Dietary carbohydrate and faecal waste in the Nile tilapia (*Oreochromis niloticus* L.)

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Chapter 1

General introduction

Overview

At a global level, aquaculture has been growing rapidly during the last few decades (FAO 2002). Since 1970, the growth rate of the aquaculture has been 8.9% per year, which is much higher than other animal food-production sectors (2.8% per year; FAO 2004). This rapid growth has resulted in competition for natural resources (i.e., land and water; Piedrahita 2003). Apart from the strong annual growth, the culture of fish in the last decades has also been strongly intensified. This intensification has also its draw backs, such as an increased environmental impact (Tacon and Forster 2003). The discharge of waste (both solid and dissolved) by effluent water from aquaculture operations can lead to eutrophication in receiving water bodies (Persson 1991), which is often characterized by excessive growth of algae and/or aquatic plants.

Aquaculture waste

The waste produced by aquacultural operations can be divided into solid and dissolved waste (Figure 1). The solid waste can be further split into settleable and suspended solids. Solid waste mainly originates from uneaten and/or spilled feed by the fish and from faeces excreted. Part of dissolved waste (i.e., COD, ammonia, phosphorous, etc.) originates from metabolites excreted by the fish (through gills and by urine). Another part of the dissolved waste originates from the disintegration/suspension of nutrients from the solid waste fraction (both settleable and suspended).

In intensive aquaculture systems, between 20 to 40% of the dietary dry matter is incorporated in the fish body, while the remaining part will be excreted (Verdegem et al. 1999). The proportion of uneaten/spilled feed ranges between 5 and 15% (Beveridge et al. 1997; Cho and Bureau 1997). The amount of faecal waste is dependent upon factors like, feed composition, fish species and temperature. The amount of faecal waste roughly ranges between 0.2 to 0.5 kg dry matter per kg feed (Chen et al. 1997).

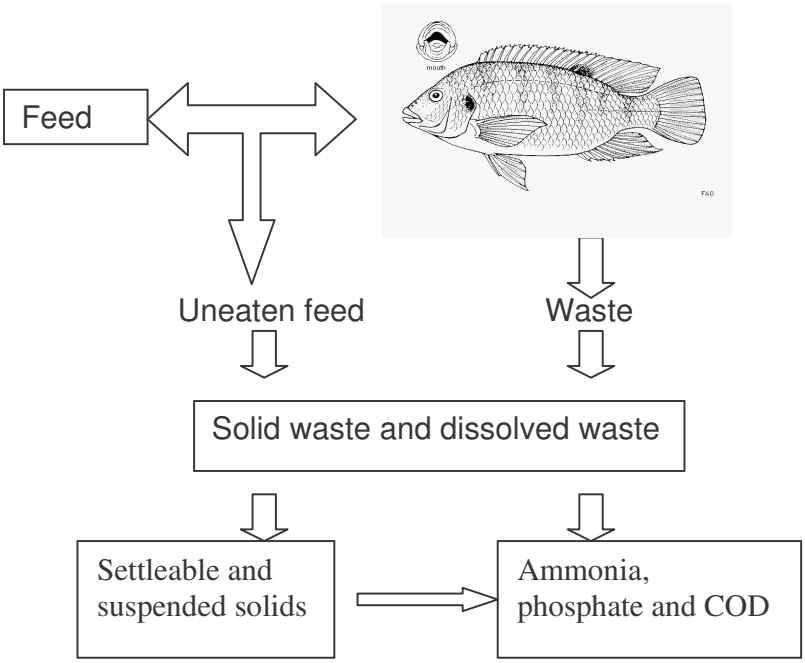

Figure 1. Scheme of waste production by fish

In all aquaculture systems, waste is partially discharged with the effluent water. However, the amount and composition of the waste discharged through effluent water differs between the various types of aquaculture systems. For instance in flow through systems all dissolved waste and suspended solids are released to the environment. Depending on the presence and efficiency of a settling basin in flow through systems, a variable part of the settleable waste is also discharged by the effluent water. In pond systems the total waste produced remains in the system and part of the organic waste matter is mineralized in situ (Verdegem et al. 2001). In "closed" recirculation systems, the amount and composition of the waste stream leaving the farm through the effluent water strongly depends on the design of the systems (i.e., type and efficiency of settling unit; presence of a denitrification unit, etc). In general, to have an efficient water quality management in recirculation systems the strategy of the design is to remove settleable solids as quick and as much as possible from the system.

Solid waste management

Accumulation of solid waste within intensive aquaculture systems has a deleterious effect on fish health (Wickins 1981) and on the efficiency of the water treatment facility (Kruner and Rosenthal 1987). The dissolved organic matter in the water column, which originates from both settleable and suspended solids, can enhance the growth of heterotrophic bacteria (Sharma and Ahlert 1977), thereby decreasing the available oxygen for fish (McMillan et al. 2003). These potential problems following from accumulation of suspended solids are especially critical in intensive recirculation systems due to the low water exchange in combination with the progressively conversion of accumulated solids into smaller particles.

Solid waste (both settleable and suspended) originated from either uneaten/spilled feed or faeces. Consequently, management of solid waste can be achieved through the control of both the amount of uneaten/spilled feed and the faecal waste production. In most fish culture operations, the contribution of faecal waste to the total solids waste production is larger than the contribution of uneaten/spilled feed (29 versus 71%; Franco-Nava et al. 2004).

Over the last decades, there have been many changes in feed technologies and feeding methods to reduce the solid waste production through uneaten/spilled feed (Enell 1995; Bergheim and Asgard 1996). Technological treatments such as extrusion and expansion have improved physical characteristics of aqua-feeds (e.g., water stability, leaching characteristics, etc.) (Kearns 1993; Willson 1994). Furthermore, the amount of uneaten feed has been reduced by improved feeding strategies (Van der Meer et al. 1997; Kwei Lin and Yi 2003; Paspatis et al. 2000).

Improvement of feed digestibility has been considered as a key factor to reduce the faeces waste. Therefore during the last decades, research on the reduction of faeces output concentrated on using highly digestible diets based on fishmeal and fish oil (Bureau and Cho 1999; Sugiura et al. 1999), excluding poorly digestible grain by-products (Cho and Bureau 2001). A second line of technological research focused on improving digestibility by applying better/new technologies (e.g., extrusion) (Kearns 1993; Wilson 1994). However, both approaches cannot solve completely the impact of faeces production by fish, because the scope of digestion in fish is limited and always a fraction of feed

remains undigested and excretes as faeces (190 g faeces per kg of feed using highly digestible diet (MNR-91H); Cho et al. (1994)). Furthermore, due to limited availability of fishmeal and fish oil in future (Hardy 1996), the plant ingredient content of aqua-feeds will increase. This change of technology will reduce the digestibility of aqua-feeds and thus increase again total faecal waste production.

Apart from controlling the total amount of faecal waste produced, management of water quality can also be achieved through controlling the removal of produced faeces. For such an effective removal, the fish faeces should have the "right" physical properties. An improvement of the faecal waste removal may be obtained through improving the consistency of the faeces. An increase in solids removal efficiency improves the proportion of settleable solids to non-settleable solids, thereby decreasing organic matter and suspended solids production within the systems. Stable faeces have a larger particle size, therefore a higher settling velocity and a lower leaching of nutrients (Westers 1991). A quick removal of solids also reduces mineralization of organic nitrogen (N) by dissolved and particulate organic matter decomposition and thereby reducing the conversion of particulate N into dissolved N. Finally, solid removal is comprehensively easier and less expensive than other water treatment operations such as filtration (Chen et al. 1993). It may be also highly effective in reducing some nutrients (like phosphorous) from the system by thickening them in relatively high-strength sludge (Piedrahita 2003).

A higher removal efficiency of settleable solids generally leads to an improved water quality within the system and subsequently to a lower wastewater discharge from the system, and then leading to a lower environmental pollution.

Factors affecting faeces characteristics

In general, there is a lack of knowledge regarding factors affecting the physical properties of faeces. However, there are some studies indicating that dietary composition can alter the faecal characteristics. Feed composition may affect the production and activity of digestive enzymes (Falge et al. 1978), retention time (Shiau et al. 1988) and physical property of digesta (Hossain et al. 2001). Binding agents that are generally used to enhance physical pellet quality (Hardy 1989) can also change the faecal stability (Storebakken 1985). Dias et al. (1998) observed visually that dietary cellulose

incorporation increased faeces cohesion in European seabass. In tilapia dietary supplementation of gelatinised starch increased the particle size of faecal solids (Han et al. 1996). Dietary inclusion of guar gum (having a high content of soluble non-starch polysaccharide) reduced the particle size of faeces (Han et al. 1996) in tilapia and increased water content of faeces in rainbow trout (Storebakken 1985). El-Shafai et al. (2004) observed in tilapia, that supplementation of 40% dried duckweed to a basal diet improved the removal efficiency of faeces from the water by Choubert collectors.

Due to the expected shortage of fish meal, increasing amounts of plant ingredients will be included in aqua-feeds to meet the protein requirements of the fish. The increased levels of plant ingredients in aqua-feeds will increase the dietary level of carbohydrate, which in turn may change the digesta characteristics, depending on the physicochemical properties of the carbohydrate. The water holding capacity, viscosity, particle size distribution and fermentability (Dreher, 1987) of the digesta may change, thereby affecting also the physical property of the faeces. In dogs, supplementation of fructo oligosaccharide reduced faecal firmness and faecal dry matter (Twomey et al. 2003). Ferguson et al. (2000) found that water content of faeces in rat increased by feeding diets containing potato starch compared to maize starch.

Hypothesis and the objectives

Fish consume feed and excrete faeces into the water column. Faeces gradually convert to three main fractions: settleable, suspended and dissolved solids. Stable faeces consists of larger particles and is relatively more resistant to water flow resulting in less suspended and dissolved solids. Due to the larger particle size, stable faeces also sink quicker and can be removed more effectively by a settling basin/unit. It is hypothesised that the removal rate/efficiency of faeces depends on the dietary composition by influencing the physical characteristics of the faeces. The overall objective of this thesis is to determine the influence of feed composition on faeces characteristics. In other words, this project should contribute to the development of aqua-feeds that increase faeces stability without reducing nutrient digestibility. Stable faeces generally lead to a proper water quality and less discharging of wastewater from system to the environment.

Outline of the thesis

The main theme of this thesis is to study the effect of different dietary fraction/ingredients on faecal characteristics and faecal waste production of tilapia kept in intensive recirculation systems. Furthermore the question whether differences in faeces characteristics, as induced by dietary composition, have an impact on water quality will be tested in recirculation systems. As mentioned before, intensification of culture systems constitutes an increased risk for negative environmental impact, declining the water quality and increasing the negative impact on animal health. This study provides information on nutritional parameters affecting faecal waste production, thereby leading to an improved water quality by improving faecal removal efficiency.

In **Chapter 2** of this thesis a number of feed ingredients, which are currently used in aqua-feeds, were evaluated regarding faecal waste production and recovery efficiency. In **Chapter 3**, two faeces collection methods (Choubert versus settling tanks) were compared for measuring nutrient digestibility and faeces recovery. These two methods were compared using high and low quality diets in terms of digestibility in order to determine if accuracy of the faecal collection is influenced by diet quality. **Chapter 4** and **5** deal with nutritional factors affecting faecal waste production and faecal removal efficiency. **Chapter 4** focuses on the fibre fraction of diets, the non-starch polysaccharide (NSP) fraction. Two different types of NSP (guar gum and cellulose), being different in water solubility, were studied. In **Chapter 5** the replacement of fat by starch, as an alternative energy source, was assessed in relation to faeces stability. Furthermore, in this chapter the mechanism by which faeces characteristics are affected were studied by measuring physical (viscosity) and chemical (dry matter and volatile fatty acids) in the digesta throughout the gastro intestinal tract. Finally in **Chapter 6** it was tested whether diets which causes differences in faecal stability (different in faecal removal efficiency but similar in digestibility), lead to differences in water quality in recirculation systems. In **Chapter 7** the overall results of this thesis and the implications are discussed.

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Chapter 2

Digestibility, faeces recovery, and related C, N, and P balances of five feed ingredients evaluated as fishmeal alternatives in *Oreochromis niloticus* L.

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Abstract

This study shows that alternatives for fishmeal in a fish diet affect not only fish growth, but also faeces stability and nitrogen and phosphorous waste production. Wheat gluten, soybean meal extract, soybean meal, duckweed, and single cell protein were evaluated as fishmeal replacement on a 15%weight/weight basis in tilapia diets. Fishmeal replacement was affecting dry matter (dm), protein, ash, and phosphorous (P) digestibility significantly. Faeces recovery (6.8-11.2%) was not significantly affected, although the amount of non-recovered faeces and total faeces showed significant differences. Duckweed and single cell protein diets resulted in the largest amounts of non-recovered and total faeces (199-210g, 224-225g dm/kg dm feed). Compared to fishmeal, the wheat gluten and soybean meal extract diets result in similar growth, but higher non-faecal nitrogen losses (471-495gN/kgN vs. 416gN/kgN). Soybean meal, duckweed, and single cell protein diets resulted in lower growth but less non-faecal loss (409-450gN/kgN). The duckweed and fishmeal diet had the highest nitrogen retention (480gN/kgN) compared to the other diets (431-451gN/kgN). Carbon retention, faecal and non -faecal losses and P retention were similar for all diets (302-358, 142-176, and 489-523gC/kgC, 606-704gP/kgP). P faecal loss was lower for all diets (329-381gP/kgP) than for the fishmeal diet (401gP/kgP).

Introduction

In the past decades several protein sources were tested as alternatives for fishmeal in finfish diets. Results of those studies for different fish species were summarized by Hardy (1996), El-Sayed (1999), and Francis et al. (2001). Most conducted studies, which are reported in literature, focused on growth related factors and feed digestibility.

There are only three studies, which are correlating total faeces recovery with feed composition (Vens-Cappell 1985; Han et al. 1996; Dias et al. 1998). Yet faeces recovery represents a physical measure of faeces stability. Faeces removal efficiency is influenced by faeces stability, which on its turn is influenced by feed composition (Vens-Cappell 1985; Han et al. 1996; Dias et al. 1998; Cripps et al. 2000). Chen et al. (1997) and Wong et al. (2000) showed that the solid removal efficiencies are proportionally to the size and stability of faeces particles. Furthermore, if fishmeal is replaced by another feed

ingredient, the feed nitrogen (N), phosphorus (P) and carbon (C) levels will change. Those changes affect the nutrient retention in fish (Machiels et al. 1986; Einen et al. 1995; Lupatsch et al. 2001), and thus automatically the nutrient released as waste in the rearing system (Brunty et al. 1997; Lupatsch et al. 1998; Lupatsch 2003). These changes influence consequently system design (Liao et al. 1974; Eding et al. 1999). Nutrient balances for C, N, and P deliver a transparent picture of retention and faecal and non-faecal loss originating from the fish. By combining nutrient balances with faeces recovery rates, the impact of a fishmeal replacement by other feed ingredients on fish and surrounding culture system can be estimated. These predictions are valuable to develop so called “low pollution diets”.

The objective of the study is to determine the digestibility, total and recovered amount of faeces, and the related nutrient balances for five feed ingredients, which can be used as alternatives for fishmeal in tilapia diets.

Material and Method

Experimental Diets

Five feed ingredients, which might serve as potential fish meal replacements, were selected based on nutritional value, and on ecological sustainability aspects. Thus the ingredients should contain a possibly high amount of good digestible protein and either be land based grown plants or grown on waste products. The selected fishmeal alternatives were wheat gluten, soybean extract, soybean meal, duckweed and a mixture of yeast and bacteria single cell protein. Wheat gluten (WGD, bulk material), soybean meal extract (SBE, Nurish 1500), and soybean meal (SBE, bulk material) have a high protein content 50-80% and high digestibility (Shiau et al. 1987; Sintayehu et al. 1996; Sugiura et al. 1998, Fontaínhas-Fernandes et al. 1999, Hardy 2000). However, soybean products can contain anti-nutritional factors (Hardy 1996; Francis et al. 2001; Vielma et al. 2002). To evaluate an influence of these anti-nutritional factors (ANF) soybean protein extract and soybean meal were compared. Duckweed (DWD) was selected, because it can be grown on fish waste water (Porath et al. 1982; Verdegem et al. 2003; El-Shafai, 2004) and be reused as fish feed (Gaigher et al. 1984; Hassan et al. 1992;

Bairagi et al. 2002; El-Shafai 2004). It represents, therefore, a renewable feed source recycling nitrogen and phosphorus waste. The duckweed (*Lemnor minor*) was obtained from a commercial culture (Levita, Baarlo, The Netherlands). Prior to incorporation in feed pellets, it was dried and grinded. Single cell protein (SCP) has been chosen as test ingredient, because it is an innovative and alternative protein source for nutritional applications, which can even be grown on waste (Tacon 1979; de Muylder et al. 1989; Kiessling et al. 1993; Anupama et al. 2000). In this experiment, a mixture of brewer's yeast and *Lactobaccilus* (BORACEL, DSM, Zaandam, The Netherlands) was tested. Fishmeal (RE 580-630) was used in the control diet (FMD). To prepare the experimental diets one of the ingredients has been added to a basal diet in a ratio of 15% weight/weight (Table 1 and 2). The obtained diets were fed as a sinking pellet of 1.5mm. To allow for digestibility studies, Diamol (Acid Insoluble Ash, AIA) was used as inert marker. Three diet samples have been collected for proximate analysis. One was taken at the beginning, one in the middle, and one at the end of the experiment as grab sample from the feed stocks. Feed samples were stored at -20°C for later analysis.

Table 1. Experimental diet formulation on % dry weight basis

Ingredient	Amount
Test Ingredient	15
Fishmeal	28.9
Corn	25.0
Wheat	17.0
Wheat bran	8.5
Fish oil	2.6
Diamol (AIA[a])	2
Premix	1

[a]AIA = acid insoluble ash

Fish and husbandry

The Wageningen University Animal Care and Use Committee approved the experiment. The study was executed at the experimental facility of the Fish Culture and Fisheries Group, Department of Animal Sciences, Wageningen University, The Netherlands. The research lasted for about eight weeks and comprised one week adaptation to the facilities, one week to feed and feeding level (starting with day 0), and six weeks sampling period (ending with day 50). Juvenile tilapias (*Oreochromis niloticus* L.) were purchased from a

commercial fish farm (Helmond, The Netherlands). One week after arrival and adaptation to the facilities, fish were weighed, and divided at random into twelve glass aquaria (40x50x35cm, 70l) with an initial stocking density of 40 fish per aquarium. These aquaria were connected to a recirculation system comprising the aquaria, a sedimentation unit, pump, sump and trickling filter. Photoperiod was 12 hours light, 12 hours dark. The fish were adapted to the experimental diets and feeding level for another week before the experimental period begun. The fish were fed on experimental diets during 49 days of the experimental period. On the sampling days (day 0 and 50) fish were not fed. Each day water quality was checked after the first feeding. The system was monitored for temperature, conductivity, pH, oxygen concentration, NH_4^+-N, NO_2^--N, and NO_3^--N concentrations. Water flow through the tanks was 8 l min^{-1}, which was checked daily with a water flow meter (Brooks, Veenendaal, The Netherlands).

Experimental procedure

The twelve aquaria were randomly assigned to one of the six experimental diets each diet in two replicates. The fish were fed by hand, four times a day: at 10:00, 12:30, 15:00, and 17:00 h. Feeding level was restricted at 17 g feed/$kg^{0.8}$*d^{-1}. Daily estimated weight gain was based on an assumed feed conversion ratio of 1.2. Before feeding, dust was sieved from the feed pellets with a 1-mm mesh. The first day after weighing and sorting, fish were fed 50% of the feeding ratio, the day after 75%, and the third day 100%. This progressive feeding level was applied to enable fish to adapt to the experimental diets and feeding regimes. Fish were weighed at the beginning (day 0) and at the end of the experiment (day 50). Weight gain was calculated as:

Weight gain = total final biomass – total initial biomass

Weight gain is in g, total final biomass = total biomass at the end of experiment in the tank (g), total initial biomass = total biomass at the beginning of the experiment (g).

Specific growth rate was calculated from the mean initial individual body weight, the mean final individual body weight and the time in between the two weighing moments:

$$SGR = 100 \times (\ln W_{final} - \ln_{initial} \times t^{-1})$$

Where W_{final} is the mean final individual body weight (g), $W_{initial}$ the mean initial individual body weight (g) and t the time period (days).

Feed conversion ratio was calculated by dividing total feed intake per tank by the total body weight gain per tank. Daily feeding rations were adjusted to the number of fish in the tanks, based on recorded mortality. Survival rate was calculated as number of fish at the end of the experiment divided by number of fish at the beginning. Relative feeding rate was calculated as:

$$R_m = (\text{feed}_{\text{consumed}} \times BW^{-0.8})\ t^{-1}$$

Where $\text{feed}_{\text{consumed}}$ is the amount of feed consumed during the experimental period (g), BW the geometric mean body weight = $e^{(\ln w\ \text{initial} + \ln w\ \text{final})/2}$ and t the experimental period (days).

Faeces were collected using six Choubert faeces collectors with a mesh size of 1000 µm (Choubert et al. 1982). This method was preferred above other collection methods because of its potentially high recovery rate and the low level of nutrient leaching (Choubert et al., 1982). The faeces were transferred from the collectors into plastic containers twice a day (9:30 and 17:00 h) and stored at –20°C for later analysis. Because only six collectors were available for twelve aquaria, collectors were shifted alternating in between two aquaria every 48h.

Analytical procedure

An initial sample of ten fish was used to analyse initial body composition. At the end of the experiment, ten fish were randomly selected from each tank during the weighing procedure to analyse final body composition. These fish were euthanized with Tricaine Methane Sulfonate and immediately stored at -20°C for subsequent analysis. Collected feed, faeces, and fish samples were analysed for dry matter, ash, crude protein, crude fat, gross energy, AIA, and phosphorus. Samples were analysed for dry matter by drying the samples for 4 h at 103°C until constant dry weight (ISO 6496, 1983), and for the ash by incineration in a muffle furnace for 4 h at 550°C (ISO 5984, 1978). Kjeldahl nitrogen was determined according to ISO 5983 (1979) procedures using a Tecator 2020 Digestor at 400°C for 4h and distillation by Tecator Kjeltec Autosampler system 1035 Anaylser (Tecator AB, Hoganas, Sweden). Kjeldahl nitrogen was translated to crude protein content by multiplication with 6.25. Crude fat was determined by Soxhlet extraction with petrol ether (EEG 18.1.84 no.15/29-30). Gross energy was determined by bomb

Table 2: Analysed composition of the tested ingredients and the experimental diets in g kg^{-1} wet weight

Ingredient	**Dry Matter**	**Ash**	**Crude Protein**	**N**	**Crude Fat**	**Carbohydrates**	**Carbon**	**Phosphorus**	**Gross Energy**	**AIA**
Wheat gluten	893.5	7.2	757.5	121.2	7.9	120.9	470.8	1.9	20.6	---
Soybean extract	911.1	42.6	815.7	130.5	2.0	50.7	466.8	6.4	20.5	---
Soybean meal	880.2	60.2	485.4	77.7	12.5	322.1	416.0	6.3	17.3	---
Duckweed	896.9	159.6	338.3	54.1	31.8	367.3	370.8	19.6	15.7	---
SingleCell Protein	948.0	128.5	405.1	64.8	2.2	412.2	404.5	17.2	16.9	---
Fish meal	903.5	142.9	577.4	92.4	87.7	95.5	422.4	23.0	16.7	---
Diet	*Dry Matter*	*Ash*	*Crude Protein*	*N*	*Crude Fat*	*Carbohydrates*	*Carbon*	*Phosphorus*	*Gross Energy*	*AIA*
WGD	870.2	76.5	347.1	55.5	75.3	371.3	410.5	9.7	17.2	1.6
SBE	872.2	82.3	357.1	57.1	74.9	357.9	409.7	10.2	17.2	1.6
SBM	868.2	84.1	304.7	48.8	70.9	408.5	400.6	10.2	16.8	1.6
DWD	863.9	98.0	274.6	43.9	73.4	417.9	390.4	12.1	16.0	1.6
SCP	866.5	95.6	291.1	46.6	73.4	406.4	394.2	11.7	16.5	1.7
FMD	868.3	98.1	325.2	52.0	83.2	361.8	400.4	13.0	16.9	1.7

WGD=Wheat gluten diet; SBE=Soybean extract diet; SBM=Soybean meal diet; DWD=Duckweed diet; SCP=Single cell protein diet; FMD=Fishmeal diet; N=Protein/6.25; Carbon=Protein*1.18*0.46+Fat*1*0.76+Carbohydrates*1.11*0.4, whereby 1.18, 1, 1.11 are the hydration factors of protein, fat and carbohydrates, and 0.46, 0.76, 0.4 is the carbon content in the hydrolyzed molecule (Machiels et al., 1986)

calorimetry (IKA-C-7000, Fa. Janke & Kunkel, IKA Analysentechnik, Heitersheim, Germany). AIA content was determined by dissolving the obtained ash in hydro chloric acid following the ISO 5985 (1981) procedures. Phosphorous was determined by a vanado-molybdate method after sample combustion at 550° C and digestion with acid (ISO 6491, 1980). Carbohydrate fraction was determined as dry matter minus fat, protein, and ash in feed and faeces.

Digestibility measurements and faeces recovery

Apparent digestibility coefficients (ADC) of nutrients and energy were determined using an internal marker (AIA) in the diets. ADCs for the different nutrients are expressed as the fractional absorption of these nutrients from diets in fish:

$$ADC_{nutrient} = 1 - (AIA_{diet} / AIA_{faeces} \times nutrient_{faeces} / nutrient_{diet}) \times 100$$

Where AIA_{diet} is the AIA in the diet (%), AIA_{faeces} the AIA in the faeces (%), $nutrient_{faeces}$ the nutrient in the faeces (%), and $nutrient_{diet}$ is the nutrient in the diet (%).

The amount of faeces recovered in relation to the total amount of faeces produced was calculated by dividing AIA recovered from the collector by AIA given with the feed:

$$\text{faeces recovery} = (AIA_{rcoverd} / AIA_{feed}) \times 100$$

Where $AIA_{recovered}$ is the AIA recovered from the collector (g kg^{-1}dm) and AIA_{feed} is the AIA given with the feed (g kg^{-1} dm).

Nutrient balance

Nutrient balances are derived from the amount of a nutrient in the feed and fish, nutrient digestibility, feed uptake and fish performance:

$$uptake_{nutrient} = \text{concentration } feed_{nutrient} \times feed_{consumed}$$

where $uptake_{nutrient}$ is the amount of nutrient taken up by the fish(g), concentration $feed_{nutrient}$ is the concentration of nutrient in the feed (g kg^{-1}) and $feed_{consumed}$ is the amount of feed consumed (g).

The fraction of digested and undigested nutrients is calculated by:

$$digested_{nutrient} = uptake_{nutrient} \times digestibility_{nutrient} / 100$$

$$undigested_{nutrient} = uptake_{nutrient} (100\text{-}digestibility_{nutrient}) / 100$$

where $digested_{nutrient}$ is the amount of nutrient digested (g), $undigested_{nutrient}$ is the determined digestibility (%).

Digested nutrients are divided into retained nutrients and non-faecal loss:

$nutrient_{retained} = W_{final} \times nutrient_{fish} - W_{initial} \times nutrient_{fish}$

$nutrient_{non\text{-}faecal\ loss} = digested_{nutrient} - nutrient_{retained}$

where $nutrient_{retained}$ is the amount of nutrient retained in the fish (g), W_{final} the final wet weight of the fish (g), $W_{initial}$ the initial wet weight of the fish (g), and $nutrient_{fish}$ is the amount of the nutrient in the fish in g kg^{-1} wet weight.

The obtained values were converted to g per kg nutrient supplied with the feed.

Statistics

In this experiment each tank is considered as one experimental unit. Data were analysed by one-way ANOVA using SPSS 11.5. The means were compared by a Turkey's Post hoc test at a five percent probability level.

Results

Fish and husbandry

Water quality remained within the appropriate ranges for fish growth during the experimental period. Probably to limitations of the experimental rearing system to process high feeding loads, on day 10 dissolved oxygen levels decreased to 3.6mg/l in one of the aquaria. To avoid possible oxygen problems, from day 13 onwards all feeding rations were kept constant. Oxygen levels remained consecutively at 4.3mg/l or higher.

Initial and final body weights and fish mortality showed no significant differences between diets (Table 3). However FCR and SGR were significantly affected by diet ($p=0.017$ and $p=0.008$). Lowest FCR and highest SGR were found for the high protein diets WGD, SBE, and FMD. Fish body composition was not significantly affected by diet ingredients for dry matter, crude protein, crude fat, phosphorous and energy (Table 4). Crude ash content of the fish was affected by diet, showing the highest contents for diets with high ash content: DWD, SCP, and FMD.

