

Carbohydrate utilisation by tilapia: a meta-analytical approach

Roel M. Maas , Marc C.J. Verdegem , Geert F. Wiegertjes and Johan W. Schrama 

Aquaculture and Fisheries Group, Wageningen University and Research, Wageningen, The Netherlands

Correspondence

Johan W. Schrama, Aquaculture and Fisheries Group, Wageningen University and Research, De Elst 1, 6708 WD Wageningen, The Netherlands. Email: johan.schrama@wur.nl

Received 12 August 2019; accepted 17 December 2019.

Abstract

Currently, studies reporting the digestibility of carbohydrates, starch and especially non-starch polysaccharides (NSP) in fish are scarce. Carbohydrate digestibility in the diet is largely dependent upon carbohydrate composition (starch vs. NSP). NSP are often considered to be indigestible and thus of no nutritional value. The present study reviews carbohydrates in fish feed, distinguishing between total carbohydrate, starch and NSP. Besides a qualitative approach, a meta-analysis was performed, compiling available data from digestibility studies on tilapia. Our meta-analysis confirms the negative effect of NSP on performance (FCR) and nutrient digestibility (crude protein, fat and energy). However, an average NSP digestibility of 24.3% was calculated in 95 cases. Out of these 95 cases, 88% of them showed a positive NSP digestibility. NSP digestibility was shown to contribute to energy digestibility. The digestion of NSP in fish is associated with fermentation in the gut, producing beneficial volatile fatty acids that are rapidly absorbed by the colonic lumen. Therefore, in diet formulation, digestibility and thus energy originating from NSP should be taken into consideration because NSP contribute to the energy needs of fish, here tilapia. Besides being an energy source, specific types of NSP may have immune-modulating and prebiotic effects and may be increasingly added to fish feed as modulators of fish health. We suggest that NSP is potentially (partly) digested by a wide range of fish species, especially by warm-water species with a long gut adapted to feeding on plant matter, as these factors favour gut fermentation.

Key words: digestibility, meta-analysis, non-starch polysaccharides, *Oreochromis niloticus*, starch.

Introduction

Many studies have been performed with tilapia (*Oreochromis* spp.), comparing digestibility across a wide range of plant ingredients used as substitutes for dietary fishmeal and oil. In general, these studies use a reference diet with a particular percentage of plant ingredients, such as soya bean meal; linseed meal; canola meal; cottonseed meal; sunflower meal; wheat middlings; corn gluten; rice bran; barley; and rye (Sintayehu *et al.* 1996; Degani *et al.* 1997; Schneider *et al.* 2004; Köprücü & Özdemir 2005; Gaber 2006; Dong *et al.* 2010; Obirikorang *et al.* 2015). In these studies, the focus is predominately on protein and fat digestibility, with little or no attention given to carbohydrate digestibility. Carbohydrates are the cheapest energy source for human, fish and other animals. Carbohydrate requirements have not been extensively investigated, compared to fat and protein requirements. It is commonly

accepted that appropriate levels of carbohydrates should be incorporated in fish diets to improve the energy availability, albeit sparingly for carnivorous fish like trout and salmon, which are less well adapted to digest complex carbohydrates. Lack of carbohydrates in fish diets will increase the catabolism of protein and lipids (Kim & Kaushik 1992; Wilson 1994), while sufficient levels of easily digestible carbohydrates allow for protein sparing effects (Kaushik & de Oliva Teles 1985; Hemre *et al.* 1993; Krogdahl *et al.* 2005).

The optimal level of dietary carbohydrates differs widely among fish species. In general, warm-water and freshwater fish are known to utilise higher levels of carbohydrates than cold-water or marine species (Wilson 1994). Omnivorous fish can handle higher levels of carbohydrates than carnivorous fish and show much higher rates of protein sparing (Hemre *et al.* 1993, 2002; Krogdahl *et al.* 2005). A review by Wilson (1994) recommends a dietary digestible

carbohydrate level of 40% for tilapia, while the optimum for marine, carnivorous and cold-water fish (i.e. Atlantic salmon, rainbow trout and plaice) is below 20%. Besides differences in optimal dietary carbohydrate levels, the way fish utilise digestible carbohydrates differs between species. The energy utilisation efficiency of digestible carbohydrates in common carp and Nile tilapia is found to be constant (linear relationship), whereas for barramundi and rainbow trout, the utilisation efficiency decreases with increasing levels of digestible carbohydrate intake (Schrama *et al.* 2018; Phan *et al.* 2019). This suggests that some fish have a limited capacity to process carbohydrates.

The total carbohydrate digestibility in the diet is largely dependent on the carbohydrate composition. The total carbohydrate digestibility depends on the starch level, the molecular complexity of the carbohydrate source and total carbohydrate level in the diet (Stone 2003). The starch fraction of the diet is considered highly digestible with apparent digestibility coefficients (ADC) of 90%, up to 99% in Nile tilapia (Amirkolaie *et al.* 2006; Leenhouders *et al.* 2007; Haidar *et al.* 2016). The ADC of the total carbohydrate fraction has been found to be as low as 30–60% (El-Saidy & Gaber 2003; Deng *et al.* 2016) and as high as 80–90% for Nile tilapia (Sintayehu *et al.* 1996; Tran-Ngoc *et al.* 2016). The large variation in the digestibility of the total carbohydrate fraction is mainly caused by differences in the total dietary fibre fraction.

The dietary fibre fraction consists of remnants of plant cells, which are non-starch polysaccharides (NSP), oligosaccharides, lignin and gums (Dhingra *et al.* 2012). The NSP forms the bulk of the total dietary fibre fraction. In this review, the term NSP is used to describe the total dietary fibre fraction, including oligosaccharides, lignins and gums. There is a wide range of NSP, differing in both characteristics and properties (i.e. solubility), while the amount and type of NSP differ among plant ingredients. NSP is generally classified into cellulose, non-cellulosic polymers (hemicellulose) and pectic polysaccharides. Cellulose is a complex polysaccharide, solely consisting of D-linked glucose units (links of 3000 or more). The non-cellulosic polymers are divided into arabinoxylans, mixed-linked β -glucans, mannans, galactomannans and glucomannans. The pectic polysaccharides mainly consist of D-galacturonic acid (GalA), which forms arabins, galactans and arabinogalactans (Choct 1997; Sinha *et al.* 2011). NSP are considered indigestible by monogastric animals, as they lack enzymes such as β -glucanases and β -xylanases to break down the long polysaccharide chains. Therefore, NSP are often considered to be of little or no nutritional value (Choct 1997; Stone 2003; Sinha *et al.* 2011).

Although considered indigestible, a study by Leenhouders *et al.* (2007) found a digestibility of up to 24% in Nile tilapia for the NSP fraction when using barley as the main

NSP source. Amirkolaie *et al.* (2005) also showed digestibility for purified cellulose and guar gum in Nile tilapia, with an ADC of 2.8% and 20.8%, respectively. A more recent study using Nile tilapia by Haidar *et al.* (2016) showed a digestibility between 41% and 73%, depending on the feeding level, for a diet enriched with dried distillers grains with solubles from wheat (DDGS).

Aim

The scarce information available shows large differences in carbohydrate and NSP digestibility in tilapia (Amirkolaie *et al.* 2006; Leenhouders *et al.* 2007; Haidar *et al.* 2016), suggesting that different factors can potentially affect the digestibility of carbohydrates and NSP. However, studies reporting carbohydrate, starch and especially NSP digestibility are scarce, which makes general statements about their digestibility difficult. Considering the above, the present study reviews carbohydrates in fish feed, distinguishing between total carbohydrates, starch and NSP. Besides a qualitative approach, a meta-analysis was performed, compiling the available data from digestibility studies with tilapia, thus calculating the theoretical total carbohydrate, starch and NSP level in the diet, as well as the digestibility of the total carbohydrate fraction and NSP. The NSP level and carbohydrate and NSP digestibility were checked for correlations with, among others, feeding level, nutrient composition of the diet and digestibility of other nutrients. The qualitative and quantitative (meta-analyses) approaches are combined in this manuscript to improve the understanding of the role and nutritional value of carbohydrates in fish feed, focusing predominately on the NSP fraction. This manuscript describes the total carbohydrate, starch and NSP fraction in separate chapters, followed by concluding remarks.

Methodology

Approach

In order to have an overview and quantify the digestibility of total carbohydrates, starch, NSP and other nutrients, existing digestibility studies concerning tilapia were compiled. From here on, CHO is used for the total carbohydrate fraction, being the dry matter minus the sum of the crude protein, fat and ash. Digestibility studies concerning tilapia were used, as several studies already showed the potential of tilapia to digest NSP. In addition, tilapia is widely used as a model species in digestibility studies, ensuring an adequate amount of data. Tilapia also has, as a herbivorous warm-water fish with a long gut, the potential for gut fermentation (Bergman 1990; Metzler-Zebeli *et al.* 2010).

Studies with common and red coloured Nile tilapia (*Oreochromis niloticus* (L.)), hybrid tilapia (*O. niloticus* × *Oreochromis aureus*) and Mozambique tilapia (*Oreochromis mossambicus*) were selected. Only studies in which CHO in the diet was given, or could be calculated from the reported dry matter (DM), crude protein, crude fat and ash or energy content, were considered. The CHO digestibility was calculated indirectly when not reported. The CHO digestibility was calculated from either (i) the mass balance using the reported ADC of DM, crude protein, fat and ash or (ii) the reported ADC of energy, crude protein and fat as described by Schrama *et al.* (2018). Studies were excluded when the CHO digestibility could not be calculated. Studies testing the digestibility under hypoxic conditions were also excluded. Only controls where fish were fed under normoxic conditions were used to exclude the effect of oxygen level on digestibility. In addition, studies in which enzymes or probiotics were incorporated in the diet were not included, as these can influence the endogenous nutrient digestibility (Castillo & Gatlin 2015; Hai 2015). Besides the nutrient composition of the diets (g kg⁻¹ DM) and the ADC, the feeding level, average weight of the fish at the start and end, duration of the feeding trial, FCR, specific growth rate (SGR) and how the diets were manufactured (extrusion, steam pelleting) were registered. These factors can potentially affect or be affected by the NSP level and CHO and NSP digestibility.

