



## Dietary non-starch polysaccharides influenced natural food web and fish production in semi-intensive pond culture of Nile tilapia

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### ABSTRACT

Dietary non-starch polysaccharides (NSP) changes the nutrient digestibility and faecal characteristics in fish. This study assessed the effect of the type of dietary NSPs on fish production and the contribution of natural food to the total fish production in semi-intensively managed tilapia ponds. Twelve ponds, each divided into three equally-sized compartments, were assigned to test the effect of the type of dietary NSPs (i.e. "PecHem-Diet", a diet with easily fermentable NSP, vs "LigCel-Diet", a diet with slowly fermentable NSP). Fish were restrictively fed, based on the crude protein content of the feed. Three feeding levels ("no = 0", "low = 9 g.kg<sup>-0.8</sup>.d<sup>-1</sup>" and "high = 18 g.kg<sup>-0.8</sup>.d<sup>-1</sup>") nested in pond were analysed in a split plot design. Initial fish biomass was 3084 g.compartment<sup>-1</sup> and the experiment lasted 56 days. With the "LigCel-Diet" biomass gain was higher (2599 vs 2192 g.compartment<sup>-1</sup>) and feed conversion ratio (FCR) was lower (1.4 vs 1.9;  $P < .001$ ) than with the "PecHem-Diet". Diet had no effect on fish survival and specific growth rate (SGR). For both diets, increasing feeding level increased ( $P < .001$ ) biomass gain, fish survival, FCR and SGR. There was a significant interaction effect ( $P < .05$ ) between diet and feeding level on FCR. Fish body composition was the same in both diets. With the "LigCel-Diet", the apparent digestibility coefficient (ADC) was higher ( $P < .001$ ) for crude protein, fat, phosphorus and calcium and lower ( $P < .05$ ) for ash compared to the other diet. Neither feeding level nor the interaction between diet and feeding level influenced the apparent digestibility coefficient (ADC) of any nutrient. Diet composition did not alter the organic matter (OM) composition of the faeces.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from the stable isotope analysis revealed that N gain in fish originated from both feed and natural food of the pond. Natural food abundance in the pond increased over time for both diets. Chlorophyll-a was higher in the pond fed with "LigCel-Diet". Fish gut content and calculated N gain indicated an enhanced contribution of natural food to fish growth in ponds fed with "LigCel-Diet". In conclusion, the type of dietary NSP determines tilapia productivity in semi-intensive managed ponds by altering food web productivity.

### 1. Introduction

Global aquaculture production doubled during the last decade (FAO, 2018). This was mainly achieved in inland ponds (Boyd, 2013). Because of the limited land area for aquaculture, growth was achieved by converting extensive into semi-intensive systems (Boyd, 2013; Tacon and Metian, 2015). This transformation required more feed to support the growth of aquaculture. As a result, total industrial feed production reached ~60 million metric ton (Tacon and Metian, 2015) and will continue to grow in the coming years. In contrast, the supply of fish-meal and fish oil, important protein and fat input in the fish diet, did not increase since 2000 (FAO, 2018). So, fish feed composition shifted from fish-based ingredients to more plant-based ingredients to meet the

demand. As a result of this change, aqua-feeds today contain more carbohydrates, including non-starch polysaccharides (NSP) than before (Meybeck and Sanchez, 2016; Wan et al., 2019).

The current knowledge on nutrient requirements of fish, summarized in NRC (1993, 2011) is predominantly based on studies in which fish were kept in aquaria or cages. In these studies, the contribution of natural food to the fish production is minimal or absent. In ponds, which today are still the most common aquaculture production system (Boyd, 2013), both diet composition and feeding level affect fish performance directly via digestion and absorption of the feed and indirectly via consumption of natural food, the latter stimulated by the feed waste acting as fertilizer. Kabir et al., (2019) showed that a diet with a protein to energy ratio below the recommended level (NRC,

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2011) increased total pond production via a stronger contribution of natural food. In a more recent study, our research team demonstrated that the type of non-protein energy (carbohydrates vs. lipid) in the diet did not affect the contribution of natural food to total pond production (manuscript submitted). It provides an opportunity to move to the production of cheaper feed using carbohydrate as the main source of dietary energy (Tacon and Metian, 2008). However, there are many carbohydrate ingredients which may have different effects on fish performance (Haidar et al., 2016)

Starch and free sugars are dietary carbohydrates, which can be hydrolysed by fish enzymes and consequently absorbed. The remaining part of the carbohydrate fraction, the non-starch polysaccharides (NSPs), comprises (among others) lignin, cellulose, hemicellulose and pectin's (Van Soest et al., 1991). NSPs are considered to have a low nutritional value for fish because of their low digestibility and also due to their anti-nutritional properties (Francis et al., 2001). Knowledge on the direct effects of dietary NSPs on fish performance is relatively scarce. However, comparison within and between studies showed that the type of NSP can have different effects on fish performance. For example, in Nile tilapia, guar gum strongly reduced growth compared to cellulose due to hampering the digestibility of protein and fat (Amirkolaie et al., 2005). Additionally, the digestibility of NSPs differs between types of ingredients (Leenhouders et al., 2008; Teuling et al., 2017). Such differences in digestibility between ingredients is more likely related to differences in fermentability of the type of NSPs (Liu et al., 2016). This suggests that the type of NSPs can alter tilapia performance directly. The type of NSPs also alters faeces composition. In recirculation aquaculture systems (RAS), solid waste needs to be removed while in ponds it can act as an in-situ fertilizer stimulating the food web. In ponds, natural food contributes substantially to fish growth (Kabir et al., 2019). Therefore, the effect of different types of dietary NSP on the fish production can be very different in ponds compared to their effects in RAS or cages. Unfortunately, information on the impact of the type of NSPs on fish performance in ponds including natural food, is absent.