Table 3. Growth and feed utilization of tilapia fed the experimental diets.

Diet	Total feed given	Individual start weight	Individual final weight	Survival rate	FCR	SGR	R_m
	g/tank	g	g	%	$kg*kg^{-1}$	$\%BWd^{-1}$	$g*kg^{-0.8}*d$
WGD	4038	55.6	156.2	97.5	1.0	2.0^{bc}	13.6
SBE	3937	54.3	153.0	98.8	1.0	2.0^{c}	13.3
SBM	4039	55.6	146.1	100.0	1.1	1.9^{abc}	13.6
DWD	4291	60.2	153.7	100.0	1.2	1.8^{a}	13.7
SCP	3997	54.8	142.1	100.0	1.1	1.9^{ab}	13.7
FMD	4085	56.4	157.0	100.0	1.0	2.1^{bc}	13.3
SEM	52.04	0.93	2.55	0.33	0.02	0.03	0.06
p	0.565	0.593	0.566	0.055	0.017	0.008	0.048

Total feed given per tank, initial individual weight, final individual final weight, survival rate, feed conversion rate (FCR), specific growth rate (SGR), and relative feeding rate per metabolic weight (R_m) as means. The experimental period lasted for 51days.
Mean within columns with a different superscript differ significantly.
WGD=Wheat gluten diet; SBE=Soybean extract diet; SBM=Soybean meal diet; DWD=Duckweed diet; SCP=Single cell protein diet; FMD=Fishmeal diet; SEM=Standard error of the mean; p=p-value; means within columns with a different superscript differ significantly

Table 4. Body composition (g per kg wet weight) of tilapia fed the experimental diets

Diet	Dry Matter	Ash	Crude Protein	Crude Fat	Phosphorus	Gross Energy
	$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$	$MJkg^{-1}$
initial	299.9	42.2	159.3	96.1	7.3	7.4
WGD	288.4	39.8^{a}	156.6	87.2	7.0	7.3
SBE	283.7	40.6^{ab}	160.1	80.7	7.3	6.8
SBM	286.8	41.3^{abc}	156.2	86.8	7.3	6.9
DWD	295.5	46.8^{d}	155.3	88.5	8.1	7.0
SCP	284.4	44.2^{bcd}	152.6	81.9	7.8	6.8
FMD	290.5	44.7^{cd}	159.6	84.5	8.0	7.0
SEM	1.63	0.78	0.96	1.29	0.14	0.07
p	0.343	0.002	0.203	0.511	0.095	0.319

WGD=Wheat gluten diet; SBE=Soybean extract diet; SBM=Soybean meal diet; DWD=Duckweed diet; SCP=Single cell protein diet; FMD=Fishmeal diet; SEM=Standard error of the mean; p=p-value; means within columns with a different superscript differ significantly

Digestibility measurements and faeces recovery

Replacement of fishmeal by the test ingredients affected digestibility of dry matter (p=0.011), crude protein (p=0.000), crude ash (p=0.039) and phosphorus (p=0.004) (Table 5). Highest dry matter and protein digestibility was determined for WGD, SBE, SBM, and FMD, lowest digestibility for DWD and SCP. Ash digestibility was significantly affected (p=0.039) and was highest for SCP (47.6%) compared to lowest

values for WGD (39.9%). Plant based diets, DWD, WGD, SBE, and SBM generally showed higher P digestibility rates than FMD or SCP. The amount of faeces recovered per kg feed was not affected by the different diets (p>0.05, Table 6). However the amount of total faeces produced and consequently the amount of non-recovered faeces per kg feed were significantly affected (p=0.011 and p=0.029). DWD and SCP resulted in

Table 5. Apparent digestibility coefficients (%) of the experimental diets

Diet	Dry Matter	Crude Protein	Crude Fat	Carbohydrates	Crude Ash	Phosphorus	Energy
WGD	80.8^{b}	92.6^{d}	96.8	74.3	39.9	65.0^{bc}	86.3
SBE	80.2^{b}	91.4^{cd}	96.9	74.5	41.7	64.5^{bc}	86.7
SBM	79.2^{ab}	90.5^{bc}	96.7	74.8	45.3	64.5^{bc}	85.7
DWD	77.6^{a}	88.9^{a}	95.9	78.0	47.1	67.2^{c}	84.3
SCP	77.5^{a}	89.1^{ab}	95.8	72.4	47.6	61.9^{ab}	84.5
FMD	79.1^{ab}	89.7^{ab}	96.8	74.4	45.4	59.9^{a}	85.3
SEM	0.40	0.40	0.19	0.68	0.94	0.74	0.36
p	0.011	0.000	0.411	0.329	0.039	0.004	0.333

WGD=Wheat gluten diet; SBE=Soybean extract diet; SBM=Soybean meal diet; DWD=Duckweed diet; SCP=Single cell protein diet; FMD=Fishmeal diet; SEM=Standard error of the mean; p=p-value, means within columns with a different superscript differ significantly

Table 6. Relation between AIA given by feed, the recovery rate, the amount of recovered faeces and of non-recovered faeces. Non recovered faeces are the differences of total faeces produced and recovered faeces.

Diet	Recovery rate %	Total Faeces produced g DM kg^{-1} feed DM	Recovered faeces g DM kg^{-1} feed DM	non-recovered faeces g DM kg^{-1} feed DM
WGD	9.5	192^{a}	18	174^{a}
SBE	8.8	198^{a}	17	181^{ab}
SBM	9.7	208^{ab}	20	188^{ab}
DWD	11.2	224^{ab}	25	199^{ab}
SCP	6.8	225^{b}	15	210^{b}
FMD	8.8	209^{ab}	18	191^{ab}
SEM	0.57	3.95	1.27	3.92
p	0.491	0.011	0.412	0.029

Amount of total faeces produced equals (1-Digestibility$_{Dry\ Matter}$)/100*1000; WGD=Wheat gluten diet; SBE=Soybean extract diet; SBM=Soybean meal diet; DWD=Duckweed diet; SCP=Single cell protein diet; FMD=Fishmeal diet; SEM=Standard error of the mean; p=p-value; means within columns with a different superscript differ significantly.

highest amounts of total faeces produced (224-225g dry matter/kg feed dry matter), WGD and SBE resulted in the lowest amounts (192-198g dry matter/kg feed dry matter). SCP resulted in the highest amount of non-recovered faeces (210g dry matter/kg feed dry

matter) while the plant based diets and FMD showed lowest amounts (174-199g dry matter/kg feed dry matter).

Nutrient balances

The C balance showed no significant effect for retention, faecal and non-faecal loss for the different diets (Table 7). Highest N retention and lowest non-faecal losses were found for FMD and DWD (480gN/kgN and 409-416gN/kgN) compared to the other diets (431-451GN/kgN, p>0.05 and 450-495gN/kgN, p=0.033). N faecal loss was highest for DWD, SCP and FMD (104-111gN/kgN) compared to WGD, SBE, and SBM (74-95gN/kgN, p=0.000). P retention was lowest for DWD, SCP, and FMD (606-633gP/kgP) compared to the other diets (640-704gP/kgP). DWD showed lower faecal losses for P (329g/kg P) compared to the other alternative diets (350-381gP/kgP). However FMD had the highest faecal loss (401gP/kgP, p=0.004).

Table 7. Carbon (C), nitrogen (N) and phosphorous (P) balance for tilapia fed with six different diets based on g per kg nutrient supplied

	C Balance			*N Balance*			*P Balance*		
Diet	*Fish*	*Faecal loss*	*Non-faecal loss*	*Fish*	*Faecal loss*	*Non-faecal loss*	*Fish*	*Faecal loss*	*Non-faecal loss*
	g kg^{-1} C	*g kg^{-1} C*	*g kg^{-1} C*	*g kg^{-1}N*	*g kg^{-1} N*	*g kg^{-1} N*	*g kg^{-1}P*	*g kg^{-1} P*	*g kg^{-1} P*
WGD	346	142	513	431	74^{a}	495^{b}	685	350^{ab}	-34
SBE	342	144	514	442	86^{ab}	471^{ab}	704	355^{ab}	-59
SBM	324	158	518	451	95^{bc}	455^{ab}	640	355^{ab}	5
DWD	325	153	522	480	111^{d}	409^{a}	625	329^{a}	46
SCP	302	176	523	442	109^{cd}	450^{ab}	606	381^{bc}	13
FMD	358	153	489	480	104^{cd}	416^{ab}	633	401^{c}	-34
SEM	6.97	4.14	7.35	7.08	4.00	9.92	16.21	7.34	15.73
p	0.188	0.167	0.877	0.244	0.000	0.033	0.587	0.004	0.490

Non-faecal loss is calculated as difference between the amounts digested nutrient and the amount of retained nutrient to close the nutrient balance. WGD=Wheat gluten diet; SBE=Soybean extract diet; SBM=Soybean meal diet; WD=Duckweed diet; SCP=Single cell protein diet; FMD=Fishmeal diet. C=Protein*1.18*0.46+Fat*1*0.76+Carbohydrates*1.11*0.4, whereby 1.18, 1, 1.11 are the hydration factors of protein, fat and carbohydrates, and 0.46, 0.76, 0.4 is the carbon content in the hydrolyzed molecule (Machiels et al., 1986); SEM=Standard error of the mean; p=p-value; means within columns with a different superscript differ significantly

Discussion

Fish showed higher specific growth rates and better FCR for the "high protein diets" WGD, SBE, and FMD compared to higher FCR and lower SGR for the "low protein diets" SBM, SCP and DWD. This result is related to the higher dry matter and protein digestibility of WGD and SBE and to lower dry matter and protein digestibilities in SBM, SCP, and DWD. In addition, these diets had also lower protein content. The high dry matter and protein digestibility of WGD is comparable to findings of Allan et al. (2000) and Sugiura et al. (1998). They found similar results for silver perch, salmon and trout respectively. In the latter study, the lower dry matter digestibility of SBM was related to indigestible components, such as fibre and starch. The higher protein digestibility of WGD and SBE reflects good protein availability compared to FMD and lower levels of anti-nutritional factors than in SBM and DWD diets (Hardy 1996; Francis et al. 2001; Vielma et al. 2002). The lower dry matter digestibility of the DWD diet, compared to SBM, is probably related to fibre content, e.g. cellulose, and other anti-nutritional factors of duckweed (Bairagi et al. 2002). The low dry matter digestibility of the SCP diet might be related to the indigestible cell wall of the bacteria or other anti-nutritional factors such as high nucleic acid content (Tacon 1979; Rumsey et al. 1991). Fish body composition was only affected significantly for ash ($p=0.002$). This coincided with significantly different ash digestibilities ($p=0.039$). FMD, SCP, and DWD contained both the highest contents in feeds and resulting fish body, and SCP and DWD showed the highest ash digestibility. The low ash digestibility and fish body content in WGD and SBE might be caused by limited availability of P. Storebakken et al. (1998) found reduced uptake of calcium and magnesium in salmon for gluten diets, comprising untreated soybean meal extracts with low P availability. In SCP the increased amount of dietary nucleotides might affect gut motility and thus promote mineral uptake to similar levels as in FMD (Davies et al. 1988). The similar ash uptake of DWD compared to FMD was surprising, because diets, which are rich in cellulose and associated products, show normally decreased mineral availability (Coudray et al. 2003). However, other sources mention that fibre content could have influenced mineral uptake positively (Davidson et al. 1998). Phytate content in DWD or other factors could have influenced mineral uptake in addition (Francis et al. 2001). Furthermore, it has been shown that excess levels of

minerals, such as calcium, lead to increased amount of minerals in bone ash (Robinson et al. 1987). The ash content in the fish body for FMD, DWD, and SCP, is finally a result of these factors. Phosphorous digestibility in fish ranges widely depending on its source 8-75% (Cheng et al. 2003; Sugiura et al. 1998). Although, phosphorous in plants is mainly present as phytin, phosphorous digestibility in plant-based diets was generally higher than in FMD or SCP. Because FMD is high in phosphorus content, relative uptake and thus apparent digestibility might decrease in fish (Coloso et al. 2003; Satoh et al. 2003).

Faeces recovery was not affected by diets (Table 6). Because differences in the feed composition are relatively small with 15%, altered faeces recovery might not have been detected. In addition, the high water flow of 8 l min^{-1} may have influenced the recovery rate. The observed recovery rates are low compared to rates of 12-99% found elsewhere (Choubert et al. 1982; El-Shafai 2004). Although, the variation among the treatments was not statistically significant, a number of observations have been made. For instance, adding duckweed to the diet resulted in a higher recovery than for the other diets. This result may be due to the ingredient composition. An increased level of cellulose leads to an increase of physical property, firmness, settlement of faeces, and larger particle size (Vens-Cappell 1985; Han et al. 1996; Dias et al. 1998). The amount of non-recovered faeces reflects differences in dry matter digestibility. WGD resulted in the least amount of non-recovered faeces and DWD and SCP in the highest amounts. These findings are supported by other studies, where inclusion of wheat gluten in tilapia feed resulted in an increased particle size of fish faeces (Han et al. 1996), or inclusion of cellulose resulted in a higher total faeces production (Dias et al. 1998).

The non-faecal loss for C is higher (513-523gC/kgC) for all diets compared to FMD (489gC/kgC), and C retention is low for SBM, DWD and SCP (302-325gC/kgC) compared to the other diets (342-358gC/kgC). This illustrates that fish has less C expenditure to grow on a fishmeal containing diet than on the other diets (Table 7). This supports the paradigm that a protein sources with a similar composition as the fish carcass composition are retained better than a protein source with a different amino acid profile (Tacon 1990; Allan et al. 2000; Storebakken et al. 2000). This effect returns in the N balances, where FMD has one of the lowest non-faecal losses (104gN/kgN). The N balances show in general higher non-faecal losses for WGD and SBE (471-495gN/kgN)

than for the other diets, especially compared to DWD (409gN/kgN). The high non-faecal N losses of WGD and SBE agree with findings of Brunty et al. (1997) that relate increased non-faecal loss of N with increased levels of protein in the feed. N retention is highest for FMD and DWD (480gN/kgN). The protein, contained in FMD, is taken up and retained better in the fish body due to its composition (Tacon 1990; Allan et al. 2000; Storebakken et al. 2000). DWD shows the opposite of the increased non-faecal loss for high protein diets WGD and SBE as low non-faecal loss for a low protein diet (Brunty et al. 1997). P retention is rather high with 606-704gP/kgP compared to values found in literature of 150-380gP/kgP (Kim et al. 1998; Lupatsch et al. 1998; van Weerd et al. 1999), for different fish species grown from 20g to 400g. However, for low dietary P levels (0.6%P), retentions as high as 600gP/kgP have been determined in trout (Coloso et al. 2003). Non-faecal P loss shows partly negative results. This might be due to minor errors in analytical procedures and applied methodologies, which are compounded in calculations used to estimate digestibilities, retentions and losses. Conversely, this low non-faecal loss is related to the described phenomenon of high relative retention for low dietary levels (Bureau et al. 1999; Coloso et al. 2003).

Taking FCR and SGR into account, wheat gluten (WGD) and soybean extract (SBE) have a high potential as fishmeal replacements on a level of 15% weight/weight. Duckweed showed a lower growth performance and overall digestibility. Yet it should not be rejected as fishmeal replacement, because it has a positive impact on faeces recovery, and yields lower non-faecal and faecal N loss. Further, the N retention in DWD is as high as in FMD and higher than in all other diets, resulting in lowest total waste loads for N supplied. For diets high in P (FMD, SCP, and DWD), P retention was low and their total waste productions/kgP was higher than for the diets with lower P content. DWD showed similar results in P retention as FMD and its faecal loss/kgP was lower than for FMD.

Conclusion

Fishmeal alternatives, such as wheat gluten, soybean products, duckweed, and single cell protein have a high potential as feed ingredients replacing fishmeal. Nutrient balances and faecal recovery data showed that “high” protein diets (WGD, SBE) result in good

fish performance for a similar replacement of fish meal on weight basis, because of their nutrient content and digestibility. However, they result as well in higher waste loads, in particular of N, to the system. Alternative products, such as duckweed, result in lower fish production, and faecal losses are higher. However, total N waste production is lower. Differences in P waste loads illustrate the need for balanced diets, which avoid an oversupply of P to the fish leading to excessive losses to the environment. The choice for fishmeal replacement should depend, therefore, on not only fish performance, but also N and P waste productions, and faeces stability, if environmental sustainable feeds are developed.

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Chapter 3

Comparison of faecal collection method with high and low quality diets regarding digestibility and faeces characteristics measurements in Nile tilapia.

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Abstract

The need for unbiased digestibility estimation has led to the development of a number of faeces collection methods. However, there is still a large variation in apparent digestibility coefficient (ADC) of diet/ingredients between these methods. This study investigated the impact of dietary quality on ADC and faeces recovery measurements obtained by two faecal collection methods (Choubert and settling tank). The fish were fed five diets: a control diet as high quality diet, two levels of dried duckweed (20 and 40%) and two levels of fresh duckweed (20 and 40%), which were added to the control diet, as low quality diets. ADC estimates were highly correlated (r> 0.95) between both faecal collection methods. For all diets the ADC estimates were higher when using settling tanks compared to Choubert collectors. For the control diet differences in ADC between two collectors were small, but by inclusion of duckweed these differences increased. The recovery % of faeces was not correlated between both faecal collection methods (r=0.22, *P*=0.41). The estimated recovery % of faeces by settling tanks was about three times higher than by Choubert. In conclusion, the differences in ADC and faeces recovery measured by two faecal collection methods depend on diet quality.

Introduction

Unbiased digestibility estimations of ingredients are essential for proper diet formulation (e.g., least cost formulation) and wastewater management (e.g., controlling/predicting faecal waste production) in aquaculture. In fish, digestibility measurements are difficult. This is reflected in the large variation in apparent digestibility coefficients (ADC) of ingredients between different studies. The applied method of faecal collection is considered as a major source for this large variation in ADC (Vandenberg and De La Noue 2001). The various faecal collection methods can be classified into three main groups (Vandenberg and De La Noue 2001): 1) collection of faeces before defecation (i.e., no contact of faeces with water) (Nose 1967; Austreng 1978; Windell et al. 1978); 2) collection of faeces from the water by a settling column (Spyridakis et al. 1989); and 3) continued removal of faeces from the water outlet by sieving (e.g., Choubert et al. 1979, 1982). The main disadvantages of direct faeces collection from the intestine (first group) are: the removal of digesta before being completely digested; and contamination

of faeces (e.g., with blood, slime, intestinal tissue, sperm and/or eggs). Both techniques cause an underestimation of ADC. Indirect faecal collection methods (second and third group) can give biased ADC estimates due to disintegration/separation of faeces and leaching of nutrients and/or indicator from the faeces. Numerous comparisons of faecal collections methods for ADC measurements have been done (e.g., Hajen et al. 1993; Storebakken et al. 1998; Weatherup and McCracken 1998; Vandenberg and Noue 2001; Hemre et al. 2003). Most comparisons were done using high quality diets (i.e., high digestibility) with a high percentage of fishmeal and fish oil. It can be hypothesized that differences in ADC between faecal collection methods depend on dietary quality (i.e., digestibility). Especially for the indirect methods, dietary composition may affect the disintegration rate of the faeces and/or the effectiveness of the collection of faeces from the water. Subjective observations of Dias et al. (1998) indicate that plant ingredients can affect the faeces physical properties (e.g., firmness, viscosity, cohesion and settling characteristics).

In addition to the amount of faeces produced, which depends on ADC, the removal efficiency of faeces from the water is important for water quality management. Chemical and physical characteristics of faeces, and thus probably dietary composition, determine the solid removal efficiency (Han et al. 1996; Dias et al. 1998; Cripps and Bergheim 2000). Total faeces recovery in combination with estimation of ADC using indirect faecal collection (e.g., screening, sedimentation) enables the estimation of the recovery % of faeces, being a measure for the solid removal efficiency of faeces. Information on the impact of dietary factors and the method of indirect faecal collection on faecal recovery is scarce.

Therefore, the effect of dietary quality on ADC and faecal recovery % estimates obtained by two types of faecal collection methods (Choubert versus settling tank) were assessed in Nile tilapia (*Oreochromis niloticus* L.).

Material and Methods

General

The current study was integrated in another study on the nutritional quality of duckweed for tilapia (El-Shafai et al.2004). Five diets were compared: a control diet and four diets with duckweed (*Lemna minor*) (Table 1). Two forms of duckweed, fresh (FDW) or dried (DDW) were included at two levels into the control diet, 20 or 40% (on a dry matter basis). Control diet and the DDW diets were pelleted. FDW was added separately to the tanks after the rest of the pelleted diet was fed. At all dietary treatments, fish were fed the same amount of control diet. In addition to the apparent digestibility (ADC) measurement by Choubert collectors (El-Shafai et al. 2004), ADC was estimated by settling tanks and also the faeces recovery % by both collection methods is reported in the current study. For a detailed description of the experimental design, see El-Shafai et al. (2004).

The experiment was carried out in accordance with Dutch law on the use of experimental animals and was approved by the ethical committee for the use of experimental animals of Wageningen University.

Table 1. The percentage of ingredients used in the control and experimental diets (Control: C; Dried duckweed: DDW; Fresh duckweed: FDW)

	Diet				
Ingredients	Control	C+20% DDW	C+40% DDW	C+20% FDW	C+40% FDW
Fishmeal	35	27.78	20.57	27.79	20.58
Dried duckweed[1]	0	20	40	0	0
Fresh duckweed[1]	0	0	0	20	40
Maize	29	23.02	17.04	23.02	17.05
Wheat	20	15.88	11.75	15.91	11.76
Wheat bran	10	7.94	5.88	7.94	5.88
Fish oil	3	2.38	1.76	2.38	1.76
Diamol	2	2	2	2	2
Premix[2]	1	1	1	1	1

[1] *Lemna minor*

[2] B-complex vitamin premix, kg^{-1} feed (Vit B1 30 mg; Vit B2 30 mg; Vit B6 30 mg; Vit B5 100mg; Vit B3 200 mg; Vit H 0.6 mg; B12 0.05mg; folic acid 15mg; Vit C 500 mg; Vit E 200 IU), Fat-soluble vitamin premix, kg^{-1} feed (Vit A palmitate 15000 IU; D-Rovimix (D3-500) 2000 IU; k3 8 mg), Macro-vitamin premix, kg^{-1} feed (inositol, 200mg; choline chloride, 1000 mg) and mineral premix, mg kg^{-1} feed (Fe 50, as $FeSO_47H_2O$; Zn 100, as Zn SO_47H_2O; Co 2.4, as $CoSO_47H_2O$; Cu 5, as $CuSO_45H_2O$; Se 1, as Na_2SeO_3; Mn 25, as $MnSO_44H_2O$; Mg 300, as $MgSO_47H_2O$; Cr 1, as $CrCL_36H_2O$; I 5, as $CaIO_36H_2O$).

Fish and housing conditions

For this study, 640 juvenile Nile tilapia with a mean initial weight of 90g were bred at the research facility. Fish were randomly assigned to one of sixteen 70-L glass aquaria (40x50x35 cm), which were all on the same recirculation system (El-Safai *et al*. 2004). The five diets were randomly assigned to 16 tanks with for tanks for control diet and three tanks for duckweed based diets. Dissolved oxygen was checked daily and ammonia, nitrite and nitrate were monitored three times per week. Those parameters were kept within optimal range for Nile tilapia. Water flow through each tank was 6 L min^{-1} and water temperature was kept at 28°C.

Faeces collection methods

In this study, two indirect faeces collection methods were compared: Choubert collectors versus settling tanks. After a 2-wk preliminary period, during which fish were allowed to adapt to the diets and the housing conditions, faeces was collected during a 5-wk period. Alternating over time, faeces from each tank was either collected by Choubert collectors or by settling tanks.

The Choubert collectors with a mesh size of 1000 µm collected faeces continuously (Choubert et al. 1982). The outflow of water from each tank flowed through metallic screens that separated faeces from the water and retained faeces in a metal tray. The settling tank used was a commercial available type, AquaOptima (AquaOptima AS, Trondheim, Norway) with a volume of 17-L and a column height of 44.0 cm and a diameter of 24.5 cm. At the bottom of the settling tank, a tap was placed at which a bottle was attached. The sampling bottles were continuously submerged into ice water to prevent bacterial decay of faeces during the collection period.

From both collection systems, faeces was collected twice a day (09.00 and 17.00 h) and stored at –20°C until analyses. Samples per collection system were pooled per tank.

Digestibility measurements

ADC of nutrients was determined by the indicator method using acid insoluble ash (AIA) as an inert marker. The AIA content of the diets was increased by adding Diamol (Diamol GM, Franz Bertram, Hamburg, Germany) (Table 1).

The five diets were ground using a 1 mm screen and thoroughly homogenized before samples for analyses were taken. Frozen faecal samples were thawed before further analyses. Chemical analyses were done in triplicate. Feed and faeces were analyzed for dry matter by drying for at least 4 h at 103°C until constant weight (ISO, 1983), for crude ash content by incineration in a muffle furnace for 4 h at 550°C (ISO, 1978) and for AIA content by dissolving the remaining ash after incineration in hydro chloric acid (ISO, 1998). Kjeldahl nitrogen was determined according to ISO (1979) using a Tecator 2020 Digestor at 400°C for 4 h and distillation by Tecator Kjeltec Autosmpler system 1035 Analyser (Tecator AB, Hoganas, Sweden). Crude protein was calculated as nitrogen content times 6.25. Organic matter was derived as dry matter minus crude ash content.

ADC of nutrients (dry matter, organic matter and crude protein) was calculated according to:

$$ADC_{nut} = (1 - [AIA_{diet}/AIA_{faeces} \times Nutr_{faeces}/Nutr_{diet}]) \times 100$$

Where ADC_{nut} = apparent digestibility coefficient of the nutrient; AIA_{diet} = dietary AIA concentration; AIA_{faeces} = faecal AIA concentration; $Nutr_{diet}$ = dietary concentration of nutrient; and $Nutr_{faeces}$ = faecal concentration of nutrient.

Faeces recovery measurements

The recovery % of faeces by both collection methods is the percentage of total faeces collected as percentage of the total amount of faeces excreted on a DM basis. The amount of excreted faeces was calculated from the measured digestibility. To estimate the recovery %, faeces were quantitatively collected and the total amount of dry matter collected was calculated. As the marker is homogeneously distributed in both feed and faeces, the recovery % was calculated, in the current experiment, as the amount of marker (AIA) recovered in the faeces divided by the amount of marker (AIA) provided by the feed. In this study faeces recovery was used as an indicator for the faeces stability. A

higher recovery % shows that the faeces particles are more firm and will disintegrate less quick by physical force (e.g., water flow) compared to low recovery %.

From the measured ADC and recovery %, the total amount of faeces produced, the amount of faeces recovered from the water and the non-recovered faeces (all expressed in g dry matter per kg of feed) was calculated. These calculations were done per aquarium for each collection method applied.

Statistical Analyses

Fish tank was considered as the experimental unit. Linear regression analyses were used to relate estimates of ADC and faeces recovery data between both collection methods applied (settling tank versus Choubert) using the GLM procedure of the SAS software (SAS, 1989).

Results

Apparent digestibility coefficients

ADC estimates were highly correlated between both faecal collections methods (Fig. 1). Pearson correlation coefficients were 0.952, 0.964 and 0.963 for ADC of crude protein, dry matter and organic matter, respectively ($P < 0.001$). The linear relationships between ADC by Choubert collectors (ADC_{CH}) and ADC by settling tanks (ADC_{ST}) were as follows for:

Crude protein $ADC_{ST} = 29.8 (\pm 5.4) + 0.68 (\pm 0.059) \times ADC_{CH}$ $r^2 = 0.91$

Dry matter $ADC_{ST} = 29.9 (\pm 3.7) + 0.66 (\pm 0.048) \times ADC_{CH}$ $r^2 = 0.93$

Organic matter $ADC_{ST} = 30.2 (\pm 3.9) + 0.66 (\pm 0.049) \times ADC_{CH}$ $r^2 = 0.93$

For all diets the ADC estimates were higher when using settling tanks compared to Choubert collectors. With the control diet the differences in ADC between both faecal collection methods were small. Inclusion of duckweed in the diets resulted in a decline in ADC of dry matter, crude protein and organic matter. With this decrease in ADC, the differences in ADC between the two faecal collection methods increased (Figure 1). This impact of dietary digestibility on the variation between both faecal collection methods was also reflected by the fact the coefficient of regression of ADC by settling tanks on

ADC by Choubert collectors differed from unity (0.68, 0.66 and 0.66 for crude protein, dry matter and organic matter, respectively).