In order to calculate a theoretical NSP digestibility, the NSP level and starch level in the diet had to be determined. This was done by reformulating all the diets with the use of the Centraal Veevoederbureau (CVB) 2016 database. The total CHO in the diet being known, as well as the ratio between NSP and starch, allowed for calculation of the total amount of NSP and starch in the diet. The NSP digestibility was calculated using (i) the CHO level in the diet; (ii) the CHO ADC; (iii) the level of NSP and starch in the diet; and (iv) an assumption for the starch ADC of 99.5% for extruded diets and 91% for pelleted diets. Section 4 will elaborate on the decision to use these ADC values for starch. An overview of the calculations used and elucidation of the composition of the data set is provided as Appendix S1.

Overview studies

Applying the described criteria, data were collected from a total of 19 published (Table 1) and six unpublished studies. The unpublished studies were completed at the Aquaculture and Fisheries Group (Wageningen University, the Netherlands). The experiments included in the data set were mostly testing multiple diets. Combined, the 25 studies provided 100 different diets with their corresponding digestibility. Among these 100 different diets, two diets

were fed at two different feeding levels and another two diets at three different feeding levels, resulting in 106 cases with known diet composition and ADC.

Table 1 gives an overview of the dietary nutrient composition, the nutrient digestibility and other related parameters (FCR, initial body weight, etc.) of the included cases. As Table 1 shows, the carbohydrate digestibility is only reported for 29 of the 106 diets, with an average ADC of 71.5%. The NSP digestibility is only presented by

Table 1 Reported average dietary nutrient composition, apparent digestibility coefficient (ADC) and experimental parameters ± SD of the included diets used for the meta-analysis

	Average	Number of diets
<i>Nutrient composition (g/100 g DM)</i>		
Dry matter (g kg ⁻¹)	92.3 ± 3.1	97
Crude protein	37.3 ± 7.0	100
Fat	10.3 ± 4.1	100
Total carbohydrates (CHO)†	43.2 ± 9.0	100
Nitrogen free extract	39.8 ± 9.4	35
Starch	27.0 ± 10.4	24
Non-starch polysaccharides (NSP)	19.6 ± 15.5	12
Crude fibre	5.2 ± 2.5	43
Energy (kJ g ⁻¹)	20.2 ± 1.0	90
Ash	9.2 ± 2.5	100
Calcium	1.42 ± 0.82	18
Phosphorus	1.06 ± 0.37	41
<i>Apparent digestibility coefficient (%)</i>		
Dry matter	74.2 ± 9.9	86
Crude protein	88.8 ± 5.6	106
Fat	87.5 ± 12.0	106
Total carbohydrates	71.5 ± 15.9	29
Nitrogen free extract	73.3 ± 13.6	21
Starch	96.2 ± 4.4	26
Non-starch polysaccharides	26.4 ± 26.8	14
Energy	80.8 ± 9.2	96
Ash	48.9 ± 10.5	73
Calcium	27.4 ± 11.7	14
Phosphorus	64.1 ± 25.6	27
<i>Experimental parameters</i>		
Feeding level (g kg ^{-0.8} day ⁻¹)	13.0 ± 4.3	98
Initial body weight (g)	72 ± 94.9	106
Feed conversion ratio (FCR)	1.34 ± 0.46	79
Specific growth rate (SGR, % day ⁻¹)	1.70 ± 0.69	80
Length of trial (day)	64 ± 56	100
<i>Manufactured by</i>		
Steam pelleting	–	62‡
Extrusion	–	44§

†Calculated on DM basis as: 100 – (crude protein + fat + ash).

‡Sintayehu *et al.* (1996), Degani *et al.* (1997), El-Saidy and Gaber (2003), El-Shafai *et al.* (2004), Schneider *et al.* (2004), Köprücü and Özdemir (2005), Amirkolaie *et al.* (2006), Amirkolaie *et al.* (2005, 2006), Gaber (2005, 2006), Leenhouders *et al.* (2007), Obirikorang *et al.* (2015).

§Dong *et al.* (2010), Saravanan *et al.* (2012), Schrama *et al.* (2012), Deng *et al.* (2016), Haidar *et al.* (2016), Tran-Ngoc *et al.* (2016).

Amirkolaie *et al.* (2006), Leenhouwers *et al.* (2007) and Haidar *et al.* (2016). These three studies show an average ADC of 26.4% for the NSP fraction, indicating the ability of tilapia to digest NSP to a certain extent. However, the sample size is small and the variation is large.

Statistics

Correlations between dietary NSP level, CHO and NSP digestibility, and factors such as nutrient composition of the diet, feeding level and FCR were explored with Pearson's correlation coefficient. Linear regression equations were estimated when the Pearson's correlation coefficient was significant. The cases (106) were used as experimental units. A one-way ANOVA was used to compare means between the production method (pelleting vs. extrusion) and starch digestibility and reported versus calculated carbohydrate digestibility. All statistics were performed using the IBM Statistical Package for the Social Sciences (SPSS) program (v 23.0, New York, NY, USA).

Total carbohydrates

Published data on total carbohydrate (CHO) digestibility are limited. The constructed data set of the current study contained digestibility values for a total of 106 reported dietary treatments (diets and feeding level). The CHO digestibility was reported (averaging 71.5%) from only 29 dietary treatments in the data set. For the remaining 77 treatments, the CHO digestibility was calculated from the known digestibility of other nutrients (for details on calculations, see Appendix S1), being on average 64.9% and not different from the reported CHO digestibility ($P > 0.10$; Fig. 1). Within the whole data set, there was a large variability in CHO digestibility, ranging from 12% to 95%, with an average of 67%. A CHO ADC of 12% was calculated for a diet high in protein and fat, including 15% cellulose (Saravanan *et al.* 2012), whereas a CHO ADC of 95% was found for a casein diet (34.5%), with only wheat starch (50.5%) as a carbohydrate source (Sintayehu *et al.* 1996).

Table 2 shows the correlation between the CHO digestibility and the experimental design, diet composition and ADC of other nutrients. Increasing mean initial body weight of the fish increases the CHO ADC ($P < 0.05$). Information on the effect of fish weight on CHO ADC is scarce. A study by Pen-Hsing and Shi-Yen (1993) indicated that digestibility of starch was higher in tilapia (*O. niloticus* × *O. aureus*) with a higher initial weight of 4.55 g, compared to fish with a lower initial weight of 0.46 g. It is a long-established fact that the gastrointestinal tract of larvae and small fish is less developed and shorter compared to adult fish (Govoni *et al.* 1986). In addition, the production of digestive enzymes is lower for smaller fish, most

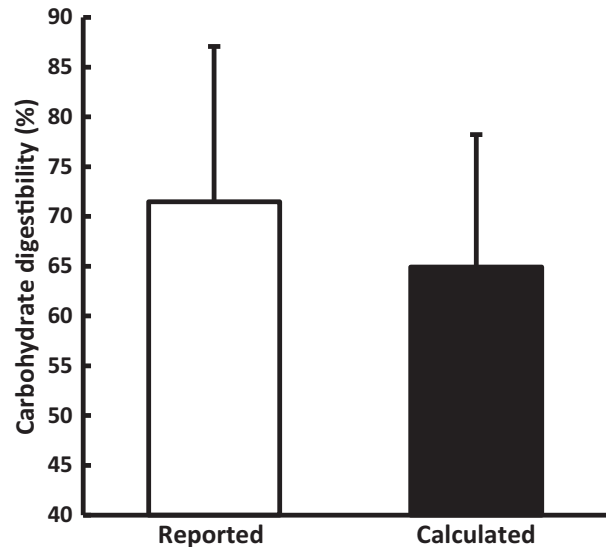


Figure 1 Comparison between the carbohydrate digestibility for the reported (29) and calculated (77) cases. Each bar shows overall mean with standard deviation represented with error bars.

likely related to the developmental stage of the digestive tract (Lauff & Hofer 1984). The feeding level did not affect the CHO ADC, which is in line with some studies (Storebakken & Austreng 1987; Azevedo *et al.* 1998) on rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar* L.). On the contrary, both Schrama *et al.* (2012) and Haidar *et al.* (2016) showed a reduction in ADC with increased feeding levels in tilapia (*O. niloticus*), for all nutrients, including CHO. In these two Nile tilapia studies, the feeding levels were approximately 1.5 and 3 times the maintenance and apparent satiation. With increasing CHO ADC, the FCR improves ($P < 0.01$), because a larger part of the CHO is utilised for growth and metabolism.

The crude protein level in the diet does not influence the CHO ADC ($P > 0.05$). The fat level and energy level in the diet are highly correlated as fat has a high energy equivalent (39.5 kJ g^{-1}), increasing the total energy content of the diet. Appropriate levels of carbohydrates should be incorporated in the diets to improve the energy availability, sparing the catabolism of protein and lipids for energy (Wilson 1994). With increasing energy levels in the diet and thereby most likely increasing fat levels, the role of carbohydrates in providing energy may become less important as sufficient fat, and thus energy, is provided. However, the CHO composition in the diet in particular has a large effect on the CHO ADC, in as much as the carbohydrate fraction consists of highly digestible starch and sugars and poorly digestible NSP. As expected, increasing levels of starch positively correlate ($P < 0.01$) with the CHO ADC, as the fraction of highly digestible starch is likely to go up with increasing