In this study, the effect of the type of dietary NSP on the productivity of tilapia cultured in ponds was assessed. It was hypothesised that the type of NSP ("hemicellulose (Hem) and pectin (Pec)" versus "cellulose (Cel) and lignin (Lig)") would influence the productivity of the natural food in the pond due to difference in their fermentability. In ruminants, it is well known that the type of NSP influence the function of the rumen (microflora) through differences in fermentability (Jha and Berrocoso, 2015). The fermentability (degradation rate) between type of NSPs declines from pectin's to hemicellulose to cellulose and is lowest in lignin (Williams et al., 2001). In this study, we wanted to explore if differences in types of dietary NSP regarding fermentability (slow vs. quick) would affect pond productivity.

## 2. Methods

Two diets, with a contrast in the type of NSPs, were tested on Nile tilapia (*Oreochromis niloticus*) in 12 outdoor ponds (six per diet) for 56 days. Each pond consisted of three equally sized compartments, to which one of three different feeding levels were assigned according to a split-plot design.

### 2.1. Diets

Two experimental diets were formulated to test the effect of non-protein energy sources on the performance of fish and on natural food in the pond. Therefore, the diets had different CHO:LIP ratios but had equal DP:DE ratios. The different CHO:LIP ratios were achieved by replacing fish oil with multiple carbohydrate sources (i.e., wheat bran, rice bran, cassava flour, and wheat flour). This mixture of carbohydrate sources was used to increase both the starch and non-starch polysaccharide content in the diets (i.e., a mixture of digestible and non-

**Table 1**

Ingredient and analysed chemical composition of the experimental Nile tilapia diets differing in the types of non-starch polysaccharides (NSP).

Ingredients	"PecHem-Diet"		"LigCel-Diet"	
	(%)		(%)	
Soybean meal	12.00			
Wheat bran	23.57			
Wheat flour	20.90		18.97	
De-oiled rice bran (DORB)	6.30		12	
Maize	18.00		17	
Canola meal	12.00			
Sunflower meal				13.3
Palm kernel				18.5
Poultry meal				10.8
Fish meal (CP > 68%)	2.00		3	
Fish oil	2.00		4	
Mono calcium phosphate (MCP)	1.50		1.50	
Lime	1.00			
Vitamin/mineral premix <sup>a</sup>	0.45		0.45	
DL Methionine (99%)	0.20		0.20	
L-Lysine (HCL 79%)			0.20	
Yttrium oxide (Y <sub>2</sub> O <sub>3</sub> )	0.08		0.08	
<b>Analysed composition</b>				
Dry matter (DM),	(g.kg <sup>-1</sup> )	917		921
Crude protein	(g.kg <sup>-1</sup> DM)	238		274
Fat	(g.kg <sup>-1</sup> DM)	58		84
Ash	(g.kg <sup>-1</sup> DM)	71		74
Phosphorus	(g.kg <sup>-1</sup> DM)	11		13
Calcium	(g.kg <sup>-1</sup> DM)	10		11
Carbohydrate <sup>b</sup>	(g.kg <sup>-1</sup> DM)	633		568
Starch	(g.kg <sup>-1</sup> DM)	323		277
NSP <sup>c</sup>	(g.kg <sup>-1</sup> DM)	265		276
Acid detergent fibre	(g.kg <sup>-1</sup> DM)	64		123
Acid detergent lignin	(g.kg <sup>-1</sup> DM)	12		21
Neutral detergent fibre	(g.kg <sup>-1</sup> DM)	189		238
Gross energy	(kJ.g <sup>-1</sup> DM)	19		20
DP:DE ratio <sup>d</sup>	(g.MJ <sup>-1</sup> )	14.2		15.8
C:N ratio <sup>e</sup>		12.3		10.8

<sup>a</sup> Commercial product.

<sup>b</sup> This is calculated as follows carbohydrate = 1000 – CP – Fat – Ash.

<sup>c</sup> NSP, non-starch polysaccharides calculated.

<sup>d</sup> Calculated based on the apparent digestibility coefficient obtained in this experiment.

<sup>e</sup> This is calculated C:N ratio considering 16% N content in the protein and 47, 70 and 50% C content in protein, fat and carbohydrate, respectively (Van De Waal and Boersma, 2012).

digestible carbohydrate sources): A "PecHem-Diet" with quick/easy bio-degradable (fermentable) NSPs versus a "LigCel-Diet" with slow bio-degradable NSPs. For creating this contrast in the type of NSPs the qualification of dietary fibres by the Van Soest method (Van Soest et al., 1991) was applied, which determines the acid detergent lignin (ADL), acid detergent fibre (ADF) and acid neutral detergent fibre (NDF). The ADL and ADF represent the lignin and cellulose part of the dietary fibre. Using the nutritional value tables of the feed ingredient database we-bapp (CVB, 2019), the "LigCel-Diet" was formulated to have a high ADL and ADF content by the inclusion of palm kernel meal and sunflower meal, in contrast to the "PecHem-Diet", while keeping higher presence of pectin and hemicellulose by including wheat bran and soya bean meal in the diet. In order to keep the DP:DE ratio equal in both diets, small alterations in the inclusion levels of protein-rich ingredients were made (Table 1). Both diets met the recommended nutrient requirements of tilapia (NRC, 2011). However, the DP:DE level was 15.6 g.MJ<sup>-1</sup>, which is below the recommended level of NRC (1993) and was done to enhance the natural food availability in the pond (Kabir et al., 2019). An inert marker, yttrium oxide (Y<sub>2</sub>O<sub>3</sub>), was included in the diets to test apparent digestibility coefficients (ADC). The experimental diets were extrusion processed to produce 3 mm diameter floating pellets at the R & D facilities of De Heus (De Heus Beheer B.V.) in Vietnam.