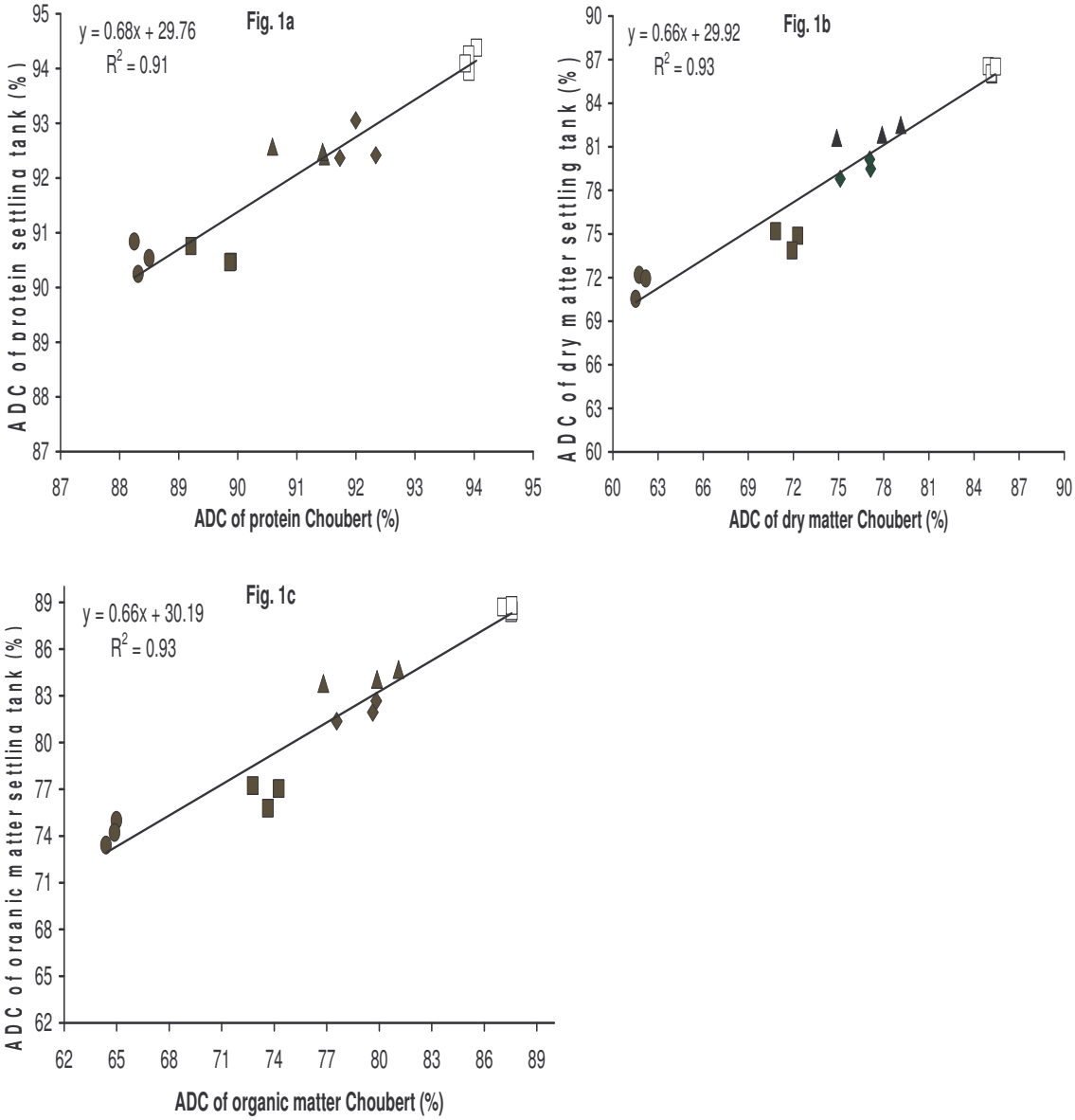

Figure 1. Comparison of apparent digestibility between Choubert and settling tank (all digestibility estimates expressed as percentage), Fig. 1a: Protein digestibility, Fig. 1b: Dry matter digestibility, Fig.1c: Organic matter digestibility.Diet symbols: □ = control; ▲= dried duckweed 20%; ■ = dried duckweed 40%; ♦ = fresh duckweed 20%; ● = fresh duckweed 40%.

Recovery % and faeces production

The total faecal waste production (expressed in dry matter per kg of feed) was also highly correlated between both faecal collection methods (r = 0.96; $P < 0.001$, Figure 2a). However, the recovery percentage of faeces was not correlated with both faecal collection methods (r = 0.22; $P = 0.41$; Figure 2b). Recovery percentage of faeces using settling tanks ranged between 39.7 and 69.0%, about three times higher than that estimated by Choubert collectors which ranged between 9.9 and 21.0%. Due to the higher recovery %, the amount of faeces recovered (expressed in dry matter per kg of feed) was higher when using settling tanks compared to Choubert collectors. Both the amount of recovered and non-recovered faeces (expressed in dry matter per kg of feed) were correlated with both collection methods (r = 0.73; $P < 0.001$, and r = 0.89; $P < 0.001$, respectively; Figure 2c and d).

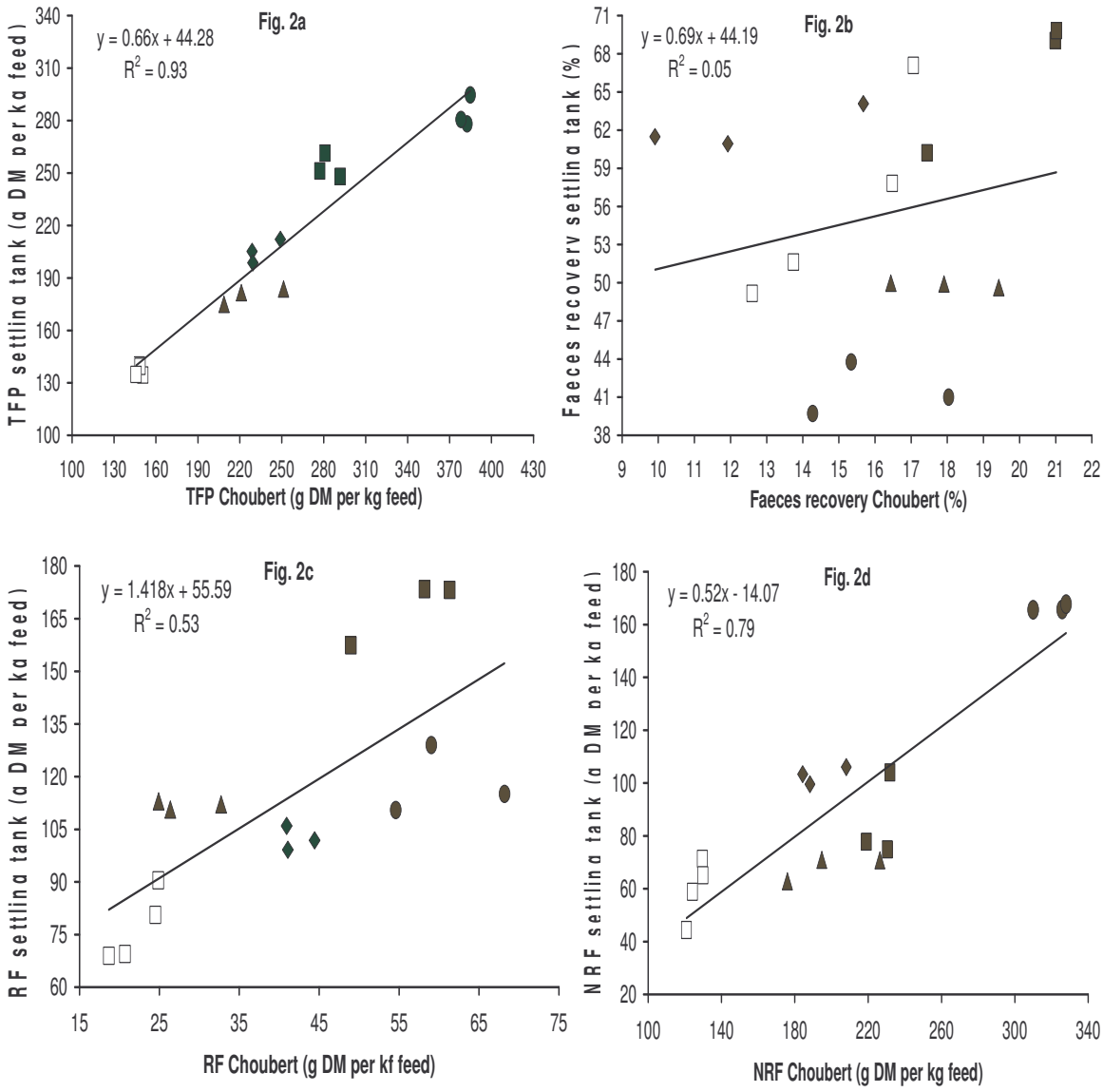

Figure 2. Comparison of recovery % and faeces production between Choubert system and settling tank: Fig. 2a: Total faeces production (TFP), Fig. 2b: Recovery %, Fig. 2c: Recovered faeces (RF), Fig. 2d: Non-recovered faeces (NRF). TFP, RF and NRF based on calculated digestibility of dry matter and recovery % and expressed as g/DM/per kg of feed.Diet symbols: □ = control; ▲= dried duckweed 20%; ■ = dried duckweed 40%; ♦ = fresh duckweed 20%; ● = fresh duckweed 40%.

Discussion

The aim of the current study was to assess the effect of dietary quality on ADC and faecal recovery % estimates obtained by two types of faecal collection methods (Choubert versus settling tank) in Nile tilapia (*Oreochromis niloticus* L.). The used settling tanks had a calculated hydraulic load of 0.222 cm S^{-1} and a retention time of 2.8 min at a water flow rate of 6 L min^{-1}. According to the predicted settling velocity figure of Timmons et al. (2001), which was adapted for tilapia assuming a specific gravity of tilapia faeces of 1.05 g/ml, particles with a size larger than 0.15 mm would have settled in the used settling tanks with the applied water flow. The Choubert collectors, which were used, had a mesh size of 1000 µm. The difference in particle size, being separated by both applied faecal collection methods, is probably the major reason for the large differences in recovery % observed in the current study (Figure 2). The recovery % by Choubert collectors in the current study (ranging from 9.9 to 21.0%) was very low compared to the recovery of 99% found by Choubert et al. (1982) in rainbow trout, *Oncoryhnchus mykiss* (Walbaum),. However, the range found in this study is in line with observations in Nile tilapia (Schneider et al. 2004; ranging from 7 to 11%) and in African catfish, *Clarias gariepinus*, (Jhari-Mangle 2001; ranging from 1 to 12%). Jhari-Mangle (2001) also showed higher recovery % by settling tanks (ranging from 10 to 36%) than by Choubert collectors.

The current study demonstrates that apparent digestibility coefficients obtained by both Choubert collectors and settling tanks are highly correlated (Figure 1) for Nile tilapia. This indicates that the ranking of diets (or ingredients) regarding their ADC is independent of the faecal collection method. However, the ADC estimates were always lower when using Choubert collectors compared to settling tanks (Figure 1). This is in line with the results of Spyridakis et al. (1989) in sea bass (*Dicentrarchus labrax*) and Vandenberg and Noue (2001) in rainbow trout. The current study also demonstrates that the dietary composition, i.e., the quality of the diet in terms of digestibility, influences the differences between the faecal collection methods. If both methods had been compared only with a high digestible diet in this study, such as the control diet, the results would have suggested that both methods would give similar ADC's. With poor digestible diets (high percentage of duckweed), the differences between the estimates of ADC became

larger between both faecal collection methods. Since in the future the inclusion of various types of plant ingredients and other alternatives for fishmeal and fish oil will increase (El-Sayed 1999), it should be realized that validation of digestibility methods with high quality diets might not be applicable to lower quality diets.

Unbiased ADC estimates of ingredients are essential for practical diet formulation regarding nutritional requirements, least cost formulation and wastewater management (e.g., controlling/predicting faecal waste production) in aquaculture. Therefore, it is important to use a faecal collection method which provides more accurate ADC estimates. Indirect faecal collection methods, like Choubert collectors and settling tanks, may overestimate ADC because of supposed nutrient leaching from faeces and decaying/fermentation of faeces. Increasing the retention time of faeces in the water resulted in increased ADC of dry matter, crude protein and lipid in rainbow trout (Windell et al. 1978) and in Chinook salmon, *Oncorhynchus tshawytscha*, (Hajen et al. 1993). Observations by Spyridakis et al. (1989) and Cho et al. (1982) suggest that leaching is quantitatively less important than decaying of faeces, especially if faeces remain undisturbed in the water. The lower ADC values, together with the lower recovery % found in the present study by Choubert, suggests that the marker may have separated from faeces during collection resulting in an underestimated ADC. This does not mean that a settling tank would yield accurate ADC values, but the higher recovery and the faeces remaining undisturbed in water probably provide more representative results. Moreover, the slopes of regression of ADC by settling tank on ADC by Choubert were almost similar for crude protein, dry matter and organic matter (Figure 1). This supports the idea that leaching of nutrients from faeces collected by a settling tank is not an important reason for an overestimation of ADC. If the leaching had a large impact on ADC, the ratio of leaching would probably not be similar for all three measured values (dry matter, crude protein and organic matter).

Stability and consistency of faeces are important factors for waste and water quality management in aquaculture systems. Recovery % of faeces from the water is therefore important. Recovery % was measured by two different collection methods. There was no correlation between the estimates of recovery % by both methods. This may partly be explained by the very low recovery % obtained with the Choubert collector. However, it

should be also realised that the results of both collection methods are related to different physical aspects of faeces. Collection by Choubert is based on the size of the faeces particles. In contrast, in the settling tank the collection of faecal particles is based on the hydraulic surface load, which is a combination of particle size as well as specific gravity. This difference between faecal collection methods may have led to the absence of a correlation between recovery % of faeces. In this study, two experimental rations contained fresh duckweed (20 or 40% of the total ration on dry matter bases). Visually no refusals of fresh duckweed were observed. However, uneaten fresh duckweed would be collected by Choubert collectors, but not by the settling tank due to floating capacity of duckweed. When excluding the data from the fresh duckweed diets, a higher correlation between the recovery % of faeces estimated by both collection methods ($r = 0.64$; $P < 0.05$) was obtained.

Conclusion

This study indicates that the validation of digestibility method with high quality diets might not be applicable to lower quality diets. The large difference in ADC obtained between the methods with poor quality diets suggests that the faeces collection method should be chosen in relation to dietary quality. The different faecal separation rate of each apparatus has probably contributed to differences in particle size being separated by the apparatus. The data suggest that a settling tank with higher recovery and faeces remaining undisturbed in water provide more accurate results.

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Chapter 4

Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus* L.)

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Abstract

Physico-chemical properties of nutrients influence physical characteristics of faeces and thus may affect waste removal efficiency. The aim of this study is to assess the effect of type of non-starch polysaccharide (NSP) on digesta viscosity, faeces recovery and nutrient digestibility in Nile tilapia. Insoluble (cellulose) and soluble (guar gum) NSPs were included separately and combined at a level of 8%, thereby formulating four experimental diets. The diets were assigned to 16 tanks with 35 fish each, with four replicates for each diet. Cellulose inclusion did not influence digesta viscosity, growth and digestibility of protein and starch and tended to increase faeces recovery (P=0.06). Guar gum inclusion increased digesta viscosity and reduced growth and digestibility of protein, fat and starch (P<0.01). Faeces recovery was reduced by 42% in diets containing guar gum. There were interaction effects (P<0.05) between cellulose and guar gum for growth and feed conversion ratio, indicating that cellulose alleviated the negative impact of guar gum. In conclusion, dietary soluble NSPs increase organic matter load in the culture system through a reduction of faeces recovery and nutrient digestibility, whereas insoluble NSPs improve removal efficiency of particles by increasing faeces recovery.

Introduction

Just as other animal production systems, aquaculture generates waste. The accumulation of pollutants can be harmful to fish and cause disease. For instance, solids which mainly come from faeces promote gill damage and reduce fish resistance to disease (Wickins, 1980). There has been a growing consensus that waste production in aquaculture should be reduced to minimize the negative impacts on the environment and maintain a better water quality for fish. Since feed is the major source of waste in aquaculture, the management of aquaculture waste should be approached through diet composition or feeding strategy.

In the past ten years, researchers have tried to reduce waste through diet formulation. They have used highly digestible protein and /or lipid ingredients (mostly fishmeal and fish oil) and excluded poorly digestible ingredients such as grain (Nijhof 1994; Cho et al. 1994; Cho and Bureau 1997). However, this alternative is not sustainable due to the expected limited availability of fishmeal in the future (Hardy 1996).

An increased consistency of faeces may improve their removal efficiency. The higher consistency of faeces may be achieved by lowering water pressure on faeces. A high water flow can break faeces into small particles, thereby increasing the proportion of dissolved and suspended solids.

Changing of faeces consistency may be also achieved by adding an indigestible nutrient such as dietary fibre, i.e. non-starch polysaccharides (NSP). NSPs can be divided into two groups: soluble and insoluble NSPs. Soluble NSPs (e.g., guar gum) form a network with water and are associated with higher viscosity of the intestinal content (Almirall et al. 1995; Choct et al. 1996). Insoluble NSPs (e.g., cellulose) behave like a sponge and their effects on digesta viscosity are relatively low (Smits and Annison 1996).

Most studies concerning the use of NSP (cellulose, guar gum, CMC) for fish (tilapia and trout) reported a growth depression and reduction of feed intake when the dietary NSP level exceeded 10 % (Hilton et al. 1983; Dioundick and Stom 1990 and Shiau and Liang1994). Moreover, the inclusion of alginate and guar gum (2.5-10%) in rainbow trout diets lowered protein and lipid digestibility (Storebakken 1985). By contrast, feeding red sea bream with carboxymethylcellulose (soluble NSP) supplementation at a level of 3, 6, 9 or 12% improved growth and feed efficiency (Morita et al. 1982). Dias et al. (1998) showed that adding two levels (10 and 20%) of cellulose did not affect digestibility of protein, growth and feed utilisation in sea bass. It seems that the physiological effects of NSPs in fish are not fully understood. Furthermore, introducing NSPs change the characteristics and recovery percentages of faeces. Dias et al. (1998) observed that the dietary cellulose incorporation increases faeces firmness. Storebakken (1985) reported that water content of faeces in rainbow trout was increased by gaur gum. While some literature is available on the effect of NSPs on digestibility and growth, there is little information on change of faeces characteristics by the use of NSPs.

The main objective of the present study is to determine the effect of type of NSP (soluble versus insoluble) on faeces recovery and to investigate how these two different types of NSPs influence digesta viscosity, nutrient digestibility and growth of Nile tilapia.

Materials and Methods

Experimental diets

In this study, two types of NSPs (cellulose and guar gum) and their combination (cellulose + guar gum) were tested in Nile tilapia. The inclusion of cellulose as insoluble NSP (0 versus 8 %) and guar gum as soluble NSP (0 versus 8%) were examined in a 2 x 2 factorial design. This led to four experimental diets which were formulated by replacing 0, 8 and 16% (cellulose + guar gum) of the basal diet with guar gum and/or cellulose.

The 0% NSP is considered as the control diet. The ingredient composition of the basal diet was similar for all four treatments. The formulation and chemical composition of the four experimental diets are presented in Table 1 and 2, respectively. The chemical composition of the control diet was slightly different from that of the experimental diets (Table 2), because of adding NSP to those diets.

All ingredients were finely ground, mixed and pelletized dry. Diamol was incorporated in the diets at a 2% inclusion level as a digestibility marker. Pellets were then dried in an air dryer at 80 °C and stored in a refrigerator until use.

Table 1. Percentage of ingredients used in the control diet and experimental diet

	Diet			
Ingredients	Control	Guar gum	Cellulose	Cell +Guar[i]
Fishmeal[a]	46.8	43.06	43.06	39.31
Soybean meal[b]	10	9.2	9.2	8.4
Maize[c]	30	27.6	27.6	25.2
Soya oil[d]	3	2.76	2.76	2.52
Palm oil	3	2.76	2.76	2.52
Cellulose[e]	0	0	8	8
Guar gum[f]	0	8	0	8
Calcium carbonate	3.5	3.22	3.22	2.94
Diamol[g]	2	1.84	1.84	1.68
Premix[h]	1	0.92	0.92	0.84
Monocalcium phosphate	0.7	0.64	0.64	0.59

[a]Danish herring meal, bran Skagen FF with a protein content of 72%
[b]Soybean meal Hipro, produced by Cargill Amsterdam, the Netherlands with a protein content of 46%
[c]Normal fed quality French maize
[d]Refined food quality Soya oil from Romi Smilfood bv Heerenveen, The Netherlands
[e]Arbocel B800, J. Rettenmaier und Sohne, Rosenberg, Germany
[f]Rudingom G555, (Product no. BG0380), Ruitenberg Ingredients, Amersfoort, The Netherlands. On product bases the composition of this guar gum is: dry matter 88%, crude protein 4%, fibres 82% and crude ash 1%. This guar gum is used in the food industry and is a cold soluble thickening agent, which give high viscosity at low dosing.
[g] Diamol GM, Franz Bertram, Hamburg, Germany
[h]Vitamin-mineral premix (Fe 50 mg/kg; Zn 100 mg/Kg; Co 100 mg/kg; Mg 300 mg/kg; Vit. B1 30 mg/Kg; Vit.B2 30mg/kg; Vit. B5 100 mg/kg; Vit. B3 200 mg/kg; Biotin 0.6 mg/kg).
[i]Cell+Guar = Cellulose + Guar gum

Experimental system and animals

The experiment was conducted for 8-weeks with tilapia with an initial body weight of 129 g. The fish were bred at the reproduction facility of the Fish Culture and Fisheries Group, Animal Sciences Department, Wageningen University, The Netherlands.

The fish were grown in the 16 glass aquaria (40x50x35cm), each with a volume of 70 litres, which were part of a closed recirculation system. The water was circulated through a bio-filter to reduce the ammonia concentration. The photoperiod regime during the experiment was 12 h light and 12 h dark.

Water quality was checked every day after the first feeding in the outflow of the system. The measured parameters were: temperature, conductivity, pH, oxygen content, $NH4^+$-N, NO_2^--N, and NO_3^--N. Altering over time, two different water flows (5 and 8 l/min) were supplied for all tanks alternately to measure the impact of water pressure on faeces recovery. The water flow for all tanks was 5 l/min for the first week. In the second

week, the water flow was raised to 8 l/min. The weekly change of water flow continued for four weeks of faeces collection. Water flow for each tank was checked daily.

Water quality remained within the appropriate ranges for fish growth during the experimental period. The water temperature was kept at 28±0.2°C during the experiment. Oxygen concentration was measured in a randomly selected tank by a digital oxygen detector and always remained above 4.5 $mg.l^{-1}$. The water pH ranged between 6.5 to 7.6 during the experiment. $NH4^{+}$-N, NO_2^{-}-N, and NO_3^{-}-N were respectively 0.45±0.18, 0.14±0.15, 112±55 $mg.L^{-1}$ over experimental period.

Experimental procedure

On day one of the experiment, fish were randomly divided over 16 tanks (35 fish per tank). The currently used stocking density was according to standard density for tilapia with an individual weight of 74.8 to 346g in commercial recirculation systems (Timmons et al. 2001). The fish were allowed to adapt to the experimental diets and to the feeding level for two weeks before the sampling period started.

Table 2. Nutrient composition of four experimental diets in g/kg or kJ/g based on dry matter basis

	Diet			
Dietary component	Control	Guar gum	Cellulose	Cell+Guar[a]
Organic matter (g/kg)	855.3	864.8	862.9	874.6
Crude protein (g/kg)	439.6	408.5	411.6	373.5
Crude fat (g/kg)	130.2	122.1	120.3	111.9
Starch (g/kg)	201.1	191.5	186.3	159.3
Sugar (g/kg)	16.8	24.3	21.7	40.5
Non-starch polysaccharide (g/kg)	67.6	118.4	123.0	189.4
Ash (g/kg)	144.7	135.2	137.1	125.4
Acid insoluble ash (g/kg)	16.7	15.8	16.1	15.0
Energy (KJ/g)	20.25	19.91	20.02	19.96

[a]Cell+Guar = Cellulose + Guar gum

The four diets were randomly assigned to 16 tanks with four tanks for each diet. The diets were given to the fish as soon as they were introduced into the tanks. The control groups received 80 g/day, the guar gum and cellulose groups received 86.9 g/day and cellulose + guar gum groups received 95 g/day. These different feeding levels were used to ascertain that all experimental groups received the same amount of basal diet (80

g/day). Consequently, the daily intake of crude protein, crude fat, starch, sugars and premix was similar between the experimental treatments (Table 3). The daily ration was divided into two feedings and fed by hand at 08:30 and 16:30. Before feeding, the pellets were sieved to remove dust and small particles.

Table 3. Daily amount of nutrient given per tank for four experimental diets in g/tank/day based on dry matter

	Diet			
Parameters	Control	Guar gum	Cellulose	Cell+Guar[a]
Daily feed given	73.0	78.9	79.8	87.0
Crude protein	32.1	32.2	32.8	32.5
Crude fat	9.5	9.6	9.6	9.7
Starch	14.7	15.1	14.9	13.9
Sugar	1.2	1.9	1.7	3.5
Non-starch polysaccharide	4.9	9.3	9.8	16.5

[a] Cell+guar= Cellulose + Guar gum

In this experiment, we used a settling tank for the collection of faeces. After a two weeks adaptation period, faeces were collected during a four weeks period. The settling tank used was a commercially available type, AquaOptima (AquaOptima AS, Trondheim, Norway) with a volume of 17L and a column height of 34.3cm and a diameter of 23.9cm. At the bottom of the settling tank, a tap was placed to which a bottle could be attached. During collection, the bottle was connected and the tap was open. Immediately after sampling the sampling bottles were submerged into ice water in order to prevent bacterial decay of faeces during the collection period. The whole content of bottle including water was collected twice a day (09.00 and 17.00 h) and stored at –20°C until analysis.

During the final week of the experiment, viscosity of digesta was measured. Fish were fed by automatic belt feeders and administration of feed was stopped two hours before sampling. Five fish were randomly selected from each tank and sacrificed by overdose (0.4 g/L) of TMS and bicarbonate (0.8 g/L). The intestine was removed and digesta collected from the proximal part (first one third of intestine). The digesta of five fish of a tank were pooled and centrifuged at 12,000 g for 10 minutes. Afterward, viscosity was immediately measured on the supernatant using a Brookfield LVDV-I+ cone/plate viscometer (Brookfield Engineering laboratories, Inc., Middleboro, MA, U.S.A). All

viscosity measurements were done at 28˚C and at shear rate of 75 to 750 S^{-1}. When measurable viscosity did not include 750 S^{-1}, data plotted as log (shear rate) versus log (absolute viscosity) giving a straight line from which the line could be extrapolated to 750 S^{-1}(Steenfeldt 2001).

Chemical analysis

Feed samples were collected at regular intervals (twice a week) during the experimental period and ground with a 1 mm screen before analysis. Freeze-dried faeces from each tank were ground with a coffee blender and thoroughly homogenised to collect representative sub-samples. All chemical analyses were done in triplicate. Feed and faeces were analysed for dry matter by drying samples for 4 h at 103°C until constant weight (ISO 1983). Ash content was determined by ashing the samples by incineration in a muffle furnace for 4 h at 550°C (ISO 1978). Acid insoluble ash was measured by dissolving ash in hydrochloric acid (ISO 1981). Crude protein (N x 6.25) was measured by the Kjeldahl method after acid digestion, according to ISO (1979). Lipid was extracted by petroleum ether extraction in a Soxhlet apparatus (ISO/DIS 1996). Starch was analysed according to the method described by Goelema et al. (1998). Sugar was calculated by measuring starch + sugar in samples and subsequent subtraction of the starch content of the samples. The analysis of starch + sugar was similar to starch measurement. The ADC of sugar was assumed 88% based on a pooled sample of four tanks. Energy content was measured by direct combustion in an adiabatic bomb calorimeter (IKA-C-7000, Fa. IKA- Analysentechnik, Weitersheim, Germany). Non-starch polysaccharides (NSP) were calculated according to the following formula: NSP= organic matter- (fat + protein +starch +sugar).

Calculations and statistical analysis

Digestibility calculation

Apparent digestibility coefficients (ADC) of nutrients in the diets were determined with the indicator method with acid insoluble ash as a marker. Apparent digestibility (%) for

nutrients in the diets is expressed as a fractional net absorption of nutrients from diets. ADC of nutrients was calculated according to:

$ADC_{nut} = (1 - [AIA_{diet}/AIA_{faeces} \times Nutr_{faeces}/Nutr_{diet}]) \times 100$

Where ADC_{nut} = apparent digestibility coefficient of the nutrient; AIA_{diet} = dietary AIA concentration; AIA_{faeces} = faecal AIA concentration; $Nutr_{diet}$ = dietary concentration of nutrient; and $Nutr_{faeces}$ = faecal concentration of nutrient.

