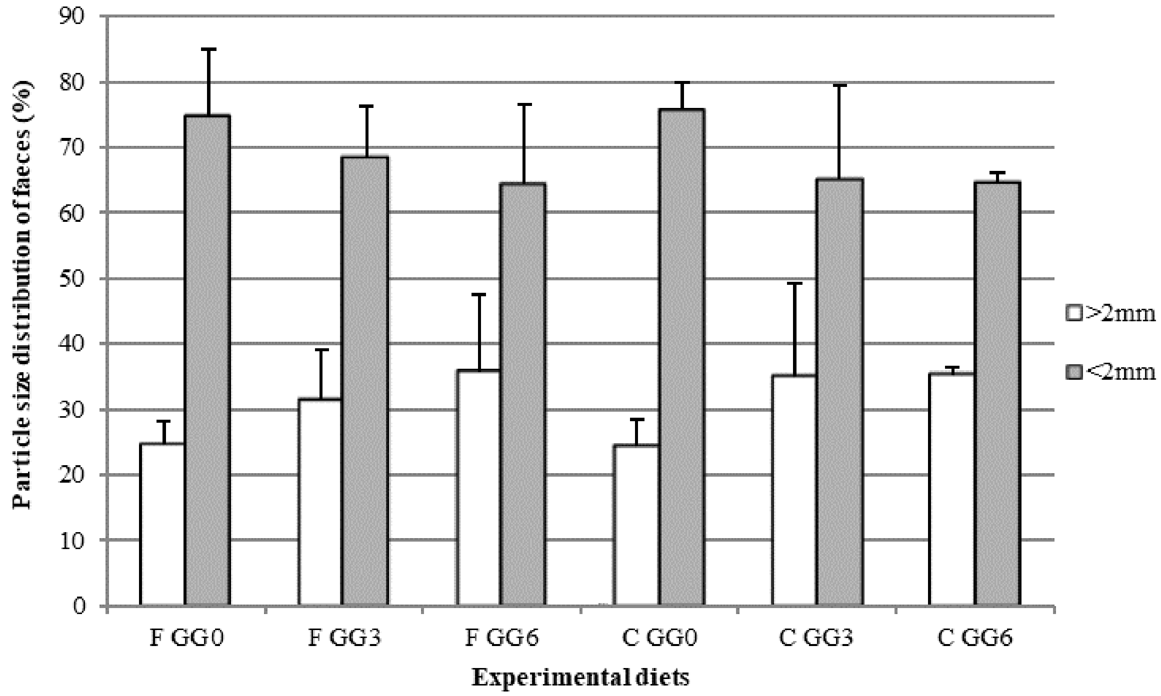

Figure 3.4 The effect of grinding screen sizes and guar gum levels on particle size distribution of faeces. P values of grinding screen sizes, GG levels and interaction are 0.825, 0.111, 0.893 for the particle bigger than 2 mm and 0.922, 0.241, 0.789 for another particle, respectively.

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 hydrolyzed 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 *et al.*, 1993b). Rye which has a high NSP content prevented the absorption of Ca, Mg, Na, and P in African catfish (Leenhouwers *et al.*, 2007b). In Nile tilapia, it decreased only Na absorption (Leenhouwers *et al.*, 2007a). 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 dry matter 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 3.2). 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 *et al.*, 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 mm 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 of 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^{-1} of diet increased the amount of faecal waste (Amirkolaie *et al.*, 2005b).

3.3.2 Faecal waste

This study revealed that increasing dietary viscosity significantly decreased the dry matter 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 *et al.*, 2005b).

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 *et al.*, 2005b, 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 occur which is indicated by increased levels of volatile fatty acids in the distal part of the intestine in broiler (Van Der Klis *et al.*, 1993a) or in fish like Nile tilapia (Leenhouwers *et al.*, 2007b) and African catfish (Van der Klis *et al.*, 1993a, Leenhouwers *et al.*, 2007b, 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 since 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, i.e., thereby standardizing the faecal pellet size already.

3.4 Conclusion

Increasing dietary viscosity by inclusion of Guar Gum 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 dry matter and carbohydrate but the elevating impact on the feed conversion ratio dependent on the dietary viscosity.

Chapter 4

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

This chapter has been submitted for publication to "*Aquaculture*" as:

Tran-Tu, L. C., Thanh-Cong, N., Verreth, J. A. J., and Schrama, J. W., 2019. **Doses response of dietary viscosity on digestibility and faecal characteristics of striped catfish (*Pangasionodon hypophthalmus*).**

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 3.0g.kg^{-1}) and Exp.2 lasted 60 days and assessed the long-term effects of three dietary GG levels (0, 0.5 and 3.0g.kg^{-1}) 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 (>2mm). increased linearly. In Exp.1, viscosity did not affect performance. However, in Exp.2, the GG inclusion level of 3.0g.kg^{-1} (1.72cP 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.

4.1 Introduction

Minimizing the effluent waste of fish farms contributes to a better environmental performance of the sector (Waite *et al.*, 2014a). 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 and 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 (Hua and Bureau, 2010; Cho and Kaushik, 1990). 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 *et al.*, 2005b; Amirkolaie, 2005; 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 *et al.*, 2005; Storebakken and Austreng, 1987; Storebakken, 1985) and bulk agents (Dias *et al.*, 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 *et al.*, 2005b), African catfish (Leenhouwers *et al.*, 2007b; Leenhouwers *et al.*, 2006) 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, the current study analysed this relation in striped catfish. Additionally, in a long-term growth trial, the impact of dietary viscosity on performance was studied.

4.2 Materials and methods

4.2.1 Experimental design, fish and diets

This study consisted of two experiments. 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^{-1}. In experiment 2 (Exp. 2) the long-term effect of three dietary viscosity levels (0, 0.5 and 3.0 g of GG per kg feed) on performance was examined during 60 days. In both experiments, treatments were run in triplicate.

The basal composition of the experimental diets was identical in both experiments (Table 1). The main ingredients used were fishmeal, soybean 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, France), which was operated at Vinh Hoan Company (Dong Thap, Vietnam). 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, Can Tho University. After extrusion, pellets were dried at 60°C for 24 hours followed by sieving and storage in a freezer until feeding. Viscosity was 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, centrifuged at 12,000*g* for 10 min and thereafter supernatant viscosity was immediately measured by Brookfield LVDV-II + cone/plate viscometer. The measured viscosity and analyzed chemical composition of the experimental diets are given in Table 2 (Exp. 1) and Table 3 (Exp. 2).

Table 1 Ingredient composition (in g kg^{-1}) of the experimental diets used in experiment 1 and 2 on as is basis

Ingredients	Amount (g kg^{-1})
Fish meal [1]	142.9
Soybean meal [2]	168.1
Rice bran [2]	151.8
Defatted rice bran [2]	162.3
Cassava [2]	160.2
Sunflower meal [3]	170.2
Premix vitamin and mineral [4]	10.0
Squid oil [5]	24.5
Inert marker [6]	10.0
Guar gum (GG) & Carboxyl methyl cellulose (CMC) [7]	10.0

[1] Kien Giang fish meal was produced by Minh Tam Co., Ltd. (Kien Giang province, Vietnam).
[2] Soybean meal, Rice bran, Defatted rice bran and Cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam).
[3] Sunflower meal was imported and supplied by de Heus LLC Co. (Vinh Long province, Vietnam).
[4] Premix vitamin and mineral (UI or mg kg^{-1}): 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., Vietnam).
[5] Squid oil was produced by Vemedim Co. (Vietnam).
[6] Inert marker being Cr_2O_3 in experiment 1 and SiO_2 in experiment 2.
[7] 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 on the GG inclusion levels (g kg^{-1}) in the diet, the ratios between GG and CMC were 0 to 10, 0.25 to 9.75, 0.5 to 9.5, 1 to 9, 2 to 8 and 3 to 7.

Striped catfish (*Pangasionodon hypophthalmus*) juveniles 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^{-1}. Within each experiment, all tanks were connected to a semi-recirculation system which was fully running only at nighttime. 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.

Table 2 Analyzed 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[1]					
	GG0	GG0.25	GG0.5	GG1.0	GG2.0	GG3.0
Dry matter (g kg^{-1})	889	902	877	889	898	889
Crude protein (g kg^{-1})	291	287	302	291	289	289
Crude fat (g kg^{-1})	58.8	57.5	58.1	57.4	57.1	58.2
Carbohydrate (g kg^{-1})	528	534	519	530	532	531
Crude ash (g kg^{-1})	113	112	112	112	113	113
Chromium oxide (g kg^{-1})	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

[1]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 per kg feed.

The photoperiod regime was approximately 12 h light and 12 h dark. Water quality was checked daily for the temperature and ranged between 28 – 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 were 3.0 and 0.7 L min^{-1} for the experiment 1 and 2, respectively. Total ammonia nitrogen TAN (0.1 – 0.3 mg L^{-1}) and NO_2^{-}-N (0 – 0.03 ppm) for both experiments were measured weekly.

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

Dietary component	Diet code[1]		
	GG0	GG0.5	GG3.0
Dry matter (g kg^{-1})	920	926	918
Crude protein (g kg^{-1})	296	295	296
Crude fat (g kg^{-1})	34.4	40.7	43.1
Carbohydrate (g kg^{-1})	555	552	548
Crude ash (g kg^{-1})	115	112	113
Feed price (thousand VND kg^{-1})[2]	13.9	14.0	14.4

[1]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 per kg feed.

[2] VND is Vietnamese Dong

4.2.2 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 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 hours in total, started at 10:00 am (1 hour after feeding) and lasted till 1 hour prior to the next feeding (8:00 am). 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. In Exp. 2 no faeces was collected. In both experiments, the uneaten feed was collected, dried at 60°C for 24 h 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^{-1}). Per diet, about 10 g of feed was weekly sampled. Faeces and fish body samples were dried at 60°C for 24 h. 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 determined by drying in the oven at 105°C until constant weight; crude protein (N x 6.25) content following the Kjeldahl method; crude ash content by placing in the furnace at 560°C for 4 h; 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 per-chloric acid (Furukawa and Tsukahara, 1966).

4.2.3 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^{-1}) by the following equation (Cho and Kaushik, 1985):

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

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 *et al.*, 2005b). The faecal waste consists of the recovered faeces and the 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 per 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 per kg 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 this 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 test. The residuals of all data were checked for normal distribution using the One-Sample Kolmogorov-Smirnov. All the tests were done by using SPSS 25.0®.

4.3 Results

4.3.1 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 = 0.996; P<0.001). Mean values per experimental diets for digestibility, performance and faecal waste characteristics are given in Table 4. Regression analysis showed that dietary viscosity did not affect fish performance during the 29-d experiment period in Exp. 1 (P>0.1; Table 5). Except for fat, all nutrient digestibility's were negatively affected by dietary viscosity (Table 5; Figure 1). For all these nutrients, the digestibility was linearly related to dietary viscosity (*i.e.*, the quadratic component was not significant). 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=0.073), although the relation was not significant.

Table 4 Effect of diet on nutrients digestibility, performance and faecal waste characteristics of striped catfish fed the experimental diets

Parameter[1]	Diet code[2]					
	GG0	GG0.25	GG0.5	GG1.0	GG2.0	GG3.0
Diet/pellet viscosity (cP)	1.58	1.63	1.72	1.81	1.96	2.16
Digestibility:						
Dry matter (%)	79.1±1.4	77.7±0.4	77.6±0.3	76.8±0.1	76.2±1.1	74.8±2.5
Crude protein (%)	89.3±0.5	88.3±0.1	88.7±0.2	87.7±0.04	87.2±0.5	86.8±1.3
Crude fat	95.3±0.7	95.8±0.2	96.7±0.5	95.3±0.7	96.1±0.2	96.7±0.6
Crude ash	44.5±4.6	38.7±0.7	39.7±0.4	36.1±0.9	35.2±1.9	31.3±5.9
Carbohydrate (%)	79.9±1.5	78.9±0.9	78.0±0.8	78.0±0.04	77.5±1.4	75.6±2.9
Performance:						
IMW (g)	95.0±0.1	95.0±0.2	95.0±0.1	95.2±0.2	95.0±0.1	95.1±0.2
FMW (g)	114±2.4	113±5.8	111±1.4	111±3.0	110±2.0	111±3.6
DWG (g d^{-1})	0.64±0.08	0.61±0.20	0.54±0.05	0.55±0.45	0.52±0.40	0.53±0.47
FCR	0.99±0.14	1.04±0.27	1.07±0.09	1.13±0.27	1.16±0.18	1.19±0.34
Survival rate (%)	98.3±2.9	98.3±2.9	100±0.0	96.7±2.9	100±0.0	98.3±2.9
Faecal waste characteristics:						
Faeces recovery (%)	44.0±1.7	44.5±3.9	40.2±1.0	36.2±3.8	32.4±2.1	30.3±1.5
TFP (g kg^{-1} feed DM)	209±14.3	223±4.3	224±3.2	232±1.0	238±11.2	252±25.2
RF (g kg^{-1} feed DM)	91.8±3.5	99.1±9.9	89.9±3.0	84.0±8.4	77.0±5.6	76.2±5.2
NRF (g kg^{-1} feed DM)	117±11.2	123±8.1	134±2.4	148±9.4	161±9.6	176±20.7
Faecal particles >2mm (%)	26.5±5.2	34.0±4.1	39.3±7.2	42.7±4.1	38.1±4.9	43.0±1.3

[1]Values (mean±SD). IMW: Initial mean weight; 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.
[2]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 per kg feed.