## 2.2. Fish, rearing and housing facilities

All male, juvenile Nile tilapia (*Oreochromis niloticus*), 14th generation WorldFish GIFT strain were collected from the Asha Hatchery, at Bagerhat, in Bangladesh, with average weight of 90 g. Twelve, 30 m<sup>2</sup>, outdoor ponds, in a field experimental station were used for this experiment. Each pond was divided into three equal compartments as described by Kabir et al. (2019).

## 2.3. Experimental procedure

Prior to the experiment, ponds were prepared following the procedure described in Kabir et al. (2020). Forty juvenile tilapia (equivalent to 4 fish per m<sup>-2</sup>) were stocked in each pond compartment. Fish were fed daily at 8.00 and 16.00 h according to their metabolic body weight. Within each pond, one of three feeding levels were applied per compartment, high (18 and 20.6 g.kg<sup>-0.8</sup>.d<sup>-1</sup> for low CHO:LIP and high CHO:LIP diet, respectively), low (9 and 10.3 g.kg<sup>-0.8</sup>.d<sup>-1</sup> for low CHO:LIP and high CHO:LIP diet, respectively) and no feeding, in a split plot design for both diets. Variation in the amount of feed between the diets under the same feeding level was due to feeding based on crude protein level of the diet. The high feeding level was comparable with normal feeding rates for semi-intensive, commercial tilapia ponds. By applying these rations, ponds were fed a similar amount of protein and energy. Sampling for pond soil and water nutrients and natural food were done at day 1, 28 and 56.

### 2.3.1. Water quality monitoring

Dissolved oxygen (DO), pH, total dissolved solid (TDS), transparency, temperature and salinity of each pond were measured daily at 6.00, 9.00, 10.00, 12.00, 14.00 and 14.30 h and NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub>, NO<sub>3</sub> and TSS was measured at day 1, 28 and 56 following the procedure described in Kabir et al. (2020).

### 2.3.2. Sampling and analysing soil and water nutrients

N, P, K and OM of soil and water were analysed from the samples collected at day 1, 28 and 56 from the experimental ponds. Procedures for sample collection, preparation and analysis were following the methods described in Kabir et al. (2020).

### 2.3.3. Sampling and analysing plankton, benthos and total bacterial count (TBC)

Phytoplankton, zooplankton, benthos, chlorophyll *a*, and soil and water for TBC samples were collected at day 1, 28 and 56. Samples were collected, prepared and analysed following the procedure described by Kabir et al. (2020).

### 2.3.4. Sampling and analysing proximate composition of fish and feed

Initial body composition was determined in 25 fish, which were randomly selected at the start of the experiment. For final body composition, five fish were randomly selected per compartment at the end of the experiment. Fish feed samples were preserved from day 1, 28 and 56. Both fish and feed samples were prepared and analysed according to the procedure described by Kabir et al. (2020).

### 2.3.5. Faeces collection and preservation

After ending the pond experiment, 180 tilapia with mean body weight of 161 (± 31) g were restocked in indoor concrete tanks for faeces collection to determine apparent digestibility. Fish were restocked in 18 indoor concrete tanks for faeces collection. The tanks had a volume of 1000 L of which 700 L were filled with water. Ten fish were allocated in each tank. All tanks were aerated. Both experimental diets were fed at 6, 9 and 12 g.kg<sup>-0.8</sup>.d<sup>-1</sup> with 3 replications per treatment. Fish were fed daily at 7.00 and 15.00 h. The first seven days, fish were fed in the tank for acclimation to tank condition and diets. Starting from day 8, faeces were collected by siphoning 3 h after each feeding for

10 days. Collected faeces were preserved in labelled plastic pots at -20 °C. Later all samples from the same tank were pooled together for chemical analysis.

### 2.3.6. Analysis of stomach contents

Fish were harvested on day 57, 19 h after the last feeding, to ensure that no pellet remained in the stomach. Fish were euthanized by an overdose of a phenoxy-ethanol solution (1.0 ml.l<sup>-1</sup>) and transported to the laboratory. In the laboratory the fish were dissected to collect the stomach and preserve it in 10% formalin. Total volume of the stomach and the number of food items were recorded. Volume of food items occupying in general and by each food group were visually estimated (Jude, 1971). Total weight of food was expressed as percentage of weight of the stomach on a wet weight basis (Gibbons and Gee, 1972). Index of relative importance (IRI) of observed natural food groups was estimated by diet to understand the relative importance of natural food group in the growth of fish following the methods described by Pinkas et al. (1971) and Prince (1975).

IRI = (%G<sub>n</sub> + %G<sub>v</sub>) × %G<sub>f</sub>. Where, G<sub>n</sub> is percentage by group number, G<sub>v</sub> is volume of group number and G<sub>f</sub> is frequency of occurrence by the group number.