Faeces recovery measurement

Faeces recovery percentage was calculated as the amount of marker (AIA) recovered in the faeces divided by the amount of marker (AIA) provided by the feed multiplied by 100. This calculation is allowed, because the marker was homogeneously distributed in both feed and faeces. Total faeces production is defined as absolute amount of faeces excreted by fish and calculated from the measured dry matter digestibility. Recovered faeces is the amount of faeces removed from the water by settling tank and calculated from faeces recovery percentage multiplied by total faeces production. Non-recovered faeces was calculated as the difference between total faeces production and recovered faeces.

Fish performance

Weight gain was determined by the difference between initial and final body weight. Specific growth rate was calculated from the natural logarithm of mean final weight minus the natural logarithm of the mean initial weight and divided by the total number of experimental days expressed as a percentage. Total feed intake per tank was calculated daily over the total experimental period. Daily growth per day was calculated by dividing the difference between initial and final body weight of all the fish from each tank by the number of experimental days. The feed conversion ratio was calculated per tank from feed intake data and weight gain.

Statistical analysis

Data are presented as means of each treatment with standard deviation. The data were verified for normality after transformation (ASIN). Preliminary analyses showed that

flow rate (5 versus 8 l/ min) and the interaction with flow rate did not affect ADC and the faeces recovery. Therefore, flow rate was omitted in the statistical analysis. All data were analyses by a 2 way ANOVA for the effect of guar gum supplementation (0 versus 8%) and the effect of cellulose supplementation (0 versus 8%). For all statistical analyses, one tank was considered as the experimental unit.

Results

Digestibility

Data on ADC in the experimental diets are presented in Table 4. There was no interaction between cellulose and guar gum in ADC estimates. Inclusion of cellulose or guar gum had a negative impact ($P<0.01$) on ADC of dry matter and organic matter. Addition of cellulose did not show any significant difference in ADC of ash, starch, protein and NSP but surprisingly cellulose supplementation slightly improved ($P<0.05$) fat digestibility. Guar gum inclusion increased the ADC of NSP, but reduced the ADC of other nutrients such as fat, starch, ash and protein ($P<0.01$).

Table 4. Apparent digestibility coefficient[a] of tilapia fed the experimental diets

	Diet				*P*-values of the factors		
Parameters	Control	Guar gum	Cellulose	Cell+Guar[b]	Cell	Guar	CellxGuar[c]
Dry matter (%)	80.0±0.5	71.1±3.0	75.2±1.0	67.3±0.8	0.001	0.001	0.619
Organic matter (%)	85.3±0.4	78.3±2.8	79.5±0.9	73.9±0.7	0.001	0.001	0.451
Crude protein (%)	91.6±0.3	84.7±3.2	91.6±0.2	85.9±0.4	0.422	0.001	0.421
Starch (%)	92.2±1.6	88.8±3.4	91.5±1.3	87.6±1.9	0.528	0.022	0.882
Crude fat (%)	95.8±0.4	93.5±2.3	96.6±0.3	94.8±0.4	0.029	0.001	0.538
Ash (%)	49.1±1.0	24.9±4.5	48.2±2.1	21.1±2.7	0.305	0.001	0.532
Non-starch polysaccharide (%)	1.7±5.1	20.8±7.2	2.8±2.1	22.5±2.0	0.471	0.001	0.874

[a]All values are means± standard deviation
[b] Cell+Guar = Cellulose + Guar gum
[c]CellxGuar = Interaction between cellulose and guar gum

In Table 5 the viscosity measurements of diets and digesta are shown. There was no interaction between cellulose and guar gum in digesta viscosity. Diets containing guar gum induced higher digesta viscosities compared to the control and cellulose diets.

These differences were not significant, due to the large variation in average digesta viscosity between the tanks.

Table 5. Viscosity measurements[a] of four experimental diets and digesta

	Diet				*P*-values of the factors		
Parameters	Control	Guar gum	Cellulose	Cell+Guar[c]	Cell	Guar	CellxGuar[d]
Diet viscosity (cP)[b]	0.9	42.9	0.9	71.7			
Digesta viscosity (cP)[b]	2.2±0.15	6.6±2.97	2.9[e]	10.2±8.7	0.587	0.169	0.718

[a]All viscosity values of digesta are means± standard deviation
[b]Centipoise
[c]Cell+Guar = Cellulose + Guar gum
[d]CellxGuar = Interaction between cellulose and guar gum
[e]Viscosity measurement for cellulose was based on one replication instead of four due to insufficient sample quantity

Faeces production and recovery

The percentage of faeces recovered by the settling tank is shown in Figure 1. There was no interaction between cellulose and guar gum on recovery. Inclusion of guar gum resulted in a significant lower recovery compared to diets without guar gum. There is a trend for higher recovery in cellulose based diet compared to diets without cellulose (*P*= 0.061).

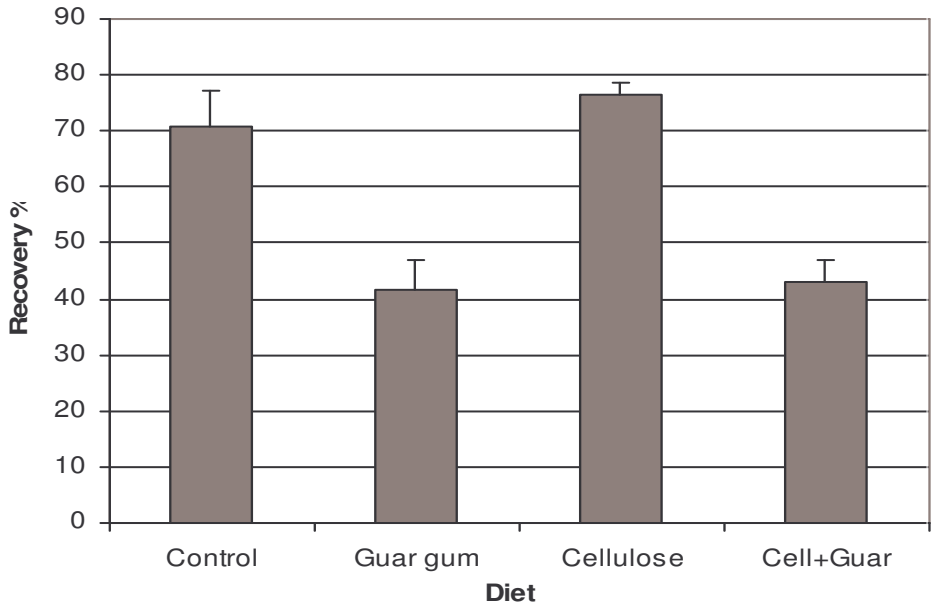

Figure 1. Faeces recovery values (%) of four experimental diets using settling tanks (Cell+Guar = Cellulose + Guar gum).The *P*-values for the main effect of cellulose, guar gum and their interaction were 0.061, 0.001 and 0.232 respectively

As shown in Figure 2, there was no interaction between cellulose and guar gum for total faeces production and non-recovered faeces. However, a significant interaction was present for the amount of recovered faeces (P=0.011), indicating that presence of cellulose influences the guar gum effect on recovered faeces. Both guar gum and cellulose increased faeces production (P<0.01) compared to diets without guar gum and cellulose. The amount of non-recovered faeces was higher (P<0.01) for guar gum based diets compared to diets without guar gum, whereas it was not influenced by cellulose supplementation.

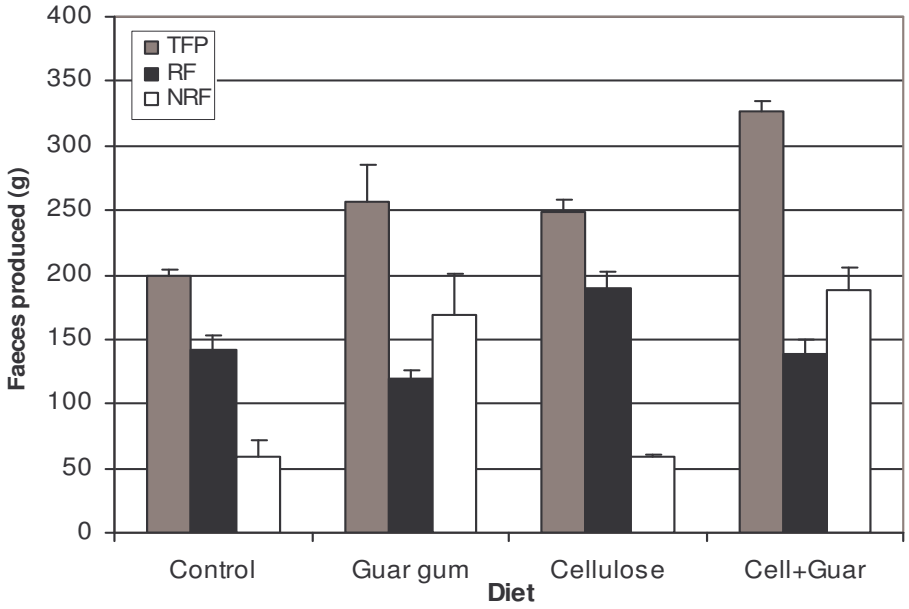

Figure 2. Total faeces produced (TFP), recovered faeces (RF) and non-recovered faeces (NRF) of four experimental diets, based on calculated digestibility of dry matter and recovery % and expressed as g/DM/per kg feed. (Cell+Guar = Cellulose + Guar gum)
The *P*-values for the main effect of cellulose, guar gum and their interaction were, respectively: 0.001, 0.001 and 0.623 for TFP; 0.001, 0.001 and 0.011 for RF; and 0.322, 0.001 and 0.323 for NRF.

Growth performance

There are significant interaction effects between guar gum and cellulose for specific growth rate and feed conversion ratio (Table 6). These interactions indicate that inclusion of cellulose alleviates the negative impact of guar gum.

Table 6. Feed conversion rate and growth performance[a] of tilapia fed the experimental diets

	Diets				*P*-values of the factors		
Parameters	Control	Guar gum	Cellulose	Cell+Guar[b]	Cell	Guar	CellxGuar[c]
Initial weight (kg)	4.45±0.16	4.52±0.17	4.55±0.14	4.56±0.11	0.399	0.573	0.673
Final weight (kg)	7.28±0.19	6.83±0.34	7.41±0.29	7.28±0.19	0.046	0.050	0.265
Weight gain (kg)	2.83±0.14	2.31±0.19	2.87±0.23	2.72±0.17	0.032	0.004	0.067
Growth (g/day/tank)	66.50±1.6	54.45±4.3	67.39±4.1	62.96±3.9	0.025	0.001	0.059
Specific growth rate (% BW/day)	1.16±0.05	0.97±0.04	1.15±0.05	1.08±0.06	0.066	0.0003	0.034
Feed conversion ratio	1.18±0.03	1.41±0.08	1.17±0.07	1.25±0.08	0.020	0.001	0.045
Survival rate (%)	100	100	100	100			

[a]All values are means± standard deviation
[b] Cell+Guar = Cellulose + Guar gum
[c]CellxGuar = Interaction between cellulose and guar gum

Discussion

Digestibility

Guar gum reduced the ADC of dry matter, organic matter, fat, protein and starch compared to diets without guar gum. This finding is similar to that of Storebakken (1985) and Fagbenro and Jauncey (1995) who observed a depression in ADC of dry matter, fat and protein in trout and tilapia that were fed diets containing guar gum. This is probably due to the increase in digesta viscosity by guar gum, which may bind the nutrients (Fagbenro and Jauncey, 1995) and limits diffusion of digestive enzymes (Choct et al. 1996). In chicken, soluble NSP reduce the ADC of starch, protein and fat due to an increased digesta viscosity (Choct et al. 1996; Refstie et al. 1999). Although in this study the difference in digesta viscosity between the treatments was not statistically significant, there was a trend for higher digesta viscosity with guar gum inclusion.

Apart from viscosity, other mechanisms can also influence nutrient digestibility. Refstie et al. (1999) observed reduced ADC of protein and dry matter in Atlantic salmon fed diets containing soybean products with a high soluble NSP content, with no associated effect on the viscosity of digesta. Refstie et al. (1999) suggested that an increased water content in the gut may have caused the reduction of ADC. Maisonnier et al. (2001) concluded that the low ADC of starch and lipid in chicken fed wheat (soluble NSP) could not be attributed only to intestinal viscosity. Other factors like an increased micro-flora population in the small intestine (Choct et al. 1996; Smits et al. 1998) may also have played a role. The stimulation of microbial growth by soluble NSP can produce toxins and deconjugation of bile salts, thereby reducing nutrient utilization (Choct 1997). It seems that the negative effect of guar gum on ADC is more severe in carnivorous and omnivorous species (e.g., rainbow trout and African catfish) than in herbivorous species (e.g., tilapia). The reduction in ADC of protein (10.4%) and fat (42.3%) by 8% guar gum inclusion compared to a control diet was fairly large for rainbow trout (Storebakken 1985) and African catfish (16.4 % reduction of ADC of protein) (Leenhouwers et al. 2004). However, in this study the reduction was considerably smaller (6.9% for ADC of protein and 2.3% for ADC of fat). This may be related to the ability of tilapia as a mainly herbivorous fish to tolerate higher levels of NSPs in diet.

Cellulose did not influence the ADC of protein and starch in tilapia. This is in agreement with findings of Dias et al. (1998) who observed that inclusion of up to 20 % of cellulose did not cause any difference in ADC of protein in sea bass. Similarly, Ulloa Rojas (2002) found that ADC of carbohydrate (starch and sugar) was not influenced by adding coffee pulp fibre (mostly cellulose) up to 106 g/kg in tilapia. Surprisingly, ADC of protein was increased by coffee pulp supplementation. Taken together, these results suggest that the addition of moderate amounts (up to 8%) of cellulose into diets does not reduce ADC of nutrients in tilapia.

Guar gum is digested at a higher rate than cellulose. Soluble NSPs are more degradable than insoluble ones (Choct et al. 1996). Slow movement of digesta in the intestine could create an ideal environment for bacterial activity and may explain the higher levels of ADC of soluble NSP (guar gum) in this study. Storebakken (1985) found that guar gum

decreased gut emptying rate in rainbow trout. Similarly Van der Klis et al.(1993) reported that carboxymethylcellulose increased the retention time of digesta in chicken. Contradictory results were reported by Shiau et al. (1988) who found that carboxymethylcellulose (a soluble NSP) increased the gastric emptying rate of tilapia. On the other hand, retention of faeces in the settling tank may have led to a higher rate of leaching of guar gum and thereby an overestimation of guar gum digestibility. More research is needed to ascertain that guar gum can be degraded in the intestine of tilapia.

Faeces production and recovery

The present results suggest that the type of NSP influences the faeces stability. Addition of a soluble NSP (guar gum) resulted in a low faeces recovery percentage. This finding is in agreement with results reported by Han et al. (1996) who found that inclusion of guar gum in the diet of tilapia led to inconsistent faeces with a small particle size. Faeces recovery in this study is used as an indicator for faeces stability. A higher faeces recovery indicates that faeces is relatively more resistant to water flow resulting in less non-recovered faeces.

Guar gum has a high viscosity and consists of long chain macromolecules. It acts as a binder in fish feed. It appears that the high water binding capacity of guar gum increases the viscosity of digesta and affects the faecal weight and volume by causing diarrhoea-like faeces that easily dissolves in water. Storebakken (1985) observed that the water content of faeces in rainbow trout was increased by guar gum inclusion. In addition, the increased dietary inclusion of soluble NSP may cause an osmotic effect as a result of the formation of low-molecular weight fermentation products (especially lactate). These products contribute to osmotic pressure of the intestine content and result in more moist faeces (Vernia et al.1988). In chicken, supplementation of soya sources with high soluble NSP resulted in wet and sticky faeces (Refstie et al. 1999). Similarly, in chicken Svihus et al. (1997) found that viscous feed ingredients were associated with a low content of dry matter of the faeces.

Cellulose has been commonly used as a non–nutritive filler in fish feeds to provide a water resistant feed and faeces (Vens-Cappell 1985; Dias et al. 1998). The inclusion of cellulose was proposed to enhance the faeces stability by providing a non- fermentable

and insoluble NSP (Kihara and Sakata 1997). However, results of present study revealed only a trend for higher faeces recovery in cellulose based diets compared to diets without cellulose. This may be due to supplementation of 30 % maize as a feed ingredient in all diets. Maize contains a high level of insoluble NSP (Bach-Knudsen 1997) and may have increased faeces recovery in the control diet, thereby masking a possible effect of cellulose on faeces recovery.

Although cellulose inclusion, in this study, increased the total faeces production, the amount of non-recovered faeces was not influenced by cellulose supplementation. Unlike cellulose, guar gum inclusion resulted in a larger amount of non-recovered faeces. An increase in non-recovered faeces enhances organic matter load in water, thereby reducing the efficiency of the water treatment facility and suspended solids removal in aquaculture. This condition can lead to a larger amount of water exchange and a higher leaching of nutrients (N and P) from faeces into the environment.

It appears that the increase of total faecal waste of the fish fed on the diets containing cellulose and guar gum, in this study, is a major drawback in terms of environmental pollution. In the future, the level of plant ingredients in fish feed is likely to increase. This situation may lead to an increased level of NSP in fish feed. Although the level of NSP applied in this study is too large to use in commercial diets, it may provide insight in the settling dynamics of faeces coming from different NSP and thereby may help in setting up an effective treatment strategy for fish farms.

Conclusion

The results obtained in the current study demonstrate that an increased digesta viscosity by soluble NSPs results in a lower growth and ADC of nutrients in tilapia. Cellulose is an inert additive and has no adverse effect on growth and ADC of fat, protein and starch. Cellulose may reduce the anti-nutritive effects of viscous NSPs.

The positive effect of cellulose on faeces recovery might have been present, but was probably masked by inclusion of insoluble NSP-rich ingredients (i.e., maize) in the control diet. Because of its high water binding capacity, guar gum reduces faeces stability and appears not to be an appropriate binder for improving faeces recovery. Further investigations are needed to verify the viscous effect of NSPs on digestive

processes and to understand the mechanism underlying the effect of different type of NSPs on faeces recovery.

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Chapter 5

Effect of type and dietary inclusion level of starch on the characteristics of digesta and faeces in Nile tilapia (*Oreochromis niloticus* L.)

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Submitted

Abstract

Higher faeces removal efficiency can help and reduce the negative impact of faecal waste on water quality and on the environment. Physicochemical properties of feed ingredients/nutrients may change the faeces characteristics and the ratio of removed to non-removed solids. The aim of this study was to assess through which mechanism physicochemical properties of the diet (viscosity, fermentation and dry matter) influence faeces characteristics and faeces removal (i.e., faeces recovery). Four experimental diets were formulated by addition of two types (gelatinized and native) and two levels (high and low) of starch to a basal diet according to a 2x2 factorial design. The diets were assigned to 16 tanks with 40 fish each, with four replicates for each diet. Replacement of native starch by gelatinized starch improved faeces removal percentage, growth and digestibility ($P<0.01$), but reduced fermentation ($P<0.05$) at the end of the intestine. Addition of gelatinized starch did not change viscosity and dry matter of the digesta at the end of the intestine. A high dietary inclusion level of starch also increased digestibility, growth and faeces removal percentage ($P<0.05$). Fermentation and dry matter content at the end of the intestine were not influenced by a high starch inclusion level, but viscosity was higher at the high level of starch inclusion. Volatile fatty acid levels in the stomach of tilapia were high in the treatments with gelatinized starch. In conclusion, intestinal fermentation induced by native starch had a negative impact on faeces removal efficiency. A higher inclusion level of starch resulted in a higher viscosity of the digesta, leading to higher faeces removal efficiency.

Introduction

Management of waste discharged from fish farms is one of the major concerns for further development of aquaculture (Naylor et al. 2001). Accumulation of pollutants such as solids deteriorates water quality (Amirkolaie et al. 2005) and can increase the incidence of disease in fish (Losordo et al. 1999). Farm effluents have to be treated or discharged to the environment. Treatment of wastewater demands large investments and sophisticated equipment. Discharge of wastewater can cause environmental pollution (Pillay 1992; Cripps and Bergheim 2000). The majority of the solid wastes of a fish farm are constituted by fish faeces (Franco-Nava et al. 2004). Therefore, the environmental impact

of fish farms can be reduced by: 1) reducing the amount of faeces produced (e.g., improving the digestibility of feeds) and 2) improving the removal of faecal waste.

Previous studies have shown that feed composition can alter the physical properties of faeces (Han et al. 1996; Dias et al.1998; Amirkolaie et al. 2005), thereby influencing the efficiency of solid waste removal (Amirkolaie et al. 2005). Dias et al. (1998) found in European seabass that dietary cellulose increased faeces firmness. Dietary inclusion of guar gum reduced faecal removal efficiency (i.e., faecal recovery) and increased the amount of non-settleable solids in Nile tilapia (Amirkolaie et al. 2005). Furthermore in Nile tilapia guar gum was partially fermented and increased the digesta viscosity (Amirkolaie et al. 2005). In rainbow trout, guar gum increased the water content of the digesta (Storebakken 1985). The mechanism by which guar gum reduces the faecal removal efficiency is still unclear, but it might be due to alterations in: 1) digesta/faeces viscosity; 2) fermentation; and 3) digesta/faeces water content.

Starch is a cheap source of energy and its inclusion in the feed influences faeces stability (Han et al. 1996). Addition of 19% gelatinized starch increased particle size of faeces in tilapia (Han et al. 1996). On the other hand, dietary starch also increased gut microbial activity in tilapia (Kihara and Sakata 1997), and this may have a negative effect on faeces stability (Fahey et al. 1990). These different observations of dietary starch in relation to faecal stability might be due to differences in the type of starch (gelatinized versus native). Native starch, which has a lower digestibility than gelatinized starch (Gallego et al. 1994; Stone et al. 2003), may increase intestinal microbial activity (Ferguson et al. 2000). Therefore, it can be hypothesised that the effect of dietary starch on the physical characteristics of digesta and faeces (e.g., solubility, viscosity and water holding capacity) and thereby also on faecal stability and its removal efficiency, is dependent on the type of starch.

In this study the mechanism through which dietary composition can influence faecal removal efficiency (faecal stability) is assessed. The effect of the amount and of the type of dietary starch on digesta and faeces viscosity, dry matter and volatile fatty acid content was studied. The two types of starch tested were gelatinized maize starch and native maize starch. These two types were chosen because they were supposed to induce different fermentation activities in the gut. In this way, the intended design would enable

to determine the impact of fermentation on faecal waste production. The Nile tilapia (*Oreochromis niloticus* L.) was used as a model species in this study.

Materials and Methods

This experiment was approved by the Ethical Committee Judging Animal Experiments (DEC) of Wageningen University.

Experimental diets

Four experimental diets (abbreviated as "GEL-LOW", "NAT-LOW", "GEL-HIGH" and "NAT-HIGH"; Table 1 and 2) were formulated according to a 2 × 2 factorial design. The first factor was the type of starch included in the diet: gelatinized maize flour ("GEL"; Suprex Corn, Cordico BV., Rotterdam, The Netherlands) or native maize flour ("NAT", Cordico BV., Rotterdam, The Netherlands) (Table 1). The gelatinized maize flour was grinded and pre-gelatinized by extrusion, whereas the native maize flour was only grinded. The second factor was the inclusion level of starch in the diets: "LOW" versus "HIGH". Fish at all dietary treatments received the same amount of gross energy and crude protein (Table 3). Therefore, maize flour was exchanged at the HIGH starch diet by plant oils in the diet (Table 1). At the LOW and HIGH starch diets, the amount of maize flour fed per fish was respectively 0.4 and 0.8 g/d (Table 3). Diets were pelleted without adding steam at a temperature < 65°C to prevent gelatinization of the native maize starch. Thereafter, pellets were cooled, dried in an air dryer with ambient air and stored at 4°C until use.

Table 1. Ingredient composition of the experimental diets on percentage dry matter basis

	Diets			
	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH
Type of maize flour	Gelatinized	Native	Gelatinized	Native
Inclusion level:	Low	Low	High	High
Ingredients:				
Gelatinized maize flour[a]	20	--	35.87	--
Native maize flour[b]	--	20	--	35.87
Fishmeal[c]	40	40	35.87	35.87
Soybean meal[d]	24.5	24.5	21.97	21.97
Soya oil[e]	4.5	4.5	0.0	0.0
Palm oil[f]	4.0	4.0	0.0	0.0
Premix	1.00	1.00	0.90	0.90
$CaCO^3$	3.30	3.30	2.96	2.96
$CaPO^4$	0.70	0.70	0.63	0.63
Diamol[g]	2.0	2.0	1.79	1.79

[a]Suprex Corn < 300 Plata (Article number 15001), produced by Codrico BV, Rotterdam, The Netherlands. This maize flour is pre-gelatinized by extrusion, has a granulation (%) with a size above 300 µm of < 5% and with 85% of the starch being gelatinized. On dry matter basis it has the following composition: fat 2.5%; starch 85%; crude fibre 0.6%; crude protein 7.0% and ash 0.6%.
[b]Maize flour Plata/Euro (Article number 11105), produced by Codrico BV, Rotterdam, The Netherlands. This maize flour is only grinded, has a granulation (%) with a size above 500 µm of < 5%. On dry matter basis it has the following composition: fat 2.5%; starch 80-90%; Crude fibre 0.6%; crude protein 7.0% and ash 0.6%.
[c]Danish herring meal, bran Skagen FF with a protein content of 70%
[d]Soybean meal Hipro, Produced by Cargill, Amsterdam with a protein content of 47%
[e]Refined soya oil, Produced by Romi Smilfood, Heerenveen, The Netherlands
[f]Refined palm oil , Produced by Romi Smilfood, Heerenveen, The Netherlands
[g] Diamol GM, Franz Bertram, Hamburg, Germany

Table 2. Nutrient composition of the experimental diets in g/kg or kJ/g based on dry matter basis

	Diets[a]			
Dietary component	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH
Dry matter (g/kg)	870.7	869.2	878.3	872.9
Organic matter (g/kg)	864.5	865.9	877.9	877.4
Crude protein (g/kg)	458.0	458.2	428.8	430.9
Crude fat (g/kg)	148.2	148.1	52.0	53.5
Starch (g/kg)	181.8	177.3	309.0	296.0
Ash (g/kg)	135.5	134.1	122.1	122.6
Acid insoluble ash (g/kg)	18.1	17.9	16.5	16.1
Energy (KJ/g)	13.6	13.3	12.1	12.5

[a]Full abbreviation see Table 1.

Experimental system and animals

Nile tilapia (*Oreochromis niloticus* L.) with an initial body weight of 45 g were used. The fish were bred at the reproduction facility of the Aquaculture and Fisheries Group, Department of Animal Sciences, Wageningen University, The Netherlands.

Fish were kept in 16 glass 70-L aquaria (40x50x35cm) on the same recirculation system at a stocking density of 40 fish per aquarium. The photoperiod regime during the experiment was 12 h light and 12 h dark.

Water quality parameters were checked three times per week after the first feeding. The measured parameters were: temperature, conductivity, pH, oxygen content, $NH4^+$-N, NO_2^--N. Those parameters were kept within optimal range for tilapia. Oxygen concentration was measured in a randomly selected tank by a digital oxygen detector and always remained above 5.5 $mg.L^{-1}$. $NH4^+$-N and NO_2^--N concentration were checked in the outflow of the system and were below 0.25 ($NH4^+$-N) and 0.19 (NO_2^--N) $mg.L^{-1}$ throughout the experiment. The water pH ranged between 6.8 to 7.7 during the experiment. Water flow for each tank was 6 $l.min^{-1}$ and checked daily during the experiment. The water temperature was kept at 26.9±15°C during the experiment.

Experimental procedure

The experiment lasted for 8 weeks and consisted of two periods: Period I and II which lasted 6 and 2 weeks, respectively. During the last week of Period I, faeces was collected and at the end of Period II (week 8) fish were sacrificed for collecting digesta samples. At the start of the experiment, fish were randomly assigned to one of the 16 tanks. The four experimental diets were randomly assigned to one of the 16 tanks, having four replicates per diet. At the LOW and HIGH starch inclusion level, each tank received 80 and 89.2 g of feed per day, respectively. These different feeding levels were used to ascertain that all experimental groups received the same amount of gross energy and crude protein per day (Table 3). However, as intended by the experimental design the fat and starch intake differed between the LOW and HIGH starch diets. During Period I the daily ration was equally divided in two and fed by hand at 09:00 h and 17:00 h. Before feeding, the pellets were sieved to remove dust and small particles.

Table 3. Daily amount feed and nutrients/ingredients given per tank to each tank.