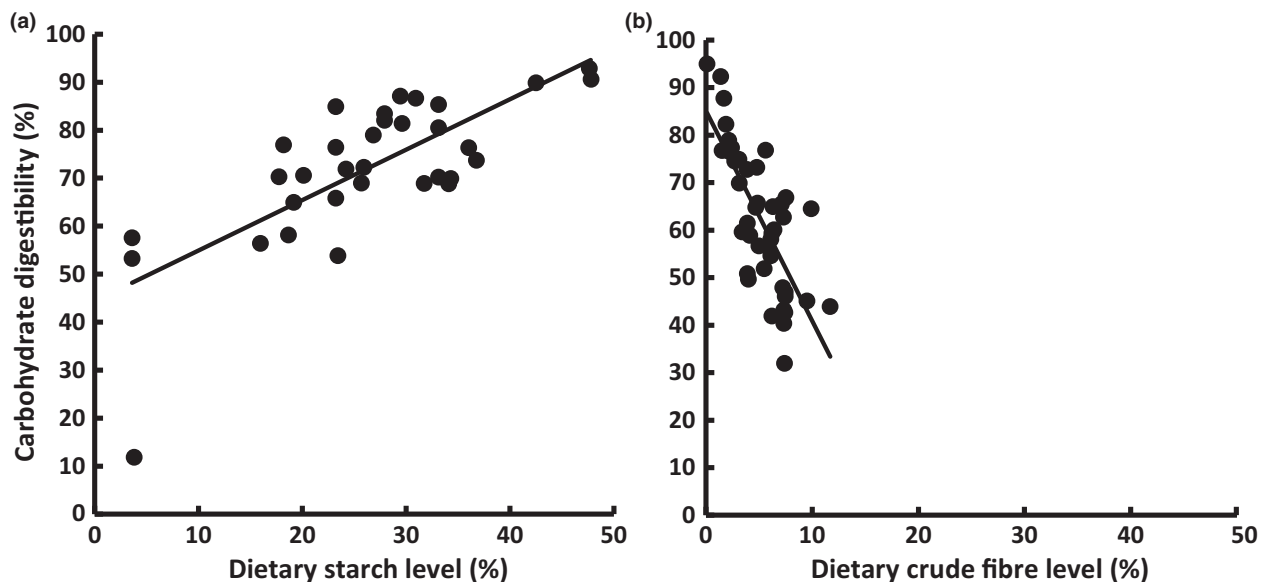
Table 2 Correlation between carbohydrate digestibility (Y variable; in %) and parameters related to the design of the experimental design, to diet formulation and to apparent digestibility coefficients of other nutrients (ADC)

X	Correlation coefficient	Number of observations	Estimated equation CHO ADC (Y)
<i>Experimental design</i>			
Body weight start (g)	0.219*	106	$Y = 64 (SE 1.7) + 0.033 (SE 0.01) \times X$
Feed intake (g DM kg ^{-0.8} day ⁻¹)	ns	98	n/s
FCR (g g ⁻¹)	-0.446**	79	$Y = 86 (SE 4.7) - 14.5 (SE 3.3) \times X$
<i>Dietary level (%)</i>			
Crude protein	ns	106	n/s
Fat	-0.375**	106	$Y = 80 (SE 3.5) - 1.32 (SE 0.32) \times X$
Energy	-0.207*	96	$Y = 125 (SE 29) - 2.9 (SE 1.4) \times X$
Total carbohydrates	0.222**	106	$Y = 51 (SE 6.7) + 0.4 (SE 0.15) \times X$
Starch	0.740**	34	$Y = 44 (SE 4.9) + 1.1 (SE 0.17) \times X$
Crude fibre	-0.748**	43	$Y = 85 (SE 3.5) - 4.4 (SE 0.61) \times X$
<i>ADC (%)</i>			
Crude protein	0.462**	106	$Y = -39 (SE 20) + 1.19 (SE 0.22) \times X$
Fat	0.630**	106	$Y = -0.2 (SE 8.2) + 0.8 (SE 0.09) \times X$
Energy	0.791**	96	$Y = -34 (SE 8.2) + 1.3 (SE 0.10) \times X$
Starch	0.525**	26	$Y = 69 (SE 8.5) + 0.06 (SE 0.09) \times X$
Ash	ns	73	n/s

* $P < 0.05$; ** $P < 0.01$.

CHO, total carbohydrates; n/s, not shown, because of being not significant; ns, not significant.

Values represent the Pearson correlation coefficient and the number of observations.

**Figure 2** Relationship between the dietary starch level and (a) dietary crude fibre level and (b) the carbohydrate apparent digestibility coefficient (%) for tilapia.

starch levels (Fig. 2a). With every 1% of extra starch in the diet, the CHO ADC increases at almost the same rate (1.05%). The level of crude fibre in the diets has a much larger effect on the CHO ADC compared to the starch level in the diet (Fig. 2b). With each 1% increase in dietary crude fibre, the CHO ADC decreases by 4.4%. Crude fibre analyses give a rough indication of the cellulose and

lignin content of the diet rather than the total NSP fraction. The diets for which the crude fibre fraction was given had, on average (43 cases), 4.8 times more NSP than the reported level of crude fibre. This high increase in poorly digestible NSP with increasing levels of dietary crude fibre explains the large impact crude fibre has on the CHO ADC.

The starch ADC is directly related to an improvement in the CHO ADC, as starch in general takes up the largest part of the carbohydrate fraction in the diet. With an increase in CHO ADC, more energy is digested in the form of CHO, thus increasing the energy ADC ($P < 0.01$). The overall trend when looking at the correlation between the CHO ADC and the ADC of the other nutrients (except ash) is that digestibility is always highly positively correlated with increasing CHO ADC. This trend, especially the correlation with protein, fat and energy, may be better explained by the quality of the diet (ingredients, formulation, processing) of the diet, rather than the CHO ADC actually having an effect on protein, fat and energy digestibility. The quality of the diet (i.e. choice of ingredients) and processing method (i.e. extrusion vs. steam pelleting) are factors known to influence the overall nutrient ADC, irrespective of the nutrient composition (Alonso *et al.* 2000; Cheng & Hardy 2003). In addition, high-quality diets are generally low in crude fibre and NSP. Therefore, the known negative effects of NSP on the digestibility of other nutrients (including CHO) are likely to be further aggravated with increasing levels of dietary crude fibre and NSP. Examples of how NSP can negatively affect nutrient digestibility are as follows: dietary viscosity, which can hamper the mixing of enzymes within the chyme and the absorption of nutrients, due to hindered effective interaction at the mucosal surface (Choct 1997); and increased faecal losses of bile acids (which are important for fat digestion), as a consequence of binding to NSP and/or an increased deconjugation of bile acids by microbial activity, stimulated by NSP (Sinha *et al.* 2011). The quality of the diet, and thus the digestibility of the nutrients (fat and protein), is likely to contribute to not only an improvement in the CHO ADC ($P < 0.01$), but also in the FCR.

Starch

As seen in Section 3 on total carbohydrates, the starch fraction is important for the CHO ADC, as starch is highly digestible in tilapia. Starches (corn, wheat, etc.) are often included to limit the amount of protein utilised for energy (Wilson 1994). For example, Wang *et al.* (2005) showed an improvement in the protein efficiency ratio in Nile tilapia when including 22–46% of starch in the diet, compared to levels of 6% or 14%. From the 26 diets reporting the ADC of starch, 10 diets were manufactured by steam pelleting with an average ADC of $91.0 \pm 2.25\%$, while the other 16 diets were manufactured by extrusion and had an ADC of $99.4 \pm 0.7\%$ (Fig. 3). These differences in starch ADC are in line with results from Amirkolaie *et al.* (2006), where starch gelatinised by extrusion showed an ADC of around 99.3%, while native starch had an ADC of around 93.8%. Similar results are shown for rainbow trout, where

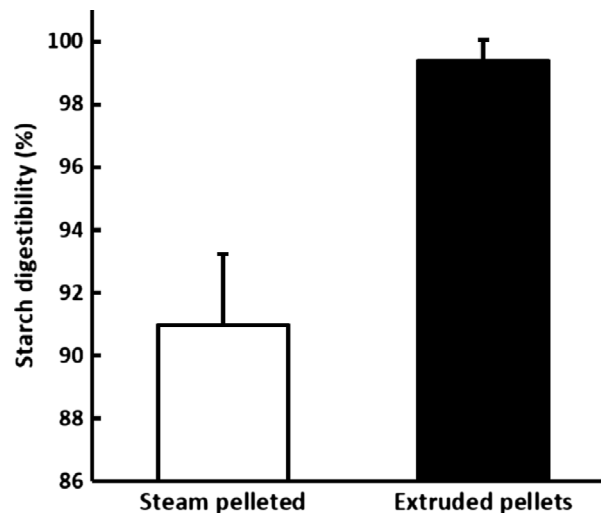


Figure 3 Effect of pellet manufacturing (steam pelleting vs. extrusion) on the starch digestibility in tilapia. Each bar shows overall mean with standard deviation represented with error bars. Bars with different lower case letters are significantly different ($P < 0.05$).

gelatinising native starch increased the ADC of starch from 38.1% to 86.5% (Bergot & Breque 1983). Cooking carbohydrates, to enhance their digestibility through gelatinisation, dates back to the 1940s for trout (Phillips & Brockway 1956).

Non-starch polysaccharides

Non-starch polysaccharides in fish feed

Nowadays plant proteins are increasingly frequently used more used to replace fishmeal in fish feeds, in order to reduce costs and keep pace with the increasing demand for high-quality protein. This has resulted in a steady decline in dietary fishmeal (and fish oil) in aquafeeds (Carter & Hauler 2000; Shepherd & Jackson 2013). A major downside of using plant ingredients such as soya, wheat, rye and legume seeds is the presence of a variety of anti-nutritional substances (ANS), often limiting the inclusion of these ingredients in aquafeeds. NSP is one of the major ANS present in plant ingredients (Choct 1997; Francis *et al.* 2001; Sinha *et al.* 2011). Multiple studies in broilers and pigs have shown that feedstuffs containing NSP negatively affect nutrient digestibility and thus limit growth (Choct & Annisson 1992; Annison 1993; Knudsen *et al.* 1993; JøRrgensen *et al.* 1996; Mroz *et al.* 2000). Similar studies in fish are scarce.