### 2.3.7. Sample collection and chemical analysis of <sup>13</sup>C and <sup>15</sup>N stable isotope

All samples were collected on day 57 (after completion of the feeding trial). Plankton were collected by pumping pond water for 5 min through a plankton net of 45 μ mesh size. Three fish from each pond compartment were isolated, euthanized by an overdose of a phenoxy-ethanol solution (1.0 ml.l<sup>-1</sup>) and transported to the laboratory. In the laboratory the fish were degutted in order to take out egested feed. Afterwards, the degutted fish were oven dried and grinded by using a bullet mill (100–200 μm) to ensure isotopic homogeneity. Dry matters from three fish was pooled together to make one composite sample per pond compartment. Then, samples were analysed for dry matter (DM) according to AOAC (1990). For total nitrogen (TN), and total carbon (TC) content, and isotopic enrichment were analysed by an EA Elemental Analyzer (Euro Vector, HEKAtech, Wegberg, Germany) coupled to an isotope ratio mass spectrometer (Delta Plus Advantage, THERMO, Bremen, Germany). Isotopic ratios were expressed relative to international standards (Vienna Pee Dee Belemnite, VPDB, for carbon and atmospheric N<sub>2</sub> for nitrogen).

## 3. Analytical procedures and calculations

### 3.1. Performance

Biomass gain (g) was calculated as the difference between the biomass stocked and biomass harvested per compartment. The specific growth rate (SGR) was calculated as  $SGR = ((\ln(\text{IndBW}_{56}) - \ln(\text{IndBW}_0)) / 56) \times 100$ ; where IndBW<sub>56</sub> and IndBW<sub>0</sub> means individual body weight at day 56 and day 0, respectively. Feed conversion ratio (FCR) was calculated per compartment using the feed given and weight gain. The percentage survival of fish per compartment was calculated as  $(N_f / N_i) \times 100$ , where N<sub>f</sub> is the final number of fish and N<sub>i</sub> the initial number at pond compartment (PC) level.

### 3.2. ADC calculation

The apparent digestibility coefficient of nutrients was measured for each tank using yttrium (Y<sub>2</sub>O<sub>3</sub>) as an inert marker. Apparent digestibility coefficients (ADCs) of crude protein, crude fat, energy and carbohydrate in the diets were calculated by using the following formula:  $\%ADC_{\text{diet}} = 100\% \times (1 - [Y_{\text{diet}} / Y_{\text{faeces}}] \times [N_{\text{faeces}} / N_{\text{diet}}])$

Here, Y<sub>diet</sub> and Y<sub>faeces</sub> are the content of the inert marker (yttrium) in the diet and faeces, respectively (g.kg<sup>-1</sup> DM); and N<sub>faeces</sub> and N<sub>diet</sub>

are the contents of the dietary nutrients in the faeces and diets, respectively ( $\text{g.kg}^{-1}$  DM).

### 3.3. Fish N gain calculation

N gain in fish was calculated by the difference between the  $N_h$  and  $N_s$ . Here,  $N_h$  is the amount of N in the harvested fish biomass and  $N_s$  is the amount of N in the biomass at start. N feed was calculated by total feed input per compartment, multiplied by the N content in feed. Contribution of feed N to fish N gain was calculated based on the ADC of CP from this study and considering average N retention efficiency (RE) of 40% (Azevedo et al., 2004) for both the diets at all feeding levels. N retained from natural food was calculated by deducting N retention from feed from the total N gain in fish.

### 3.4. Calculation of isotope ratios

Isotope ratios were compared by using a  $\delta^H X$  value, obtained by using Formula 1 (Fry, 2006; Peterson and Fry, 1987).

$$\delta^H X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 (\text{‰}) \quad (\text{Formula 1})$$

Here, H the atomic mass, X the atom,  $R_{\text{sample}}$  the isotope concentration of the sample and  $R_{\text{standard}}$  a standard value which is for  $^{15}\text{N}/^{14}\text{N}$  based on the concentration in the air (0.0036765) and for  $^{13}\text{C}/^{12}\text{C}$  based on PeeDee Belemnite (0.011180) (Fry, 2006).

### 3.5. Statistical analysis

The data were analysed using the IBM SPSS software package version 23. All data, except water quality and ADC, were analysed in a split plot design using the procedure general linear model (GLM). Effect of diet has been tested against the variation between ponds while the effects of feeding level and diet by feeding level interaction were tested against the variation between compartments within the pond. Univariate analysis was done to see the effect of diet on water quality at pond level only. Effect of diet, feeding level and their interaction on ADC of nutrients were tested by a two-way ANOVA following the procedure general linear model (GLM). When a significant interaction effect was present, post hoc multiple comparisons of means using Tukey's multiple range test was performed.

## 4. Results

Average individual body weight (BW) at stocking was 77 g, unaffected by diet and feeding level. At pond level, biomass gain was 18.5% higher ( $P < .05$ ) with the "LigCel-Diet", while FCR was 25% lower ( $P < .001$ ), compared to the other diet. Biomass harvested, biomass gain per compartment, individual gain, fish survival, FCR and

growth rate increased with feeding level ( $P < .001$ ; Table 2). The interaction effect between diet and feeding levels influenced FCR ( $P < .05$ ), and also tended ( $P < .1$ ) to influence biomass harvested and biomass gain (Table 2). With increasing feeding level, the difference between both diets became larger.

The apparent digestibility (ADC) of ash, crude protein, fat, phosphorus and calcium were affected by the type of dietary NSP. The ADCs of these nutrients were higher at the "LigCel-Diet" than at the "PecHem-Diet". There was a tendency ( $P < .1$ ) for higher ADC of carbohydrate at the "PecHem-Diet" and for energy an opposite tendency was observed. Feeding level and the interaction between feeding level and diet did not influence any of the nutrient ADCs (Table 3).

The type of dietary NSP did not affect body composition, but feeding level influenced protein and ash content ( $P < .05$ ; Table 4). Protein and ash content increased with feeding level. The interaction between diet and feeding level did not affect body composition (Table 4).