	Diets[a]			
Dietary component	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH
Total feeding level (as fed, g/d)	80.0	80.0	89.2	89.2
Maize flour (g/d)	16.0	16.0	32.0	32.0
Dry matter (g/d)	69.7	69.5	77.7	77.9
Crude protein (g/d)	31.9	31.9	33.3	33.6
Starch (g/d)	12.7	12.3	24.0	23.1
Crude fat (g/d)	10.3	10.3	4.0	4.2
Energy (kJ/d)	947.3	924.8	939.8	973.2

[a]full abbreviation see in table 1

In this experiment, a settling tank was used to collect faeces. Faeces was collected during the last week of Period I. The settling tank used was a commercial type, AquaOptima (AquaOptima AS, Trondheim, Norway) with a volume of 17L, a column height of 34.3cm, and a diameter of 23.9cm. At the bottom of the settling tank, a tap was placed to which a bottle was attached. During collection, the bottle was connected and the tap was open. The sampling bottles were continuously submerged into ice water, to prevent bacterial decay of faeces during the collection period. The whole content of bottle, including water, was collected twice a day (08.30 h and 16.30 h) and stored at –20°C until analysis.

During Period II, fish were fed by automatic belt feeders, 24 h a day. The daily amount of feed given was equal to the feeding level in Period I. In the final week of the experiment, all fish from each tank were sacrificed by overdose (0.4 g/L) of TMS and bicarbonate (0.8 g/L). For each tank, the automatic feeding was stopped 2 h before sacrificing the fish. Individual body weight, liver weight, empty stomach and intestine weight were measured in five randomly selected fish per tank. Digesta of 25 randomly selected fish per tank was collected in four sections of the gastro intestinal tract: the stomach and the proximal, middle, and distal part of the intestine (Intestine I, II, and III respectively). The division of the intestine was done based on having equal lengths per intestine section. Collected digesta from each section were pooled for all 25 fish within the tank. Digesta was collected for dry matter, volatile fatty acid (VFA) and viscosity measurements. The digesta of 25 fish per tank were pooled.

Chemical analysis

Feed samples were collected at regular intervals (twice a week) during the experimental period and ground using a 1 mm screen before analysis. Freeze-dried faeces from each tank were ground using a coffee grinder and thoroughly homogenised to obtain representative sub-samples. All chemical analyses were done in triplicate. Feed and faeces were analysed for dry matter by drying samples for 4 h at 103°C until constant weight (ISO 6496 1983). Ash content was determined by incineration in a muffle furnace for 4 h at 550°C (ISO 5984 1978). Acid insoluble ash was measured by dissolving ash in hydrochloric acid (ISO 5985 1981). Crude protein (N x 6.25) was measured by the Kjeldahl method after acid digestion, according to ISO 5983 (1979). Lipid was extracted by petroleum ether extraction in a Soxhlet apparatus. Energy content was measured by direct combustion in an adiabatic bomb calorimeter (IKA-C-7000, Fa. IKA-Analysentechnik, Weitersheim, Germany). Starch was analysed according to the method described by Goelema et al. (1998).

For viscosity measurements, fresh digesta were centrifuged at 12,000 g for 10 minutes. Viscosity was immediately measured on the supernatant using a Brookfield LVDV-I+ cone/plate viscometer (Brookfield Engineering laboratories, Inc., Middleboro, U.S.A). All viscosity measurements were done at 28˚C and at a shear rate of 75 to 750 S^{-1}. When measurable viscosity did not include 750 S^{-1}, data were linearized by logarithmic transformation giving a straight line from which the line could be extrapolated to 750 S^{-1} (Steenfeldt, 2001).

For volatile fatty acid (VFA) measurements, 0.5 g of digesta was added to 1 ml distilled water and 50 µl of 85% phosphoric acid and stored at -20°C until analysis. Digesta samples were centrifuged for 10 min at 10000 *g*. The concentrations of acetic, propionic, iso-butyric, butyric, ico-valeric and valeric acid were measured by gas chromatography using GLC (Packard 419 glass column, CE instruments, Milan, Italy), filled with Chromosorb 101, carrier gas N_2 saturated with methanoic acid, at 190 ^{o}C, with iso-caproic acid as an internal standard. VFA concentrations were expressed in mmol per g of fresh digesta.

Dry matter of digesta was determined by using a 2 g sample from stomach and three parts of intestine according to (ISO 6496 1983).

Calculations and statistical analysis

Digestibility calculation and faecal removal measurement

Apparent digestibility coefficients (ADC) of nutrients in the diets were determined using the indicator method with acid insoluble ash as a marker. Apparent digestibility (%) for nutrients in the diets is expressed as a fractional net absorption of nutrients from diets. ADC of nutrients were calculated according to:

$$ADC_{nut} = (1 - [AIA_{diet}/AIA_{faeces} \times Nutr_{faeces}/Nutr_{diet}]) \times 100$$

Where ADC_{nut} = apparent digestibility coefficient of the nutrient; AIA_{diet} = dietary AIA concentration; AIA_{faeces} = faecal AIA concentration; $Nutr_{diet}$ = dietary concentration of nutrient; and $Nutr_{faeces}$ = faecal concentration of nutrient.

Since the marker is homogeneously distributed in both feed and faeces and as the faeces is collected after removal in a settling tank, the faeces removal percentage (i.e., faeces recovery) was calculated as the amount of marker (AIA) found in the faeces divided by the amount of marker (AIA) provided by the feed. From the measured ADC of dry matter and the faeces removal percentage, the total amount of faeces produced, the amount of faeces removal from the water and the non-removed faeces (all expressed in g dry matter per kg of feed) were calculated.

ADC and faeces removal analyses were done on faeces collected during the final week of first period (week 6 of the experiment).

Fish performance

Biomass gain was determined by the difference between total initial and final body weight. Feed conversion ratio was calculated per tank from feed intake data and weight gain. Relative organ weight was calculated by dividing organ weight to body weight expressed as a percentage.

Statistical analysis

Data are presented as means of each treatment with standard deviation. The percentage data were verified for normality after transformation (ASIN). All data were analysed by a 2 way ANOVA for the effect of starch type (gelatinized versus native) and the effect

of starch level (low versus high). For all statistical analyses, tank was considered the experimental unit.

Results

Digestibility and growth

Nutrient ADC data are given in Table 4. There was no interaction effect between type and level of starch on ADC of all nutrients, except for crude ash ($P < 0.05$). Exchanging gelatinized starch by native starch increased the crude ash ADC and this increase in ash ADC was larger at the LOW starch diets compared to HIGH starch diets. Type of starch (GEL versus NAT) affected ADC of all nutrients ($P < 0.001$). In contrast to crude ash ADC, ADC of all other nutrients were higher at the GEL diets than at the NAT diets. The dietary inclusion level of starch had no impact on crude protein and starch ADC, but influenced all other nutrients ($P < 0.05$). All ADC values, excluding crude protein and starch, were increased when the starch inclusion level of the diets increased.

All performance parameters were influenced by the effect of starch type as well as the effect of dietary starch level, but no interaction effect was present (Table 5). Growth was higher ($P < 0.001$) and FCR lower ($P < 0.01$) for fish fed the gelatinized starch compared to those fed native starch. Increasing the starch content of the diets resulted in an increased growth ($P < 0.001$) and a lower FCR ($P < 0.01$).

A high dietary inclusion level of starch increased ($P < 0.01$) relative liver weight. An interaction effect was present for the empty stomach weight between starch type and dietary starch inclusion level ($P < 0.05$; Table 5). The relative intestinal weight tended to be higher for native starch compared to gelatinized starch ($P=0.08$) and tended to decline when the inclusion level of starch increased ($P=0.09$; Table 5).

Table 4. Apparent digestibility coefficients (ADC) ±SD in tilapia fed with two types of maize starch (gelatinized; GEL versus native; NAT) and two levels of maize starch (LOW versus HIGH).

Parameters	Diets[a]				*P*-values of the factors		
	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH	Type	level	Type*level
Dry matter (%)	81.63±0.87	79.36±0.72	84.05±0.69	82.25±0.32	0.001	0.001	0.530
Organic matter (%)	88.30±0.48	85.33±0.50	90.25±0.37	87.08±0.25	0.001	0.001	0.635
Ash (%)	51.50±2.16	55.73±1.87	51.00±1.95	60.76±1.48	0.001	0.032	0.012
Protein (%)	92.48±0.47	91.05±0.16	92.80±0.52	91.25±0.30	0.001	0.215	0.761
Starch (%)	99.18±0.00	93.78±0.00	99.48±0.00	93.93±0.01	0.001	0.211	0.667
Energy (%)	90.95±0.66	88.78±0.72	92.20±0.50	90.05±0.67	0.001	0.002	0.966
Fat (%)	94.95±0.23	93.88±0.24	96.35±0.05	94.98±0.34	0.001	0.001	0.236

[a]full abbreviation see in table 1

Table 5. Growth performance and organ characteristics ±SD in tilapia fed with two types of maize starch (gelatinized; GEL versus native; NAT) and two levels of maize starch (LOW versus HIGH).

Growth Parameters	Diet[a]				*P*-values of the factors		
	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH	Type	level	Type*level
Initial weight (g)	45.9±0.9	43.7±0.8	45.4±2.2	45.6±0.9			
Final weight (g)	147.5±2.2	141.4±1.6	153.5±2.9	148.5±1.1	0.002	0.001	0.634
Biomass gain (g)	4066±90	3912±40	4325±30	4114±74	0.001	0.001	0.382
FCR	0.94±0.03	0.97±0.02	0.98±0.02	1.03±0.02	0.006	0.002	0.602
Organ characteristics							
Relative liver weight (%)	1.8± 0.15	1.72± 0.27	2.44± 0.48	2.15± 0.14	0.149	0.005	0.680
Relative stomach weight[b] (%)	0.30± 0.02	0.36± 0.06	0.41± 0.07	0.32± 0.04	0.479	0.181	0.016
Relative intestine weight[b] (%)	1.90± 0.24	2.22± 0.13	1.90± 0.12	1.98± 0.31	0.082	0.094	0.289

[a]full abbreviation see in table 1
[b]measurements were done on empty stomach and intestine

Faeces removal and digesta characteristics

There was no interaction between starch type and starch inclusion level for faeces removal percentage neither for faeces production parameters (Figure 1 and 2). The faeces removal percentage was higher ($P < 0.01$) with the gelatinized starch diets than with the native starch diets. Furthermore, increasing the dietary inclusion level of starch increased ($P<0.05$) the faeces removal percentage for both starch types. Total faeces production was affected by both the effect of starch type and starch inclusion level (Figure 2). Faeces production was high at the native starch diets compared to the gelatinized starch diets.

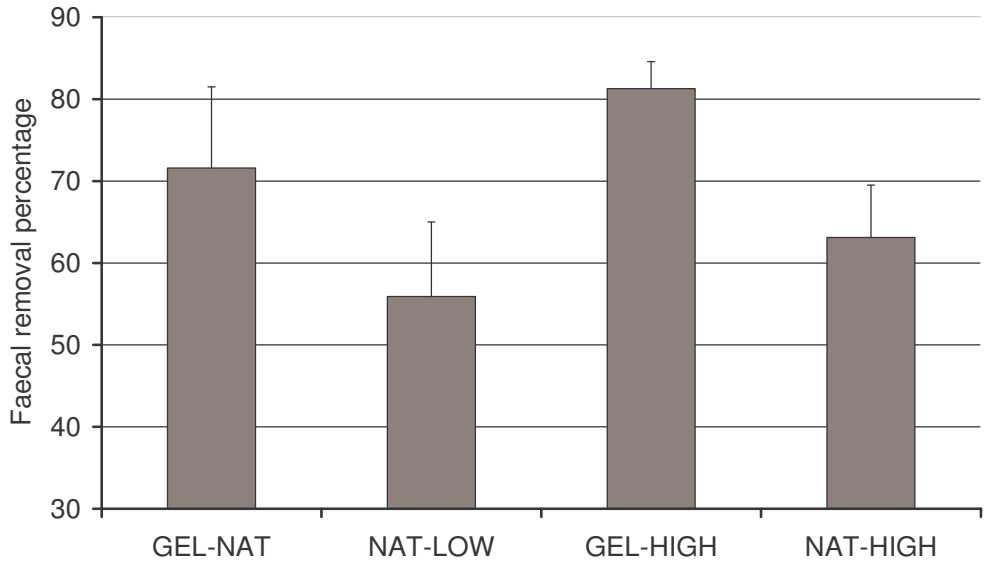

Figure 1. Faecal removal percentage ±SD in tilapia (g DM per kg of feed) fed with two types of maize starch (gelatinized; GEL versus native; NAT) and two levels of maize starch (LOW versus HIGH).The *P*-values for the main effect of type and level was, respectively: 0.001 and 0.048. There was no significant interaction effect between type and level of starch.

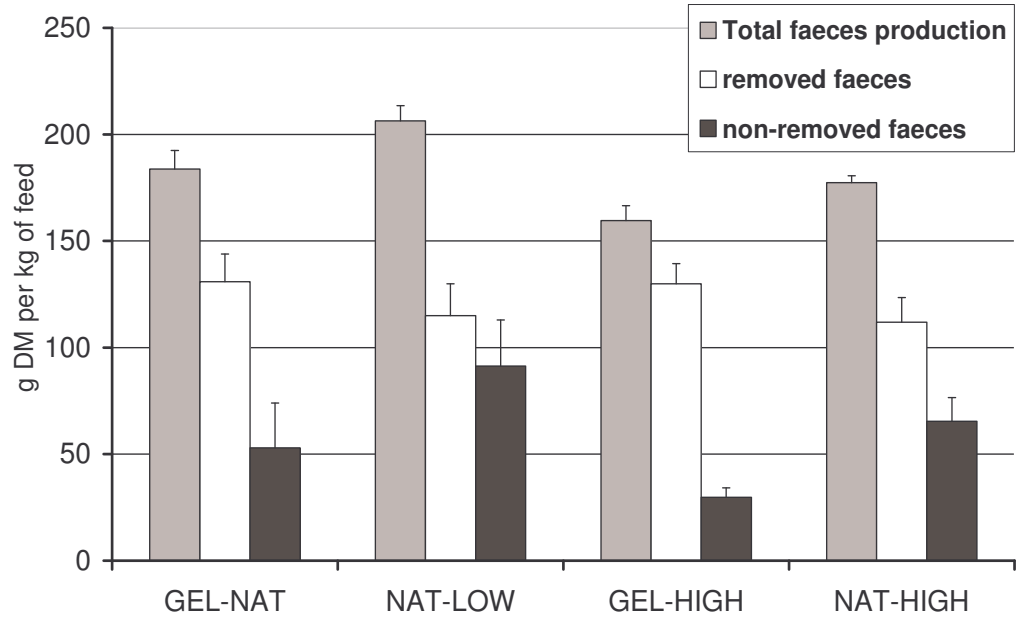

Figure 2. Total faeces production, removed faeces and non-removed faeces (g/DM per kg of feed) ±SD in tilapia fed with two types of maize starch (gelatinized; GEL versus native; NAT) and two levels of maize starch (LOW versus HIGH).The *P*-value for the main effect of type and level was, respectively: 0.001 and 0.001 for total faeces production; 0.018 and 0.747 for removed faeces and 0.001 and 0.011for non-removed faeces. There was no significant interaction effect between type and level of starch.

More faeces was produced at the low starch inclusion level compared to the high level. The amount of removed faeces was only affected by the type of starch. More faeces was removed at the gelatinized than at the native starch diets. Both the effect of starch type and inclusion level of starch influenced the amount of non-removed faeces. More faeces remained in the water at the native starch diets than at the gelatinized diets. Furthermore, the amount of non removed faeces decreased when the inclusion level of starch in the diets increased.

Viscosity measurements of diets and digesta are shown in Table 6. Digesta viscosity was not affected by the type of starch, except for stomach where viscosity was significantly higher for gelatinized starch compared to native starch. Diets containing a higher level of starch tended to induce a higher digesta viscosity than diets containing lower starch levels. Both in the stomach and the intestine I, type and level of starch affected the dry matter content of the digesta. However, type or level of starch did not influence dry matter content of digesta in intestine II and III.

Total VFA production was higher at native starch versus gelatinized starch, except for the stomach where gelatinized starch lead to higher VFA compared to native starch (Table 7). In the intestine I and II, higher total VFA concentrations were found with high inclusion of starch. In all experimental diets, acetic acid was the most abundant VFA throughout the gastro intestinal tract of tilapia and this is followed by a low concentration of propionic acid and butyric acid.

Table 6. Digesta characteristics ±SD in stomach and intestine of tilapia fed with two types of maize starch (gelatinized; GEL versus native; NAT) and two levels of maize starch (LOW versus HIGH).

	Diet[a]				*P*-values of the factors		
Viscosity (cP)	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH	Type	level	Type*level
Diets	3.59	2.04	5.55	1.70			
Stomach	1.50± 0.16	1.30± 0.14	1.95± 0.20	1.43± 0.08	0.001	0.002	0.058
Intestine I	1.77± 0.21	1.88± 0.27	1.75± 0.32	2.05± 0.08	0.099	0.539	0.439
Intestine II	1.55± 0.20	1.70± 0.31	1.8±0 0.35	2.08± 0.08	0.128	0.031	0.622
Intestine III	1.67± 0.39	1.89± 0.44	2.15± 0.49	2.27± 0.37	0.443	0.067	0.814
Digesta dry matter (g/kg)							
Stomach	170.5± 9.3	232.2±12.5	159.8± 9.8	200.1±139	0.001	0.005	0.118
Intestine I	139.5 ±4.5	144.2 ±3.2	120.6 ±7.5	138.1 ±2.3	0.001	0.001	0.018
Intestine II	114.2 ±4.7	117.1 ±3.6	110.9 ±2.2	113.1 ±7.7	0.356	0.234	0.893
Intestine III	119.3±11.6	119.8 ±5.7	111.9±10.3	115.1 ±8.7	0.708	0.204	0.787

[a]full abbreviation see in table 1

Table 7. Volatile fatty acid concentration[a] (mmol per kg fresh digesta) ±SD in stomach and intestine of tilapia fed with two types of maize starch (gelatinized; GEL versus native; NAT) and two levels of maize starch (LOW versus HIGH).

	Diet[b]				*P*-values of the factors		
	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH	Type	level	Type*level
Stomach							
Acetic acid	9.1±1.4	3.9±0.4	9.2±1.3	3.6±0.7	0.001	0.877	0.668
Propionic acid	1.4±0.1	0.4±0.2	1.4±0.2	0.2±0	0.001	0.190	0.311
Butyric acid	0.2±0.1	0.2±0.1	0.1±0	Not detected			
Total	10.7±1.4	4.5±0.6	10.7±1.5	3.8±0.7	0.001	0.697	0.575
Intestine I							
Acetic acid	6.6±0.7	7.6±2.5	8.0±0.7	10.4±1.3	0.047	0.018	0.396
Propionic acid	0.2±0.1	0.3±0.1	0.5±0.2	0.3±0.1	0.249	0.317	0.104
Butyric acid	Not detected	Not detected	Not detected	Not detected			
Total	6.8±0.8	7.9±2.6	8.5±0.7	10.7±1.4	0.057	0.017	0.489
Intestine II							
Acetic acid	10.3±0.7	14.6±2.6	11.9±2.0	16.2±1.0	0.001	0.093	0.973
Propionic acid	0.2±0	0.2±0.1	0.3±0.1	0.2±0	0.443	0.113	0.022
Butyric acid	Not detected	Not detected	Not detected	Not detected			
Total	10.5±0.5	14.8±2.6	12.2±2.0	16.4±1.0	0.001	0.077	0.899
Intestine III							
Acetic acid	14.2±2.3	17.5±0.6	14.7±1.7	18.3±2.2	0.004	0.513	0.878
Propionic acid	0.3±0.1	0.2±0.1	0.3±0	0.2±0.1	0.160	0.652	0.651
Butyric acid	0.2±0.1	0.1±0	0.2±0.1	0.1±0			
Total	14.7±2.4	19.4±3.9c	15.2±1.7	18.6±2.3	0.011	0.852	0.629

[a] iso-butyric, iso-valeric and valeric acid were not detected in the samples
[b]full abbreviation see in table 1
[c] valeric acid was detected in one of four replicates and added to the total VFA

Discussion

Digestibility

In comparison to native starch, gelatinized starch improved the ADC of dry matter, organic matter, protein, starch and fat. This finding is similar to that reported by Mohapatra et al. (2003) who found a positive correlation between the percentage of gelatinized carbohydrate (starch and dextrin) and the ADC of protein and carbohydrate in *Labeo rohita* fry. Similar results were also reported by Forneris et al. (1993), Gallego et al. (1994), Cheng and Hardy (2003) in respectively carp, European eel and rainbow trout. Gelatinization changes the physical structure of starch granules from crystalline to a gel structure which promotes efficiency of interaction with α-amylases (Englyst and Cummings 1987). Unlike the positive effect of extrusion on ADC of nutrients, ADC of ash was lower in gelatinized diets compared to native ones. Similarly, Cheng and Hardy (2003) found that extrusion reduced the ADC of minerals such as Mg, P, S, Cu, Fe, Mn, and Zn in rainbow trout. We believe that extrusion may have resulted in formation of indigestible mineral-nutrient complexes, leading to a reduced ADC of ash.

Faeces characteristics and its removal

The present study showed that the differences in digesta characteristics (fermentation and viscosity) induced by dietary type and level of starch can change faeces removal percentage.

Intestinal fermentation appears to play an important role in manipulation of faeces characteristics. Amirkolaie et al. (2005) showed that addition of 8% guar gum as a partially fermentable substrate reduced faeces removal efficiency in tilapia. Similarly, Twomey et al. (2003c) observed that in dog an increased fermentation induced by high levels of fructooligosaccharide resulted in loose faeces. The results of the current study show that the higher VFA values at the end of intestine (II and III) resulting from native starch, coincide with a lower faecal removal percentage. This supports the idea that also in tilapia an increase in fermentation reduces faeces stability.

Literature provides contradicting views on the mechanism through which fermentation may influence faecal characteristics. Vernia et al. (1988) and Twomey et al. (2003a)

observed that higher fermentation products in humans and dog fed on dietary soluble non-starch polysaccharide resulted in moist faeces. In the present study the increased concentration of VFA in the digesta did not decrease the mean dry matter content of the digesta at the end of the intestine. So, it seems that the lower dry matter content of faeces resulting from dietary soluble non-starch polysaccharides is caused by the higher water binding capacity of these products rather than by osmotic pressure of fermentation products. Similar to our results, Ferguson et al. (2000) and Kishida et al (2001) observed that an increased VFA concentration in the intestine of rat fed on starch-based diets did not increase water content of digesta.

Apart from water content of faeces, fermentation may also influence faeces stability through other mechanisms. The gas produced by intestinal microbes may be trapped inside the faeces strand, reducing the density of faeces and thus reducing the settling of solids. In addition excessive fermentation in the intestine cleaves undigested macro-molecules (Bach Knudsen et al. 2001), which otherwise may act as a binder to make faeces particles stick together. This condition can reduce the faeces particle size and its removal efficiency. Twomey et al. (2003a,b) observed that in dog the addition of enzyme to the diet for breaking down soluble non-starch polysaccharide molecules to smaller polymers reduced faecal stability. This supports the idea that degradation of indigestible macro-molecules such as non-starch polysaccharide or starch protein matrix reduces the particle size distribution of faeces and its settleability.

In addition to fermentation, viscosity also influences faeces characteristics. A slightly higher digesta viscosity induced by dietary composition will lead to the production of more structured faeces by increasing elastic resistance in the digesta (Onsoyen et al. 1992). In the current study, a higher faeces removal at high dietary level of starch is coincided with an increased viscosity of digesta suggesting that a slight increase in viscosity may improve faeces removal in tilapia. Brinker et al. (2005) reported that increase in digesta viscosity by inclusion of 0.3 % guar gum as a dietary binder improved faeces stability in rainbow trout.

Water content of digesta (especially in intestine III) is generally an indicator for faeces physical characteristics. An increased water content of the digesta represents delicate faeces which may be easily disintegrated by the water flow. However, the results of the

current study show that neither dietary starch type nor level affect the moisture content of the digesta in the intestine III. Therefore, differences in faeces removal percentage cannot be explained by moisture content of digesta.

Digesta characteristics

The VFA data suggest that starch serves as a substrate for the intestinal micro-flora throughout the gastro intestinal tract of tilapia. A relatively higher VFA concentration induced by native starch at the end of the intestine supports the idea that a fraction of starch escapes digestion (resistant starch) and becomes available for fermentation by intestinal microbes. Similarly in rat, Ferguson et al. (2000) and Kishida et al. (2001) observed that inclusion of high-amylose maize starch (containing resistant starch) resulted in a lower ADC of nutrients and a higher VFA production compared to standard maize starch. Surprisingly, large levels of VFA for gelatinized starch were found in the stomach. According to Mountfort et al. (2002), who measured VFA concentration in three marine herbivorous species, fermentation in the stomach is much lower than in the intestine. However, the current study suggests that highly digestible diets like the one with gelatinized starch, can cause a relatively high fermentation in the stomach of tilapia.

Viscosity was slightly higher for the high starch inclusion level. It seems that exchanging of fat by starch increases digesta viscosity in tilapia. An increase in digesta viscosity may have enlarged the intestinal transit time (van der Klis et al. 1993), allowing a greater time for exposure of nutrients to the digestive process, or, creating an ideal environment for bacterial activity. All this may explain a higher ADC at the high starch inclusion level, thereby leading to a better growth in tilapia.

In conclusion, degradation by gut microbes seems to be major factor by which faeces removal efficiency is influenced. Fermentation in digesta and faeces increases the proportion of small particles and dissolved solids by cleavage of undigested binder macromolecules or by gas production which reduce faeces density. The negative effects of native starch on faeces removal efficiency may extend to those of other fermentable dietary ingredients. Therefore, detrimental effects of fermentability on faeces removal may be predicted during formulation of a diet. A large microbial degradation also can take place in the stomach of tilapia depending on the feed quality. Replacement of fat by

starch improved faeces stability by increasing digesta viscosity. This suggests that inclusion of starch at different forms and levels have beneficial effect on faeces stability.

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Chapter 6

Faecal physical properties influence water quality in a recirculation system

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Submitted

Abstract

In aquaculture, removal of solid waste is important in managing water quality. It can improve water quality within systems and reduce the amount of effluent discharged from aquaculture operations. Improvement of faecal physical properties will increase faecal removal percentage (i.e., faecal recovery). The aim of this study is to assess whether differences in faecal removal efficiency, induced by dietary composition, have significant impact on water quality. Tilapia (*Oreochromis niloticus*) weighing 94.2 g were fed diets containing 8% cellulose or 8% guar gum for six weeks. Fish were housed in four identical recirculation systems and each diet was replicated two times. Total suspended solids were measured weekly and dissolved oxygen, ammonia, nitrite and nitrate-Nitrogen were measured daily throughout the experiment. Supplementation of cellulose increased faeces removal and reduced total suspended solids. Dissolved oxygen was higher in the systems containing fish fed cellulose. Ammonia and nitrite concentrations were higher in the systems with guar gum fed fish, although these differences were not significant. Nitrate concentration was significantly higher in systems containing fish fed cellulose. In conclusion, feed composition can reduce suspended solids by increasing faecal removal percentage, thereby improving water quality of the recirculation system.

Introduction

Recirculation aquaculture systems (RAS) may offer an alternative to pond aquaculture due to little usage of land and water. In RAS, fish can be raised intensively under controlled environmental conditions. RAS also allow fish production throughout the year and production sites can be built closer to markets. Currently because of profits associated with RAS, its potential is not only for high valued fish species like salmon or sea bass but also for lowered valued ones like tilapia (Shnel et al. 2002).

Maintaining a desirable water quality is of primary importance in RAS. Poor water quality can reduce growth and increase the incidence of disease (Losordo et al. 1999). The accumulation of solids in RAS increases stress in fish (Klontz et al. 1985; Braaten et al. 1986), reduces nitrification rate (Kruner and Rosenthal 1987) and clogs bio-filters (Muir 1982). High levels of solids provide a suitable substrate for proliferation of pathogenic organisms (Liltved and Cripps 1999) and may damage gill tissue (Wickins

1981). Moreover, the solids discharged from RAS form a major environmental concern because they can cause eutrophication of receiving waters such as lakes or rivers (Pillay 1992; Cripps and Bergheim 2000).

Rapid and efficient solid waste removal is a key factor both to maintain water quality in RAS and to reduce the impact of aquaculture wastewater on the environment. Both uneaten feed and faeces are contributing to solids production, but faeces is the main source of solids in RAS (e.g., in seabass 71 versus 29%; Franco-Nava et al. 2004). The amount of faecal waste production can be reduced through changes in diet composition, for example the use of highly digestible feed ingredients (e.g., fishmeal and fish oil). However, in the future potential shortage and high prices of fishmeal and fish oil will limit the use of these feed ingredients in aqua-feeds (Kaushik 1990; Hardy 1996).