Between the experimental diets, the faeces recovery ranged from 29.2 to 48.9% (Table 4; Figure 2a). Faeces recovery declined linearly with increasing dietary viscosity ($P<0.001$). 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 ($P=0.084$; Table 5). 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 5). 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 5). 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 ($P<0.01$). Increasing the dietary viscosity increased the percentage of particles larger than 2mm, but this relationship with particle size tended to be quadratic ($P= 0.059$; Figure 2b). With increasing dietary viscosity levels, the improvement of particle size became smaller (*i.e.*, curved off).

Table 5 The estimated relationships[1] 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[2]	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	0.59	<.001	---
Crude protein	Y = 95.1 (±1.3) – 4.0 (±0.73) × X	0.65	<.001	---
Crude fat	Y = 93.2 (±1.5) + 1.5 (±0.81) × X	0.19	0.073	---
Crude ash	Y = 66.8 (±6.8) – 17.9 (±3.7) × X	0.59	<.001	---
Carbohydrate	Y = 89.4 (±3.0) – 6.3 (±1.6) × X	0.48	0.001	---
Performance:				
FMW (g)	111 (±0.73)	---	0.188	---
DWG (g d^{-1})	0.57 (±0.03)	---	0.181	---
FCR	1.1 (±0.05)	---	0.197	---
Survival rate (%)	98.6 (±0.54)	---	0.883	---
Faecal waste characteristics:				
Faeces recovery (%)	Y = 85.6 (±5.8) – 26.4 (±3.2) × X	0.81	<.001	---
Faeces revovery (%)	Y = 197 (±61) – 147 (±65) × X + 32 (±18) × X^2	0.85	---	0.084
TFP (g kg^{-1} feed DM)	Y = 110 (±25) + 65.8 (±14) × X	0.59	<.001	---
RF (g kg^{-1} feed DM)	Y = 153 (±14) – 36.9 (±7.9) × X	0.58	<.001	---
NRF (g kg^{-1} feed DM)	Y = – 42.8 (±23) + 103 (±12) × X	0.81	<.001	---
Faecal particles >2mm (%)	Y = – 0.80 (±13) + 21.0 (±6.9) × X	0.37	0.008	---
Faecal particles > 2mm (%)	Y = – 263 (±129) +306 (±140) × X – 76.2 (±37) × X^2	0.51	---	0.059

[1]Only relationships (both linear and quadratic), which were significant ($P<0.05$) or tended to be significant ($P<0.10$) are given.
[2]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.

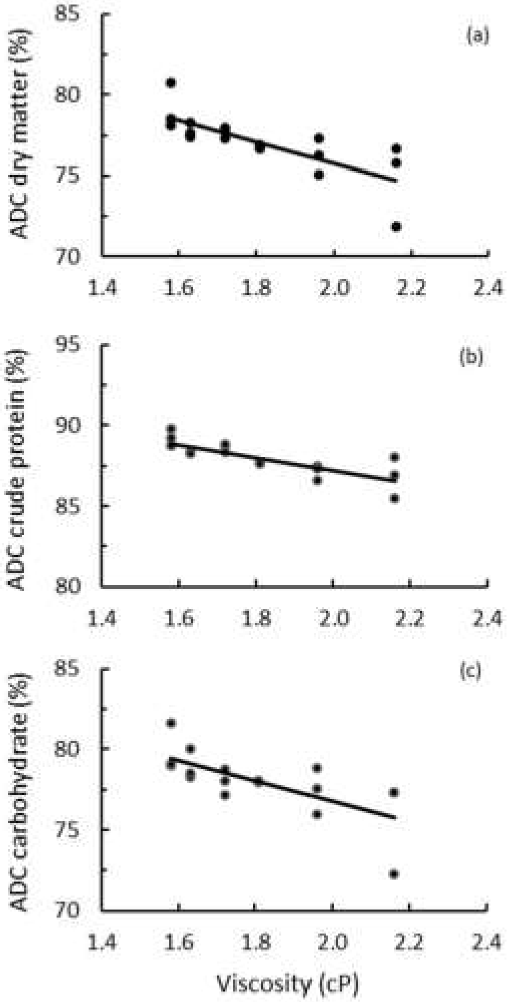

Figure 1 The linear relationships between dietary viscosity (cP) measured on the pellets of the experimental diets and nutrient digestibility (%) of: dry matter (a), crude protein (b), and carbohydrate (c).

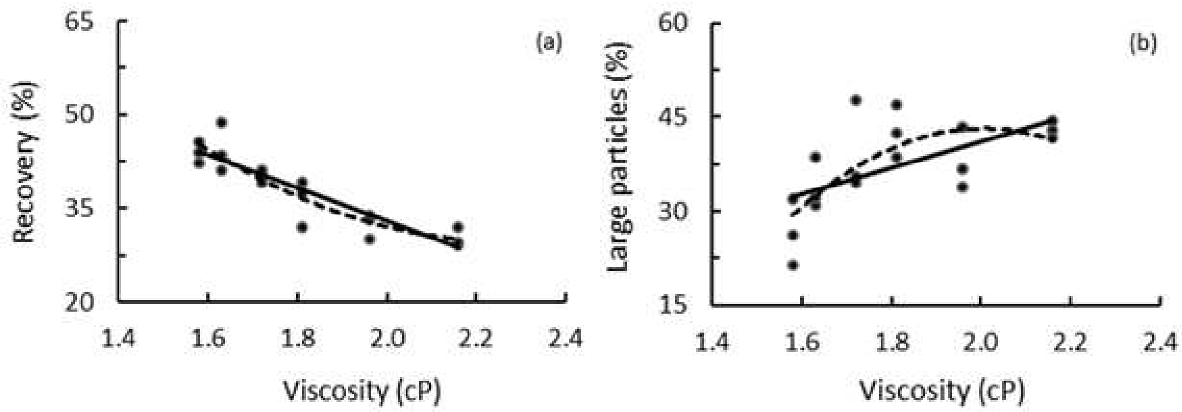

Figure 2 The relationships (both linear and quadratic) between dietary viscosity (cP) measured on the pellets of the experimental diets and: the recovery of the faeces from the water by settling (in %) (a); and the percentage of faecal particles being larger than 2 mm measured in faeces of striped catfish collected by settling (b)

4.3.2 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 6). Growth was similar at the diets with a GG inclusion of 0 and 0.5 g kg^{-1}, but reduced at the diet with 3.0 g kg^{-1} ($P<0.05$). Consequently, also FCR was higher at the highest GG inclusion level compared to the other two diets (Table 6). 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 6) 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^{-1} compared to the other two experimental diets.

Table 6 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)

Parameter	Dietary code[1]		
	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})	2.31±0.26^{b}	2.44±0.27^{b}	1.28±0.16^{a}
Feed intake (g DM $fish^{-1}$ day^{-1})	2.59±0.46	3.03±0.48	2.33±0.13
Feed conversion ratio	1.11±0.09^{a}	1.24±0.17^{a}	1.84±0.17^{b}
Survival rate (%)	86.7±7.6	93.3±11.5	98.3±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	4.12±0.70^{c}	3.03±0.16^{b}	2.05±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 ($P<0.05$).

[1]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 per kg feed.

4.4 Discussion

4.4.1 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 ($P<0.001$, except for fat). Similar negative effects on the nutrient digestibility of striped catfish was found between diets with a contrast in dietary viscosity (low versus 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 (Fagbenro and Jauncey, 1995; Amirkolaie *et al.*, 2005b), rainbow trout (Storebakken, 1985), African catfish (Leenhouwers *et al.*, 2007b; Leenhouwers *et al.*, 2006), Atlantic salmon (Kraugerud *et al.*, 2007), and also in broiler chickens (Van der Klis *et al.*, 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 *et al.*, 2014). These effects of dietary viscosity are often explained by: 1) 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; 2) viscous NSP absorb water and alter the chyme into a gel, which hamper the mixing of endogenous enzymes and thereby reduce hydrolysis of nutrients; 3) the gelling of chyme reduces diffusion of nutrients thereby reducing their absorption and increasing faecal nutrient losses, such as bile acids, amino acids; 4) the increased viscosity of the chyme may alter/damage the gut mucosa, thereby decreasing the absorption of hydrolysed nutrients by the enterocytes (Sinha *et al.*, 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 *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 *et al.*, 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. Also, the requirement for water for fat emulsification may play a role, since viscous substances are known to bind water. However in Nile tilapia (Amirkolaie *et al.*, 2006), African catfish (Harter *et al.*, 2013) and also in striped catfish (Tran-Tu *et al.*, 2019, submitted article) 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 *et al.*, 2007a; Shiau *et al.*, 1988) or snakehead (Janphirom *et al.*, 2010). However there are also various studies where the effects of Exp. 1 in the current study (Table 5) are not observed like in: African catfish (Harter *et al.*, 2015; Leenhouwers *et al.*, 2006), Nile tilapia (Leenhouwers *et al.*, 2007a); 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 6), a reduced growth, protein utilization, fat retention and an increased FCR ($P<0.05$) was found but only at the diet with highest viscosity. Next to differences in experimental duration (60 d in Exp. 2 *vs* 29 d Exp. 1), also the feeding level may have caused the absence of effects in Exp. 1 (restricted feeding versus *ad. libitum* feeding in Exp. 2).

Regarding the cost of feed per kg of fish gain, Exp. 2 (Table 6) 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^{-1} GG did not affect the growth of African catfish (Leenhouwers *et al.*, 2006). Similar observations were found in rainbow trout with 3 g kg^{-1} GG inclusion (Brinker, 2009; Brinker, 2007), and in striped catfish with 0.5 g kg^{-1} GG inclusion (this study Exp. 2). In contrast, in snakehead dietary GG inclusions of 0.1 g kg^{-1} decreased fish growth (Janphirom *et al.*, 2010), and in Nile tilapia, a dietary level of 3 g kg^{-1} GG declined the digestibility of nutrients (Fagbenro and Jauncey, 1995). Also in broiler chickens, GG inclusion of 1.0 g kg^{-1} 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^{-1} GG but a decreased digestibility was shown in the diet with 10 g kg^{-1} 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 depend 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.

4.4.2 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 *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 *et al.*, 2005; Brinker, 2007; Brinker, 2009; Brinker and Friedrich, 2012). 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 *et al.*, 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.

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 *et al.*, 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 *et al.*, 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 *et al.*, 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 versus 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.

4.5 Conclusion

In striped catfish, digestion of macro-nutrients, 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.

Chapter 5

Effect of dietary viscosity on digesta characteristics and progression of digestion in different segments of the gastrointestinal tract of striped catfish (*Pangasionodon hypophthalmus*)

This chapter has been published as:

Tran-Tu, L.C., Bosma, R.H., Verstegen, M.W.A., and 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

Abstract

The physical and chemical characteristics of chyme have been shown to related to apparent faecel nutrient digestibility, also in fish. This study assessed the effect of dietary viscosity on chyme characteristics in different segments of the gastrointestinal tract (GIT) of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878). Moreover, its effect on the progression of nutrient digestion throughout the GIT was studied. Six diets were produced, that had moderate differences in dietary viscosity by exchanging carboxymethylcellulose for guar gum (0, 0.25, 0.5, 1, 2, or 3 g kg^{-1} of diet). Eighteen tanks, each stocked with 20 fish of 95g, were used (three replicate tanks per diet). Diets were randomly assigned to tanks and fed for 29 days. Thereafter fish were euthanized for the collection of chyme from four different segments of the GIT: stomach, proximal, mid and distal intestine. The chyme characteristics, dry matter content, viscosity and osmolarity were affected by the interaction effect of diet and GIT-segment. This implies that the impact of dietary viscosity on chyme characteristics differs between GIT-segments. Chyme viscosity increased with increasing dietary viscosity, but this mainly occurred in the stomach and distal intestine. Dry matter content of chyme was mainly affected (increased with inclining dietary viscosity) in the stomach. The digestibility of both dry matter and crude protein was different between GIT-segments and increased from stomach towards distal intestine. Moreover, these nutrient digestibilities were influenced by dietary viscosity being negatively related. Already in the stomach increased dietary viscosity reduced the disappearance of nutrients (*i.e.*, digestibility). These differences in digestibility were persistent and constant throughout the total GIT.

5.1 Introduction

Dietary viscosity is dependent on ingredient composition. For example, the grains of the main cereals (wheat, barley, oat, rye, maize) alter the dietary viscosity differently (Leenhouwers *et al*., 2007a). Alterations in dietary viscosity are predominantly related to the soluble non-starch polysaccharide (NSP) content (Sinha *et al*., 2011). In practical aqua feed production, binders with viscous properties are sometimes used to increase pellet quality (Storebakken, 1985). Next to that, binders (*e.g.* guar gum) have the potential to enhance faecal pellet stability in some fish species like rainbow trout (Brinker, 2007). However, this impact is not uniform across fish species. For instance, in Nile tilapia (Amirkolaie *et al*., 2005) and also in striped catfish (Tran-Tu *et al*., 2017), increasing dietary viscosity by guar gum inclusion negatively affected faecal stability.