Non-starch polysaccharides also influences chyme viscosity. Dietary soluble NSP increases the viscosity, thereby decreasing the passage rate and diffusion rates of digestive enzymes and substrates, whereas insoluble NSP increases the passage rate. NSP may influence the gut

morphology, physiology and mucus layer, affecting the endogenous secretion of water, proteins, electrolytes and lipids. These changes can lead to reduced nutrient digestibility (Choct 1997; Choct & Kocher 2000; Sinha *et al.* 2011). In pigs, for example, inclusion of cellulose in the diet resulted in the shortening of villus length. Shortening of villi results in a loss of intestinal surface area. As a result, absorption of nutrients is decreased (Sinha *et al.* 2011). A lower passage rate, as a result of increased levels of soluble NSP in the diet, may decrease the oxygen tension in the gut, favouring anaerobic microbiota. An increase in anaerobic microbiota can enhance the production of toxins and the deconjugation of bile salts, which are important for fat digestion (Carey *et al.* 1983; Choct 1997; Sinha *et al.* 2011). However, information about the effect of NSP on the metabolism in fish is scarce.

The calculated dietary NSP level in the present meta-analyses averaged $17.5\% \pm 8.6$, with a minimum of 1.4% and a maximum of 37.9%. This large variation in dietary NSP level is simply due to the choice of ingredients, that is using soya bean meal as a protein source versus an isolate or concentrate, like soya protein isolate or pea protein concentrate. In line with the above theory, increasing the levels of dietary NSP was shown to negatively correlate with the digestibility of crude protein, fat and energy ($P < 0.001$; Table 3). Figure 4 shows the relationship between the dietary NSP level and energy digestibility; for each per cent increase in dietary NSP, the energy ADC decreases by 0.7%. Similarly, as in Figure 4, Haidar *et al.* (2016) showed that increasing the dietary NSP level causes a decrease in both the energy digestibility and the protein digestibility ($P < 0.05$). In line with a lower availability of nutrients, due to a decrease in nutrient digestibility, the FCR increases by 0.03 for every per cent increase in dietary NSP (Fig. 4).

Non-starch polysaccharide digestibility

Digestive enzymes, such as α -amylase, disaccharidase and α -glucosidase, in combination or independently, are able to hydrolyse α -glycoside bonds (Stone 2003). NSP, however, generally remains undigested in monogastric animals. The enzymes needed to hydrolyse the β -glycosidic bonds of the long NSP chains such as cellulose, β -xylanases, β -glucanases and β -galactases are scarce or non-existing in the gastrointestinal tract (GIT) of fish (Kuz'mina 1996; Choct 1997; Stone 2003; Sinha *et al.* 2011). Cellulase activity has been reported in several carp species, for example bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*; Chakrabarti *et al.* 1995; Li *et al.* 2009; Banerjee *et al.* 2016). In tilapia, this information is rare; however, a study by Saha *et al.* (2006) measured cellulase activity from isolated bacterial strains from the gut of tilapia (*Oreochromis mossambica*), measuring a maximum cellulase activity of 67 U mL^{-1} . In Banerjee *et al.* (2016), besides cellulase, xylanase activity and xylanase-producing microbial symbionts were detected in the proximal and distal intestine of six freshwater carp species. The assumption was that the xylanase- and cellulase-producing microbiota were autochthonous as the fish were starved for 48 h prior to taking the GI tracks, after which the GI tracts were cleansed with sterilised (0.9%) saline. German and Bittong (2009) suggest that fish ingest cellulase- and xylanase-producing microbes while feeding on detritus derived from plant matter, rather than these enzymes being produced by the autochthonous microbiota. Whether the cellulase- and xylanase-producing microbiota are endogenous, and/or whether the gut is colonised by ingested microbes while feeding on detritus, is unknown. Cellulase and xylanase are responsible for the breakdown of cellulose and xylans, respectively, which are the major NSP in plant matter (Sinha *et al.* 2011; Banerjee *et al.* 2016).

Table 3 Correlation between the total non-starch polysaccharide (NSP) level in the diet (X variable in the equation, in %) and fish performance parameters and apparent digestibility coefficients (ADC) of other nutrients

Y	Correlation coefficient	Number of observations	Estimated equation (Y) using NSP level (X)
<i>Performance</i>			
FCR	0.500**	79	$Y = 0.9 \text{ (SE 0.10)} + 0.03 \text{ (SE 0.006)} \times X$
SGR (% day ⁻¹)	ns	80	n/s
<i>ADC (%)</i>			
Crude protein	-0.491**	105	$Y = 100 \text{ (SE 2.3)} - 0.7 \text{ (SE 0.12)} \times X$
Fat	-0.500**	105	$Y = 95 \text{ (SE 1.1)} - 0.3 \text{ (SE 0.06)} \times X$
Energy	-0.662**	95	$Y = 93 \text{ (SE 1.6)} - 0.7 \text{ (SE 0.08)} \times X$
Starch	ns	26	n/s
Ash	ns	72	n/s

** $P < 0.01$.

n/s, not shown, because of being not significant.

Values represent the Pearson correlation coefficient and the number of observations.

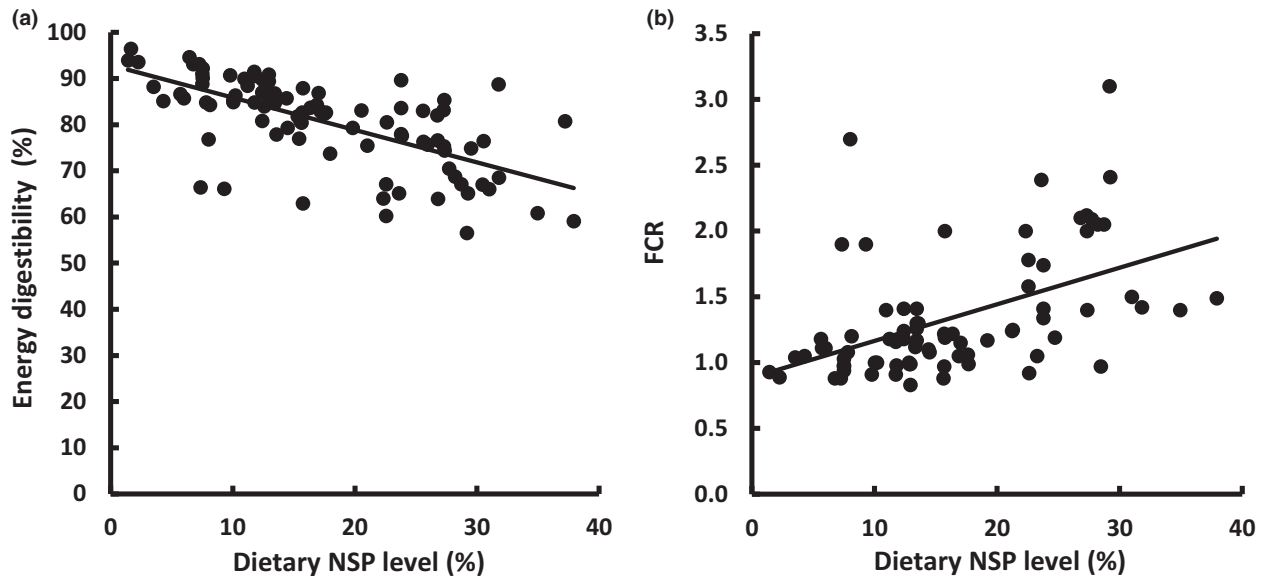


Figure 4 Relationship between dietary non-starch polysaccharide level and (a) the energy digestibility and (b) the feed conversion ratio for tilapia.

Although present in the gut, the activity of cellulase is generally low ($<70 \text{ U mL}^{-1}$; Chakrabarti *et al.* 1995; Banerjee *et al.* 2016). Besides that, the hydrolysis of cellulose is generally slow and incomplete and therefore possibly only occurs on a small scale in the gut (Schwarz 2001).

Although long considered as indigestible in fish, a few studies now show that part of the NSP fraction can be digested by tilapia (Amirkolaie *et al.* 2005; Leenhouders *et al.* 2007; Haidar *et al.* 2016). The wide range of NSP used in these studies differed in characteristics and properties, including endogenous digestibility. Amirkolaie *et al.* (2005) showed significant differences in the NSP digestibility when comparing guar gum (oligosaccharides) and cellulose, indicating that cellulose, with an ADC of 2.8%, is more inert than guar gum, which has an ADC of 20.8%. Similar results were found by Leenhouders *et al.* (2007), where replacement of 40% of a reference diet with either maize, wheat, barley or rye resulted in significant differences in NSP digestibility. Solubility of the NSP is an important factor affecting the digestibility of the NSP fraction (Sinha *et al.* 2011). Leenhouders *et al.* (2007) made a distinction in NSP digestibility based on its solubility (soluble vs. insoluble), indicating negative values for the digestibility of the insoluble NSP fraction, independent of the NSP source (maize, wheat, barley and rye), with an ADC of up to 60% for the soluble NSP fraction. This is in line with the poor digestibility found for cellulose (insoluble), compared to guar gum (soluble), in Amirkolaie *et al.* (2005). In the present meta-analysis, the feeding ($\text{g DM kg}^{-0.8} \text{ day}^{-1}$) had no effect on the NSP digestibility. On the contrary, Haidar *et al.* (2016) showed that with increasing feeding levels, feeding the same diet, the ADC of

NSP decreased drastically. This suggests a threshold in the amount of NSP that can be digested.

From the present meta-analysis, an average NSP digestibility of 24.3% was found from the 95 cases presented. From these 95 cases, in only 11 cases was there a negative ADC, indicating that no NSP was digested. This indicates that in 83 of these cases NSP digestibility can be assumed, of which 12 had an ADC above 50%. In three studies (Amirkolaie *et al.* 2005; Leenhouders *et al.* 2007; Haidar *et al.* 2016), where NSP ADC was reported, the NSP ADC averaged 26.4% for a total of 14 cases, which is highly comparable to the NSP ADC of 24.3% found by meta-analyses. In cases where NSP ADC reported a negative value, if this value was hypothetically increased to 0%, then the ADC value would increase to 28.4%. In other monogastric animals, like pigs and poultry, it is well established that fermentability varies considerably among different types of NSP, with, for instance, lignin being very resistant and pectins usually undergoing complete fermentation (Williams *et al.* 2001, 2017). In tilapia this also seems to be the case based on the limited amount of literature on NSP ADC. For example, in a study by Maas *et al.* (2019), different experimental diets contained contrasting types of NSP, by incorporating wheat bran, sunflower meal or citrus pulp to a reference diet. The ingredients were chosen for their contrast in NSP composition; wheat bran being relatively rich in hemicellulose, sunflower meal rich in cellulose and citrus pulp rich in pectins. The pectin-rich citrus pulp diet showed the highest NSP digestibility at 31.7%, followed by the sunflower (18.8%) and wheat bran (17.1%) diets.