Manipulation of diet composition may also alter faecal physical properties. Han et al. (1996) reported that addition of guar gum (i.e., a soluble non-starch polysaccharide) reduced particle size distribution of faeces. Dias et al. (1998) observed that inclusion of an insoluble non-starch polysaccharide like cellulose increased faeces firmness. Dietary inclusion of guar gum reduced faecal removal and increased the amount of non-settleable solids in tilapia compared to cellulose (Amirkolaie et al. 2005). An increased faeces stability will increase the ratio of settleable to non-settleable solids and thereby improve faecal removal percentage, furthermore it converts to suspended and dissolved solids at a lower ratio.

So far, the relationship between water quality in RAS and faecal physical properties has not been studied. The objective of the present paper is to investigate whether differences in faecal physical properties, that are induced by dietary composition (guar gum versus cellulose), have significant impact on water quality in RAS containing tilapia (*Oreochromis niloticus* L.).

Materials and Methods

This experiment was approved by the Ethical Committee judging Animal Experiments (DEC) of Wageningen University.

Experimental diets, animals and system

In the current study two diets were used: a guar gum versus a cellulose diet. In both these experimental diets, guar gum (soluble non starch polysaccharide) and cellulose (insoluble non starch polysaccharide) were added to a basal diet at a level of 8%. The formulation and proximate composition of the two experimental diets are presented in Table 1.

Table 1. Ingredient and proximate composition of the experimental diets

Parameters	Guar gum (%)	Cellulose (%)
Fishmeal[a]	43.1	43.1
Soybean meal[b]	9.2	9.2
Maize[c]	27.6	27.6
Soy oil[d]	2.76	2.76
Palm oil	2.76	2.76
Cellulose[e]	0	8.0
Guar Gum[f]	8.0	0
Calcium carbonate	3.22	3.22
Diamol[g]	1.84	1.84
Premix[h]	0.92	0.92
Monocalcium phosphate	0.64	0.64
Proximate composition (% on DM basis)		
Organic matter	86.6	86.7
Ash	13.4	13.3
Crude protein	41.1	41.0
Gross energy (MJ/kg)	20.0	19.9

[a]Danish herring meal, bran Skagen FF with a protein content of 72%
[b]Soybean meal Hipro, produced by Cargill Amsterdam with a protein content of 46%
[c]Normal fed quality French maize
[d]Refined food quality Soya oil from Romi Smilfood bv Heerenveen, The Netherlands
[e]Arbocel B800, J. Rettenmaier und Sohne, Rosenberg, Germany
[f]Rudingom G555, (Product no. BG0380), Ruitenberg Ingredients, Amersfoort, The Netherlands. On product bases the composition of this guar gum is: dry matter 88%, crude protein 4%, fibres 82% and crude ash 1%. This guar gum is used in the food industry and is a cold soluble thickening agent, which give high viscosity at low dosing.
[g] Diamol GM, Franz Bertram, Hamburg, Germany
[h]Vitamin-mineral premix (Fe 50 mg/kg; Zn 100 mg/Kg; Co 100 mg/kg; Mg 300 mg/kg; Vit. B1 30 mg/Kg; Vit.B2 30mg/kg; Vit. B5 100 mg/kg; Vit. B3 200 mg/kg; Biotin 0.6 mg/kg).

These two diets were selected out of four diets, which were studied in a previous experiment (Amirkolaie et al. 2005). The selection of these two diets were based on the following criteria: 1) having a similar apparent dry matter digestibility (i.e., amount of faecal waste production), 71 and 75% for the guar gum and cellulose diet respectively; and 2) differing as much as possible in faecal removal efficiency, 42 and 76% for guar gum and cellulose respectively (Amirkolaie et al. 2005). Diamol (Acid Insoluble Ash:

AIA) was incorporated in the diets as a digestibility marker. All ingredients were finely grounded, mixed and dry pelletized. Pellets were thereafter dried in an air dryer at 80°C and stored in a refrigerator until use. Data on fish performance, apparent nutrient digestibility, faecal removal and dietary and digesta viscosity have been presented by Amirkolaie et al. (2005).

The fish (Nile tilapia, initial body weight of 94.2g) were full-sibs and bred at the reproduction facility of the Fish Culture and Fisheries Group (De Haar Vissen), Wageningen University, The Netherlands.

Four identical separate RAS were used. Each RAS system contained a 75-L fish tank, a 68-L trickling filter containing filter material of BIO-M (Fleuren, Someren, The Netherlands) with a specific surface area of 200 $m^2.m^{-3}$ and a 17-L settling tank (AquaOptima AS, Trondheim, Norway). Each system also had a 17-L sump tank from where a submerged pump (Universal pump, model 1260) supplied the trickling filter with a water flow of 5-6 $l.min^{-1}$. Before running the experiment, the systems were disinfected with halamid (Axcentive, Barneveld, The Netherlands) for 24 hours. Preliminary runs were conducted to activate the bio-filter by addition of ammonia–chloride and sodium-nitrate for a period of 34 days.

Water temperature was kept within 27-28°C by two 300W heaters. The photoperiod regime during the experiment was 12 h light and 12 h dark.

Experimental procedure and analytical techniques

The experiment was conducted for six weeks. On day one of the experiment, fish were randomly divided over four systems (30 fish per system) and weighed. Twenty randomly selected fish were sacrificed by overdose of Tricaine Methane Sulfonate (0.8 $g.l^{-1}$; TMS Crescent Research Chemicals, Phoenix, Arizona, USA) and stored at -20°C for later analysis of initial body composition. The two diets were randomly assigned to four systems, thus replicating each diet two times. Both cellulose and guar gum groups received the same amount of feed per day throughout the study. On day one, each system received 40 g feed. The feeding level was gradually increased during the experiment (Fig. 1) to allow fish to adapt to the experimental diets. In the guar gum treated group, on day 9 and 2 days within the last week of the experiment ammonia and

nitrite levels exceeded the optimal range for tilapia (Timmons et al. 2001). On those days, feeding level was decreased for all four systems to avoid possible health problems. Fish were fed once a day at 09:30 by hand. Before feeding, the pellets were sieved to remove dust and small particles.

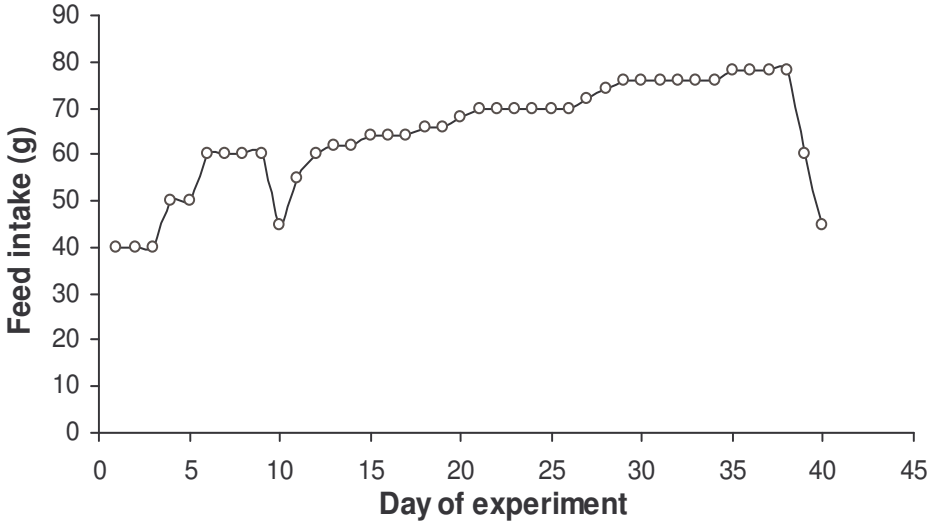

Figure 1. Daily feed ration (g/system) of tilapia during the experiment. Feed ration was identical in all systems

Faeces were collected by settling tanks throughout the experiment. Faeces were collected in sampling bottles which were attached to the bottom of settling tank. During 24-hours faecs collection, the sampling bottles were continusly sunmerged into ice water to prevent bacterial decay of faeces during the collection period. The whole content of the bottle including water was collected twice a day (08:30 and 17:00) and stored at–20°C until analysis. Samples were pooled per system for the whole experimental period.

Total ammonia–N (TAN), total nitrite-N (NO_2^--N) and total nitrate-N (NO_3^--N) were measured weekly at 9:00 by a Skalar auto-analyser (ISO 8464 1990). In the present study, nitrogen concentration was measured in a mixed sample composed of equal aliquots for the outlet of the fish tank and the outlet of the settling tank, because in a commercial RAS, microbial processes may occur within the settling tank, causing both nitrification and de-nitrification. Ammonia, nitrite and nitrate were also measured daily before feeding with an Aquamerck Quick test (Aquamerck, Merck, Darmstadt, Germany) to monitor the performance of the systems for adjustment of daily feeding level. Total solids

(TS) and total suspended solids (TSS) were measured weekly at 9:00 at the outlet of the fish tank and at the outlet of the settling tank using the method of Clesceri et al. (1998). For measurement of TS, a 200-mL water sample was evaporated in a weighed dish and dried until constant weight in an oven at 102°C. The increase in weight of the dish represents the TS. For measurement of TSS, a 300-mL water sample was filtered onto a pre-weighed and pre-dried Whatman GF/A glass micro- fibre filter with pore size of 1.6µm. The filter was then oven dried at 70°C for 24 hours. TSS was calculated by comparing the initial and final weight of filter.

Oxygen concentration was measured daily before feeding at the outlet of the fish tank by a digital oxygen detector (model oxi-340i; WTW, Weilheim, Germany). Besides daily oxygen measurements, 24-hours oxygen measurements were conducted on day 35 of the study to evaluate within-day variation of oxygen between the treatments. Every two hours, oxygen was measured at inlet and outlet of the fish tanks. The water pH was measured daily before feeding using a WTW pH meter (model pH 340; WTW, Weilheim, Germany). pH was maintained between 7-7.5 by the addition of natrium bicarbonate ($NaHCO_3$). From each system 30 litre of water was exchanged with fresh water on day 22, 29, 31 of the experiment to keep NO^-_3-N concentration below 220mg.L^{-1} during the experiment.

To make a nitrogen mass balance, water samples were taken from each system at the start and end of the experiment and at the moment of water exchange (day 22, 29, 31) from each system. Nitrogen associated with the system was measured at the end of experiment by collecting the whole water (125L) and solids of the system in a bucket. The bucket content was weighed and stirred by a pump to attain maximum homogeneity. Two 1 L sub-samples per system were obtained using 1 L plastic bottles.

At the end of the experiment, random samples of five fish from each system were collected and stored at -20°C for final analysis of body composition.

Chemical analysis

Feed samples were collected twice a week during the experimental period and ground using a 1-mm screen before analysis. Frozen-fish were ground and mixed homogenously.

Freeze-dried faeces from each system were ground using a coffee blender and thoroughly homogenised. Feed and faeces were analysed for dry matter by drying samples for 4 h at 103°C until constant weight (ISO 6496 1983). Acid insoluble ash (AIA) was measured by dissolving ash in hydrochloric acid (ISO 5985 1981). Crude protein (N x 6.25) was measured by the Kjeldahl method after acid digestion (ISO 5983, 1979). Energy content was measured by direct combustion in an adiabatic bomb calorimeter (IKA-C-7000, Fa. IKA- Analysentechnik, Weitersheim, Germany). All chemical analyses were done in triplicate.

Digestibility calculation and faecal removal measurement

Apparent digestibility coefficients (ADC) of nutrients in the diets were conducted according to the following formula:

$$ADC_{nut} = (1 - [AIA_{diet}/AIA_{faeces} \times Nutr_{faeces}/Nutr_{diet}]) \times 100$$

where ADC_{nut} = apparent digestibility coefficient of the nutrient; AIA_{diet} = dietary AIA concentration; AIA_{faeces} = faecal AIA concentration; $Nutr_{diet}$ = dietary nutrient concentration; and $Nutr_{faeces}$ = faecal nutrient concentration.

As the marker is homogeneously distributed in both feed and faeces, the faecal removal percentage (i.e., faecal recovery) was calculated as the amount of marker (AIA) removed in the faeces divided by the amount of marker (AIA) provided by the feed. From the measured ADC and faecal removal percentage, the total amount of faeces produced, the amount of faeces removed from the water and the non-removed faeces (all expressed in g dry matter per kg of feed) were calculated per system.

Fish performance

Biomass gain (BG) was calculated as the difference between total initial and final body weight. Specific growth rate (SGR) was calculated from the natural logarithm of the mean final weight minus the natural logarithm of the mean initial weight and divided by the total number of experimental days expressed as a percentage per day. Feed conversion ratio (FCR) was calculated by dividing total feed intake per system to total weight gain during the experiment.

Nitrogen mass balance

Total nitrogen input, output, uptake by fish and accumulation in the culture system during the experiment were measured according to the following formula:

$N_{feed} = N_{fish} + N_{faeces\ removal} + N_{accumulation\ in\ the\ system} + N_{water\ exchange} + N_{unexplained}$

Nitrogen supplied by feed (N_{feed}) was the only nitrogen input to the system. Nitrogen retention in the fish (N_{fish}) was calculated by comparing initial and final nitrogen content of fish. Nitrogen output in the exchange water ($N_{water\ exchange}$) was calculated by multiplying the nitrogen concentration with the total exchanged water volume. Nitrogen accumulation ($N_{accumulation\ in\ the\ water}$) in the system was calculated by comparing nitrogen contained in the system on day one and day 42 of the experiment. Nitrogen faecal removal ($N_{faecal\ removal}$) was calculated by multiplying the total removed faeces with the nitrogen concentration of the faeces. Total removed faeces is the amount of faeces which is collected by settling tank during six weeks experiment.

Statistical analysis

The system was considered as the experimental unit. Data are presented as means of each treatment with standard deviation. To test the effect of different diets (type of non starch polysaccharide) on the nitrogen balance, digestibility, faecal removal percentage and growth, the data were tested by the student *t* tests assuming equal variance between the treatments.

Since the weekly water quality measurements were considered as repetitions, the effect of diets on water quality was assessed by repeated measurement analysis. All analyses were carried out by Proc GLM of SAS (version 9.1).

Results

Growth, digestibility and faecal removal percentage

Growth performance and feed conversion were similar between the treatments (Table 2). Guar gum supplementation increased ADC of dry matter, but decreased ADC of protein compared to cellulose (Table 3). Faeces removal percentage tended (P=0.07) to be higher for the cellulose diet compared to the guar gum diet. Inclusion of cellulose

resulted in significant higher total amount of faeces produced compared to guar gum inclusion, but the absolute amount of non-removed faeces was higher for the guar gum based diet (65.0 versus 48.8 g DM per kg of feed).

Table 2. Performance of tilapia fed two types of non starch polysaccharide (guar gum versus cellulose).

	Diet		
Parameters	Guar gum	Cellulose	*P*-value
Initial body weight (g)	94.0± 1.3	94.4 ±3.8	0.90
Final body weight (g)	159.9±3.8	162.8± 7.4	0.67
Biomass gain (kg/tank)	1.98± 0.07	2.05± 0.11	0.51
Specific growth rate (%/day)	1.26± 0.02	1.30± 0.01	0.23
Feed conversion	1.30± 0.05	1.26± 0.07	0.51

Table 3. Apparent digestibility coefficient and faeces removal of tilapia fed two types of non starch polysaccharide (guar gum versus cellulose) in recirculation systems.

	Diet		
Parameters	Guar gum	Cellulose	*P*-value
Dry matter digestibility (%)	80.0± 0.5	77.0± 0.3	0.02
Crude protein digestibility (%)	87.3± 0.3	91.2± 0.2	0.003
Faeces removal (%)	67.6± 0.9	79.0± 4.3	0.07
Total faeces produced (g DM per kg feed)	200.2± 5.1	229.7± 3.0	0.02
Removed faeces (g DM per kg feed)	135.2± 1.7	181.3±7.5	0.01
Non-removed faeces (g DM per kg feed)	65.0± 3.4	48.4± 10.5	0.17

Water quality

The average of TS during the experimental period was not affected by type of diet (Table 4). However, supplementation of guar gum increased the average level TSS in the outlet of fish tank ($P<0.05$) and in the outlet of settling tank ($P=0.06$) (Table 4). Weekly measurements of TSS in the outlet of settling tank were almost similar between the two treatments up to week four of the study (Fig. 2) and then showed higher values for the guar gum based diet.

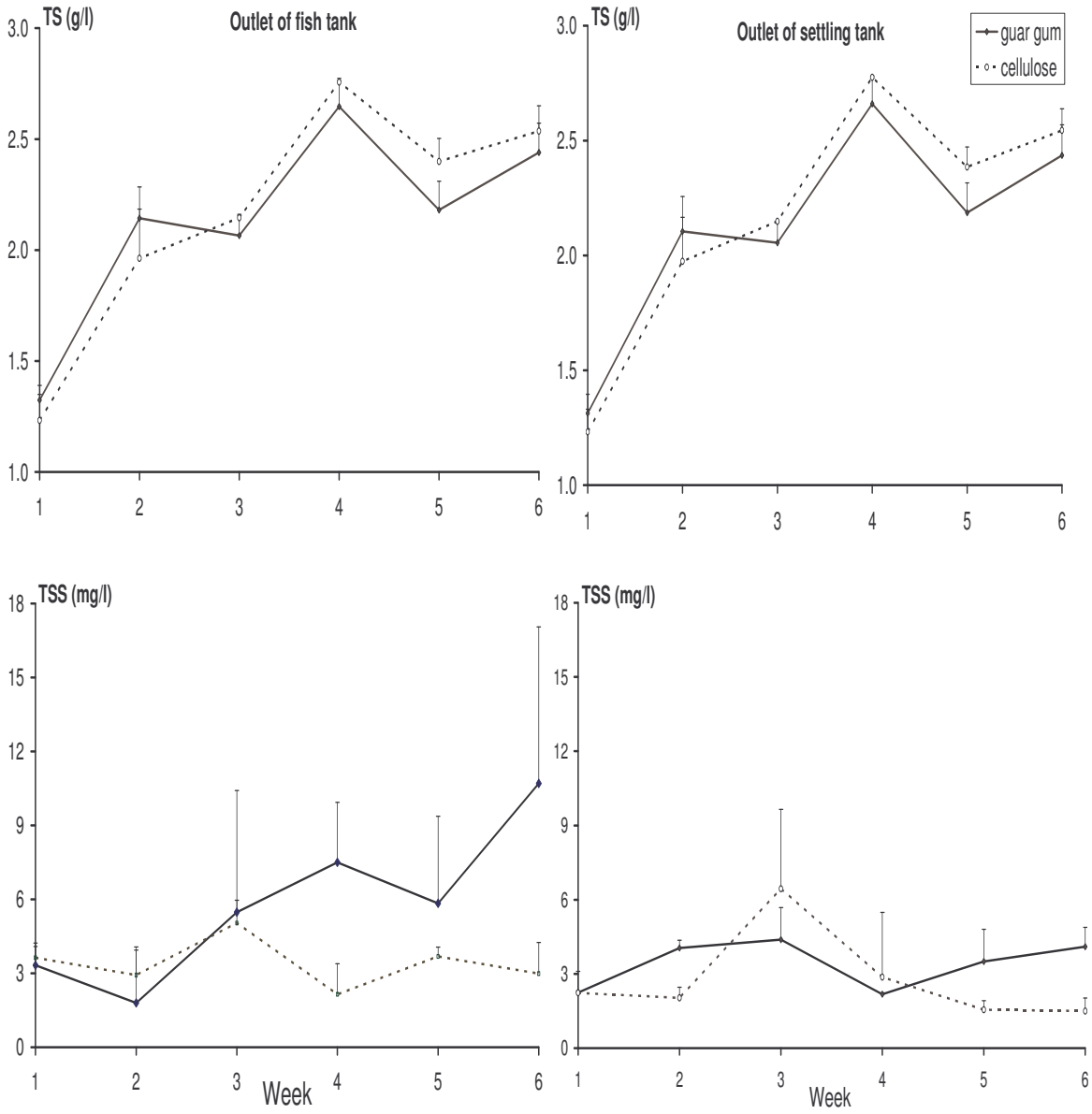

Figure 2. A comparison of total solids (TS) and total suspended solids (TSS) for tilapia fed with two types of non starch polysaccharide in a recirculation system. In the repeated measurement analysis the following P-values were found for the effect of diet, week and their interaction respectively: 0.699, 0.001 and 0.212 for TS (outlet of fish tank); 0.247, 0.001 and 0.247 for TS (outlet of settling tank); 0.004, 0.462 and 0.340 for TSS (outlet of fish tank); 0.063, 0.106 and 0.269 for TSS (outlet of settling tank).

Overall, dissolved oxygen (DO) was lower (P= 0.03) in the systems containing fish fed guar gum compared to cellulose during the experimental period (Fig. 3 and Table 4). Figure 4 shows 24-hours oxygen measurements in the inlet and outlet of the fish tank. At

both sampling sites, DO was affected significantly by type of diet and time of measurement. There was a consistent higher DO concentration for the cellulose diet at

Table 4. Effects of two types of non starch polysaccharide (guar gum versus cellulose) on water quality parameters. For each treatment, water quality data for all sampling moments were averaged (n= 6 measurements that were done weekly for each water quality parameter).

	Diet		P-value
Parameters	Guar gum	Cellulose	Diet
Out let of fish tank			
Total solids (g/l)	2.1±0.1	2.2±0.1	0.70
Total suspended solids (mg/l)	5.8±0.2	3.4±0.1	0.004
Oxygen (mg/l)	5.7±0.1	6.1±0.1	0.03
Out let of settling tank			
Total solids (g/l)	2.1±0.1	2.2±0.1	0.60
Total suspended solids (mg/l)	3.4±0.2	2.8±0.2	0.06
Mixed sample[a]			
Ammonia-N(mg/l)	0.29±0.03	0.27±0.03	0.68
Nitrite-N (mg/l)	0.95±0.50	0.53±0.28	0.41
Nitrate-N (mg/l)	132.9±0.5	145.1±2.7	0.03

[a]measurement based on an equally mixed sample from outlet of fish tank and outlet of settling tank

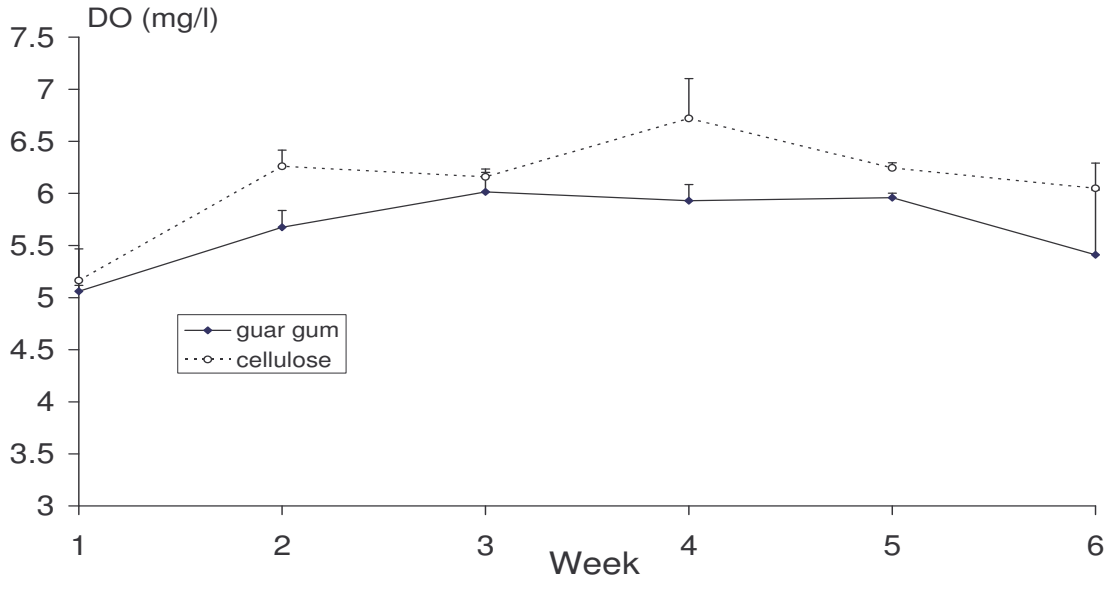

Figure 3. A comparison of dissolved oxygen (DO) for tilapia fed diet with two types of non starch polysaccharide. In the repeated measurement analysis the following P-values were found for the effect of diet, week and their interaction respectively : 0.031, 0.001 and 0.424.

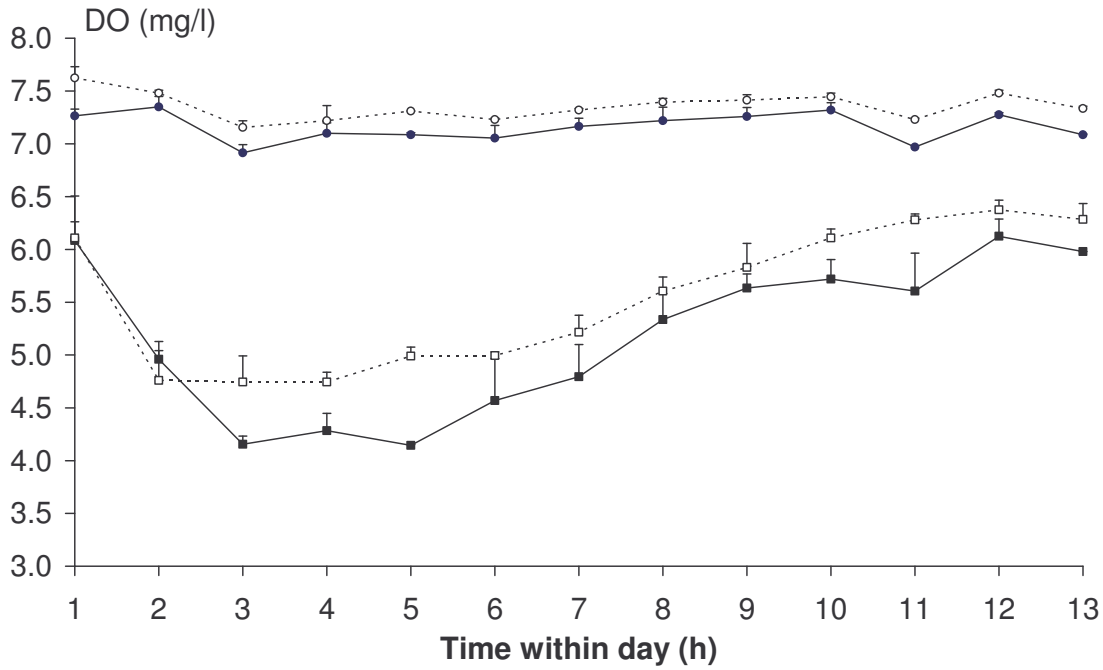

Figure 4. Within day variation of dissolved oxygen (DO) in recirculation system as affected by two types of non starch polysaccharide (guar gum versus cellulose) measured at inlet of fish tank (●= guar gum; ○= cellulose) and outlet of fish tank (□=guar gum ■=cellulose). In the repeated measurement analysis the following P-values were found for the effect of diet, week and their interaction respectively : 0.053, 0.001 and 0.114 for inlet of fish tank; 0.039, 0.001 and 0.087 for outlet of fish tank.

both sites. At both treatments, DO at the outlet of the fish tank dropped markedly to around 4.1-4.7 mg.L^{-1} three hours after feeding (12:00) and then gradually recovered to around 6 m.L^{-1} at 04:00 (Fig. 4). The reduction of DO was more pronounced in the guar gum fed systems.

Data on ammonia, nitrite and nitrate concentration throughout the experiment are shown in Table 4 and Fig. 5. There is a significant interaction effect between diet and week of measurement for ammonia showing that differences in ammonia concentration between the diets depend on the moment during the experimental period. Average ammonia and nitrite values over the experimental period were higher (0.29 versus 0.27 mg.L^{-1} for ammonia-N and 0.95 versus 0.53mg.L^{-1} for nitrite-N) in the system with guar gum fed fish although these differences were not significant. There was no consistent difference between diets in weekly measurement of ammonia and nitrite, but these values began to

differ distinctively from week 5 of the experiment (Fig. 5). The average nitrate concentration was higher ($P< 0.05$) for the cellulose-fed systems (Table 4).

Nitrogen balance

Nitrogen discharged in the exchange water during the experiment was significantly higher in the systems containing fish fed cellulose (Table 5). A higher quantity of nitrogen was removed via faecal collection in the systems with a diet at 8% guar gum inclusion. Unexplained nitrogen was accounted 23.1 and 19.8 % for guar gum and cellulose based diet. These estimates however were not significantly different.