Dietary viscosity, depending on the level, can negatively affect the nutrient digestibility (ADC) in pig (Longland *et al*., 1993), chicken (Van der Klis *et al*., 1993b) and fish (Leenhouwers *et al*., 2006; Refstie *et al*., 1999). For example, increasing levels of guar gum inclusion reduced the ADC of protein in African catfish (Leenhouwers *et al*., 2006), Nile tilapia (Amirkolaie *et al*., 2005), rainbow trout (Brinker, 2007; Brinker, 2009) and striped catfish (Tran-Tu *et al*., 2017). The decreased ADC with increased dietary viscosity is suggested to be due to binding and reduced mixing of endogenous enzymes with the digesta when chyme viscosity is increased (Leenhouwers *et al*., 2006; Storebakken, 1985).

Next to changes in chyme viscosity, different types of cereal grains also induced differences in dry matter (DM) content in chyme of African catfish (Leenhouwers *et al*., 2007b) and Nile tilapia (Leenhouwers *et al*., 2007a). Amirkolaie *et al.* (2006) found in Nile tilapia negative correlations between chyme DM content in the stomach as well as proximal intestine with nutrient digestibility values. This might imply that water content (or fluxes) in the proximal part of the GIT play a role in digestion as well. Information on effects of diets on the water content of chyme is scarce. Recently, it was shown that dietary fat replacement by starch altered the intestinal water fluxes and chyme DM content in African catfish (Harter *et al*., 2015). In striped catfish, no information is available on the impact of dietary viscosity on chyme characteristics inside the GIT.

Digestion and absorption in animals, measured as apparent faecal digestibility, are related to physico–chemical conditions in the GIT (*e.g.* viscosity, dry matter, osmolarity, and pH) (Van der Klis *et al*., 1993a). However, most studies on nutrient digestion in fish do not measure the aforementioned characteristics in the GIT. Furthermore, in fish, few studies have reported on the (cumulative) digestion of nutrients throughout the GIT (i.e., in different compartment, stomach, proximal, mid and distal intestine), like was done for rainbow trout by (Austreng, 1978). Recently, Harter *et al*. (2015) showed that exchanging dietary starch for dietary fat affected the ADC of protein already in the stomach. Apart from the Harter (2015) study, there is no information on the relation between chyme properties and digestion in different locations of fish's GIT. This study assesses the impact of increasing dietary viscosity (by addition of guar gum) on chyme characteristics and digestion (protein and dry matter) in different segments of the GIT (stomach, proximal, mid and distal intestine) of striped catfish. In the current study moderate increases in dietary viscosity were studied by supplementing 0 up to 3 g kg^{-1} guar gum to quantify the potential impact of viscos pellet binders on digestion of practical striped catfish diets.

5.2 Materials and Methods

5.2.1 Experimental diets

The experiment lasted 29 days. This study aimed to examine the effect of moderate changes in dietary viscosity on chyme characteristics and digestion. Therefore, six levels of guar gum (GG) were added to the basal diet: 0, 0.25, 0.5, 1.0, 2.0 and 3.0 g kg^{-1}. The basal diet mimicked a practical striped catfish diet. The main ingredients of the basal diet were fishmeal, soybean meal, rice bran, defatted rice bran, cassava, sunflower meal and carboxyl methyl cellulose (CMC) as a pellet binder (Table 1). The supplemented GG replaced CMC in the basal diet. The chemical composition of the diets is given in Table 2. The basal ingredient mixture (with the superscript numbers 1, 2 and 3 in the Table 1) was grinded using a mesh size of 0.8 mm with a hammer mill (Stolz, France) at Vinh Hoan Company (Dong Thap, Vietnam). After the grinding this ingredient-mixture, the formulated amounts of vitamin–mineral premix, inert marker (Cr_2O_3), GG, CMC and squid oil were added for each treatment and thereafter properly mixed. The mixtures for each treatment were extruded through the 3 mm-die using a single screw-extruder. This resulted in pellets with a diameter of approximately 4.5 mm. The extruder was manufactured by the Centre for Technology Research and Application, College of Engineering Technology, Can Tho University. After extrusion, the pellets were dried at 60°C for 24 h, followed by sieving and storage in a freezer until feeding. The meal mixtures prior to extrusion and also the extruded pellets were sampled and analysed for viscosity.

Table 1 The amounts (in g kg^{-1}) of ingredients used in the basal diet to test six levels of guar gum (0, 0.25, 0.5, 1.0, 2.0 and 3.0 g kg^{-1})

Ingredients	Amount (g kg^{-1})
Fish meal [1]	142.9
Soybean meal [2]	168.1
Rice bran [2]	151.8
Defatted rice bran [2]	162.3
Cassava [2]	160.2
Sunflower meal [3]	170.2
Premix vitamin and mineral [4]	10.0
Squid oil [5]	24.5
Chromium oxide	10.0
Binders [6]	10.0

[1] Kien Giang fish meal was produced by Minh Tam Co., Ltd. (Kien Giang province, Vietnam).
[2] Soybean meal, Rice bran, Defatted rice bran and Cassava were imported and supplied by Vinh Hoan Co. (Dong Thap province, Vietnam).
[3] Sunflower meal was imported and supplied by de Heus LLC Co. (Vinh Long province, Vietnam).
[4] Premix vitamin and mineral (UI or mg kg^{-1}): 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., Vietnam).
[5] Squid oil was produced by Vemedim Co. (Vietnam).
[6] Binders: the combination between Guar gum (GG) and Carboxyl methyl cellulose (CMC); 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^{-1}) in the diet, the ratios between GG and CMC were 0 to 10, 0.5 to 9.5, 1 to 9, 2 to 8 and 3 to 7.

Table 2 The analysed chemical composition of six experimental diets on dry matter basis

Dietary component	Diet code					
	G0	G0.25	G0.5	G1.0	G2.0	G3.0
Dry matter (g kg^{-1})	889	902	877	889	898	889
Crude protein (g kg^{-1})	291	287	302	291	289	289
Crude fat (g kg^{-1})	58.8	57.5	58.1	57.4	57.1	58.2
Carbohydrate (g kg^{-1})	528	534	519	530	532	531
Crude ash (g kg^{-1})	113	112	112	112	113	113
Chromium oxide (g kg^{-1})	8.9	8.8	8.9	8.6	9.4	9.4

5.2.2 Experimental system and animals

Striped catfish (*Pangasionodon hypophthalmus*) juveniles with an initial body weight of 95.1±0.14 g $fish^{-1}$ were bought from a local hatchery. The fish was a mixed-sex population. The juveniles were randomly distributed into eighteen plastic digestibility tanks (Tran-Tu *et al.*, 2017) of 170L each. Per tank 20 fish were stocked. The tanks were filled with 80% with water and were constantly aerated (one air stone per tank). Tanks were on a semi-recirculation system; the water flow over each tank was set at 3 L min^{-1}. During daytime, half of the outflowing water per tank was replaced by de-chlorinated tap water, which had been stored in a 2 m^3 tank with continuous aeration.

The photoperiod regime was approximately 12 h light and 12 h dark. Water quality was checked daily and maintained for temperature (28 – 31°C), pH (7.3 – 7.8), oxygen concentration (5.6 – 6.5 ppm) and water flow (3 L min^{-1}). Total ammonia nitrogen TAN (0.1 – 0.3 mg L^{-1}) and NO_2^--N (0 – 0.03 ppm) were measured and monitored weekly.

5.2.3 Experimental procedure

Prior to the start of the experiment, fish were fed a mixture of the six experimental diets for two weeks to allow adaptation to the dietary ingredients. During the 29-day experimental period, fish were fed restrictively. The mean initial fish weight between the experimental tanks ranged between 94.9 and 95.4 g $fish^{-1}$. Each tank received the same amount of feed from their assigned experimental diet (20g per tank) at 09:00 am. Thirty minutes after giving the last feed, the uneaten feed was collected, dried at 60°C for 24 h and weighed.

During the last day of the experiment, eight hours after feeding, the fish in each tank were netted and then anaesthetized by immersion in a benzocaine solution. This solution was prepared by dissolving 1 g of benzocaine into 3 mL acetone and thereafter this mixture was diluted with fresh water up to 10 L. This solution was maintained at approximately 20°C. After 10 minutes of immersion, all fish were well sedated and the fish were weighed as a group. Eighteen sedated fish from each tank were sacrificed by putting them in ice water (2:1). After being euthanized fish were dissected for the collection of the digesta in the gastrointestinal tract (GIT). Before and after dissection, each fish was weighed. Each GIT was divided into four segments: stomach and three equal parts of the gut, representing proximal, mid and distal gut. Per tank and per segment the collected chyme was pooled (i.e. of 18 fish). The proximal, mid and distal intestine are considered to be comparable to the duodenum, jejunum and ileum, respectively in broilers. One part of the pooled digesta samples was centrifuged at 12000*g* for 10 minutes. Afterwards, we immediately measured the pH (Mettler Toledo), viscosity (cP, analysed by the Brookfield LVDV-II + cone/plate viscometer) and osmolarity (mOsm L^{-1}, Fiske One-Ten Osmometer) in the supernatant of each sample. The viscosity of the pellets after grinding and of the dietary meal mixtures (prior to extrusion) was done according to Leenhouwers *et al.* (Leenhouwers *et al*., 2006) as follows: one gram of these samples was mixed with 4 mL of demi-water, incubated for 30 minutes at 28°C, thereafter centrifuged at 12000*g* for 10 minutes before measuring the viscosity (cP). The remaining part of the pooled digesta samples per segment and tank was dried and stored for later analysis to determine dry matter and protein digestibility.

5.2.4 Sample analysis

About 10 g of each feed was sampled weekly during the experimental period. The feed was ground for analysis of DM, chromium oxide, crude protein, crude fat, crude ash. The chyme samples were dried at 60°C for 24 h and then analysed as similar as feed but only for DM, chromium oxide and crude protein. Before analysis, both feed and chyme samples were ground by coffee blender and stored in the freezer -20°C. The chemical analyses were done in triplicate. 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 x 6.25) content following the Kjeldahl method; mineral (ash) content by placing in the furnace at 560°C for 4 h; and crude fat content by solvent extraction. Except for the moisture content, the analysed parameters were calculated on DM basis. The carbohydrate content in the sample was calculated by the equation: 100 – (crude protein + crude ash + 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 per-chloric acid (Furukawa and Tsukahara, 1966).

5.2.5 Data analysis

The Apparent Digestibility Coefficients of nutrient (Nutrient.ADC) like DM and crude protein was measured by the marker method using chromic oxide as inert marker (Cho and Kaushik, 1985):

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

Where: - Marker_{diet} is the concentration of chromium oxide in the diet (g 100 g^{-1} DM).

- Marker_{chyme} is the concentration of chromium oxide in the chyme (g 100 g^{-1} DM).
- $\mathrm{Nutrient}_{diet}$ is the concentration of nutrient in the diet (g 100 g^{-1} DM).
- $\mathrm{Nutrient}_{chyme}$ is the concentration of nutrient in the chyme (g 100 g^{-1} DM).

All results were introduced in a database (MS-Excel®) and mean and standard deviation (SD) of each treatment were calculated. Data expressed in percentage were transformed into ASIN results. After that, all data were checked for normal distribution using the One-Sample Kolmogorov-Smirnov test and on the homogeneity of variances using Levene's test. Data were analyzed using a split-plot approach for the effects of diet (inclusion level of GG), gut segment (stomach, proximal, mid and distal intestine) and their interaction with the gut segment being nested within the tank (i.e. plots). The main effect of diet was tested against the between tank variation within diets. Statistical analyses were done by using SPSS 23.0®. In the case of significant main effects, post hoc comparison of means was done by the Tukey test.

5.3 Results

5.3.1 Dietary and chyme viscosity

The viscosity of experimental diet mixtures was measured in samples taken before and after extrusion (Figure 1). In both types of samples, dietary viscosity gradually increased with increasing dietary GG inclusion. However, the viscosity of extruded pellets was lower than that of meal mixtures prior to extrusion.