Comparable to the digestibility of the CHO fraction, the digestibility of the NSP fraction increases with an increase

in initial body weight ($P < 0.001$; Table 4). The digestion of NSP in fish occurs through fermentation in the gut (Bergman 1990; Metzler-Zebeli *et al.* 2010). The gastrointestinal tract of larvae and adult fish differs largely, with a more complex morphology and histology for adult fish (Govoni *et al.* 1986). As the gut is the site of action of gut fermentation, a more developed gut in bigger fish could explain for the higher NSP digestibility with increasing fish size. Increasing NSP digestibility significantly ($P < 0.05$) contributes to an improvement in FCR, as seen by the negative correlation coefficient. The digestibility of NSP decreases with increasing levels of crude fibre ($P < 0.05$). Crude fibre analyses give a rough indication of the cellulose and lignin content of the diet rather than the total NSP fraction. Amirkolaie *et al.* (2005) showed that cellulose is almost completely inert to digestion by tilapia. Increasing levels of almost inert dietary crude fibre may therefore decrease the total NSP ADC. The energy and fat digestibility increased with increasing NSP digestibility ($P < 0.01$) in tilapia. With an increase in NSP digestibility, the NSP level in the gut is likely to go down and with it the potential negative effects NSP has on the gastrointestinal tract and nutrient utilisation. A NSP ADC of 24.3% (average found in present study), would result in an increase in the energy ADC by 3% compared to having no NSP digestibility in tilapia.

Fermentation and volatile fatty acids

The digestion of NSP in fish, as shown in the present meta-analysis and in studies by Amirkolaie *et al.* (2005), Haider

et al. (2016) and Leenhouwers *et al.* (2007) using tilapia, occurs through fermentation in the gut. Other than starch and sugars, which are generally directly digested in the stomach, NSP are mainly fermented in the gut. During the fermentation of NSP and carbohydrates, short-chain fatty acids (SCFA) and the gases H_2 , CO_2 and CH_4 are formed through microbial anaerobic glycolysis (Bergman 1990; Metzler-Zebeli *et al.* 2010). The SCFA are often referred to as volatile fatty acids (VFA). With fermentation of carbohydrates, 73% of the C fraction can be converted into completely metabolisable VFA, whereas the remainder is lost (CO_2 ; Williams *et al.* 2001). The VFA produced can rapidly be absorbed by the colonic lumen. In monogastric animals, around 95–99% of the VFA produced are absorbed before reaching the rectum (Von Engelhardt *et al.* 1989; Schepach 1994). The principle end products of VFA from carbohydrate fermentation are acetate, propionate and butyrate and to a lesser extent formate, valerate, caproate and the branched chain acids isobutyrate and isovalerate (Williams *et al.* 2001; Macfarlane & Macfarlane 2003). Levels of the intermediate lactic acid, which is commonly found in monogastrics like pigs (Argenzio & Southworth 1975), have not been reported in fish, but most likely, they have not been measured. However, with the use of an *in vitro* gas production technique, using freshly collected intestinal content from Nile tilapia, lactic acid was measured (Leenhouwers *et al.* 2008). Hereby, four different substrates were used (glucose, wheat starch, arabinoxylan, whole wheat), the substrate used strongly influenced the amount of lactic acid produced ($P < 0.001$). 11.06 mM

Table 4 Correlation between digestibility of non-starch polysaccharide (NSP) (Y variable; in %) and parameters related to the design of the experiment, to diet formulation and to apparent

X	Correlation coefficient	Number of observations	Estimated equation NSP ADC (Y)
<i>Experimental design</i>			
Body weight start (g)	0.967**	95	$Y = 17 (SE 3.8) + 0.1 (SE 0.03) \times X$
Feed intake (g DM $kg^{-0.8} day^{-1}$)	ns	87	n/s
FCR (g g^{-1})	-0.245*	70	$Y = 43 (SE 11) - 17 (SE 7.6) \times X$
<i>Dietary level (%)</i>			
Crude protein	ns	95	n/s
Fat	-0.287**	95	$Y = 45 (SE 8.0) - 2.1 (SE 0.74) \times X$
Energy	ns	85	n/s
Total carbohydrates	0.213*	95	$Y = -8 (SE 15) + 0.7 (SE 0.35) \times X$
Starch	ns	27	n/s
Crude fibre	-0.379*	41	$Y = 57 (SE) - 7 (SE 2.6) \times X$
<i>ADC (%)</i>			
Crude protein	ns	95	n/s
Fat	0.319**	95	$Y = -46 (SE 22) + 0.8 (SE 0.25) \times X$
Energy	0.412**	86	$Y = -92 (SE 28) + 1.4 (SE 0.34) \times X$
Starch	ns	29	n/s
Ash	0.395**	67	$Y = -9 (SE 10) + 0.7 (SE 0.21) \times X$

* $P < 0.05$; ** $P < 0.01$.

n/s, not shown, because of being not significant.

Digestibility coefficients of other nutrients (ADC). Values represent the Pearson correlation Coefficient and the number of observations.

(g⁻¹ organic matter weighed in) of lactic acid was measured for glucose and 2.82 mM for wheat starch, while there was almost no lactic acid found for the substrates arabinoxylan and whole wheat (<0.04 mM). The total VFA (acetic + propionic + butyric + isobutyric + valeric + isovaleric acid) level measured for the glucose amounted to 3.01 mM, which is 3.7 times lower compared to the amount of lactic acid (11.06 mM). The *in vitro* study of Leenhouders *et al.* (2008) makes the assumption plausible that lactic acid can be produced in Nile tilapia. It should be noted that both glucose and wheat starch are not commonly incorporated in aqua feeds, of the 25 studies included in the present analyses only one used wheat starch as an ingredient (Sintayehu *et al.* 1996). If other starches, like maize starch, result in similar levels of lactic acid, as compared to wheat starch, is currently unknown.

The fermentation in fish takes place throughout the gut with increasing microbial activity from the proximal to the distal end of the hindgut, conjointly with increasing levels of VFA towards the proximal end (Mountfort *et al.* 2002; Amirkolaie *et al.* 2006; Leenhouders *et al.* 2007). This is comparable with pigs, where easily accessible compounds are utilised in the upper part of the GIT, whereas complex low-soluble substrates are fermented and utilised in the lower GIT sections (Knudsen *et al.* 2012). In general, the composition of the VFA produced remains within a range of 60–75% acetate, 15–25% propionate and 10–15% butyrate for monogastric animals fed conventional diets (Bugaut 1987). However, in humans the rate of breakdown of individual NSP and the ratio of fermentation products (acetate, butyrate & propionate) vary depending on the type of polysaccharide present (Macfarlane & Macfarlane 2003). Similar characteristics have been seen in cows, where the amount of VFA and the ratio between acetate, butyrate and propionate differed depending on the cereal used, when comparing hay (100%) versus a concentrate of cracked corn (81%), soya bean (9%) and timothy hay (10%; Russell 1998). Hitherto, little is known about the ratio of the VFA produced through fermentation in fish compared to pigs and poultry. Comparable with other monogastrics, Leenhouders *et al.* (2007) and Amirkolaie *et al.* (2006) found high levels of acetate (± 16.4 mM on fresh digesta) and minor levels of propionate (± 0.4 mM) and butyrate (± 0.2 mM) in the distal gut of tilapia. An overview of studies measuring VFA in the distal gut of different fish species is given in Table 5, for species with predominately herbivorous feeding habits. Most of these studies measured VFA levels in different gut segments, showing increasing total VFA levels towards the proximal end (Clements *et al.* 1994; Clements & Choat 1995; Mountfort *et al.* 2002; Amirkolaie *et al.* 2005; Fidopiastis *et al.* 2006; Leenhouders *et al.* 2007). The VFA composition is comparable among studies with proximate

levels of 60–95% acetate, 5–25% propionate and 1–15% butyrate.

In an *in vitro* experiment, using the contents of the distal gut from common carp (*C. carpio* L.), cumulative and composition differences in VFA products were found when the contents were inoculated with different substrates (oligosaccharides; Kihara & Sakata 2002). With NSP fermentation, the concentration, and thus the anti-nutritional effects of the NSP fraction, will be lowered. With NSP fermentation, the digestibility of the NSP and thus the carbohydrate fraction will improve, resulting in lower faecal waste and improved feed efficiency (Sinha *et al.* 2011). Besides these practical implications, the microbiota, intestinal- and animal health can be influenced by the production of VFA. The amount and composition of the fermentation end products will influence the animal, microbiota and intestine in different ways as they differ in characteristics and properties (Bergman 1990; Claus *et al.* 2007). Acetate and propionate enter the blood passively due to a concentration gradient and are transported to the liver (Williams *et al.* 2001; Montagne *et al.* 2003). Acetate is used as energy substrate for muscle tissue via acetyl-coenzyme A syntheses (Clements *et al.* 1994). In the liver, propionate is used for gluconeogenesis, turning propionate into glucose, which provides an important source of energy for metabolic activities in the colon. Butyrate is primarily used as a direct source of energy by the colonocytes, providing energy for its metabolic activities and stimulating epithelial cell proliferation. This is mainly known for pigs and poultry. In fish, such data are lacking. By supplying energy in different ways, the contribution of VFA to the energy in the gut and body can be substantial (Von Engelhardt *et al.* 1989; Williams *et al.* 2001; Montagne *et al.* 2003). VFA was shown to inhibit growth of some bacterial pathogens in the gut of rabbits and pigs when in an acidic environment, similar effects on pathogens induced by VFA production in fish may occur as well (May *et al.* 1994; Montagne *et al.* 2003).