Table 5. Nitrogen balance for tilapia fed with two different type of non starch polysaccharide in a recirculation system. (Data in parentheses expressed as percentage of N in the feed)

	Diet		
N-distribution	Guar gum	Cellulose	*P*-value
N in feed (g)	150.9	151.7	
N retained in fish (g)	46.8±2.6	50.1±5.1	0.500
	(31.0± 1.7)	(33.0±3.4)	
N accumulated in water (g)	26.3±0.7	27.7±0.8	0.205
	(17.4± 0.5)	(18.3± 0.5)	
N water exchange (g)	29.5±0.6	33.1±0.4	0.019
	(19.6± 04)	(21.8± 0.3)	
N faeces removal (g)	13.5±0.1	10.8±0.4	0.012
	(8.9± 0.0)	(7.1± 0.3)	
N unexplained (g)	34.8±2.4	30.0±4.3	0.303
	(23.1± 1.6)	(19.8± 2.8)	

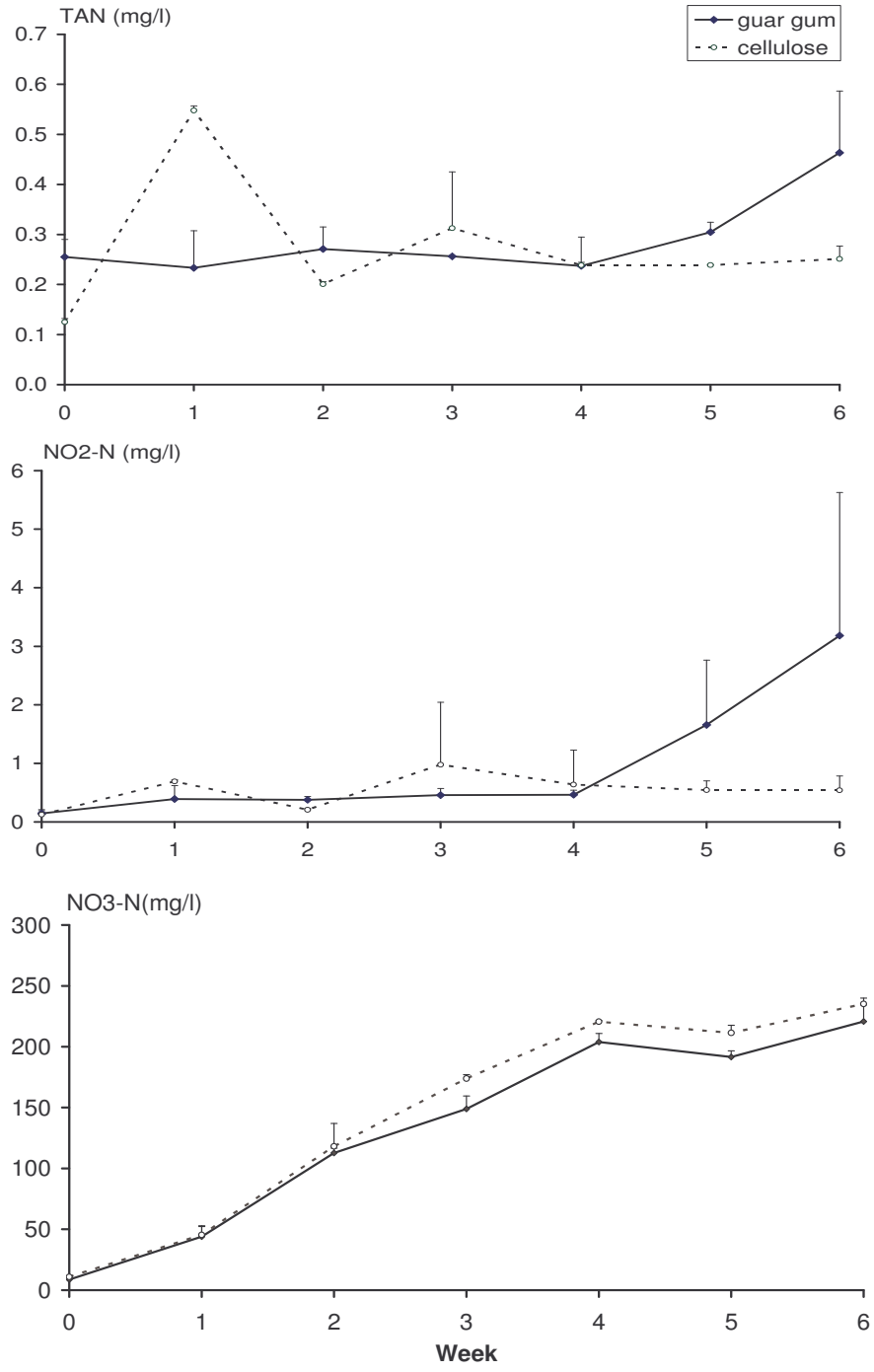

Figure 5. A comparison of total ammonia nitrogen (TAN), nitrite-N and nitrate-N produced for tilapia fed with two types of non starch polysaccharide (guar gum and cellulose) in a recirculation system. In the repeated measurement analysis the following P-values were found for the effect of diet, week and their interaction respectively: 0.675, 0.001 and 0.001 for ammonia; 0.407, 0.086 and 0.109 for nitrite; 0.025, 0.001 and 0.389 for Nitrate-nitrogen. Measurements were based on an equally mixed sample from the outlet of the fish tank and the outlet of the settling tank.

Discussion

Digestibility and faecal removal

The higher values of ADC of dry matter in guar gum diets compared to cellulose diets are in contrast to our earlier finding that showed nearly a similar dry matter ADC between cellulose and guar gum (Amirkolaie et al. 2005). These different findings may be caused by differences in faeces collection methods. In this study, all particles resided on the wall of settling tank were settled to the bottom of the tank by a brush. This condition may affect marker/nutrient ratio especially in soluble non starch polysaccharide which is more readily soluble in water and thus higher values for ADC.

Supplementation of guar gum reduced faecal removal percentage compared to cellulose diet. Guar gum as a soluble NSP appears to have a high water binding capacity (Smits and Annison 1996). The action of soluble non starch polysaccharide on faeces consistency is probably twofold. First there is direct effect on the bulk of faeces production because of the fraction that remains undigested in the intestine. This portion can increase water content of the faeces (Schneeman 1999). Second, soluble non starch polysaccharide sources tend to be more readily degraded or fermented by bacteria in the large intestine (Schneeman 1999). The fermentation products (especially lactate) contribute to osmotic pressure of the intestine content and result in more moist faeces (Verina et al. 1988). In contrast, cellulose is an insoluble molecule and has a moderate capacity to bind water. Due to low fermentation (Kihara and Sakata 1997), cellulose may have acted as a frame to make faeces particles stick together.

The reduction of faeces removal induced by guar gum was not statistically significant ($P=0.07$). This is not comparable to the results from our previous experiment (Amirkolaie et al. 2005) showing that dietary supplementation of 8% guar gum in tilapia decreased substantially the faeces removal percentage by 42%. The smaller reduction of faeces removal by guar gum inclusion in this experiment can be caused by differences in water flow between this (5-6 $l.min^{-1}$) and the other experiment (7.5 $l.min^{-1}$). A higher water flow can reduce settling rate of smaller particles which are mainly originated from guar gum diet (Han et al. 1996) and thereby increasing the difference in faeces removal between two experiments. As cellulose increases faeces firmness (Dias et al. 1998) and

thereby faecal particle size, an increased water flow (from 5.5 to 7.5 l.min^{-1}) only slightly reduces faeces removal percentage in cellulose treatment.

Moreover, the previous study was conducted in 16 aquaria connected to the same recirculation system. This condition may increase the risk of introducing particles through inflow water and therefore inaccurate measurement of faeces removal percentage.

Two replicates for each diet in the current study versus four replicates in our previous study may also explain a higher P-value (P=0.07) and no significant difference between two diets.

Water quality

In the current study, a higher removal percentage of faeces in cellulose fed system was associated with release of less suspended solids, higher oxygen level and lower ammonium and nitrite. These differences in water quality support the idea that improvement in faecal stability can reduce waste generation in RAS.

There is no difference in TS production between the two treatments. It appears that the estimates of TS represent the concentration of dissolved minerals in the water rather than the quantity of faeces. The similar measurements of TS in two different locations (outlet of fish tank and outlet of settling tank) support the idea that the percentage of faeces in TS is almost negligible. If faeces was a major part of TS, the quantity of TS would probably be higher in the outlet of the fish tank compared to the outlet of the settling tank.

The production of TSS was increased by feeding the diet containing guar gum. This may be due to the decrease in faeces cohesion which leads to the removal a smaller proportion of faeces from the system compared to cellulose. This finding is in agreement with results observed by Han et al. (1996) who found that inclusion of 9% guar gum in the diet of tilapia reduced the particle size distribution of solid waste. There is more evidence showing that feed composition affects particle size and/or gravity of suspended solids and thereby the retention efficiency. Patterson et al. (2003) concluded that heavier particles in the system water in a salmon farm are mainly composed of wheat-related heavy cellulose particles which are initiated from the undigested portion of the feed. Chen et al. (1993)

observed that the retention of solids largely depends on particle size and specific gravity which are related to the organic nature of solids. Suspended solids are composed of two parts, organic and inorganic. The organic part is known as volatile suspended solids (Timmons et al., 2001) and contributes to heterotrophic oxygen consumption (Sharma and Ahlert 1977). Inorganic part of solids contributes in the formation of sludge (Timmons et al. 2001). Relatively lower dissolved oxygen in the guar gum fed systems might have been caused by a higher organic matter load in the systems, particularly on the bio-filter. Golz et al. (1999) found that oxygen limitation increased with solids residence time in the bio-filter. Sharma and Ahlert (1977) stated that the dissolved organic matter can promote the growth of heterotrophic organisms that compete for dissolved oxygen and space with nitrifying bacteria. This decomposition process consumes oxygen and decreases the oxygen available to the fish (McMillan et al. 2003). In the current study, a slow-recovery of dissolved oxygen after feeding in the system with guar gum fed fish supports the idea that a higher organic load in water caused by suspended solids increased the population of heterotrophic bacteria.

In the present study, ammonia and nitrite in the guar gum systems peaked on the final weeks of the experiment when the highest feeding level was applied. This further suggests that organic matter accumulation indeed reduced the nitrification process. Figueroa and Silverstein (1992) observed that ammonia removal efficiency decreased by increasing of organic load in the water. Similarly Golz et al. (1999) reported that longer solids retention time on the bio-filter reduce the apparent nitrification.

Decomposition of the suspended solids in the bio-filter may increase nutrients in the system by solubilization of organic matter (Golz et al. 1999). In this study, the soluble non starch polysaccharide may have caused higher levels of dissolved solids, and thus indirectly to dissolved organic carbon in the systems as a result of an increased (C:N) ratio. This condition may decrease the growth of nitrifying bacteria resulting in a longer period for starting up and complete nitrification due to competition for dissolved oxygen and space in the bio-film (Okabe et al. 1996).

There was a constant lower nitrate concentration in the systems with guar gum fed fish throughout the experiment. That may be caused by a higher de-nitrification in guar gum systems due to organic matter load (Schuster and Stelz 1998). The numerically higher

unexplained nitrogen in guar gum treatment may support the higher de-nitrification. However, it can not be neglected that also protein digestibility was increased by cellulose treatment. This may have increased the ammonia excretion by fish (Rychly 1980; Brunty et al. 1997), thereby leading to a constant higher level of nitrate in the cellulose treatment.

Nitrogen balance

Due to a similar feed intake, both treatments received the same amount of nitrogen. Between 31 to 33% nitrogen was incorporated into harvested fish (Table 5). The remainder was released into the system as waste. There was a significant higher loss of nitrogen through exchange water in the cellulose fed systems. The inclusion of cellulose was supposed to reduce nitrogen level in the water by increasing the faeces removal percentage. However, the results are not supporting this hypothesis. This may be due to a higher ADC of protein in cellulose diet compared to guar gum. This decreased faecal nitrogen removal (2.7g) in cellulose treatment by the low amount of nitrogen excreted via faeces and therefore increased the nitrogen input to the systems.

The nitrogen accumulated in the system was similar for both treatments. A larger suspended solids production in the guar gum fed systems might have increased solid-residence and thereby leading to higher sediment inside the systems. This may increase nitrogen accumulation inside the systems and compensate the lower quantity of water nitrogen in the guar gum systems.

Conclusion

The water quality data from this study indicate that differences in feed composition can significantly reduce suspended solids production by improving physical property of faeces. This condition increases nitrification rate and dissolved oxygen. A quick faeces removal from the system lessens the amount of solids undergoing bacterial decomposition, thus reducing the organic loading discharge into the surrounding environment. Although, the supplementation of soluble non starch polysaccharide in this study was high compared to commercial levels (below 1.5%; Refstie et al.,1999), replacement of fishmeal by plant ingredients will increase the non starch polysaccharide content of aqua-feeds and thereby changes faeces characteristics. Developing diets to

maximize faeces removal percentage may play an important role in maintaining water quality in intensive systems.

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Chapter 7

General discussion

Introduction

During the past two decades, the waste produced by aquaculture operations posed increasingly problems for the outside environment (Macmillan et al. 2003a). Concurring with the continuous trend to intensify the production process, the waste produced by the fish complicates also the management of the water quality within the system (Piedrahita 2003; Macmillan et al. 2003b). As a result, "managing aquacultural wastes" has become an important focus area in aquacultural research (Cho and Bureau 1997 and 2001).

Aquacultural waste is composed of dissolved and solid components. The dissolved fraction (mainly consisting of excreted nitrogen, phosphorous and dissolved organic matter) can be treated in water purification units. The solid fraction is usually collected and disposed. Faeces is the main source of solids waste in a fish farm (Franco-Nava et al. 2004). The impact of faecal waste can be reduced by using highly digestible feed ingredients or by improving the removal efficiency of the faeces.

The removal efficiency of the faeces can be improved by a better faeces consistency, since this will increase the ratio of removed to non-removed faeces. When relative more faeces can be removed from the water, the suspended solids and dissolved solids load within the system will decrease, thereby creating better water quality conditions in the production systems.

In the present study, the underlying hypothesis was that feed composition, and more specifically, the type and quantity of plant ingredients in the feeds may have profound effects on the quantity and quality of faeces being produced. Indeed, plant ingredients have generally a lower digestibility compared to fishmeal, leading to more faeces being produced (Table 1). Further, plant ingredients may contain varying levels of starch and/or of non-starch polysaccharides (NSP), which may increase bacterial degradation in the intestine, thereby reducing the stability of the faeces. In conclusion, the composition of the diet and more specifically, the use of plant ingredients may affect faeces production and faeces stability, thereby influencing the water quality within the system and potentially, the waste discharge to the outside environment.

Table 1. Total faeces production and digestibility in tilapia fed on plant based diets

Diets	Inclusion level	ADC of dry matter (%)	Total faeces produced (g DM kg feed)	References
Fishmeal	15%	79.1	209	Chapter 2
Soybean extract	15%	80.2	198	Chapter 2
Dried duckweed	15%	77.6	224	Chapter 2
Dried duckweed	40%	71.1	283	El-Shafai et al. (2004)
Acha meal	30%	60.5	395	Fagbenro et al. (2000)
Sorghum meal	30%	63.9	361	Fagbenro et al. (2000)

The main objective of this thesis was to determine the effect of dietary composition on faeces characteristics and faeces production in tilapia. Moreover, this project investigated whether differences in faecal characteristics, induced by dietary composition, have a significant impact on water quality in recirculation system.

How to measure faecal physical characteristics

To analyse the faeces characteristics, a proper approach for this measurement had to be developed first. Therefore, in this study, the removal efficiency of faeces by a collector (Settling tank and/or Choubert collector) was proposed as an indicator for faeces stability. The faeces removal efficiency is based on the difference between the collected quantity of faeces by a collector to the total faeces produced by fish. A higher faeces removal shows that the faeces particles are firmly fixed together which is not easily crashed down, indicating a higher faeces stability. The selection of faeces collectors in the current study was based on the common solids removal systems in Recirculating Aquaculure Systems (RAS). The used settling tank was used as a model for a settling basin in RAS. The applied Choubert system functions similar to a drum filter. To our knowledge, the use of faeces removal efficiency as a parameter for faeces stability is quite new. Han et al. (1996) determined particle size distribution of solids and used this as a measure for faeces stability. In a study on European seabass, Dias et al. (1998) measured visually the physical characteristics of the faeces such as firmness, cohesion and settleability as a measure for faeces stability. Quantitive faeces collection method is easier than that of particle size measurement (Han et al. 1996) and more precise compared to visually observation method (Dias et al. 1998). However, this method only measures faeces

removal efficiency, but suspended solids load to the system still unclear. In the future, a stationary suspended solids filtration facility or a drum filter, which can be connected directly to the fish tank, may allow to measure particles and COD load to the system.

The relationship between faeces production and faeces removal

In Chapter 4 and 5, we proved that an increased faeces stability enhances the ratio of removed faeces to non-removed faeces. In Chapter 6, it was shown that this increased faeces stability reduced also the conversion of faeces to suspended and dissolved solids. Indeed, as shown in Figure 1, an increased faeces removal (from 50 to 90%) may reduce also the non-removed fraction of the faeces (from 250 to 50 g). However, apart from the overall removal efficiency, the total faeces production (as indicated by the dry matter digestibility) remains important. The main concern is that developing diets to maximize faeces stability may reduce the nutrient digestibility, thereby increasing the total solid waste output. An increased total faeces production will increase proportionally the amount of non-removed faeces. If the extra solid waste cannot be collected by improving the faeces removal efficiency, the increase of total solid waste can be a major drawback in terms of water quality and environmental protection.

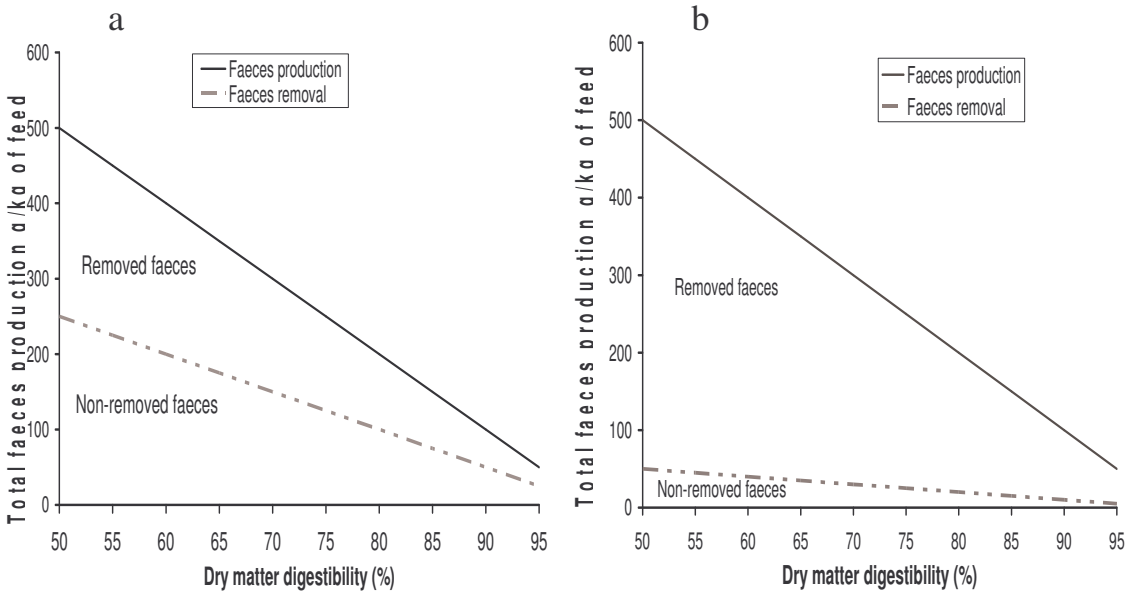

Figure1. Comparison of removal and non-removal fraction of faeces at two faeces removal efficiencies (a: 50% and b: 90%)

This condition raises the question to which extent improving of the faeces stability (faeces removal %) can compensate for the extra faeces produced and not removed. Differences in the amount of non-removed faeces between a control (e.g., a high quality diet) and a cellulose-based diet (as a plant based diet) can be reduced by improving the faeces removal percentage (Figure 2). For instance the production of non-removed faeces in the control diet will be similar to the cellulose-based diet if the faeces removal percentage for the cellulose-based diet would increase from 70 to 75%. This is precisely what happened in our experiments (Chapter 4), where inclusion of cellulose did not increase the amount of non-removed faeces compared to the control diet, due to an increase in faeces removal efficiency.

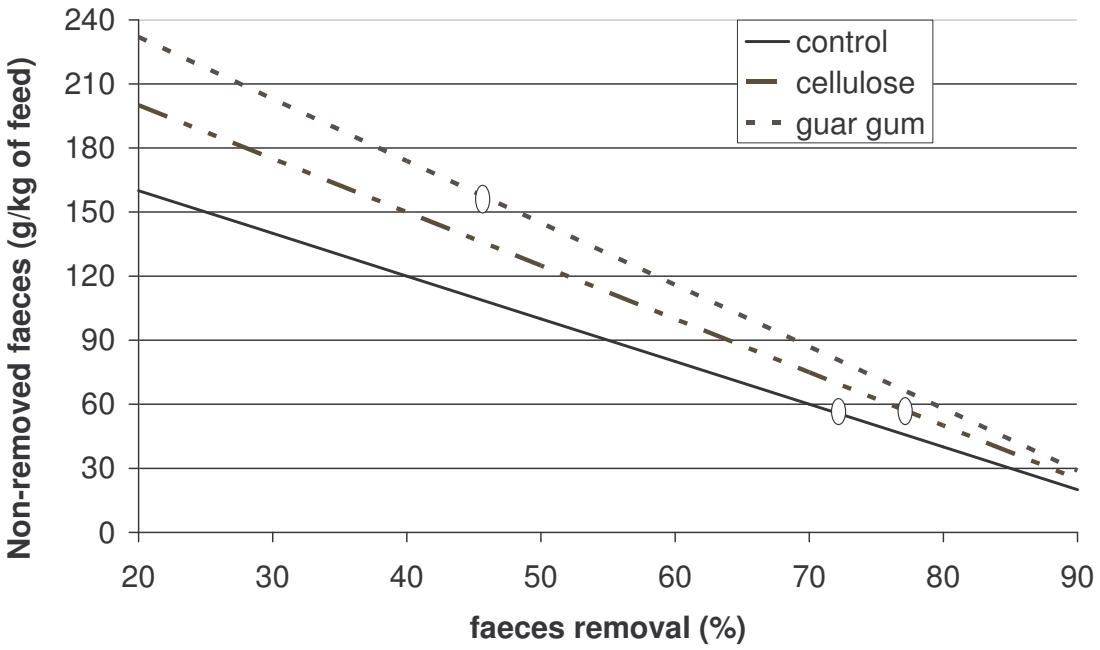

Figure 2. Hypothetical relation between faeces removal percentage and non-removed faeces (digestibility data are from Chapter 4, circles (0) represents the real position of the experimental diets on the graph)

Does the carbohydrate fraction affect faeces stability?

Faeces mainly consists of undigested carbohydrate from grain (starch and NSP) and of minerals (Cho et al. 1994; Cho and Bureau 2001). Undigested protein and fat are usually

low since in fish feeds, dietary protein and fat are generally highly digestible (Cho and Bureau 2001). The results of this thesis showed that undigested fractions of carbohydrate in faeces changes the faeces characteristics. These changes depend on the physicochemical properties of carbohydrates (see also Chapter 4 and 5). This is in agreement with results of Storebakken (1985), Han et al. (1996) and Dias et al. (1998) who reported that the carbohydrate fraction can alter the faeces characteristics in respectively rainbow trout, tilapia and European seabass.

Dietary inclusion of soluble NSP reduced faeces stability (Chapter 4). This finding is similar to that of Storebakken (1985) and Han et al. (1996) in respectively rainbow trout and tilapia and Lewis et al. (1994) and Twomey et al. (2003) in dog who studied the impact of soluble NSP on faeces characteristics. Addition of soluble NSP to the diet also increases the viscosity of the intestinal digesta (see Chapter 4 in our study and Leenhouwers et al. 2004) and the retention time of the digesta (Van der Klis et al. 1993), thereby increasing microbial fermentation (De Lange 2000). Degradation of the undigested dietary ingredients by the intestinal micro-flora increased the water content of the digesta (Verina et al. 1988) and reduced the pH of the digesta and faeces (Row 1997; Twomey et al. 2003).

Insoluble NSP with moderate water holding capacity (Robertson and Eastwood 1981) and less fermentability (Kihara and Skata 1997) appears not to interfere with digesta characteristics such as solubility, fermentation and water content. Even more, since insoluble NSP decreases the retention time of the digesta (Dias et al. 1998), they may improve the faeces stability by reducing the time available for fermentation in the gut (Smits and Annison 1996; Chapter 4).

There is limited information available on the physicochemical and functional effects of dietary starch on digesta and faeces. Han et al. (1996) observed that in tilapia inclusion of 19% of gelatinized starch improved the faeces stability and the faeces particle size. It seems that indigestible starch (resistant starch) has a great impact on the digesta characteristics. The fraction of starch which escapes digestion will be fermented by the intestinal microbes in rat (Kishida et al. 2001; Ferguson et al. 2001) and also in tilapia (Chapter 5). In rat, a diet containing high levels of resistant starch decreased moderately

the transit time and increased the faecal bulk compared with the control diet (Jenkins et al. 1998; Fergusen et al. 2000; Kishida et al. 2001).

The physicochemical effects such as fermentation and viscosity of resistant starch on digesta are almost similar to those of soluble NSP (Cummings 1995). However, unlike soluble NSP, fermentation, induced by dietary maize starch, does not increase the water content of the digesta in tilapia (Chapter 5) nor in rat (Kishida et al. 2001; Ferguson et al. 2001). Increasing the starch content of the diet increases the viscosity of the intestinal digesta (Chapter 5). This higher viscosity increase the shear resistance of the faeces and significantly inhibit particle break down (Brinker et al. 2005).

All this may explain that an increased dietary carbohydrate fraction changes the digesta characteristics, thereby affecting the removal efficiency of the faeces.

Mechanisms through which carbohydrates affect faeces characteristics

It seems that each carbohydrate fraction influences the faeces characteristics through a specific route, depending on the physicochemical properties of the fraction. The viscous nature of soluble NSP increases the water content of the digesta as they pass through the intestinal tract (Fahey et al. 1992). An increase in digesta viscosity also leads to a higher retention time and may enhance the activity of the intestinal bacteria (Smits 1996). The osmotic pressure induced by fermentation products and by soluble carbohydrate molecules which escape digestion lead to a higher water content of the digesta (Vernia et al. 1988), resulting in delicate faeces (Figure 3). On the other hand, viscosity alone also increases the shear resistance and structural stability of the faeces, resulting in a higher faeces removal (Chapter 5; Brinker et al. 2005). The overall impact of soluble NSP on faeces removal depends on the interaction between fermentation and viscosity of the digesta.