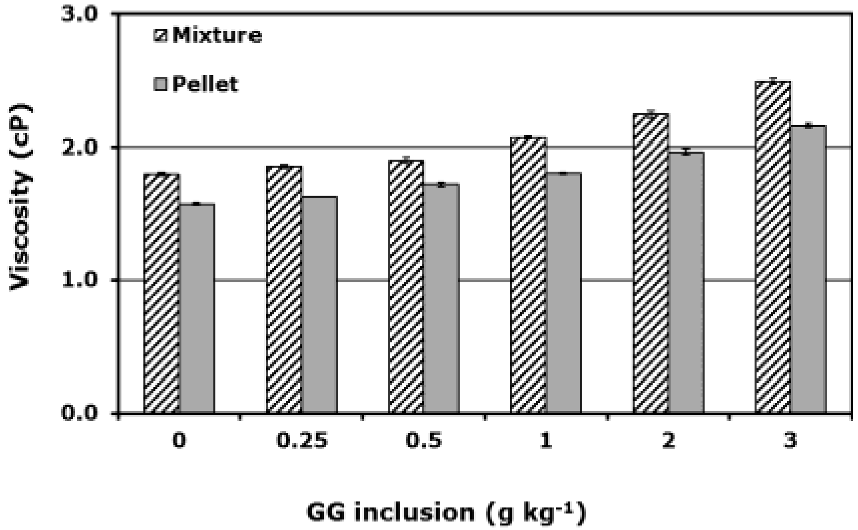

Figure 1 Effect of dietary guar gum (GG) inclusion levels on the viscosity of the ingredient meal mixtures prior to extrusion and in extruded pellets. GG was exchanged for carboxyl methyl cellulose (CMC) in the experimental diets

Table 3 Effect of dietary guar gum levels on chyme viscosity (cP) of striped catfish fed the experimental diets

Guar gum inclusion levels (g kg^{-1})	GIT segments				Pooled mean
	Stomach	Proximal intestine	Mid Intestine	Distal Intestine	
0.0	1.69±0.09	1.54±0.24	1.34±0.06	1.64±0.16	1.55^{a}
0.25	1.72±0.05	1.45±0.10	1.38±0.05	2.03±0.15	1.64^{ab}
0.5	1.96±0.10	1.58±0.13	1.43±0.08	1.70±0.21	1.67^{ab}
1.0	1.67±0.18	1.55±0.09	1.40±0.03	2.17±0.49	1.70^{ab}
2.0	1.88±0.16	1.63±0.08	1.50±0.15	1.89±0.18	1.72^{ab}
3.0	2.15±0.04	1.54±0.17	1.43±0.07	2.23±0.37	1.84^{b}
Pooled mean	1.84^{b}	1.55^{a}	1.41^{a}	1.94^{b}	

P values: Segments P<0.001, dietary guar gum inclusion (GG) P=0.079, Segments*GG P=0.022. Values are presented mean+SD and different letters of pooled means are significantly different (P<0.05).

Averaged over dietary treatments, the viscosity of chyme in striped catfish differed between GIT segments (P<0.001); being highest in the stomach and distal intestine (Table 3). Averaged over GIT segments, viscosity tended to differ between the dietary treatments

(P<0.10); being reflected by an increase in chyme viscosity at higher GG inclusions. However, the interaction effect between GIT segment and GG inclusion on chyme viscosity was significant (P<0.05), which indicated that the effect of GG inclusion differed between the GIT segments. In the stomach, chyme viscosity increased with GG inclusion. Whereas in the proximal and mid intestine this effect of GG inclusion on chyme viscosity seemed absent, but in the distal intestine the effect of GG inclusion seemed to reappear (Table 3).

5.3.2 Chyme dry matter (DM)

Data on chyme dry matter (DM) as affected by dietary GG inclusion at different locations in the GIT are given in Table 4. Averaged over diets, chyme DM was highest in the stomach (247 g kg^{-1}) and lowest in the proximal intestine (138 g kg^{-1}). Chyme DM was influenced by the interaction effect of GG inclusion and GIT segment (P=0.003). The impact of dietary GG inclusion seemed more pronounced in the stomach then in the other parts of the GIT. With increasing dietary inclusion of GG the chyme DM content declined in the stomach.

Table 4 Effect of dietary guar gum levels on chyme dry matter (g kg^{-1}) of striped catfish fed the experimental diets

Guar gum inclusion levels (g kg^{-1})	GIT segments				Pooled mean
	Stomach	Proximal intestine	Mid Intestine	Distal intestine	
0.0	266±22.9	131±26.9	163±4.8	164±9.2	181^{a}
0.25	233±15.1	151±1.5	157±6.6	150±5.1	170^{a}
0.5	259±12.7	135±21.8	141±34.6	131±14.9	166^{a}
1.0	233±15.1	151±1.5	157±6.6	150±5.1	173^{a}
2.0	225±7.6	145±11.4	158±5.8	163±7.4	173^{a}
3.0	239±12.7	129±9.4	149±6.2	140±25.1	164^{a}
Pooled mean	247^{c}	138^{a}	153^{b}	147^{ab}	

P values: Segments P<0.001, dietary guar gum inclusion (GG) P=0.424, Segments*GG P=0.003. Values are presented mean+SD and different letters of pooled means are significantly different (P<0.05).

5.3.3 Chyme osmolarity

The osmolarity of the chyme was affected by dietary GG inclusion (P<0.05), GIT segment (P<0.001) as well as by their interaction (P<0.05). The osmolarity of the chyme declined as digesta passed through the GIT (Table 5). Averaged over GIT segments, the osmolarity of the chyme increased with inclining dietary GG inclusion. This GG effect was present in the stomach, diminished in the proximal and mid intestine and seemed to reappear in the distal part of the intestine (Table 5).

Table 5 Effect of dietary guar gum levels on chyme osmolarity (mOsm kg^{-1}) of striped catfish fed the experimental diets

Guar gum inclusion levels (g kg^{-1})	GIT segments				Pooled mean
	Stomach	Proximal intestine	Mid Intestine	Distal intestine	
0.0	468±53.1	469±12.5	388±8.7	334±30.2	415^{ab}
0.25	565±16.9	422±17.4	393±7.6	312±3.1	423^{ab}
0.5	515±24.8	465±34.0	386±31.8	326±8.4	423^{ab}
1.0	497±31.8	453±14.8	374±23.0	309±21.0	408^{a}
2.0	532±94.0	446±12.3	401±19.0	340±29.0	430^{ab}
3.0	585±37.1	459±16.3	396±19.0	376±11.6	454^{b}
Pooled mean	527^{d}	452^{c}	390^{b}	333^{a}	

P values: Segments P<0.001, dietary guar gum inclusion (GG) P=0.043, Segments*GG P=0.045. Values are presented mean+SD and different letters of pooled means are significantly different (P<0.05).

5.3.4 Chyme pH

The pH of the chyme was different between all GIT segments (P<0.001; Table 6). Averaged over all diets, pH was lowest (4.85) in the stomach and highest (6.94) in the proximal intestine and from there on slightly decreased to 6.46 in the distal part of the intestine. The interaction effect between GG inclusion and GIT segment was present (P<0.05). Overall differences between diets were predominantly present in the stomach. At the higher GG inclusion levels, the decline in chyme pH seemed to occur earlier (mid intestine).

Table 6 Effect of dietary guar gum levels on chyme pH of striped catfish fed the experimental diets

Guar gum inclusion levels (g kg^{-1})	GIT segments				Pooled mean
	Stomach	Proximal intestine	Mid intestine	Distal intestine	
0.0	4.97±0.24	7.00±0.19	7.04±0.22	6.47±0.13	6.37^{a}
0.25	4.86±0.08	6.95±0.22	6.94±0.19	6.42±0.12	6.27^{a}
0.5	4.86±0.08	6.95±0.22	6.94±0.19	6.42±0.12	6.29^{a}
1.0	5.11±0.40	6.98±0.14	6.57±0.27	6.52±0.06	6.30^{a}
2.0	4.46±0.12	6.76±0.08	6.55±0.04	6.45±0.06	6.05^{a}
3.0	4.71±0.09	6.95±0.18	6.58±0.41	6.51±0.10	6.19^{a}
Pooled mean	4.85^{a}	6.94^{d}	6.73^{c}	6.46^{b}	

P values: Segments P<0.001, dietary guar gum inclusion (GG) P=0.120, Segments*GG P=0.025. Values are presented mean+SD and different letters of pooled means are significantly different (P<0.05).

5.3.5 Chyme digestibility

For both DM and CP ADC, there was no interaction effect between GG inclusion level and GIT segment (P>0.1, Table 7 and 8). Thus, the observed main effect of dietary GG inclusion on DM (P<0.001) and CP ADC (P<0.05) was constant throughout the GIT. In other words, the declined ADC of DM as well as of CP with increasing inclusion levels of GG were already present in the stomach and remained present up to the distal intestine. Averaged over diets, the digestibility of DM and CP increased from the stomach towards the distal intestine (P<0.001). Averaged over all diets, the apparent digestibility coefficient (ADC) was already 9.9% for DM and 10.3% for CP in the stomach and increased to 64.7% for DM and 79.9% for CP in the distal intestine.

Table 7 Effect of dietary guar gum levels on chyme DM digestibility (%) of striped catfish fed the experimental diets

Guar gum inclusion levels (g kg^{-1})	GIT segments				Pooled mean
	Stomach	Proximal intestine	Mid intestine	Distal intestine	
0.0	13.1±2.8	49.1±4.8	57.8±0.3	69.7±0.7	47.4^{c}
0.25	11.2±0.7	47.0±3.1	57.9±2.7	66.5±1.0	45.7^{bc}
0.5	11.6±2.0	49.6±2.3	55.1±1.9	67.6±2.7	45.9^{bc}
1.0	7.6±2.8	44.7±3.7	55.1±4.5	62.3±3.9	42.4^{ab}
2.0	7.9±3.9	39.8±2.3	53.6±1.4	63.0±1.6	41.1^{a}
3.0	7.9±1.5	40.4±1.1	51.3±5.4	59.2±2.3	39.7^{a}
Pooled mean	9.9^{a}	45.1^{b}	55.1^{c}	64.7^{d}	

P values: Segments P<0.001, dietary guar gum inclusion (GG) P<0.001, Segments*GG P=0.470. Values are presented mean+SD and different letters of pooled means are significantly different (P<0.05).

Table 8 Effect of dietary guar gum levels on chyme protein digestibility (%) of striped catfish fed the experimental diets

Guar gum inclusion levels (g kg^{-1})	GIT segments				Pooled mean
	Stomach	Proximal intestine	Mid intestine	Distal intestine	
0.0	11.9±5.9	59.5±5.7	74.8±0.9	83.3±1.4	57.4^{ab}
0.25	11.0±0.6	59.5±4.8	75.6±1.6	80.0±1.2	56.6^{ab}
0.5	14.2±1.3	64.3±2.4	76.2±1.6	82.0±1.3	59.2^{b}
1.0	12.8±6.5	59.7±9.0	75.2±4.3	79.5±2.7	56.8^{ab}
2.0	5.6±4.4	54.6±10.7	73.1±2.9	78.7±3.1	53.0^{ab}
3.0	6.6±2.7	50.8±3.0	68.7±2.5	75.9±1.9	50.5^{a}
Pooled mean	10.3^{a}	58.1^{b}	74.0^{c}	79.9^{d}	

P values: Segments P<0.001, dietary guar gum inclusion (GG) P=0.048, Segments*GG P=0.781. Values are presented mean+SD and different letters of pooled means are significantly different (P<0.05).

5.4 Discussion

This study assessed the impact of dietary viscosity on nutrient digestion and chyme characteristics at different locations in the GIT of striped catfish. In current commercial stripped catfish diets in Vietnam, dietary viscosity was on average 1.47 cP and ranges from 1.36 to 1.55 cP (n=7; unpublished data). The basal diet in the current study (0 g kg^{-1} GG inclusion) was aimed to representative for commercial catfish diets; having a viscosity of 1.58 cP, which was slightly higher than intended during the formulation. The exchange of CMC with GG resulted in a gradual increase in dietary viscosity (Fig. 1). Increasing the dietary viscosity (i.e., GG inclusion) in the current study coincided with a trend in an increased viscosity of chyme averaged over all GIT segments. This is in agreement with the literature on various animals' species. In African catfish (Leenhouwers *et al*., 2006; Leenhouwers *et al*., 2007b), Nile tilapia (Amirkolaie *et al*., 2005) and broiler chicken (Van der Klis *et al*., 1993a) it is shown that increasing dietary viscosity by the dietary soluble NSPs level increased digesta viscosity. Altering dietary viscosity by changing the ingredient composition of the feed, like gelatinized maize flour versus native maize flour (Amirkolaie *et al*., 2006) and rye (Leenhouwers *et al*., 2007a) also altered chyme viscosity in Nile tilapia. The absolute values of digesta' viscosity found in this study for striped catfish are comparable to those in Nile tilapia fed different types of maize starch (Amirkolaie *et al*., 2006). However the current values are lower than those reported in African catfish fed different dietary GG levels (Leenhouwers *et al*., 2006; Leenhouwers *et al*., 2007b), but this is most likely due to the low GG inclusion levels in the current study (<3 g kg^{-1} versus >40 g kg^{-1}). The viscosity of chyme altered throughout the GIT in striped catfish (Table 3). The pattern in the various GIT segments was similar to that observed in Nile tilapia (Amirkolaie *et al*., 2006; Leenhouwers *et al*., 2007a). Digesta had a slightly lower viscosity in the proximal intestine than in the stomach. From proximal to the distal intestine, chyme viscosity gradually increased, reaching the highest value in the distal part. The decline in viscosity in stomach and proximal intestine might relate to moisturizing of the feed pellets/digesta. Whereas the increase in viscosity from proximal to distal is the result of digestion and absorption of nutrients like protein, fat and starch, which results in an increased concentration of viscous NSP as digesta passes through the intestine. These processes of water and nutrient fluxes at different locations in the GIT might explain the observed interaction effect between the gut segment and dietary GG level in the present study.