NSP as functional feed ingredients

Non-starch polysaccharides, including β -glucans, mannan-oligosaccharides (MOS), galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS; Nawaz *et al.* 2018), but also pectins (Wiese 2019), may contribute as prebiotics to animal health by preventing pathogen adhesion, stimulating immune maturation and gut barrier function, and serving as fermentable substrates for gut bacteria. The increased production of intermediate metabolites, such as SCFA, can assist in balancing the immune system. In aquaculture, the NSP most frequently used as a prebiotic is probably β -glucans, their effects are often evaluated as modulations of innate immune responses by measuring gene expression or enhanced phagocytosis, reactive oxygen species, lysozyme

Table 5 Overview of studies measuring volatile fatty acids in the distal gut of different fish species, values are means in mm L^{-1} (fresh sample)

Species		Acetate	Propionate	Butyrate†	Reference
Nile tilapia	<i>Oreochromis niloticus</i>	16.5	0.5	0.3	Leenhouders <i>et al.</i> (2007)
		16.2	0.3	0.2	Amirkolaie <i>et al.</i> (2006)
African catfish	<i>Clarias gariepinus</i>	25.7	1.1	2.0	Leenhouders <i>et al.</i> (2007)
Silver drummer	<i>Kyphosus syndneyanus</i>	37.5	12.8	1.3	Mountfort <i>et al.</i> (2002)
Butterfish	<i>Odax pullus</i>	20.8	4.7	1.0	<i>Id.</i>
		23.5	7.7	5.2	Clements <i>et al.</i> (1994)
Herring cale	<i>Odax cyanomelas</i>	24.6	1.9	2.0	<i>Id.</i>
Sea carp	<i>Crinodus lophodon</i>	16.4	4.5	1.3	<i>Id.</i>
Marblefish	<i>Aplodactylus arctidens</i>	8.3	4.1	1.3	Mountfort <i>et al.</i> (2002)
Zebra-perch	<i>Hermosilla azurea</i>	15.3	4.0	0.6	Fidopiastis <i>et al.</i> (2006)
Surgeonfish	<i>Acanthurus</i> spp.	9.7	1.7	0.8	Clements and Choat (1995)‡
Striated surgeonfish	<i>Ctenochaetus striatus</i>	5.1	0.3	0.9	<i>Id.</i>
Ridolfi fish	<i>Naso</i> spp.	18.1	4.2	1.2	<i>Id.</i>
Surgeonfish	<i>Zebrasoma</i> spp.	24.3	7.1	2.7	<i>Id.</i>
Parrotfish	<i>Scarus</i> spp.	5.5	0.4	0.3	<i>Id.</i>
Blue sea chub	<i>Kyphosus cinerascens</i>	28.7	7.8	3.5	<i>Id.</i>
Bicolor angelfish	<i>Centropyge bicolor</i>	35.7	8.6	2.2	<i>Id.</i>
Rabbitfish	<i>Siganus</i> spp.	14.2	1.8	0.6	<i>Id.</i>

†Clements and Choat (1995) and Fidopiastis *et al.* (2006) measured both isobutyrate and butyrate; the sum is shown in the table as butyrate.

‡Clements and Choat (1995) measured volatile fatty acids in the gut of a total of 32 fish species; for fish of the same family, the average values are calculated; *Acanthurus* spp., *Naso* spp., *Zebrasoma* spp. and *Scarus* spp.

activity and, more recently, trained immunity (Petit & Wiegertjes 2016). These prebiotic products are added to fish feed, usually at low inclusion rates of <1% as functional feed ingredients (Dalmo & Bøgdal 2008). It should be noted that the β -glucans used as prebiotics generally originate from yeast, which has a different structure than the β -glucans from cereals and legumes. To which extent β -glucans from cereals and legumes have the same mode of action as those from yeast is unclear.

Non-starch polysaccharides may be increasingly added to fish feed as modulators of fish health but, for most if not all NSP, it remains undecided whether their modulatory effects are direct or gut microbiota mediated. In a preliminary study on the normal gut microbiota of common carp we could detect an abundant presence of *Bacteroides* bacteria, well known for their capacity to degrade and ferment carbohydrates, in line with earlier studies of cellulase activity (Chakrabarti *et al.* 1995; Li *et al.* 2009; Banerjee *et al.* 2016). *In vitro* fermentation of a commercially available β -glucan functional feed ingredient indicated an increased presence of propionate, a SCFA with immunomodulatory properties, whose presence possibly helps explain earlier-noted immunomodulatory effects, such as inhibition of the expression of several pro-inflammatory genes (Falco *et al.* 2014).

Direct immune-modulatory effects of NSP could be achieved via recognition of, for example, β -glucans, by receptors present locally on cells in the gut. Yet, despite the frequent application of β -glucans in aquaculture practice,

the exact receptors and downstream signalling remain to be described. In fish genomes, no clear homologue of Dectin-1 could be identified, a member of the C-type lectin receptor (CLR) family and the best-described receptor for β -glucans in mammals. Still, a recent transcriptome analysis of genes expressed in common carp macrophages, stimulated with β -glucan, highlighted differential regulation of a signalling pathway typical of CLR activation. Subsequent genome analysis identified a large number of candidate β -glucan receptor genes encoding for proteins, with at least one C-type lectin domain (CTLD) typical of the CLR family. This large number could be narrowed down to a few genes with a typical sugar-binding motif in their CTLD, but these were not expressed in macrophages, the innate immune cell types often associated with recognition of β -glucans (Petit *et al.* 2019a). Of course, β -glucan receptors in the gut are not necessarily exclusively expressed on macrophages and could also be found on other locally present (non-)immune cells.

Possibly the most interesting immune-modulating effects of NSP, such as β -glucans, could be their effect on the innate immune cells associated with the concept of trained immunity, a form of innate immune memory best described in mice and humans. A literature review for indications of trained immunity in fish, supported the notion that the innate immune system of teleost fish can be trained and that effects could be long-lived (Petit & Wiegertjes 2016). This was based on indications for at least one out of three of the following criteria, considered characteristic of

this phenomenon: (i) providing protection against a secondary infection in a T- and B-lymphocyte-independent manner; (ii) conferring increased resistance upon re-infection; and (iii) relying on key roles for innate immune cell types such as natural killer cells and macrophages. A recent follow-up study *in vitro* could confirm that common carp macrophages can indeed be trained, with evidence of metabolic reprogramming as well as heightened phagocytosis, production of reactive oxygen species and expression of inflammatory genes (Petit *et al.* 2019b). Overall, the longevity, non-specificity and presumed evolutionary conservation of immune-modulating effects of NSP associated with trained immunity, could be especially interesting to aquaculture practise.

NSP degrading enzymes

The use of enzymes, like phytase, xylanase and β -glucanase in pigs and poultry, is a common way to break down NSP in order to enhance VFA production and to improve the digestibility of the feed (Bedford & Schulze 1998). Recently, the use of exogenous carbohydrase enzymes in aqua feeds is attracting more attention. Multiple effects have been attributed to exogenous carbohydrase supplementation like improved feed intake, improved growth rate, increase in endogenous digestive enzymes and nutrient digestibility (Goda *et al.* 2012; Castillo & Gatlin 2015; Maas *et al.* 2018). Approximately 200 enzymes from of a microbial origin, out of around 4000 enzymes known, are commercially used. From these 200 enzymes, only 20 are produced on an industrial scale (Li *et al.* 2012). These include a wide range of carbohydrases, including enzymes that cut part of the polymeric carbohydrates by hydrolysis. Examples of commercially available carbohydrases include (β -)glucanase, (α -)xylanase, amylase, cellulase, pectinase and galactosidase. Of these enzymes, β -glucanase and xylanase make up more than 80% of the commercial market (Adeola & Cowieson 2011; Castillo & Gatlin 2015). There is a large variety in NSP (cellulose, arabinoxylans, mixed-linked β -glucans, mannans, galactomannans, glucomannans, arabins, galactans, arabinogalactans) from different sources, differing in characteristics and properties (Choct & Kocher 2000; Sinha *et al.* 2011). NSP have different characteristics and chemical structures, therefore it is likely that not all enzymes have the same effect on diets with different NSP contents. Maas *et al.* (2019) showed that the effectiveness of phytase and xylanase on growth performance and nutrient digestibility is dependent on the NSP composition. Therefore, it is important that the formulation of the enzyme(s) is complementary to the diet composition (Officer 2000; Maas *et al.* 2019). An important benefit of the carbohydrase enzyme is that they reduce the negative effects NSP have on the fish and its gut (i.e. affecting the chyme viscosity). Reducing the

negative effects of NSP, and breaking down the polysaccharides into readily available oligomers and monomers for fermentation, will improve nutrient utilisation (Vahjen *et al.* 2007; Castillo & Gatlin 2015).

Concluding remarks

In this manuscript, an average total carbohydrate (CHO) digestibility of 67% was calculated for tilapia. The large differences in CHO digestibility between studies are mainly attributed to the dietary inclusion level of NSP, because NSP is poorly digested compared to starch. NSP digestibility averaged 24.3% across studies. The presented meta-analysis allows us to assume that NSP digestibility occurred in many tilapia studies (88%). If we assume that 100% of the starch was digested, then this would still result in a positive value for NSP digestibility. This clearly demonstrates that NSP is not inert for tilapia but is digestible. However, between studies and diets the variation in digestibility is high. By knowing the factors affecting NSP digestibility, predictions may be made for NSP ADC. This study shows that NSP ADC increased with increasing fish weights and with dietary carbohydrate levels, whereas increasing dietary fat levels as well as dietary crude fibre levels decreased NSP digestibility. Information on the aforementioned factors enabled a qualitative prediction of NSP digestibility, but for a quantitative prediction of NSP digestibility from dietary and fish-related factors, more knowledge is required. In other words, more *in vivo* studies are required, in which the CHO and NSP digestibility is actually measured and/or indicators of the degree of fermentation of NSP are used.

In the present study, the CHO fraction not consisting of starch and sugars was considered as NSP. This approach was used in order to be able to quantify the amount of NSP. However, it is not entirely correct to treat the different types of NSP as one, and to allocate certain properties to NSP as group. As seen in this manuscript, the variation in NSP digestibility is large, which is most likely partly related to the different characteristics of the NSP fractions. In addition, specific NSP, like β -glucans and MOS, may have immune-modulatory effects and contribute to animal health as a prebiotics. Therefore it is important to create more insight into the effects and characteristics of individual types of NSP.