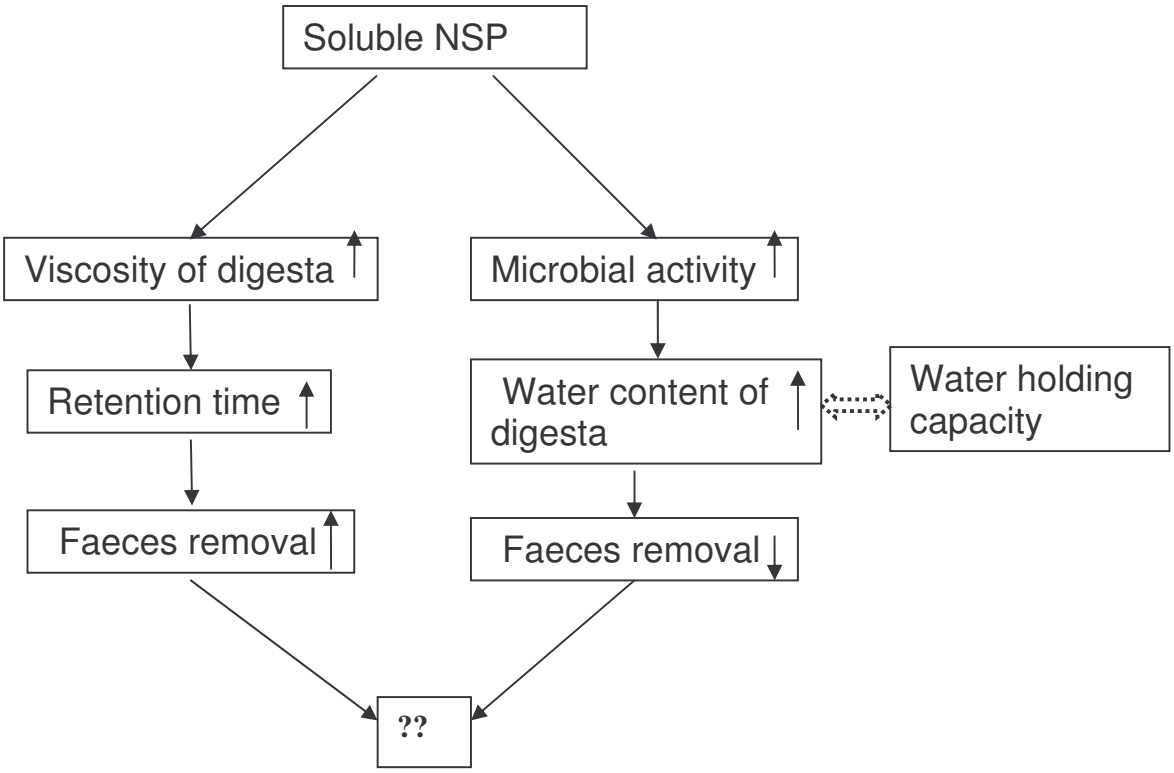

Figure 3. The mechanism by which soluble NSP may affect faeces removal efficiency in tilapia

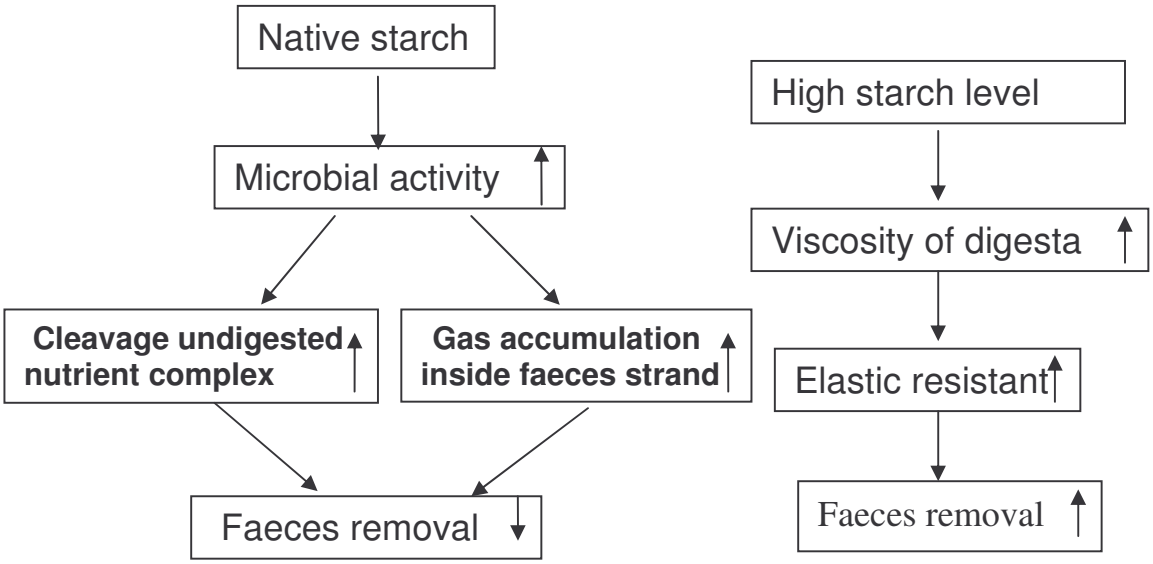

Figure 4. The mechanism through which starch may affect faeces removal efficiency in tilapia

In contrast to the situation with NSP, the physicochemical effects of starch on the faeces characteristics are not associated with a change in water content of the digesta (Chapter 5). In tilapia, starch can serve as a fermentation substrate (Kihara and Sakata 1997; Chapter 5) and can also induce a higher viscosity in the gastro-intestinal tract (Chapter 5). The undigested fraction of starch (mainly available in native starch) is degraded at the end of the intestine, reducing the faeces particle size and the faeces particle density (Figure 4). However, addition of a higher level of starch to the diet improves faeces removal through a higher shear resistance of the faecal pellet, caused by an increased viscosity. It seems therefore that with starch containing diets, the faeces removal efficiency is determined by the balance between positive effects of viscosity and negative effects of fermentation (Table 2).

Table 2. The relationship between digesta characteristics and faeces removal in tilapia fed with two types of maize starch (gelatinized; GEL versus native; NAT) and two levels of maize starch (LOW versus HIGH) (Chapter 5).

	Diets			
Dietary component	GEL-LOW	NAT-LOW	GEL-HIGH	NAT-HIGH
Intestinal Fermentation	Low	High	Low	High
Intestinal viscosity	Low	Low	High	High
Faeces removal %	71.6	55.9	81.3	63.1

The previous discussion shows that the intestinal microflora is largely responsible for the degradation of digesta and faeces, and that it may excrete a negative effect on the faeces stability (Chapters 4 and 5). The microflora can also influence the viscosity of the digesta due to degradation of macro-molecules (Annison 1993; Bedford 1996). The negative impact of fermentation on the faeces stability or on the digestibility of given feed ingredients can be monitored by determining the in vitro fermentability, thereby predicting in vivo fermentation of the intestinal content in fish.

Is higher faeces removal always associated with lower ADC?

The relationship between ADC of dry matter and faeces removal shows that faeces removal increases linearly with dry matter digestibility for both NSP and starch (Figure 4). In other words an increase in faeces removal is associated with a lower total faeces production (Chapter 4, 5 and 6). This suggests that the physicochemical characteristics of the diet, which may reduce the faeces stability (e.g., fermentability), may also cause a lower nutrient digestibility. Similarly, Smits and Annison (1996) observed that compared to conventional chickens, in germ-free broiler chickens the anti-nutritive effect of viscous rich ingredients (carboxymethyl cellulose) was absent.

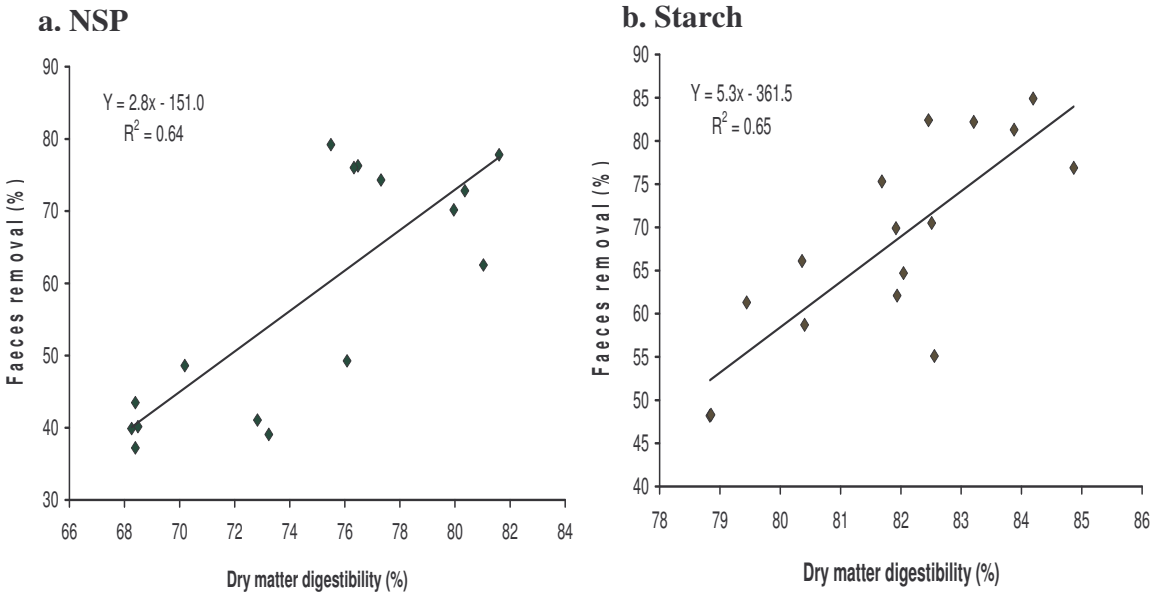

Figure4. The relationship between dry matter digestibility and faeces removal; a; NSP supplementation, b: starch supplementation

However, the relations shown in Figure 4 are very specific since they are based on feed ingredients tested in the present study (starch and NSP) only and using a settling tank as faeces collector. This condition cannot be generalized for all feed ingredients. It appears that the addition of less fermentable ingredients changes the positive relationship between dry matter digestibility and faeces removal efficiency.

Conclusion

The removal efficiency of faeces as measured by the Choubert collector and/or by a settling tank is a good measure for faeces stability. The proper faeces collection method should be chosen in relation to the dietary composition. However, a settling tank gives a higher faeces removal efficiency and therefore seems to provide more representative results. Manipulation of the diet composition changes the faeces characteristics in tilapia. Fermentation and viscosity of the digesta are two main parameters affecting the faeces stability. A detailed investigation on fermentability and viscosity of different ingredients provided insight in characterisation of faeces, thereby giving suggestions for diet formulation geared to maximize solids removal. An increase in faeces removal efficiency reduces the organic matter load to the system, thereby improving the water quality in the system.

Future research on the effects of feed composition on faeces characteristics can help and test the effect of osmotic pressure of the intestinal content (possibly induced by dietary minerals) on the water content of the digesta. The interactions between feed composition and fish species, and their consequences on digesta characteristics can be also a subject for future work.

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Summary

Samenvatting

Public concerns about environmental pollution are putting increasing pressure on fish farms to treat their wastewater before release. The waste produced by the fish can also deteriorate water quality within the system. Aquaculture waste can be divided into solid and dissolved waste. Dissolved waste can be treated by a water purification unit and solid waste can be removed by a settling basin and/or filtration. Faeces is the main source of solid waste in a fish farm. Improvement of nutrient digestibility is as a key factor to reduce the production of total solid. However, the scope of digestion is limited and total faeces production will increase in future due to an increased plant ingredient content of fish feed. An increased consistency of faeces may improve its removal efficiency, thereby improving water quality.

The objective of this research is to determine physical characteristics of the faeces and total faeces production in relation to feed composition in Nile tilapia. Moreover, also the question whether these differences in faecal characteristics have a significant impact on the water quality in a recirculation system was addressed.

The first step of this study (Chapter **2**) aimed to determine the total faeces production and faeces stability in tilapia fed on five ingredients. These five feed ingredients were soybean meal, soybean protein concentrate, wheat gluten, duckweed and single cell protein. Feed ingredients did not change significantly faeces removal percentage and settleable faeces production. However, total faeces production and non-settleable solids were influenced by feed ingredients. Duckweed and single cell protein resulted in a larger faeces production due to a lower dry matter digestibility. The low faeces removal (6.8 to 11%) raised questions about the reliability of the Choubert collector for measuring faeces removal percentage and nutrient digestibility.

In the third Chapter, two faeces collection methods (settling tank versus Choubert collector) were compared for measuring digestibility and faeces characteristics. Digestibility estimates were highly correlated between both faeces collection methods. For a control diet (as a highly digestible diet) differences in digestibility between the two collection methods were small, but when a low quality diet was used (duckweed based diet), these differences increased. The average faeces removal percentage was about three times higher in a settling tank than in a Choubert collector. Yet, there was no clear

correlation between the type of collectors, the type of feed and removal percentage. Based on these methods we concluded that more representative data would be obtained by using a settling tank.

In Chapter **4** and **5**, we focused on the effects of nutritional factors (particularly carbohydrate fractions) on faeces characteristics. Chapter **4** deals with the impact of two different types of non-starch polysaccharide (NSP) on faeces characteristics. In future, NSP content of fish feed will increase because it can be expected that plant ingredients will increase to replace fishmeal. Soluble NSP increased digesta viscosity and reduced faeces removal by 42%. Insoluble NSP did not change digesta viscosity and faeces removal. Soluble NSP decreased faecal waste removal efficiency through a reduction of faeces removal and nutrient digestibility.

In Chapter **5**, the mechanisms through which dietary composition can influence faecal removal efficiency were assessed. Two types of dietary starch (native versus gelatinized), each at two levels (high versus low) were chosen to induce different fermentation levels in the intestine of tilapia. Replacement of native starch by gelatinized starch improved faeces removal percentage, growth and digestibility, but reduced fermentation at the end of the intestine. Viscosity and dry matter of digesta did not change at the end of the intestine by addition of gelatinized starch. A high dietary inclusion level of starch (both types) also increased digestibility, growth and faeces removal percentage. Fermentation and dry matter content at the end of the intestine were not influenced by a high starch inclusion level, but viscosity was higher at the high level of starch inclusion. Volatile fatty acid levels in the stomach of tilapia were high in the treatments with gelatinized starch. In conclusion, intestinal fermentation induced by native starch has a negative impact on faeces removal efficiency. A higher inclusion level of starch resulted in a higher viscosity of digesta, lead to a higher faeces removal efficiency.

Based on the results in Chapter **4** (a big difference in faecal removal between cellulose and guar gum based diet but similar digestibility), in Chapter **6** the effects of differences in faeces removal efficiency on water quality was assessed. Supplementation of guar gum reduced faeces removal and increased total suspended solids. Dissolved oxygen was lower in the systems containing fish fed guar gum. Ammonia and nitrite concentrations were higher in the systems with guar gum fed fish, although these differences were not

significantly different. Nitrate concentration in the water was significantly higher in the systems with fish fed with a diet at 8% cellulose inclusion. An increased faeces removal from the system lowers organic matter load in the system, thereby increasing nitrification and dissolved oxygen levels.

This thesis proved that manipulation of feed composition can improve the physical properties of faeces, thereby increasing faeces removal efficiency. Due to a relatively lower digestibility, carbohydrates are the most abundant fraction in faeces and thus have a significant impact on the digesta characteristics and faeces removal efficiency. Soluble NSP reduced faeces removal efficiency and increased the viscosity of digesta and faeces. Gelatinized starch improved faeces removal and reduced intestinal fermentation. Increasing dietary starch level induced a higher intestinal viscosity and improved faeces removal. Degradation of digesta and faeces by gut microbes cleaves macro-molecules. It might be a major factor to reduce faeces stability. The viscosity of digesta supports the shear resistance of faeces and seems to improve the faeces stability. An increased faeces removal from the system decreases the amount of organic load in the system, thereby improving water quality.

Wegens mogelijke negatieve gevolgen voor het milieu worden viskwekerijen steeds meer genoodzaakt om hun afvalwater te behandelen. Afvalstoffen geproduceerd door vissen kunnen de waterkwaliteit van het kweeksysteem zelf ook negatief beïnvloeden.

Afval afkomstig van viskwekerijen wordt geclassificeerd als vast en opgelost afval. Opgelost afval kan behandeld worden door middel van waterzuiveringsinstallaties. Vast afval wordt verwijderd door bezinkingsbassins en/of door filtratie. Mest geproduceerddoor vissen is de grootste bron van afval in een viskwekerij. Het verbeteren van de verteerbaarheid van het voer is belangrijk voor het reduceren van het totaal geproduceerde vaste afval. Echter, de mate van mogelijke verbetering in verteerbaarheid is beperkt. Bovendien kan de totale mestproductie toenemen in de toekomst door een toename aan minder goed verteerbare plantaardige ingrediënten in het visvoer. Daarentegen zou een toename in mestconsistentie de efficiëntie van mestverwijdering kunnen bevorderen en daardoor waterkwaliteit verbeteren.

Het doel van dit proefschrift is het bestuderen van effecten van voersamenstelling op fysieke eigenschappen van mest en totale mestproductie in Nijl tilapia. Bovendien zal de invloed van verschillen in mestconsistentie op waterkwaliteit in recirculatiesystemen onderzocht worden.

In Hoofdstuk 2 van dit proefschrift is totale mestproductie en mestconsistentie onderzocht in tilapia gevoerd met vijf verschillende ingrediënten, namelijk sojaschroot, soja eiwit concentraat, tarwe gluten, eendekroos en single cel eiwit. Deze ingrediënten hadden geen invloed op het percentage mest dat verwijderd kon worden en bezonken was. Echter, totale mestproductie en niet-bezinkbaar afval werden wel beïnvloed door het voer. Eendekroos en single cel eiwit gaven een hogere mestproductie vanwege een lagere droge stof verteerbaarheid. Het lage percentage mest dat verwijderd kon worden (6.8-11%) riep wel vragen op aangaande de betrouwbaarheid van de Choubert collectors om mestverwijderingspercentage en nutriëntenverteerbaarheid te meten.

In Hoofdstuk 3 zijn twee mestverzamelingsmethoden (bezinkingstank versus Choubert collector) vergeleken voor het meten van nutriëntenverteerbaarheid en mesteigenschappen. Schattingen voor verteerbaarheid waren hoog gecorreleerd tussen deze methodes. Voor het controle voer (hoog verteerbaar) waren de verschillen in verteerbaarheid klein tussen de collectiemethodes, maar voor minder goed verteerbare

voeders (eendekroos) waren de verschillen groter. Het gemiddelde mestverwijderingspercentage was ongeveer drie maal zo hoog in een bezinkingstank vergeleken met een Choubert collector. Er was geen eenduidige correlatie tussen type collector en type voer aan de ene kant en mestverwijderingspercentage aan de andere kant. Gebaseerd op deze methoden hebben we geconcludeerd dat meer representatieve resultaten worden verkregen bij gebruik van de bezinkingstank.

In Hoofdstuk 4 en 5 zijn we nader ingegaan op de effecten van voedingsfactoren (met name koolhydraatfracties) op mesteigenschappen. Hoofdstuk 4 onderzocht het effect van twee typen niet-zetmeel polysacchariden (NSP) op mesteigenschappen. Het NSP-gehalte in visvoeders zal in de toekomst stijgen door het toenemende gebruik van plantaardige ingrediënten in visvoer. Wateroplosbare NSP verhoogden chymus viscositeit en verlaagden mestverwijdering met 42%. Wateronoplosbare NSP hadden geen invloed op chymus viscositeit en mestverwijderingspercentage. Concluderend, oplosbare NSP verlagen de efficiëntie van mestverwijdering door middel van een verlaging in mestverwijdering en nutriëntenverteerbaarheid.

In Hoofdstuk 5 zijn mechanismen onderzocht die effecten van voersamenstelling op mestverwijderingsefficiëntie zouden kunnen verklaren. Twee soorten zetmeel (natief versus ontsloten) in twee concentraties (hoog versus laag) werden gebruikt om verschillen in fermentatie-activiteit in de darm te induceren. Vervanging van natief zetmeel door ontsloten zetmeel leidde tot een verbetering in mestverwijderingspercentage, groei en verteerbaarheid, maar tot een verlaging in fermentatie in het achterste gedeelte van de darm. Viscositeit en droge stof gehalte van chymus veranderden niet in het achterste gedeelte van de darm door toevoegen van ontsloten zetmeel. Een hoge concentratie zetmeel (beide typen) in het voer bevorderde ook groei, verteerbaarheid en mestverwijderingspercentage. Fermentatie en droge stof gehalte aan het einde van de darm werden niet beïnvloed door een hoog zetmeelgehalte, maar viscositeit was verhoogd bij het hoge inclusieniveau (beide typen). Vluchtige vetzuren in de maag van tilapia waren hoog in de behandelingen met ontsloten zetmeel. Concluderend, fermentatie geïnduceerd door natief zetmeel had een negatief effect op mestverwijderingspercentage. Een hoger gehalte aan zetmeel in het voer leidde tot een hogere chymus viscositeit met als gevolg een hogere efficiëntie van mestverwijdering.

Gebaseerd op de resultaten in Hoofdstuk 4 (grote verschillen in mestverwijdering tussen cellulose en guar gum voeders, maar gelijke verteerbaarheid), zijn in Hoofdstuk 6 de effecten van verschillen in mestverwijderingsefficiëntie op waterkwaliteit onderzocht. Toevoeging van guar gum reduceerde mestverwijdering en verhoogde de totale in suspensie zijnde vaste stof. Het zuurstofgehalte was lager in het systeem met vissen die guar gum gevoerd werden. Ammonia en nitriet concentraties waren hoger in de guar gum-gevoerde systemen, maar deze verschillen waren niet significant. Nitraat concentraties waren significant hoger in de systemen waarin vissen met cellulose gevoerd werden. Concluderend, een verhoogde mestverwijdering uit het systeem verlaagt de organische stof belasting en verhoogt daarbij nitrificatie en zuurstofgehalte.

Dit proefschrift heeft bewezen dat veranderingen in voedersamenstelling de fysieke eigenschappen van mest kunnen beïnvloeden en daardoor de efficiëntie van mestverwijdering kunnen veranderen. Vanwege een relatief lage verteerbaarheid vormen koolhydraten de grootste fractie in mest en dus hebben ze een significante invloed op chymus eigenschappen en mestverwijderingsefficiëntie. Wateroplosbare NSP verlagen de efficiëntie van mestverwijdering en verhogen de viscositeit van chymus en mest. Ontsloten zetmeel verhoogt mestverwijdering en verlaagt fermentatie in de darm. Een verhoging van het zetmeelgehalte in het voer leidt tot een hogere viscositeit en verbeterde mestverwijdering. Afbraak van chymus en mest door darmmicroben verbreekt de structuur van micromoleculen. Dit kan een belangrijke factor zijn om meststabiliteit te verbeteren. De viscositeit van de chymus lijkt de stabiliteit van mest te verhogen. Een toename in mestverwijdering uit het systeem verlaagt de organische belasting en verbetert daarbij de waterkwaliteit.

List of publications

Journals

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

Amirkolaie, A.K., El-Shafai, S.A., Eding, E.H., Schrama, J.W., Verreth, J.A.J. (2005) Comparison of faecal collection method with high and low quality diets regarding digestibility and faeces characteristics measurements in Nile tilapia, Aquaculture Research 36, 578-585.

Amirkolaie, A.k., Leenhouwers, J. I., Verreth, J. A. J., Schrama, J. W., (2005) Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile Tilapia (*Oreochromis niloticus* L.). Aquaculture Research 36, 1157-1166.

Amirkolaie, A.K, N. Duijster, Leenhouwers, J. I, J. W. Schrama, J. A. J. Verreth (2005) Faecal physical properties influence water quality in a recirculation system. Submitted.

Amirkolaie, A.K, J. W. Schrama, J. A. J. Verreth (2005) Effect of type and dietary inclusion level of starch on digesta and faeces characteristics in Nile Tilapia (*Oreochromis niloticus* L.). Submitted.

Proceedings

Amirkolaie, A.k., Leenhouwers, J. I., Verreth, J. A. J., Schrama, J. W. (2004) Effect of type of dietary fibre (DF) on faeces characteristics , digestibility and fish performance in tilapia (*Oreochromis niloticus*): In: 11th International Symposium on Nutrition and Feeding in fish, Phuket, Thailand. Book of Abstracts 218.

Amirkolaie, A.K, Schrama, J. W., Verreth, J. A. J. (2004) Comparison of digestibility and faeces recovery in tilapia (*Oreochromis niloticus*) using two methods of faeces collection. In: Aquaculture Europe 2004, Biotechnologies for quality, Barcelona, Spain. Extended Abstracts. 121.

Amirkolaie, A.K, N. Duijster, J. W. Schrama, and J. A. J. Verreth (2005) Faecal physical properties influence water quality in a recirculation system, World aquaculture 2005, Bali, Indonesia , Book of Abstract 34.

Schneider, O., **Amirkolaie, A.K**., Vera-Cartas, J., Eding, E.H., Schrama, J.W., Verreth, J.A.J. (2004) C, N, and P balances of five feed ingredients evaluated as fish meal alternatives in tilapia diets, Barcelona, Spain. Extended Abstract 725

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Abdolsmad Keramat Amirkolaie
Wageningen, September 8, 2005

Curriculum Vitae

Abdolsmad Keramat Amirkolaie was born on September 21, 1971, in Amirkola (Babol), in the north of Iran. He completed his elementary and intermediate school in Amirkola. He then went to the neighborhood city (Babol) to complete his high school education in Natural Science.

After high school in 1989, he joined to the Faculty of Natural Resources at Tehran University. He completed his Bachelor in Fisheries and Environmental Science in 1993. Later on, he served in the Iranian army for two years obligatory military service. He passed successfully the entrance examination for Master of Science and started his MSc in 1995 at Tarbiat Moddars University. He was the first honor student at the entrance exam to the MSc course. He completed his MSc in the field of aquaculture with a thesis titled by "*Life food preference of sturgeon fry in earthen pond"*. In his thesis, he characterized the phone and quantity of live food in the diet of sturgeon fry.

With a national examination in 1997, he was awarded a scholarship to continue his education toward PhD in the abroad universities. However, he started his PhD course four years later in 2001. Before PhD, he was working at Mazandarn University, department of aquaculture as a temporary lecturer. He was responsible for giving lecture at two courses and for providing material for practical. He joined to the Fish culture and Fisheries group in Jun, 2001. His research work focused on "characterization of faecal waste in the Nile tilapia in relation to feed composition". He finished his PhD after four years and four months by a fund from Ministry, Research, Science and Technology in Iran. He will return back to home and resume work at Mazandaran University.

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Training and Supervision Plan		**Graduate School WIAS**
Name PhD student	Abdolsamad Keramat Amirkolaie	
Project title	The effect of dietary carbohydrate on faeces characteristics in Nile tilapia	
Group	Fish culture and fisheries group	
Daily supervisor	Dr. Johan W. Schrama	
Supervisor	Prof. dr. Johan A.J. Verreth	
Project term	from 01-06-2001	until 01-06-2005
Submitted	date: 1-5-2005	first plan / midterm / **certificate**

EDUCATION AND TRAINING (minimum 21 cp, maximum 42 cp)		
The Basic Package (minimum 2 cp)	year	cp*
WIAS Introduction Course (mandatory)	2003	1.0
WIAS course "Biology underpinning animal sciences: Broaden your horizon"	2003	1.0
Subtotal Basic Package		**2.0**
Scientific Exposure (conferences, seminars and presentations, minimum 5 cp)	year	cp
International conferences *(minimum 2 cp)*		
11th International symposium on nutrition and feeding in fish, Thailand, May 2-7	2004	0.8
Aquaculture Europe, Barcelona, Spain, October 20-23	2004	1.0
World Aquaculture 2005, Bali, Indonesia, May 9-14	2005	1.0
Seminars and workshops		
WIAS Science Day 2002, 2003, 2004 and 2005	2002-2005	0.8
WIAS seminar plus "Fats and Seafood for Health", Wageningen, The Netherlands	2003	0.2
WIAS seminar "Shaping the embryo; Dynamics of early vertebrate development", Wageningen	2003	0.2
Workshop " Challenge for Mediterranean aquaculture". Barcelona, Spain	2004	0.2
WIAS seminar "Vitality in fish". Wageningen, The Netherlands	2005	0.2
Presentations *(minimum 4 original presentations of which at least 1 oral, 0.5 cp each)*		
Poster presentation at "WIAS Science Day 2003"	2003	0.5
Poster presentation at "Fin fish nutrition 2004"	2004	0.5
Poster presentation at "Aquaculture Europe 2004"	2004	0.5
Oral presentation at "World Aquaculture 2005"	2005	0.5
Oral presentation at "WIAS Science Day 2005"	2005	0.5
Subtotal International Exposure		**6.9**
In-Depth Studies (minimum 4 cp)	year	cp
Disciplinary and interdisciplinary courses		
VLAG/WIAS course "Ecophysiology of the gastrointestinal tract"	2005	1.0
Aqualabs Training Course in Fresh Water Aquaculture and Environment (Szarvas, Hungary).	2005	1.2
Advanced statistics courses*(optional)*		
WIAS course “Design of Animal Experiment”. November 25-27, Wageningen	2002	0.6

Workshop "Experimental design and methodologies for nutritional studies on finfish and crustaceans". Phuket, Thailand	2004	0.2
Undergraduate courses		
Aquaculture production systems (E450-219)	2001	4.0
Fish nutrition (E450-217)	2001	4.0
Statistical methods (A100-243)	2001	3.0
Subtotal In-Depth Studies		**14.0**
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Written English	2002	2.0
WIAS Course Techniques for Scientific Writing	2003	0.8
Use of Laboratory Animals	2003	3.0
Time planning and project management	2004	1.0
Subtotal Professional Skills Support Courses		**6.8**
Research Skills Training (apart from carrying out the PhD project, optional)	year	cp
Preparing own PhD research proposal (optional, maximum 4 cp)	2003	4.0
Subtotal Research Skills Training		**4.0**
Didactic Skills Training (optional)	year	cp
Supervising MSc theses *(maximum 1 cp per MSc student)*		
Supervisor of Niel Duijster	2004	1.0
Supervisor of Job Munten	2004	1.0
Subtotal Didactic Skills Training		**2.0**
Management Skills Training (optional)	year	cp
Organisation of seminars and courses		
WIAS Science Day 2004	2004	1.0
Subtotal Management Skills Training		**1.0**
Education and Training Total (minimum 21 cp, maximum 42 cp)		**36.7**

*One credit point (cp) equals a study load of approximately 40 hours.

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