The water-holding capacity (*i.e*., DM content) of chyme and faeces can be affected by diet composition(Sinha *et al*., 2011), especially by soluble NSPs content (*e.g.*, by dietary inclusion of GG). Parallel to Nile tilapia (Leenhouwers *et al*., 2007b) and broilers (Van der Klis *et al*., 1993a; Van der Klis *et al*., 1993b), the impact of dietary composition on chyme DM content differed between gut segments in striped catfish. In striped catfish, the impact of dietary GG inclusion seemed more pronounced in the stomach then in the other parts of the GIT. Only in the stomach, chyme DM content seemed to decline with dietary viscosity. In Nile tilapia fed diets containing different cereal grains (i.e., different types and levels of NSPs), significant differences in chyme DM content were present in the stomach, middle and distal intestine, but not in the proximal intestine (Leenhouwers *et al*., 2007a). In the broiler, dietary soluble NSPs only influenced the DM content of digesta in the lower jejunum and the upper ileum (Van der Klis *et al*., 1993a). In many freshwater fish species, differences in DM content of digesta are present between different GIT segments. The current study showed that in striped catfish the DM content of chyme was highest in the stomach and relatively equal between the different parts of the intestine. The current observation parallels the observed patterns in chyme DM content within the GIT of rainbow trout (Bucking and Wood, 2006), Nile tilapia (Amirkolaie *et al*., 2006; Leenhouwers *et al*., 2007a) and African catfish (Harter *et al*., 2013). Absolute differences in DM content of chyme between studies were largest for the stomach and relatively small for all parts of the intestine. *E.g.* inside the stomach, the DM content of chyme was approximately 330 g kg^{-1} for 350-g rainbow trout (Bucking and Wood, 2006), 280 g kg^{-1} for 70-g Nile tilapia (Leenhouwers *et al*., 2007a), 191 g kg^{-1} 45-g for Nile tilapia (Amirkolaie *et al*., 2006) and 243 g kg^{-1} for 1-kg African catfish (Harter *et al*., 2013). This large variability between studies in DM content inside the stomach is predominantly due to differences in sampling time post-prandial. With time after eating, DM content inside the stomach declines (Bucking and Wood, 2006; Harter *et al*., 2013; Saravanan *et al*., 2013).

Chyme osmolarity varies between fish, with the largest between species variability being present in the stomach and proximal part of the intestine. The current values of chyme osmolarity of striped catfish in the first three segments of the GIT are higher than those of African catfish (Harter *et al*., 2013). However, the current values for striped catfish are comparable to those of rainbow trout (about 525 mOsm in the stomach and 400 mOsm for proximal and mid intestine) after 8 hours feeding (Bucking and Wood, 2006). Towards the distal part of the intestine, differences between fish species become smaller. The chyme osmotic pressure found in the distal intestine of striped catfish in the current study is similar to values in African catfish (Harter *et al*., 2013). Differences between studies in osmolarity in the stomach most likely related to differences in sampling time after the last meal and maybe diet composition. The current study showed that chyme osmolarity in striped catfish was influenced by dietary composition (i.e. dietary viscosity), with the largest differences being present in the stomach. Since the mineral composition of the six experimental diets was identical the differences in osmolarity in the stomach is due to the difference in water fluxes or ion fluxes in the first part of the gastrointestinal tract. The observed differences in

DM content of chyme and osmolarity in the stomach were not correlated in the current study. This suggests that the diets induced differences in either ion secretion or uptake inside the stomach.

The chyme digestibility values (for both dry matter and protein) found in this study reflect the tendency of negative impact from increasing dietary viscosity (i.e., GG inclusion) already in the stomach; this effect accumulated throughout the GIT and was most clear in the distal intestine. This can be explained by the water absorbance and viscosity from GG. Firstly, when GG absorbs water from the chyme and swells, this will prevent the dilution of the digesta and will affect the digestion (Sinha *et al*., 2011). Secondly, the viscosity may hinder also the contact between digestive enzymes and substrates (Sinha *et al*., 2011). Similar results were found on African catfish fed different dietary GG levels (Leenhouwers *et al*., 2006), or on broiler chickens fed maize diets with GG inclusion (Maisonnier *et al*., 2001). The mean values of chyme DM and protein digestibility in the stomach are approximately 10%, which is higher than expected. The latter can be caused by the faster disappearance rate of the liquids compared to the solids in the digesta from the stomach (Maurer, 2015). The literature on rainbow trout (Austreng *et al*., 1987) and on chicks (Bielorai and & Hurwitz, 1973) showed similar values. After passage through the stomach, slightly more than 45% of feed was immediately digested in the proximal segment.

5.5 Conclusion

This study demonstrated that in striped catfish, moderate changes in dietary viscosity leads to alterations in chyme viscosity, but predominantly in the stomach and in the distal part of the intestine. Increasing dietary viscosity leads to reduced DM content in faeces (distal intestine). Increasing dietary viscosity negatively affects the digestibility of protein and DM. Already in the stomach increased dietary viscosity reduces the disappearance of nutrients (*i.e*., ADC). These differences in digestibility are persistent and constant throughout the total GIT.

Chapter 6

General discussion

6.1 Introduction

Aquaculture is the fastest growing animal food sector. One of the main drivers of this growth has been the intensification of the production system. As a consequence of this growth and intensification, waste and waste driven environmental impacts have also increased. A key concern for further sustainable growth of aquaculture is proper management of this waste (Dauda *et al.*, 2018). Faeces is a major source of waste in aquaculture production systems. It is composed of settleable and suspended solid material (Dauda *et al.*, 2018). A fraction of both types of waste can dissolve in the water column. For better management of aquaculture (faecal) waste, it is crucial to reduce the mass production of faeces (referred to as "quantity" in this thesis) by improving the digestibility of the feed. Another option is to collect and dispose the faecal waste as completely and quickly as possible from the water. However, the success of this faecal removal depends largely on the faecal characteristics (referred to as "quality" in this thesis). Very small and/or suspended solid particles are difficult to filter out from the water. Suspended faecal particles may also disintegrate very quickly in the water. Altering the quality of the faeces may be necessary to enable a successful faecal particles removal from the water.

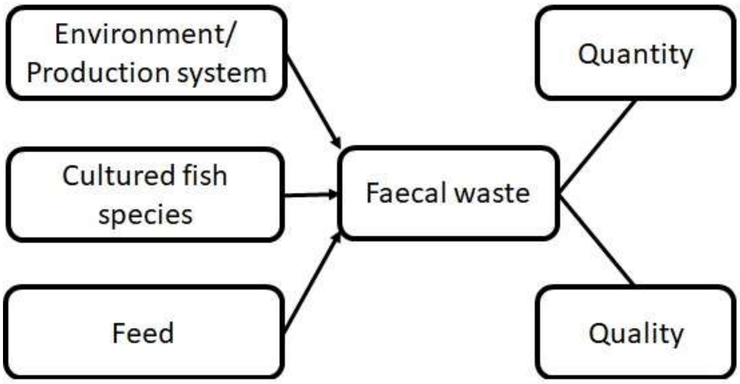

Figure 6.1 Factors affecting quantity and quality of faecal waste and thereby the effectiveness of waste management in aquaculture.

Several factors affect the quantity and quality of faecal waste and its management (Figure 6.1). Production systems with stagnant water or flowing water will have different impacts on the waste. Different production systems also have different capacities to deal with waste. For example, in a recirculating aquaculture system (RAS), a large part of the waste can be removed by the purification unit. In an intensive aquaculture pond, the bottom soil with microbes can neutralise only a limited quantity of waste.

Compared to other animal production sectors, the number and type of farmed species in commercial aquaculture is large and diverse. The faecal characteristics differ between the species as their digestive physiology and feeding habits are different. For example, trout produces round faeces, while tilapia produces string faeces and striped catfish produces diarrhoea-like faeces. These features (or characteristics) of faeces strongly influence the faecal waste recovery/removal rate. As a result of different food and feeding habits, the ability of fish to utilize/digest different types of diets (plant *vs.* animal ingredients) varies as well between fish species. Thus, changing the diet composition can also alter the quality and quantity of faecal waste. As the faecal waste management is related to the quality and quantity of the faeces, both the species and diet types can influence the degree to which faecal waste can be managed and/or mitigated in aquaculture.

This thesis assessed the impact of nutritional interventions on both the quantity and quality of faecal waste produced by striped catfish. The most ideal nutritional interventions would be those that reduce the quantity and at the same time improve the quality of faeces.

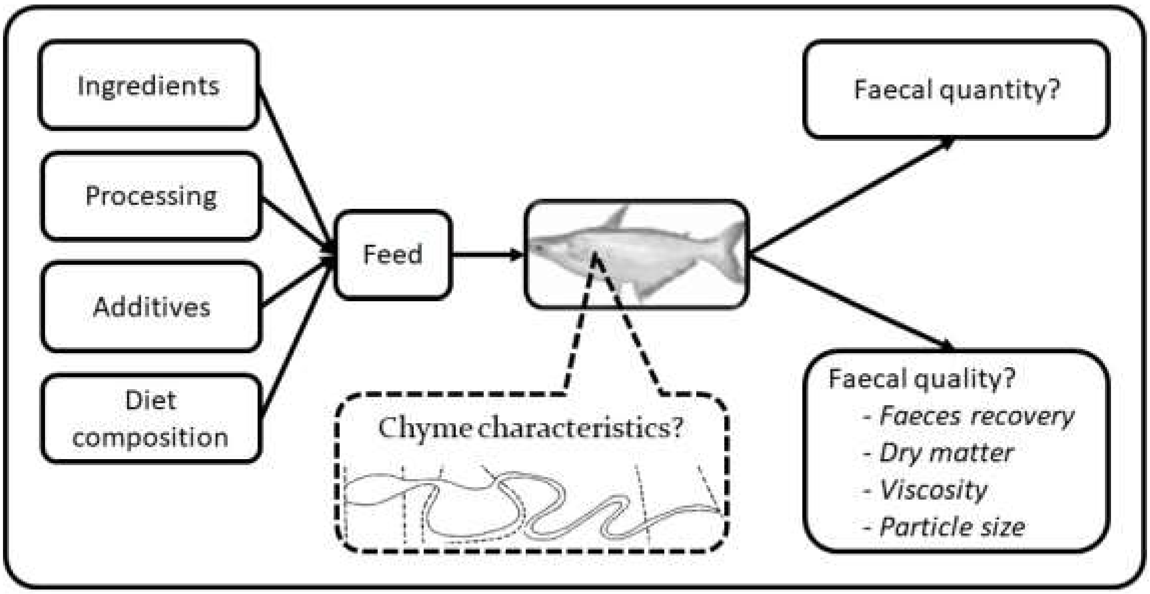

Figure 6.2 Classification of feed-related factors that influence quantity and quality of the faecal waste and thereby the effectiveness of waste management in aquaculture.

The feed-related factors that influence the quantity and quality of the faecal waste are presented in Figure 6.2. In this thesis, it was observed that the dietary ingredient composition affected both the quantity and quality of faecal waste of striped catfish (Chapter 2). Ingredient processing altered the faecal quantity, but may also influence the faecal quality (Chapter 3). Increasing dietary viscosity due to the inclusion of binders increased faeces production (Chapter 4), because the kinetic/location of digestion in the gastro-intestinal tract was altered (Chapter 5).

6.2 Factors affecting the faecal quantity and quality

By definition, the quantity of faecal waste fully relates to the apparent digestibility of the dry matter (ADC-DM). The better the ADC-DM is, the lower the amount of faeces. The quality of the faecal waste is associated with the characteristics (*e.g.* dry matter, viscosity, particle size of faeces, density *etc.*), which determine the recovery rate of the faeces depending on the collection methods used (settling *vs* sieving). The quantitative and qualitative assessment of faecal waste in this research was done using settling systems. We chose this approach to mimic the sludge settlement in RAS (Eding *et al.*, 2006). This design is also feasible for the waste treatment (sludge removal) in striped catfish ponds, cultured in the Mekong Delta, Vietnam. A central parameter for faecal quality in this thesis was the faecal recovery rate by settling. In Chapter 3, we demonstrated that this parameter was reproducible for the reference diet in the three consecutive experiments. This implies that the noticed treatment effects on the recovery rate were not by coincidence.

Both quantity and quality of faeces are influenced by three related factors (Figure 6.1), *i.e.* environment/system, fish, and feed. This thesis focused only on the feed related factors. In general, the effect of these three factors on the faecal quantity and quality are not well studied yet in fish, let it be that the optimal combination is known. More and broader research might be warranted to improve waste management for more sustainable aquaculture.

6.2.1 Fish related factors

The digestibility of ingredients and of complete diets depends on the type of fish species (*e.g.*, carnivore, herbivore *etc.*). For example, the digestibility of soybean meal, a common fish feed ingredient, differed between rainbow trout, snakehead, striped catfish, and tilapia (Table 6.1). This implies that the amount of faeces produced from the same ingredient can differ strongly between fish species; ranging from 177 to 320 g DM kg^{-1} ingredient. The difference between fish species is larger among plant-protein sources compared to animal-protein sources (*e.g.*, fishmeal). Previous studies (Hossain *et al.*, 1997; Sampaio-Oliveira and Cyrino, 2008; Vandenberg *et al.*, 2012; Da *et al.*, 2013; Che *et al.*, 2017) searched to improve the digestibility of plant-protein sources for carnivorous fishes in order to reduce the feed costs and to source alternative ingredients. Therefore, when we want to reduce the faecal quantity, the question of how we can improve the digestibility of a feed or ingredient is one of the main factors to study.