The comparison of the carbon ( $^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ ) stable isotope ( $\delta\text{C}:\delta\text{N}$ ) signature of the experimental diets, fish and natural food web items are presented in Fig. 2. Diets were equal in  $\delta\text{C}$  and had a small difference in  $\delta\text{N}$ . The  $\delta\text{C}$  and  $\delta\text{N}$  content of the food web items (plankton as well as periphyton) overlapped strongly, did not differ between ponds fed the different diets and overlapped with the signature of both diets. The  $\delta\text{C}$  and  $\delta\text{N}$  content of fish did not differ between both experimental diet treatments.

Irrespective to diets, nutrients (N, P, K and organic matter) of pond soil and water changed ( $P < .001$ ) over the time (Table 5). The N content of the pond water was higher ( $P < .05$ ) with the "PecHem-Diet" compared to the "LigCel-Diet", and tended to be higher for water organic matter content ( $P < .1$ ). The 3-way interaction effect between sampling time, diet and feeding level influenced the soil N content, while the interaction effect between sampling time and diet affected the water N content (Table 5). Furthermore, the interaction effect between sampling time and diet influenced the water organic matter content (Table 5). For all these interaction effects with time, the effect between treatments (diets) was largest at the last sampling moment.

Sampling time influenced chlorophyll *a*, phytoplankton (abundance & diversity), zooplankton and benthos abundance and total bacterial count in both pond water and soil (Table 6). Chlorophyll *a* content of water was higher with the "LigCel-Diet". The difference in Chlorophyll *a* increased with time, indicated by the significant interaction effect between sampling time and diet. The interaction between sampling time and diet also tended ( $P < .1$ ) to influence benthos abundance and total count of soil bacteria. The interaction effect between sampling time and feeding level influenced ( $P < .05$ ) the total bacterial count of water and tended to influence ( $P < .1$ ) the soil bacteria count as well. Total soil bacteria count also showed a tendency ( $P < .1$ ) to be influenced by the interaction effect between sampling time and feeding level.

The effect of diet and feeding level on stomach fullness with natural

**Table 2**  
Effect of type of dietary non-starch polysaccharides (NSP) and feeding level on performance of tilapia.

	Units	"PecHem-Diet"			"LigCel-Diet"			Pooled SEM	P-values		
		FLO	FL1	FL2	FLO	FL1	FL2		D	FL	D*FL
Initial individual BW	g	77	77	77	77	77	77	0.7	ns	ns	ns
Biomass stocked	$\text{g.comp}^{-1}$	3097	3068	3089	3073	3083	3092	30	ns	ns	ns
Biomass harvested	$\text{g.comp}^{-1}$	3669	5466	6695	3824	5738	7483	139	*	***	#
Individual BW Gain	g	46	84	104	51	87	114	7.3	ns	***	ns
Biomass gain	$\text{g.comp}^{-1}$	572	2398	3607	752	2654	4391	150	*	***	#
Survival	%	76	86	93	77	88	98	3.0	ns	***	ns
FCR	$\text{g.g}^{-1}$		1.40	2.45		1.13	1.73	0.076	***	***	*
Growth Rate	$\text{g.d}^{-1}$	0.8	1.5	1.8	0.9	1.6	2.0	0.10	ns	***	ns

"PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP, FLO = no feeding, FL1 = low feeding level, FL2 = high feeding level, D = diet and FL = feeding level, D\*FL = diet and feeding level interactions, BW = body weight, FCR = feed conversion ratio, Comp = compartment, P values: ns (not significant,  $P > .1$ ), # ( $P < .1$ ), \* ( $P < .05$ ), \*\*\* ( $P < .001$ ).



**Table 3**

Effect of type of dietary non-starch polysaccharides (NSP) and feeding level on apparent digestibility coefficient (ADC) in tilapia.

	Units	"PecHem-Diet"			"LigCel-Diet"			Pooled SEM	P-values		
		FLO	FL1	FL2	FLO	FL1	FL2		D	FL	D*FL
Crude ash	%	-16	-06	-30	-01	-05	-02	7.0	*	ns	ns
DM	%	61	65	60	63	63	65	2.0	ns	ns	ns
Crude protein	%	77	79	77	81	81	82	1.0	***	ns	ns
Fat	%	87	87	86	90	91	91	1.0	***	ns	ns
Energy	%	67	70	67	69	70	72	2.0	#	ns	ns
Carbohydrate	%	61	66	62	58	59	62	2.0	#	ns	ns
P	%	39	42	40	51	53	54	2.0	***	ns	ns
Ca	%	-4	3	-5	10	14	14	4.0	***	ns	ns

"PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP, FLO = no feeding, FL1 = low feeding level, FL2 = high feeding level, D = diet and FL = feeding level, D\*FL = diet and feeding level interactions, P values: ns (not significant,  $P > .1$ ), # ( $P < .1$ ), \* ( $P < .05$ ), \*\*\* ( $P < .001$ ).

**Table 4**

Effect of type of dietary non-starch polysaccharides (NSP) and feeding level on body composition of tilapia.