This study demonstrates that NSP is partially digestible by tilapia, but the process behind it remains unclear. NSP is expected to be fermented through microbial anaerobic glycolysis, forming short-chain fatty acids. However, how the glycosidic bonds of the long NSP chains are hydrolysed is unclear, as enzymes like cellulase, β -xylanases, β -glucanases and β -galactases are scarce, or non-existing, in the gastrointestinal tract of fish. It is unclear whether and to what extent other fish species can digest NSP. The ability of omnivorous

fish to utilise higher levels of carbohydrates, compared to carnivorous fish, is well known. Besides tilapia, Leenhouwers *et al.* (2007) showed the ability of African catfish (*Clarias gariepinus*) to digest NSP with an ADC of up to 56%. Like tilapia, the African catfish is a warm-water species adapted to feeding on plant matter. This suggests that NSP is potentially digestible by a wide range of fish species, especially warm-water species with a long gut adapted to feeding on plant matter, as these factors favour gut fermentation.

To conclude, the current meta-analysis confirms the negative effect NSP has on the performance (FCR) and nutrient digestibility (crude protein, fat and energy) of tilapia. NSP is not inert to digestion in tilapia, with an average ADC of 24.3%. The body weight and dietary fat level, total carbohydrates and crude fibre are factors that relate to NSP digestibility, in addition to the functional properties of the NSP fraction, that is structure and molecular complexity. NSP could be interesting as functional feed ingredients in aquaculture practice, especially due to NSP associated immune-modulatory effects related to trained immunity. NSP digestibility was shown to contribute to energy digestibility in tilapia. Therefore, in tilapia diet formulations the digestibility, and thus energy originating from NSP, should be taken into consideration as it contributes to the energy needs of the fish.

Acknowledgements

This work is part of the Netherlands Organisation for Scientific Research (NWO) research programme 'Green Aquafeeds' (project number 870.15.100). Danisco Animal Nutrition, a division of DuPont Industrial Biosciences, partly funds this NWO project.

References

- Adeola O, Cowieson A (2011) Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *Journal of Animal Science* **89**: 3189–3218.
- Alonso R, Aguirre A, Marzo F (2000) Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry* **68**: 159–165.
- Amirkolaie AK, Leenhouwers JI, Verreth JA, Schrama JW (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 AK, Verreth JA, Schrama JW (2006) Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture* **260**: 194–205.
- Annisson G (1993) The role of wheat non-starch polysaccharides in broiler nutrition. *Crop and Pasture Science* **44**: 405–422.
- Argenzio R, Southworth M (1975) Sites of organic acid production and absorption in gastrointestinal tract of the pig. *American Journal of Physiology-Legacy Content* **228**: 454–460.
- Azevedo PA, Cho CY, Leeson S, Bureau DP (1998) Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources* **11**: 227–238.
- Banerjee S, Mukherjee A, Dutta D, Ghosh K (2016) Non-starch polysaccharide degrading gut bacteria in Indian major carps and exotic carps. *Jordan Journal of Biology Sciences* **147**: 1–10.
- Bedford M, Schulze H (1998) Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews* **11**: 91–114.
- Bergman E (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* **70**: 567–590.
- Bergot F, Breque J (1983) Digestibility of starch by rainbow trout: effects of the physical state of starch and of the intake level. *Aquaculture* **34**: 203–212.
- Bugaut M (1987) Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **86**: 439–472.
- Carey MC, Small DM, Bliss CM (1983) Lipid digestion and absorption. *Annual Review of Physiology* **45**: 651–677.
- Carter C, Hauler R (2000) Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture* **18**: 299–311.
- Castillo S, Gatlin DM (2015) Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: a review. *Aquaculture* **435**: 286–292.
- Centraal Veevoeder Bureau (2016) *CVB Veevoedertabel 2016*. Federatie Nederlandse Diervoederketen, Wageningen.
- Chakrabarti I, Gani MA, Chaki KK, Sur R, Misra KK (1995) Digestive enzymes in 11 freshwater teleost fish species in relation to food habit and niche segregation. *Comparative Biochemistry and Physiology Part A: Physiology* **112**: 167–177.
- Cheng ZJ, Hardy RW (2003) Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* **9**: 77–83.
- Choct M (1997) Feed non-starch polysaccharides: chemical structures and nutritional significance. *Feed Milling International* **191**: 13–26.
- Choct M, Annisson G (1992) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* **67**: 123–132.
- Choct M, Kocher A (2000) Non-starch carbohydrates: Digestion and its secondary effects in monogastrics. Paper presented at the PROCEEDINGS-NUTRITION SOCIETY OF AUSTRALIA.
- Claus R, Günthner D, Letzguß H (2007) Effects of feeding fat-coated butyrate on mucosal morphology and function in the small intestine of the pig. *Journal of Animal Physiology and Animal Nutrition* **91**: 312–318.

- Clements K, Choat J (1995) Fermentation in tropical marine herbivorous fishes. *Physiological Zoology* **68**: 355–378.
- Clements K, Gleeson V, Slaytor M (1994) Short-chain fatty acid metabolism in temperate marine herbivorous fish. *Journal of Comparative Physiology B* **164**: 372–377.
- Dalmo RA, Bøgvold J (2008) β -glucans as conductors of immune symphonies. *Fish & Shellfish Immunology* **25**: 384–396.
- Degani G, Viola S, Yehuda Y (1997) Apparent digestibility of protein and carbohydrate in feed ingredients for adult tilapia (*Oreochromis aureus* \times *Oreochromis niloticus*). *The Israeli Journal of Aquaculture* **49**: 115–123.
- Deng J, Chen L, Mai K, Mi H, Zhang L (2016) Effects of replacing soybean meal with rubber seed meal on digestive enzyme activity, nutrient digestibility and retention in tilapia (*Oreochromis niloticus* \times *Oreochromis aureus*). *Aquaculture Research* **48**: 1767–1777.
- Dhingra D, Michael M, Rajput H, Patil R (2012) Dietary fibre in foods: a review. *Journal of Food Science and Technology* **49**: 255–266.
- Dong XH, Guo YX, Ye JD, Song WD, Huang XH, Wang H (2010) Apparent digestibility of selected feed ingredients in diets for juvenile hybrid tilapia, *Oreochromis niloticus* \times *Oreochromis aureus*. *Aquaculture Research* **41**: 1356–1364.
- El-Saidy DM, Gaber M (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. *Aquaculture Research* **34**: 1119–1127.
- El-Shafai SA, El-Gohary FA, Verreth JA, Schrama JW, Gijzen HJ (2004) Apparent digestibility coefficient of duckweed (*Lemna minor*), fresh and dry for Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research* **35**: 574–586.
- Falco A, Miest JJ, Pionnier N, Pietretti D, Forlenza M, Wiegertjes GF et al. (2014) β -Glucan-supplemented diets increase poly (I: C)-induced gene expression of Mx, possibly via Tlr3-mediated recognition mechanism in common carp (*Cyprinus carpio*). *Fish & Shellfish Immunology* **36**: 494–502.
- Francis G, Makkar HP, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* **199**: 197–227.
- Fidopiastis PM, Bezdek DJ, Horn MH, Kandel JS (2006) Characterizing the resident, fermentative microbial consortium in the hindgut of the temperate-zone herbivorous fish, *Hermosilla azurea* (Teleostei: *Kyphosidae*). *Marine Biology* **148**: 631–642.
- Gaber MM (2005) The effect of different levels of krill meal supplementation of soybean-based diets on feed intake, digestibility, and chemical composition of juvenile Nile Tilapia *Oreochromis niloticus*, L. *Journal of the World Aquaculture Society* **36**: 346–353.
- Gaber MM (2006) The effects of plant-protein-based diets supplemented with yucca on growth, digestibility, and chemical composition of Nile Tilapia (*Oreochromis niloticus*, L) fingerlings. *Journal of the World Aquaculture Society* **37**: 74–81.
- German DP, Bittong RA (2009) Digestive enzyme activities and gastrointestinal fermentation in wood-eating catfishes. *Journal of Comparative Physiology B* **179**: 1025–1042.
- Goda AMA, Mabrouk HA-HH, Wafa MAE-H, El-Afifi TM. (2012) Effect of using baker's yeast and exogenous digestive enzymes as growth promoters on growth, feed utilization and hematological indices of Nile tilapia, *Oreochromis niloticus* fingerlings. *Journal of Agricultural Science and Technology* **B2**(1B).
- Govoni JJ, Boehlert GW, Watanabe Y (1986) The physiology of digestion in fish larvae. *Environmental Biology of Fishes* **16**: 59–77.
- Hai NV (2015) Research findings from the use of probiotics in tilapia aquaculture: a review. *Fish and Shellfish Immunology* **45**: 592–597.
- Haidar MN, Petie M, Heinsbroek LTN, Verreth JAJ, Schrama JW (2016) The effect of type of carbohydrate (starch vs. non-starch polysaccharides) on nutrients digestibility, energy retention and maintenance requirements in Nile tilapia. *Aquaculture* **463**: 241–247.
- Hemre GI, Lie Ø, Sundby A (1993) Dietary carbohydrate utilization in cod (*Gadus morhua*): metabolic responses to feeding and fasting. *Fish Physiology and Biochemistry* **10**: 455–463.
- Hemre GI, Mommsen T, Krogdahl Å (2002) Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition* **8**: 175–194.
- Jørgensen H, Zhao X-Q, Knudsen KEB, Eggum BO (1996) The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. *British Journal of Nutrition* **75**: 379–395.
- Kaushik SJ, de Oliva Teles A (1985) Effect of digestible energy on nitrogen and energy balance in rainbow trout. *Aquaculture* **50**: 89–101.
- Kihara M, Sakata T (2002) Production of short-chain fatty acids and gas from various oligosaccharides by gut microbes of carp (*Cyprinus carpio* L.) in micro-scale batch culture. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **132**: 333–340.
- Kim J, Kaushik SJ (1992) Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **106**: 161–169.
- Knudsen KEB, Jensen BB, Hansen I (1993) Digestion of polysaccharides and other major components in the small and large intestine of pigs fed on diets consisting of oat fractions rich in β -D-glucan. *British Journal of Nutrition* **70**: 537–556.
- Knudsen KEB, Hedemann MS, Lærke HN (2012) The role of carbohydrates in intestinal health of pigs. *Animal Feed Science and Technology* **173**: 41–53.
- Köprücü K, Özdemir Y (2005) Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **250**: 308–316.
- Krogdahl Å, Herme GI, Mommsen T (2005) Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. *Aquaculture Nutrition* **11**: 103–122.