The differences in digestibility between species (Table 6.1) imply that there is a genetic basis for this trait. Thus, selection for digestibility might be a way to alter faecal quantity within a fish species. The potential for selection on digestibility is substantiated by the fact that the gut length in Nile tilapia is heritable (h^2 total gut length is 22% and for relative gut length 9%) (Charo-Karisa, 2006). However, selection for digestibility will be time-consuming, difficult to measure in individual fish and therefore costly. Another option to improve digestibility might be through nutritional training of fish larvae and/or alevins. In this method, also known as "early programming", the larvae/alevins will be fed specific feeds to train their digestive enzymatic activities with the goal to adapt the digestive system to diets in their later life. An example of "early programming" was shown by Geurden *et al.* (2013). In that study rainbow trout exposed to high levels of carbohydrates at the larval stage, accepted and utilized plant-based diets better as juveniles. Due to the low quality of faeces in striped catfish (Table 6.2), both "early programming" as well as genetic selection for lower faeces production might be considered as strategies for improving the sustainability of striped catfish culture.

Table 6.1 Apparent digestibility coefficients (%) of soybean meal (solvent extracted) in different fish species

Species	Apparent digestibility coefficients (ADC, %)					Source
	Dry matter	Crude Protein	Gross energy	Crude fat	Crude ash	
Rainbow trout	68.8	92.8	76.8	-	-	Kaushik *et al.* (1995)
Snakehead	68.0	83.4	70.4	89.7	-	Yu *et al.* (2013)
Striped catfish	82.3	93.1	84.7	59.6	72.6	Chapter 2, Table 2.4
Nile tilapia	78.0	92.2	83.1	93.0	42.9	Tran-Ngoc *et al.* (2017)

Parallel to the faecal quantity, large differences are observed between fish species regarding faecal quality. This is reflected in the different faecal removal efficiencies for different species using settling systems (Table 6.2). Compared to tilapia and carp, striped catfish showed the poorest faeces removal efficiency, just 20 to 52%. This might be related to the variation in types of faeces between these species: *e.g.* regarding form (round *vs* string) or consistency (solid *vs* diarrhoea-like). Striped catfish is a diarrhoea-like faeces producer. Other examples of species with diarrhoea-like faeces and consequently low recovery rates by settling are sole and seabream (Schrama, personal communication). The large differences in type of faeces egested might imply that factors, which improve the quality of faeces in one species: *e.g.*, rainbow trout; (Brinker *et al.*, 2005; Brinker, 2007; Brinker, 2009), will not automatically be effective in another species (*e.g.*, striped catfish). In parallel to faecal quantity, the variability between fish species in faecal characteristics suggest also a genetic basis and therefore the potential of selection. The underlying mechanisms, which result in the differences in the faecal quality among species, are not well known. To improve the faecal removal rate by altering the faecal quality, therefore requires more knowledge on the digestive physiology of fish.

Table 6.2 The removal efficiency of faeces by settling in different fish species

Fish species	Range in removal efficiency (%)	Source
Nile tilapia	56-80	Amirkolaie *et al.* (2006)
Common carp	68-74	Prabhu *et al.* (2019)
Pangasius	20-52	This thesis (Chapter 2, 3 & 4)

6.2.2 Environment/system related factors

The feed digestibility in fish (and thus the faecal quantity) is affected by many environmental factors. Increased temperatures or salinities, for example, as induced by climate change, may influence the physiological and biochemical activities in fish (Mazumder *et al.*, 2014) thereby also influencing the digestion of feed. Also, other physical features of the farming environment, *e.g.* dissolved oxygen, N-NO_3, N-NO_2, NH_3, *etc.*, may alter the digestion process. There are several potential solutions dealing with the above factors. Deepening the pond or covering the surface can attenuate temperature changes. Irrigation systems and dikes can help preventing or minimizing salinity intrusion in the areas exposed to seawater rise. Aeration can improve the oxygen level in the farmed pond. In striped catfish culture, some farmers deepened their pond to have more water volume and thus a higher mass of oxygen available to the fish. However, dissolved oxygen (DO) gets depleted with increasing water depth (Lefevre *et al.*, 2011), possibly because of within pond bacterial breakdown of the small particle size, diarrhoea-like faecal waste of the fish. The depleted DO makes fish swimming in the top layers of the water column (Lefevre *et al.*, 2011) and may cause the increase crowding. The advantage of having a deeper pond gets thereby largely lost. There is few information available on whether water quality parameters associated with deepening the pond have changed the quantity of faeces in the pond. Monitoring the discharged amount at the outlet might give an indication for this hypothesis, but is also very difficult given the fact that a large part of the faecal waste might be decomposed within the pond before being discharged. Anyway, we infer that alteration of the physical conditions of the farm in addition to changing the diet and ingredients may contribute to a better faecal waste management.

Most of the physico-chemical water related factors will not affect the faecal quality, but the conditions caused by the configuration of a production system can affect. For example, faeces recovery differed depending on the design of the system, *e.g.*, whether the faeces was removed by sieving or sedimentation (Amirkolaie *et al.*, 2005a). Therefore, in general, system conditions such as water flow rate, aeration, or the solids settling conditions may influence the rate of faecal pellet degradation. A sedimentation system as applied in RAS may be considered for striped catfish farming to increase the effectiveness of faeces removal by settling. In recent years, a few large-scale hatcheries of striped catfish started to use RAS. However, their main goal is to reduce the water exchange and minimize disease outbreaks (Liem *et al.*, 2016) rather than faecal waste management. Application of this concept for grow-out farming is economically less viable for small and medium scale producers (Phuong, personal communication), which constitutes the majority of the striped catfish farming community.

6.2.3 Feed related factors

Several factors related to feed formulation (*e.g.,* ingredient, processing, additives, *etc.*) strongly affect the quantity and quality of faecal waste (Chapter 1, Figure 1.2).

6.2.3.1 Dietary ingredients

Ingredient choices are very important for faecal waste management (Chapter 2, Table 2.3 and 2.4). Yet, the impact on faecal waste management is not a criterion used in feed formulation. Feeds are formulated using least-cost programming. This means that ingredients are selected, based on their nutrient composition and to meet the set nutritional requirements for the lowest feed price. Consequently, price changes of ingredients will lead to different dietary ingredient compositions. For example, due to the limitation of fish meal, plant-based ingredients are increasingly used nowadays (Waite *et al.*, 2014b). The current plant-based feeds are certainly cost-effective, but they may also increase the amount of faecal waste. If we want to enhance faecal waste management, quality ingredients need to be used, but then feeds become also more expensive. From a sustainability point of view, alternative ingredients such as left-over streams and/or by-products from other food systems may be beneficial because they reduce the feed/food competition and fit very well in a circular food production approach, despite their impact on the faecal waste production. Striped catfish is an omnivore. Therefore, inclusion of by-products of other food systems can be tested in this species as well. In that case, the trade-off between the profitability and sustainability for future aqua-feed production needs to be analysed carefully.

6.2.3.2 Processing

Ingredient processing and feed manufacturing technology can alter the faecal quantity. We saw that the screen mesh size used for grinding has an effect on the amount of faecal waste through its influence on the digestibility (Chapter 3, Table 3.3). This effect of processing (grinding screen size) on the digestibility was also found in pigs (Callan *et al.*, 2007; Moreira *et al.*, 2009). In tilapia, the gelatinisation degree of maize starch also altered the faecal recovery rate (Amirkolaie *et al.*, 2005a). Up to now, the current study (in striped catfish) is the only research done on the effect of process conditions (grinding screen sizes) on faecal quality (Chapter 2). Other factors, *e.g.* extruding conditions, may affect the faecal quantity and quality as well. However, to date, this is not well-studied in fish including striped catfish.

6.2.3.3 Additives

The main functions of feed additives are among others: increasing the digestibility; improving the feed palatability; or improving the physical characteristics of feed pellets, *e.g.* pellet durability (by using a binder). Additives such as digestive enzymes or probiotics increase the digestion. It is possible to reduce the amount of faecal waste by enzyme inclusion to improve digestion of carbohydrates (Maas *et al.*, 2018). Commonly used pellet binders are either carbohydrates (*e.g.*, guar gum, carrageenan), proteins (*e.g.*, gelatine, wheat gluten), or minerals (*e.g.*, clay). Pellet binders, which are indigestible, may also be applied to improve faecal pellet quality. For example, in trout, indigestible pellet binders with viscous properties have been proposed to improve faecal stability (Brinker *et al.*, 2005). In this thesis, steering dietary viscosity by indigestible binders was tested in striped catfish using guar gum (Chapter 3 and 4).

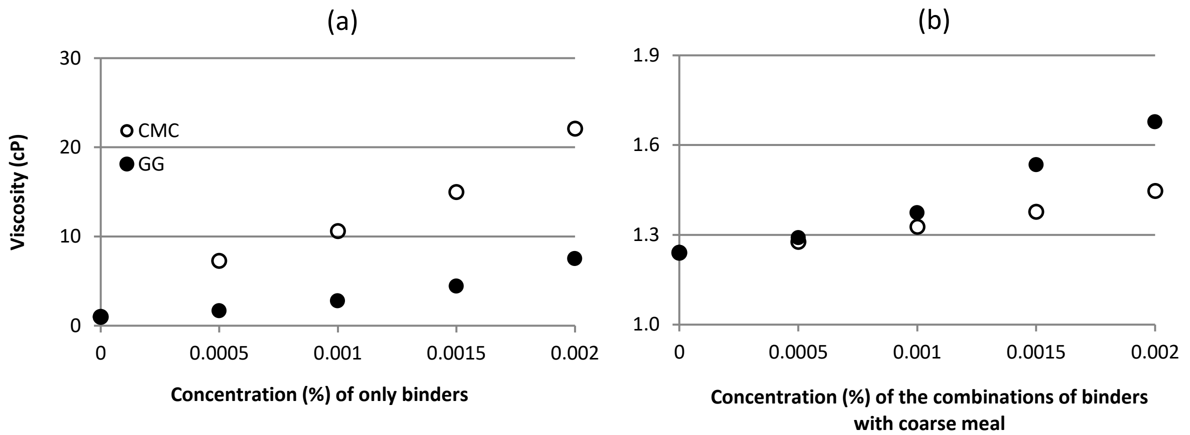

Figure 6.3 The differences in viscosity between the two types of binders, guar gum (GG) and carboxyl methyl cellulose (CMC) as pure products dissolved in water (a) and mixed with a standard mixture of ingredients (grinded at the mesh size 1.0 mm) and then dissolved in water and incubated at 28°C for 30 minutes (b).

We measured the effects of different inclusion levels of guar gum (GG) on the quantity and quality of faecal waste. When formulating the dietary treatments, for the basal diet we used a common formulation as used in striped catfish farming. This practical formulation included CMC. We saw that the dietary viscosity increased with increasing GG levels in the diet (Chapter 3, Figure 3.2a). This is due to the viscous characteristic of the binders. Actually, the viscosity differed between the two binders, but even more, the viscosity decreased differently when they were mixed with the mixture of ingredients (Figure 6.3). For some fish species, binders (*e.g.* GG) can help to improve the faecal quality, such as in rainbow trout (Storebakken, 1985; Storebakken and Austreng, 1987; Brinker and Friedrich, 2012). However, in striped catfish (Chapter 3), the inclusion of binders which increase the dietary viscosity, hampered not only the digestion, but also the faecal recovery. This was also observed in tilapia (Amirkolaie *et al.*, 2005b).

Apart from the faecal recovery, in this study the proportion of large particle sizes (>2 mm) of the total amount of faecal waste being recovered tended to increase with increasing dietary viscosity by GG inclusion (Figure 6.4). If the faecal pellets are larger, this will alter the settling velocity. In general, the binders, *i.e.* GG and CMC, which both have viscous properties, help to stabilise the feed pellets and this goes together with an increased dietary viscosity. Figure 6.3a shows that CMC has a higher viscosity then GG in its pure form. However, when CMC and GG are mixed within the feed mixture, the increase in dietary viscosity with increasing inclusion is higher for GG than for CMC (Figure 6.3b). This reveals that the use of CMC as a binder in fish feed is not a good choice when one wants to increase the stability of the faecal pellet. This also implies a lack of knowledge of how a binder reacts with feed processing. It is clear that in striped catfish, binders such as CMC or GG should not be used in the diet because, although they increase the particle size of faecal pellets, they reduce the digestibility and faeces recovery.

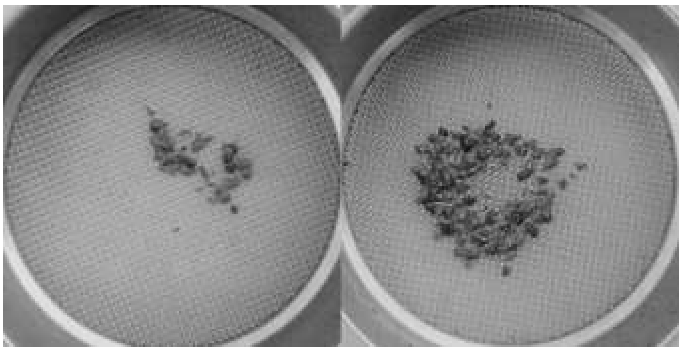

Figure 6.4 The large particle size (>2 mm) of faeces pellets in striped catfish fed low (left) and high (right) dietary viscosity.