	Units	"PecHem-Diet"			"LigCel-Diet"			Pooled SEM	P-values		
		FLO	FL1	FL2	FLO	FL1	FL2		D	FL	D*FL
DM	g.kg <sup>-1</sup>	280	288	308	280	294	281	7.5	ns	#	ns
Protein	g.kg <sup>-1</sup>	151	153	163	153	157	155	2.4	ns	*	ns
Fat	g.kg <sup>-1</sup>	49	51	57	50	53	52	2.0	ns	ns	ns
Ash	g.kg <sup>-1</sup>	52	58	63	55	62	60	2.4	ns	*	ns

"PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP, FLO = no feeding, FL1 = low feeding level, FL2 = high feeding level, D = diet and FL = feeding level, D\*FL = diet and feeding level interactions, P values: ns (not significant,  $P > .1$ ), # ( $P < .1$ ), \* ( $P < .05$ ), \*\*\* ( $P < .001$ ).

food, both volumetric and gravimetric, is given in Table 7. Volumetrically, the presence of natural food in the stomach was higher ( $P < .05$ ) and gravimetrically it tended to be higher ( $P < .1$ ) at the "LigCel-Diet" compared to the "PecHem-Diet". Gravimetrically, the interaction effect between diet and feeding level tended to influence the presence of natural food in the fish stomach, showing an increased stomach fullness at the higher feeding levels at the "LigCel-Diet". IRI, the indicator of relative importance of natural food group in the diet of fish, from the stomach content observation for both the diets showed that phytoplankton, zooplankton and crustaceans, respectively, are the important natural food groups for tilapia for both diets (data not shown).

All the measured physical parameters of pond water quality were unaffected by diet (i.e., type of the dietary NSPs) and were within the accepted level for tilapia cultured in ponds (Table 8).

## 5. Discussion

In this study, the effect of type of dietary non-starch polysaccharides (NSP) on the productivity of tilapia cultured in ponds was assessed. It

was hypothesised that the type of NSP regarding fermentability (slow vs. quick; e.g., "hemicellulose and pectin's" versus "cellulose and lignin") would influence the productivity of the pond food web. The experimental results demonstrate that the type of dietary NSPs can influence pond productivity in tilapia mono-culture. This impact on productivity seems to be related to the enhancement of the natural food in ponds fed with the "LigCel-Diet", as differences were observed in concentration of water chlorophyll-a, benthos abundance and total count of soil bacteria, and natural food content in fish stomach (Tables 6 and 7).

The differences in pond productivity between diets (e.g., biomass gain; Table 2) was not due to a different input of nutrients via feeding. Ponds were all fed the same amount of protein (nitrogen) based on the analysed dietary crude protein content (Fig. 2). Although the C:N ratio of both diets were almost equal, the energy (C input) given to ponds at the "PecHem-Diet" was slightly higher compared to ponds at the "LigCel-Diet" due to a small numerical difference in C:N ratio. Studies on dietary protein to energy ratio by Kabir et al. (2019) demonstrated that lowering this ratio (i.e., increasing the C:N ratio) increased pond

**Table 5**

Effect of type of dietary non-starch polysaccharides (NSP) and feeding level on soil and water nutrients.

	Nutrients	Units	"PecHem-Diet"			"LigCel-Diet"			Pooled SEM	P-values						
			ST1	ST2	ST3	ST1	ST2	ST3		ST	D	FL	D*FL	ST*D	ST*FL	ST*D*FL
Water	Nitrogen	mg.l <sup>-1</sup>	13	22	24	13	19	22	0.7	***	*	ns	ns	*	ns	ns
	Phosphorus	mg.l <sup>-1</sup>	37	34	38	39	35	39	1.6	***	ns	ns	ns	ns	ns	ns
	Potassium	mg.l <sup>-1</sup>	68	42	59	70	45	63	1.5	***	*	#	ns	ns	ns	ns
	Organic matter	mg.l <sup>-1</sup>	256	445	489	266	376	445	14	***	#	ns	ns	***	ns	ns
Soil	Nitrogen	mg.l <sup>-1</sup>	0.08	0.09	0.08	0.08	0.10	0.09	0.002	***	ns	ns	ns	ns	ns	*
	Phosphorus	mg.l <sup>-1</sup>	582	505	694	762	748	924	111	***	ns	ns	ns	ns	ns	ns
	Potassium	mg.l <sup>-1</sup>	733	526	627	742	581	676	19	***	*	ns	ns	ns	ns	ns
	Organic matter	g.l <sup>-1</sup>	15.6	17.9	17.3	16.2	18.5	17.8	0.31	***	ns	ns	ns	ns	ns	ns

"PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP, FLO = no feeding, FL1 = low feeding level, FL2 = high feeding level, ST = sampling time (day), D = diet and FL = feeding level, D\*FL = diet and feeding level interactions, P values: ns (not significant,  $P > .1$ ), # ( $P < .1$ ), \* ( $P < .05$ ), \*\*\* ( $P < .001$ ).

**Table 6**

Effect of type of dietary non-starch polysaccharides (NSP) and sampling time (ST) averaged over feeding levels natural food items in ponds (expressed per compartment).

	Units	"PecHem-Diet"			"LigCel-Diet"			Pooled SEM	P-values						
		ST1	ST2	ST3	ST1	ST2	ST3		ST	D	FL	D*FL	ST*D	ST*FL	ST*D*FL
Chlorophyll a	µg.l <sup>-1</sup>	4	3	5	6	4	16	2	***	*	ns	ns	*	ns	ns
Phytoplankton abundance	Ind.ml <sup>-1</sup>	235	419	697	252	422	913	119	***	ns	ns	ns	ns	ns	ns
Phytoplankton diversity	group.l <sup>-1</sup>	12	11	13	11	12	14	0.7	*	ns	ns	ns	ns	ns	ns
Zooplankton abundance	Ind.ml <sup>-1</sup>	137	90	195	94	82	134	31	*	ns	ns	ns	ns	ns	ns
Zooplankton diversity	group.l <sup>-1</sup>	7	6	7	7	7	6	0.50	ns	ns	ns	ns	ns	ns	ns
Benthos abundance	Ind.l <sup>-1</sup>	52	83	82	70	62	81	16	*	ns	ns	ns	#	ns	ns
Benthos diversity	group.l <sup>-1</sup>	2.7	3.0	2.7	2.9	2.7	2.8	0.22	ns	ns	ns	ns	ns	ns	*
Water bacteria	CFU.ml <sup>-1</sup>	478	704	2458	464	723	2447	165	***	ns	#	ns	ns	*	ns
Soil bacteria	CFU.ml <sup>-1</sup>	606	1455	3849	472	1578	4481	166	***	ns	ns	ns	#	#	ns

"PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP, FLO = no feeding, FL1 = low feeding level, FL2 = high feeding level, ST = sampling time (day), D = diet and FL = feeding level, D\*FL = diet and feeding level interactions, Ind. = Individual, P values: ns (not significant, P > .1), # (P < .1), \* (P < .05), \*\*\* (P < .001).