- Kuz'mina VV (1996) Influence of age on digestive enzyme activity in some freshwater teleosts. *Aquaculture*, **148**: 25–37.
- Lauff M, Hofer R (1984) Proteolytic enzymes in fish development and the importance of dietary enzymes. *Aquaculture* **37**: 335–346.
- Leenhouwers JI, Ortega RC, Verreth JA, Schrama JW (2007) Digesta characteristics in relation to nutrient digestibility and mineral absorption in Nile tilapia (*Oreochromis niloticus* L.) fed cereal grains of increasing viscosity. *Aquaculture* **273**: 556–565.
- Leenhouwers J, Pellikaan W, Huizing H, Coolen R, Verreth JA, Schrama JW (2008) Fermentability of carbohydrates in an in vitro batch culture method using inocula from Nile tilapia (*Oreochromis niloticus*) and European sea bass (*Dicentrarchus labrax*). *Aquaculture Nutrition* **14**: 523–532.
- Li H, Zheng Z, Cong-xin X, Bo H, Chao-yuan W, Gang H (2009) Isolation of cellulose-producing microbes from the intestine of grass carp (*Ctenopharyngodon idellus*). *Environmental Biology of Fishes* **86**: 131–135.
- Li S, Yang X, Yang S, Zhu M, Wang X (2012) Technology prospecting on enzymes: application, marketing and engineering. *Computational and Structural Biotechnology Journal* **2** (3): e201209017.
- Maas RM, Verdegem MC, Dersjant-Li Y, Schrama JW (2018) The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. *Aquaculture* **487**: 7–14.
- Maas RM, Verdegem MC, Schrama JW (2019) Effect of non-starch polysaccharide composition and enzyme supplementation on growth performance and nutrient digestibility in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* **25**: 622–632.
- May T, Mackie R, Fahey G, Cremin J, Garleb K (1994) Effect of fiber source on short-chain fatty acid production and on the growth and toxin production by *Clostridium difficile*. *Scandinavian Journal of Gastroenterology* **29**: 916–922.
- Macfarlane S, Macfarlane GT (2003) Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society* **62**: 67–72.
- Metzler-Zebeli BU, Hooda S, Pieper R, Zijlstra RT, van Kessel AG, Mosenthin R *et al.* (2010) Nonstarch polysaccharides modulate bacterial microbiota, pathways for butyrate production, and abundance of pathogenic *Escherichia coli* in the pig gastrointestinal tract. *Applied and Environmental Microbiology* **76**: 3692–3701.
- Montagne L, Pluske J, Hampson D (2003) A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology* **108**: 95–117.
- Mroz Z, Moeser A, Vreman K, Van Diepen J, Van Kempen T, Canh T, Jongbloed A (2000) Effects of dietary carbohydrates and buffering capacity on nutrient digestibility and manure characteristics in finishing pigs. *Journal of Animal Science* **78**: 3096–3106.
- Mountfort DO, Campbell J, Clements KD (2002) Hindgut fermentation in three species of marine herbivorous fish. *Applied and Environmental Microbiology* **68**: 1374–1380.
- Nawaz A, Irshad S, Hoseinifar SH, Xiong H (2018) The functionality of prebiotics as immunostimulant: evidences from trials on terrestrial and aquatic animals. *Fish & Shellfish Immunology* **76**: 272–278.
- Obirikorang KA, Amisah S, Fialor SC, Skov PV (2015) Digestibility and postprandial ammonia excretion in Nile tilapia (*Oreochromis niloticus*) fed diets containing different oilseed by-products. *Aquaculture International* **23**: 1249–1260.
- Officer DI (2000) Feed enzymes. In: D'Mello JPF (ed) *Farm Animal Metabolism and Nutrition*, pp. 405–426. CABI Publishing, Wallingford, UK.
- Pen-Hsing T, Shi-Yen S (1993) Carbohydrate utilization versus body size in tilapia *Oreochromis niloticus* × *O. aureus*. *Comparative Biochemistry and Physiology Part A: Physiology* **104**: 585–588.
- Petit J, Wiegertjes GF (2016) Long-lived effects of administering β -glucans: indications for trained immunity in fish. *Developmental and Comparative Immunology* **64**: 93–102.
- Petit J, Bailey EC, Wheeler RT, de Oliveira CA, Forlenza M, Wiegertjes GF (2019a) Studies into β -glucan recognition in fish suggests a key role for the C-type lectin pathway. *Frontiers in Immunology*, **10**: 280.
- Petit J, Embregts CWE, Forlenza M, Wiegertjes GF (2019b) Evidence of trained immunity in a fish: conserved features in carp macrophages. *The Journal of Immunology* **203**: 216–224.
- Phan L, Groot R, Konnert GDP, Masagounder K, Figueiredo-Silva AC, Glencross BD *et al.* (2019) Differences in energy utilisation efficiencies of digestible macronutrients in common carp (*Cyprinus carpio*) and barramundi (*Lates calcarifer*). *Aquaculture* **511**: 734238.
- Phillips AM Jr, Brockway DR (1956) The nutrition of trout: II. Protein and carbohydrate. *The Progressive Fish-Culturist*, **18**: 159–164.
- Russell J (1998) The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production in vitro. *Journal of Dairy Science* **81**: 3222–3230.
- Saha S, Roy RN, Sen SK, Ray AK (2006) Characterization of cellulase-producing bacteria from the digestive tract of tilapia, *Oreochromis mossambica* (Peters) and grass carp, *Ctenopharyngodon idella* (Valenciennes). *Aquaculture Research* **37**: 380–388.
- Saravanan S, Geurden I, Figueiredo-Silva A, Kaushik S, Haidar M, Verreth JA *et al.* (2012) Control of voluntary feed intake in fish: a role for dietary oxygen demand in Nile tilapia (*Oreochromis niloticus*) fed diets with different macronutrient profiles. *British Journal of Nutrition*, **108**: 1519–1529.
- Scheppach W (1994) Effects of short chain fatty acids on gut morphology and function. *Gut* **35**(1 Suppl): S35–S38.
- Schneider O, Amirkolaie AK, Vera-Cartas J, Eding EH, Schrama JW, Verreth JA (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.
- Schrama JW, Saravanan S, Geurden I, Heinsbroek LT, Kaushik SJ, Verreth JA (2012) Dietary nutrient composition affects digestible energy utilisation for growth: a study on Nile tilapia (*Oreochromis niloticus*) and a literature comparison across fish species. *British Journal of Nutrition* **108**: 277–289.
- Schrama JW, Haidar MN, Geurden I, Heinsbroek LT, Kaushik SJ (2018) Energy efficiency of digestible protein, fat and carbohydrate utilisation for growth in rainbow trout and Nile tilapia. *British Journal of Nutrition* **119**: 782–791.
- Schwarz W (2001) The cellulosome and cellulose degradation by anaerobic bacteria. *Applied Microbiology and Biotechnology* **56**: 634–649.
- Shepherd C, Jackson A (2013) Global fishmeal and fish-oil supply: inputs, outputs and markets. *Journal of fish biology* **83**: 1046–1066.
- Sinha AK, Kumar V, Makkar HP, De Boeck G, Becker K (2011) Non-starch polysaccharides and their role in fish nutrition – a review. *Food Chemistry* **127**: 1409–1426.
- Sintayehu A, Mathies E, Meyer-Burgdorff KH, Rosenow H, Günther KD (1996) Apparent digestibilities and growth experiments with tilapia (*Oreochromis niloticus*) fed soybean meal, cottonseed meal and sunflower seed meal. *Journal of Applied Ichthyology* **12**: 125–130.
- Stone DAJ (2003) Dietary carbohydrate utilization by fish. *Reviews in Fisheries Science* **11**: 337–369.
- Storebakken T, Austreng E (1987) Ration level for salmonids: I. Growth, survival, body composition, and feed conversion in Atlantic salmon fry and fingerlings. *Aquaculture* **60**: 189–206.
- Tran-Ngoc KT, Dinh NT, Nguyen TH, Roem AJ, Schrama JW, Verreth JAJ (2016) Interaction between dissolved oxygen concentration and diet composition on growth, digestibility and intestinal health of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **462**: 101–108.
- Vahjen W, Osswald T, Schäfer K, Simon O (2007) Comparison of a xylanase and a complex of non starch polysaccharide-degrading enzymes with regard to performance and bacterial metabolism in weaned piglets. *Archives of Animal Nutrition* **61**: 90–102.
- Von Engelhardt W, Rönna K, Rechkemmer G, Sakata T (1989) Absorption of short-chain fatty acids and their role in the hindgut of monogastric animals. *Animal Feed Science and Technology* **23**: 43–53.
- Wang Y, Liu YJ, Tian LX, Du ZY, Wang JT, Wang S (2005) Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture Research* **36**: 1408–1413.
- Wiese M (2019) The potential of pectin to impact pig nutrition and health: feeding the animal and its microbiome. *FEMS Microbiology Letters* **366**: fnz029.
- Williams BA, Verstegen MW, Tamminga S (2001) Fermentation in the large intestine of single-stomached animals and its relationship to animal health. *Nutrition Research Reviews* **14**: 207–228.
- Williams BA, Grant LJ, Gidley MJ (2017) Gut fermentation of dietary fibres: physico-chemistry of plant cell walls and implications for health. *International Journal of Molecular Sciences* **18**: 2203.
- Wilson R (1994) Utilization of dietary carbohydrate by fish. *Aquaculture* **124**: 67–80.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Calculation details of meta-analysis on digestibility of carbohydrates in tilapia.