6.3 Interaction between the dietary composition and gastrointestinal physiology

Very little research is done regarding the interaction between the dietary composition and gastrointestinal physiology. In this study, we saw that inclusion of binders in the diet (guar gum) which change digesta viscosity, also altered the quantity and quality of faecal waste. Therefore, in Chapter 5, the impact of dietary binder inclusion (dietary viscosity) on the interaction between the digesta physical conditions and nutrient digestion was assessed as digesta pass through the gastrointestinal tract (GIT) of the fish. The dietary viscosity altered the characteristics of digesta like viscosity, dry matter and osmolarity. However, the impacts on viscosity and dry matter content were predominantly present in the stomach, vanished in the first and mid intestine and then reappeared to a certain extend in the distal intestine. Most likely this is due to the fact that GG is indigestible and that with progression of the digestion of nutrients (crude protein, fat and starch) the concentration of GG increases from proximal, mid to the distal part of the GIT. Increasing dietary viscosity negatively affects the digestibility of protein and dry matter. Because of the increased viscosity, the disappearance of dry matter and crude protein was already present in the stomach. This "reduced digestion" of protein and dry matter in the stomach remained present as digesta progressed through the GIT. In other words, no compensation in the more distal parts of the GIT seemed to occur. So far in fish there is limited knowledge on how physicochemical conditions (*e.g.*, osmoregulation and water balances) interfere with nutrient digestion/absorption. A more reliable and robust understanding of the kinetics of digestion may facilitate the introduction of alternative ingredients in fish feeds, but also help to mitigate/manage faecal waste in aquaculture.

6.4 Controversy between faecal quantity, faecal quality, waste management, and profitability

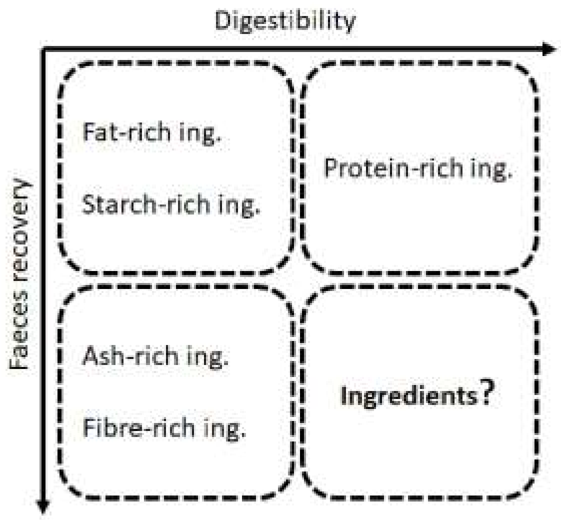

Figure 6.5 A generalized summary of the results of Chapter 2 how different types of ingredients (ing.) determine the final amount of non-recovered faeces (NRF) of a feed for striped catfish.

This study showed that the quantity (digestibility) and quality (recovery rate) of faeces is the outcome of the different ingredients included in a fish feed (Chapter 2, Table 2.3 and 2.4). The amount of non-recovered faeces (NRF) per kg feed can be steered by diet formulation. In Figure 6.5, a generalized summary is given how different types of ingredients affect the final NRF of a striped catfish feed. High inclusion levels of fat/oils and starch-rich ingredients gave the highest amount of NRF, because of a high quantity of faeces together with a low recovery. The NRF of a diet (and thus also at farm level) can be reduced by either increasing the DM-ADC of the feed (*e.g.*, by adding protein-rich ingredients), but also by increasing the recovery rate of faeces produced by a feed (*e.g.*, by adding fibre-rich ingredients). However, combining both aspects to minimize the NRF seems at first sight not very easy. However, figure 6.6 shows that such a strategy is possible, because the impact of ingredients on faecal quantity (*i.e.*, DM-ADC) and faecal quality (*i.e.*, recovery rate) are not related to each other within the cluster of 15 ingredients tested in Chapter 2. The number of tested ingredients was small and therefore the absence of a correlation might not exist if more or other types of ingredients would be tested. Enlargement/development of a bigger database, which characterizes digestibility and faeces recovery of a variety of ingredients, is also important to valorise the potential of diet formulation to minimize NRF.

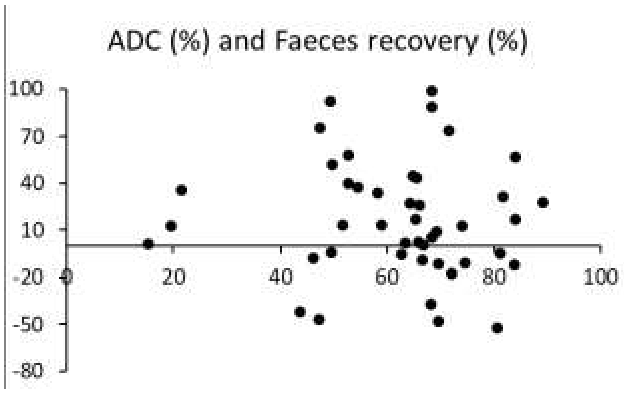

Figure 6.6 The relationship between dry matter digestibility (*i.e.*, the faecal quantity) and the faeces recovery (*i.e.*, faecal quality) of 15 ingredients tested in Chapter 2. ADC (in %) on X axis and faecal recovery on Y axis (in %).

In practice, formulation of diets with a low NRF by increasing the digestibility of the feed will drive up its price because such ingredients are often more expensive. However, the big advantage is that less faecal waste will be produced. Implementing such low NRF feeds may be an option to mitigate the environmental impact of striped catfish culture. However, its success will depend on various factors. For example, the question is whether farmers are willing to pay for such a good digestible feed or will rather prefer to pay levees for the higher amount of discharged faecal waste when using a low digestible feed. In addition, if the production costs of the fish increase, because of using feeds with low amounts of NRF, can these increased prices be transmitted through the value chain and eventually be paid by the consumer? Ngoc *et al.* (2016) demonstrated symmetric price transmission for frozen pangasius fillets throughout the value chain, which would offer farmers an equal bargaining power when negotiating price increases due to increased production costs. Therefore, it is very well possible that striped catfish consumers would be willing to pay for these extra costs if it was clear that the environment in Vietnam would benefit from it or if it would contribute to a more sustainable form of catfish production. However, in the value chain of striped catfish products, the profit margins that the farmers get are very meagre and are impacted by small changes in the production cost (De Silva and Phuong, 2011). Therefore, farmers do not prefer to increase production cost. Yet, in spite of this, production costs doubled in less than 5 years from around 0.82 up to 1.40 USD kg^{-1} (US$ 1 ~ VND 15,900) during the period of 2007 to 2011 (Hong *et al.*, 2015). Similarly, the application of a high-tech system like RAS, (which has the advantage to well manage the waste), was not well adopted by Vietnamese catfish farmers because of its high investment requirements (Phuong, personal communication). Therefore, balancing increased production cost *vs* improved waste management remains a challenge (Figure 6.7). This challenge can be improved by creating a fair farming environment through proper government policies regarding striped catfish farming in the Mekong delta. This suggests that inclusion of faecal quality/characteristics as part of a national feed act can help the users to make the right choice.

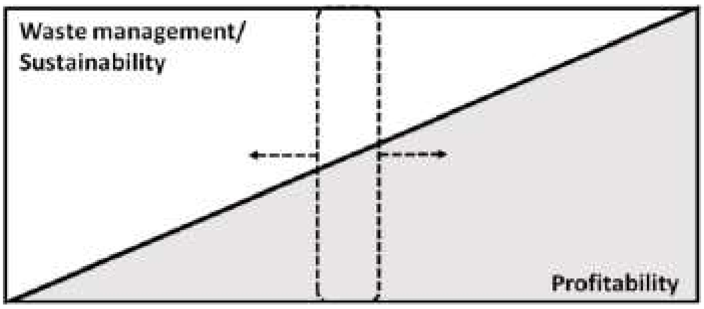

Figure 6.7 The controversy between waste management/sustainability and profitability.

6.5 Implications of the research

The results of this study on faecal quantity and quality are important for faecal waste management. So far, in this thesis the chemical composition of the faeces has not been addressed. Yet, the latter information may be highly important for possible later destinations of the collected faecal waste. For example, in a high-tech system like RAS the collected faeces can be used as carbon source for a denitrification unit, but its effectivity will strongly depend on the faecal composition (Meriac *et al.*, 2014). Similarly, in the case of striped catfish, the composition of the faecal waste will be important when it is used as fertilizer in agriculture (Hossain *et al.*, 2016). Table 6.3 shows that the chemical composition of striped catfish faeces is altered by dietary ingredients composition. Depending on how collected faecal waste is valorised and depending on the potential profit from it, one might optimize diets for having a proper chemical composition of the faeces, next to its quantity and quality.

Table 6.3 Chemical compositions (dry matter basis) of faecal waste of striped catfish fed different dietary ingredients (data from Chapter 2)

	Reference	Fishmeal	Soybean meal	Defatted rice bran	Cassava
Dry matter (g kg^{-1})	910	937	921	874	942
Crude protein (g kg^{-1})	271	245	251	142	78.2
Crude fat (g kg^{-1})	25.9	18.5	25.7	40.9	5.13
Crude ash (g kg^{-1})	248	234	363	390	638
Carbohydrate (g kg^{-1})	455	503	361	428	279
Gross energy (KJ g^{-1})	11.7	10.6	13.3	11.8	13.2

Moreover, the current study shed some light to what extent farmers can make a decision with regard to the controversy of sustainability *vs* profitability (Figure 6.6). This research also highlights which stakeholders to be involved in the development of future policy and farm management guidelines. Current thinking about aquaculture waste management usually focuses on removal and treatment of the faecal waste. However, faecal waste still contains valuable nutrients. These nutrients (Table 6.3) can be altered by the dietary composition and the digestibility of the diet. For example, using a different ingredient composition may be used to alter the C:N ratio in the faeces. In pond aquaculture, the C:N ratio is important for the productivity of the natural food web (microalgae and bacteria), which can be used as an additional food resource for the farmed fish (Kabir, 2019). Further research needs to optimize the faecal waste management in a more comprehensive approach to balance economic and environmental benefits.

6.6 Conclusions

Overall, the following conclusions can be drawn from this study.
Regarding faecal quantity, the main conclusions are:

- Ingredient composition of a feed strongly determines the amount of faecal waste produced on a diet.
- Faecal quantity can be altered by feed technology/feed processing conditions (*e.g.,* grinding screen size).
- For striped catfish, feed and faecal pellet binders, which increase the dietary viscosity, should not be used in the diet because it reduces the dry matter digestibility even at very low inclusion levels.
- The negative impact of dietary viscosity on digestion in striped catfish appears very proximal in the gastro-intestinal tract (stomach). This negative impact on digestion is not compensated after evacuation of the chyme from the stomach.

Regarding faecal quality, the main conclusions are:

- Stability of faecal waste is determined by the ingredient composition of the feed consumed by the fish.
- Faeces recovery efficiency decreases with increasing dietary viscosity when specific binders/additive like guar gum are used.
- Technological processing (*e.g.* grinding) may influence the faecal stability (*e.g.* recovery rate).

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Summary

The rapid growth of aquaculture in the world is the result of a combination of increasing the area for production and of intensification. The latter means that the inputs in the system have increased and concomitantly more waste is released from the production system. This release of aquaculture effluents rich in nitrogen and phosphorus into the open water will decrease the quality of the recipient surface waters and associated ecosystems. Also, the loss of nutrient-rich waste leads to both economic and environmental inefficiency. To enable the continued growth of aquaculture, the nutrient efficiency of the production system should be improved. Wasted nutrients can be recovered into new valuable biomass, such as rice or vegetables for human food, duckweed for animal feed, or microalgae for functional food or medicines. In this way, aquaculture can contribute to circular processes in the agro-food industry. However, the possibility of reusing waste largely depends on the waste removal efficiency which on its turn is linked with the type and the amount of waste produced. In this thesis, striped catfish was taken as a model species, to study the options for aquaculture waste management.

To manage the faecal waste production in striped catfish, two important approaches are available: 1) reducing the amount of waste, 2) getting the waste out of the farming system quickly and completely. This study investigated the nutritional interventions to alter/improve faecal pellet quality (which could lead to better and more complete removal of faecal waste) and the enhancement of the stability of egested faeces. Up till now, it is not clear if nutritional interventions targeted to reduce the amount of faecal waste can go along with improving the faecal quality. The main aim of this research was to study the impact of different nutrition interventions on both the quantity and quality of faecal waste produced by striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878). The most ideal nutritional interventions would be those that reduce the quantity and at the same time, improve the quality of faeces.

In **chapter 2**, we determined the characteristics of waste production and digestibility of dietary ingredients for striped catfish. This study was conducted with three experiments: Exp 1, protein-rich ingredients (local fishmeal, defatted soybean meal, meat and bone meal, rapeseed meal, and full fat soybean meal); Exp 2, ingredients relatively rich in non-starch polysaccharides (defatted rice bran, rice bran, wheat bran, palm kernel meal, and dried distillers grains with solubles); and in Exp 3, oil and starch-rich ingredients (fish oil, soybean oil, wheat, broken rice, and cassava) were tested. Within each experiment, one reference diet and five test diets were tested in triplicate. Dietary ingredients strongly affected both the quality and quantity of faecal waste of striped catfish. High fibre content ingredients increased the quality but decreased the quantity of faeces. Protein-rich sources improved

the digestibility while fat sources hampered the digestion (excluding for fat) and both these ingredients decreased the faecal quality. Also starch rich ingredients hampered the digestibility.