A list of phytoplankton, zooplankton and benthos recorded during the experiment has been presented in the Supplementary Table 1.

**Table 7**

Effect of type of dietary non-starch polysaccharides (NSP) and feeding level on the amount of natural food content in the stomach of tilapia.

	Units	"PecHem-Diet"			"LigCel-Diet"			Pooled SEM	P-values		
		FLO	FL1	FL2	FLO	FL1	FL2		D	FL	D*FL
Volumetric occurrence of natural food	%	26	23	21	31	28	29	2.5	*	ns	ns
Gravimetric occurrence of natural food	%	30	21	24	22	32	31	3.8	#	ns	#

"PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP, FLO = no feeding, FL1 = low feeding level, FL2 = high feeding level, D = diet and FL = feeding level, D\*FL = diet and feeding level interactions, P values: ns (not significant, P > .1), # (P < .1), \* (P < .05).

**Table 8**

Effect of type of dietary non-starch polysaccharides (NSP) and feeding level on pond water quality.

	Units	"PecHem-Diet"	"LigCel-Diet"	Pooled SEM	P-values for Diet
Dissolved oxygen (at morning)	mg.l <sup>-1</sup>	5.4	5.4	0.0	ns
Temperature	°C	30	30	0.1	ns
pH	-	7.6	7.6	0.0	ns
Transparency	cm	33	33	0.5	ns
Water depth	cm	107	109	3.6	ns
Salinity	ppt	1.9	2.0	0.42	ns
Total suspended solid	mg.l <sup>-1</sup>	325	323	23	ns
Total dissolved solid	mg.l <sup>-1</sup>	4121	4062	196	ns
NO <sub>2</sub>	mg.l <sup>-1</sup>	0.011	0.012	0.004	ns
NH <sub>4</sub>	mg.l <sup>-1</sup>	0.19	0.17	0.053	ns

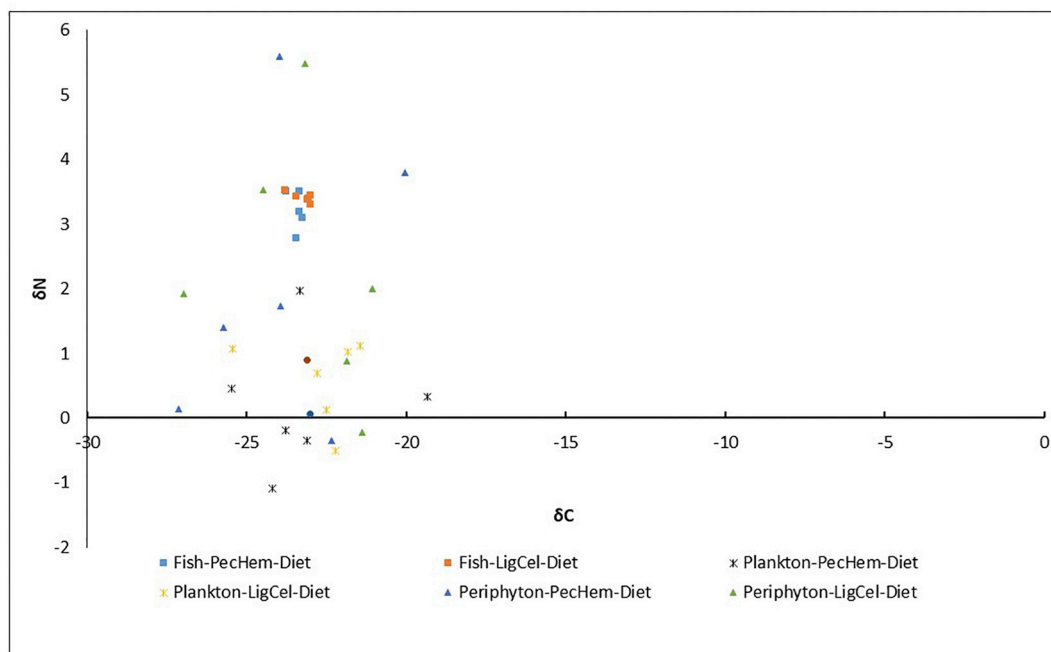
"PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP, FLO = no feeding, FL1 = low feeding level, FL2 = high feeding level, D = diet and FL = feeding level, D\*FL = diet and feeding level interactions, P values: ns (not significant, P > .1).

productivity by enhancing the food web. Consequently, the small difference in dietary C:N ratio between the experimental diets might have reduced the observed impact of type of dietary NSP in the current study. Next to N, P input via the feed into the ponds was identical between diets.