In **chapter 3**, we tested the impacts of the ingredients' particle size and the dietary viscosity on digestion, performance and faecal waste management of this fish. The experiment had a 2x3 factorial design: two feed mesh particle sizes, by grinding the ingredient mixture at two screen sizes (0.8 versus 1.0 mm); and three dietary viscosity levels, which were created by exchanging carboxyl methyl cellulose by guar gum (GG) (0, 3, and 6 g of GG kg^{-1} of diet). The six diets were assigned to 18 tanks, each connected to three faecal settling tanks. All aquaria were stocked with 20 fish of 82 g $fish^{-1}$ on average. 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 the 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.

In **chapter 4**, we analysed the dose-response relationship between dietary viscosity and nutrient digestibility and faecal waste characteristics in striped catfish and the long-term impacts of viscosity on the 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 amounts of guar gum (GG; 0, 0.25, 0.5, 1.0, 2.0 or 3.0 g kg^{-1}), 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^{-1}). In Exp.1, digestibility of nutrients (except crude fat) decreased linearly with dietary viscosity. With increasing viscosity, the removal efficiency of faeces from the water reduced linearly also, whereas the total amount of faeces produced and the fraction of big sized faecal particles (>2mm) increased linearly. In Exp.1, viscosity did not affect performance. However, in Exp.2, the GG inclusion level of 3.0 g kg^{-1} (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.

In **chapter 5**, we assessed the relationship between the physical and chemical characteristics of chyme and apparent faecal nutrient digestibility. In particular, this study determined the effect of dietary viscosity on chyme characteristics in different segments of the gastrointestinal tract (GIT) of striped catfish, and the progression of nutrient digestion throughout the GIT. Six diets were produced, that had moderate differences in dietary viscosity by exchanging carboxymethylcellulose for guar gum (0, 0.25, 0.5, 1, 2, or 3 g kg^{-1} of diet). Eighteen tanks, each stocked with 20 fish of 95 g, were used (three replicate tanks per diet). Diets were randomly assigned to tanks and fed for 29 days. Thereafter fish were euthanized for the collection of chyme from four different segments of the GIT: stomach, proximal, mid and distal intestine. The chyme characteristics, dry matter content, viscosity, and osmolarity were affected by the interaction effect of diet and GIT-segment. This implies that the impact of dietary viscosity on chyme characteristics differs between GIT-segments. Chyme viscosity increased with increasing dietary viscosity, but this mainly occurred in the stomach and distal intestine. Dry matter content of chyme was mainly affected (increased with rising dietary viscosity) in the stomach. The digestibility of both dry matter and crude protein was different between GIT-segments and increased from the stomach towards the distal intestine. Moreover, these nutrient digestibility values were influenced by dietary viscosity being negatively related. Already in the stomach increased dietary viscosity reduced the disappearance of nutrients (*i.e.*, digestibility). These differences in digestibility were persistent and constant throughout the total GIT. In conclusion, moderate increases in dietary viscosity alter predominantly stomach and distal chyme viscosity. They increase chyme DM content in the distal intestine and reduce protein and DM digestibility, which are already different between diets from the stomach onward.

In **Chapter 6,** the main outcomes of the studies of this thesis were summarized and discussed in the context of the impact of the nutrition interventions on both the quantity and quality of faecal waste produced by striped catfish. The most ideal nutritional interventions reduced the quantity and increased the quality of faeces.
Overall, the following conclusions can be drawn from this study.
Regarding faecal quantity, the main conclusions are:

- Ingredient composition of a feed strongly determines the amount of faecal waste produced on a diet.
- Faecal quantity can be altered by feed technology/feed processing conditions (*e.g.,* grinding screen size).
- For striped catfish, feed and faecal pellet binders, which increase the dietary viscosity, should not be used in the diet because it reduces the dry matter digestibility even at very low inclusion levels.
- The negative impact of dietary viscosity on digestion in striped catfish appears very proximal in the gastro-intestinal tract (stomach). This negative impact on digestion is not compensated after evacuation of the chyme from the stomach.

Regarding faecal quality, the main conclusions are:

- Stability of faecal waste is determined by the ingredient composition of the feed consumed by the fish.

- Faeces recovery efficiency decreases with increasing dietary viscosity when specific binders/additive like guar gum are used.

Technological processing (*e.g.* grinding) may influence the faecal stability (*e.g.* recovery rate).

Acknowledgements

The journey from Can Tho – a small city in the Mekong Delta of Vietnam – to Wageningen (another beautiful green village in the Netherlands (NL)) was long cherished. The journey from the Campus to the Aula (just less than 4km) however, was long and challenged. Luckily, I reach the destination now. I am grateful to so many people who were part of this journey, kept their trust in me and supported in all extent to make it successful.

Special thanks to my local supervisors from Vietnam, Prof. Nguyễn Thanh Phương and Ass. Prof. Đỗ Thị Thanh Hương, who introduced me for a PhD, even at the mid-level of my career. I am in debt grateful to Ass. Prof. Trần Thị Thanh Hiền and her husband Cao Văn Hoạt. Not only for my PhD and career at Cantho University but much more beyond that which influenced my personal life, regularly encouraging me when I was in NL.

It is hard for me to write about my supervisors from the Aquaculture and Fisheries group (AFI) of Wageningen University and Research (WUR). The words can't express my gratitude to my supervisors. They are contributors to my academic maturity. Dr. Johan Schrama put his efforts to train me, through the four articles and my thesis. I know that I disappointed him many times. He is very strict but if you follow his guidelines you will be very good. Prof. Johan Verreth, I will never forget the events he was reading my documents spending sleepless nights and giving feedback on my text in WhatsApp during his traveling. You will be always my mentors.

Roel Bosma, I will gratefully remember you, the first Dutch person I learned to know, and also the person who introduced me to the PhD scholarship. I am so thankful, not only for the parties at your home but also for your care. I am indebted to Prof. Martin Verstegen and his wife Mariet. He helped me to revise my first article and she helped me to improve my English pronunciation. Both of them helped me to regain confidence and I love them as my grandparents. Ep Eding and Leon Heinsbroek, I was very happy to welcome you in Vietnam. I thank for your help to improve the experimental system I had to work with in Vietnam and to develop the methodology to measure the particle sizes of faeces. Ronald Booms – thanks a lot. You helped me in teaching me the lab work protocol. I am also grateful to Dr. Marc Verdegem for his advice in statistical analysis (SPSS). Gera den Dikken, Eugenia Halman and Annet Willink, all of you were magicians, surprisingly solving all problems of each PhD student. I am very grateful to all of you. Thanks also to Marjon Hinssen for your kind assistance. I am also grateful to Prof. Geert Wiegertjes for supporting me by providing the last three month's allowance.

The day I went to Wageningen, I saw Kim Tran who welcomed me in Dijkgraaf: thanks to her for everything. I am grateful to Mahmoud Haidar, my office neighbor. I would like to thank

Marit Nederlof, my Dutch roommate, for her kindness. I also thank my "big" brother Nhứt Nguyễn and my "project mate" Ngọc Phạm for our connections in the past, now and in the future. I cannot forget the nice PhD trip to China in 2019, thanks for the committee, supporters and attenders, Gauthier Konnert, Folasade Elesho, Apriana Vinasyiam, Thuật Phan, Yale Deng, Zhang Yaquing, Koletsi Paraskevi, Thomas Staessen, Roel Mass, Twan Stoffers and Kazi Kabir. Whenever I remember that trip, I remember all of you. In AFI I have never felt alone because of the presence of all my friends, Happy Peter, Davood Karimi, Restiana Wisnu, Gang Liu, Devi Hermsen, Joost Van Loo and Tính Trần. We had a nice time together, weekend lunches, Sunday walking, movie nights, day trips, birthday trips, coffee breaks, AFI drinks and much more. When I am leaving Wageningen, we will certainly keep contact through WhatsApp. Special thanks to Daniel, Felicidade, Nazri, Soleha, Lei Mao, Simon and Jingyan for being my good friends in Zodiac. I would like to thank Mike who was Martin's student, for his worries and cares about me. Betina Muelbert, I am grateful for our nice time together.

I am very gratefully to Mrs. Hương van der Schans and her husband Johan van der Schans for their kindness. Almost all PhD students from the Animal Nutrition Group and certainly the Vietnamese students in Zodiac are lovely calling her "mama Huong". I am also grateful to Mrs. Vì Brands and Mr. Bình for their warm delicious traditional Vietnamese meals.

Our Vietnamese friends made a small Vietnamese community in Wageningen. We celebrated most of the Vietnamese festivals and organized many weekends and traveling holiday events. I am grateful to all of you, Dr. Dũng Trần, Trung, Long, Liên, Nhung, Tiền, Nam, Lộc, Yến, Huy, Trường, Huyền, Phượng, Hoàng, Ngân, and Hiếu, for making my stay in Wageningen joyful.

The role of my mom, Lê Thanh Thủy and my dad, Trần Quốc Thọ is unmeasurable. I am very grateful to you for always standing behind me. My parents always inspired me to go for higher education and learning. Your best wishes were always my strength. While I was away from home, my younger sister, Ngân Trần and her husband Dũng Trần, had to take family responsibilities to some extent – I am grateful to you as well. I also thank my big family members and my groupmates, Tuân Hùynh and Tư Bỉnh for your care during the hard time when my dad passed away.

Thanks to my old department (Nutrition and Food Processing) colleagues, Dr. Phú Trần, Dr. Thịnh Nguyễn, Đào Nguyễn, Hà Nguyễn, Thủy Lê, Hạ Nguyễn, Thu Trương, Tùng Vương, Trí Nguyễn, Tiến Nguyễn, Khá Nguyễn, Phong Nguyễn, Toàn Nguyễn, and Khánh Nguyễn for being with me during my experimental time in Vietnam. Special thanks to Ass. Prof. Nguyễn Văn Thu for the first small talk about viscosity in animal nutrition. I am grateful to all my project team members in Vietnam, Lam, Trường, Dững, Nhiên, Phúc, Công, Hằng and Tâm; the farmers, Mr. Lâm and his wife Mrs. Diễm, who sold me the fish and ingredients any time when I needed it. I am also very grateful to Thắng Nguyễn and Nguyên Trần, with your useful help for finishing my experiments and timely having feed samples to analyse in Wageningen.

I would like to thank SUPA project, Vinh Hoan company, Provimi company, Cargill company, DeHeus company, College of Aquaculture and Fisheries – Cantho University, and Aquaculture and Fisheries group – Wageningen University and Research for supporting my PhD.

I gratefully thank to my very best friend Kazi Kabir for his useful help, patience and care. He is my thesis cover designer for free. I also thank my paranymphs, Folasade Elesho and Koletsi Paraskevi for their warm care. You will be always in my mind.

About the author

Tran Le Cam Tu was born on 10 October 1982 in Can Tho, in the Mekong Delta, in the south of Vietnam. After completing the higher secondary school, she studied Aquaculture Engineering, at the College of Aquaculture and Fisheries (CAF) in Cantho University (CTU) and passed BSc in 2004. After her BSc graduation, she assisted in practical teaching and bachelor's thesis advising in the nutrition of aquatic animals at CAF – CTU and was a member of the Youth Union Committee of the College of Aquaculture and Fisheries in Cantho University from 2006 to 2008. Between 2008 and 2010, she followed an MSc study in Aquaculture and Aquatic Resources Management at the Asian Institute of Technology, Thailand, where she graduated in 2010 with an MSc thesis on the energy partitioning of striped catfish. Since 2012, Tran Tu is employed as a lecturer of Aquatic Animal Nutrition at CanTho University. As such, she participated in many small Vietnamese national projects about shrimp nutrition and/or freshwater fish nutrition. She also participated in the ACIAR (Australian Center for International Agriculture Research) project about "Improving Feeds and Feeding for small scale Aquaculture in Vietnam and Cambodia" from 2004 to 2007. Tran Tu started her PhD at the Aquaculture and Fisheries Group of Wageningen University at the end of 2012. The study had a break of 2 years after her father passed away in 2014. She restarted her PhD at the end of 2015. During her career, she published science papers both in Vietnamese and English.

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Colophon
The experimental work reported in this study was carried out at Cantho University, Vietnam. The research described in this thesis was financially supported by the SuPa project (in full: "Waste management for Pangasius culture in the Mekong Delta of Vietnam") (WU/AFI/VRF10/WSSD/1), a bilateral Dutch-Vietnamese Public Private Partnership in the area of Fisheries coordinated by the Ministry of Economic Affairs, Agriculture and Innovation (EL&I) from the Netherlands, the Vietnamese Ministry of Agriculture and Rural Development (MARD) and the WIAS Graduate School of Wageningen University and Research, together with a consortium of Dutch and Vietnamese companies. Additional financial support was provided by the companies Provimi (now belonging to the Cargill group), DeHeus and Vinh Hoan and by Wageningen University and Research (Aquaculture and Fisheries Group). The printing of this thesis was financially supported by Wageningen University.

Cover and layout design: Kazi Kabir and Balayet Hossain|balayet.graydesigner@gmail.com
Photos and figures thesis: Tran Tu
Printed by: Proefschriftmaken.nl | Digiforce Vianen