To determine if the effects of the type of dietary NSP on pond productivity were due to differences in nutrient digestibility (ADC), the ADC of macro-nutrients were determined in fish kept in tanks without the presence of the natural food web (Table 3). The measured ADC of macronutrients showed that diets were not only different regarding the type of NSP, but also regarding their digestibility. The observed differences in ADC between both diets in protein and fat are most likely due to the fact that diets were largely different regarding the

ingredients that provided the dietary fat and crude protein (Table 1). It is well known, that ingredient composition is a major determinant in feed quality, i.e., digestibility (Glencross et al., 2007). However, the higher crude protein and fat ADC with the "LigCel-Diet" might also be due to a direct effect of the type of dietary NSPs. Water soluble NSP, mostly originating from pectin's and hemicellulose, affect dietary viscosity (Leenhouwers et al., 2007). Various studies in fish have demonstrated that increasing dietary viscosity can negatively affect digestibility of other macronutrients (Amirkolaie et al., 2005; Tran-Tu et al., 2019; Tran-Tu et al., 2018). Opposite to crude protein and fat ADC, carbohydrates tended to have a higher ADC with the "PecHem-Diet" compared to the "LigCel-diet", which is fully in line with the higher fermentability of pectin's and hemicellulose compared to cellulose and lignin. This is also in line with findings in tilapia that diets/ingredients rich in pectin's and hemicellulose have higher carbohydrate and NSP ADC compared to cellulose rich diets (Amirkolaie et al., 2005; Haidar et al., 2016; Maas et al., 2019). Faecal starch content was not measured in this study, but when assuming a constant starch ADC of 98% for both diets, the calculated NSP ADC in the current study was 17% at the "PecHem-Diet" and 22% at the "LigCel-Diet". This shows, like in other studies in Nile tilapia (Haidar et al., 2016; Leenhouwers et al., 2008; Maas et al., 2019), that NSP are not inert. Besides, the available phosphorus resulting from the low ADC with the "PecHem-Diet" (Table 3) might have also influenced fish performance. The difference in macronutrient ADC were small between diets, but could still have played a role in the observed difference in pond productivity due to an altered faeces composition having a fertilization effect on the natural food and/or direct uptake of nutrient (especially protein) for growth.

In Fig. 3 the N gain from feed and food web was calculated identical to Kabir et al. (2019). Over the whole experimental period, the total N gain per pond was 284 with the "PecHem-Diet" and 308 g with the "LigCel-Diet", of which 46.3 and 44.8%, respectively, originated from feed-N. The difference in N-gain at pond level between both diets was for 71% related to a higher contribution coming from the food web with



**Fig. 1.** Effect of type of dietary non-starch polysaccharides (NSP) on the distribution of  $\delta C:\delta N$  in feed, plankton, periphyton and fish body. where, Fish-“PecHem-Diet” = fish fed with “PecHem-Diet”, Fish-“LigCel-Diet” = fish fed with “LigCel-Diet”, Plankton-“PecHem-Diet” = plankton samples collected from the pond fed with “PecHem-Diet”, Plankton-“LigCel-Diet” = plankton samples collected from the pond fed with “LigCel-Diet”, Periphyton-“PecHem-Diet” = periphyton samples collected from the pond fed with “PecHem-Diet”, Periphyton-“LigCel-Diet” = periphyton samples collected from the pond fed with “LigCel-Diet”, PecHem-Diet” = a diet with quick/easy bio-degradable (fermentable) NSP, “LigCel-Diet” = a diet with slow bio-degradable (fermentable) NSP,

the “LigCel-Diet”. This indicates that the type of dietary NSP can influence the productivity of the pond food web. The higher productivity of the food web at the “LigCel-Diet” is in line with the observed higher water chlorophyll  $\alpha$  content (Table 6), the abundance of benthos and soil bacteria (Table 6) and stomach fullness with natural food (Table 7).

Analysis of  $^{13}C$  and  $^{15}N$  stable isotope (Fig. 1) also indicate that fish consumed nutrients not only from the feed but also from other sources in the pond (i.e. natural food). The IRI indicates that phytoplankton (or algae) was the most important group of natural food found in the stomach of the fish for both diets. Higher chlorophyll- $a$  levels in ponds fed with “LigCel-Diet” thus indicate that the dominant food group was more abundant in the ponds fed with this diet. Because algae is the primary producer in a pond, more likely they had also a positive impact on the other parts of the food web in the pond. The better growth performance of tilapia in the non-fed compartments of pond fed with “LigCel-Diet” (Table 2), also indicates the importance of pelagic natural food to growth of tilapia in aquaculture ponds. So, natural food, more specifically the pelagic food web led to the difference in the fish performance.

Still the question remains how different types of dietary NSP steer the natural food in the pond. Enhancement of the natural food in a pond by fertilization through feed supplementation depends among others on the amount and composition of both the uneaten feed and the produced faeces by the fish. The C:N ratio of the nutrient input (Asaduzzaman et al., 2010; Avnimelech, 1999) is considered to be a key factor for natural food enhancement in fish ponds. In Fig. 4, the calculated organic matter composition of faeces produced by both diets (derived from nutrient ADC values in Table 3) is shown. The C:N ratio in the feed (12.3 vs 10.8) were slightly different but in the faeces this ratio was comparable (17.1 vs 17.5). Overall, organic matter composition of the faeces was similar (Fig. 4). The total amount of the faeces produced, calculated based on feed ration and ADC of DM, was higher with the “PecHem-Diet” than with the other diet (DM 1531 vs 1269 g). One would expect that the higher amount of faeces at the “PecHem-Diet” would be positive for stimulating the food web because this enlarged

especially the C input in the ponds. However, the type of NSP might also affect the stability of the faeces. Amirkolaie et al. (2005) showed that soluble vs. insoluble NSP (guar gum vs. cellulose) altered the stability/characteristics of the faeces. The soluble NSP diets had more diarrhoea like faeces. Therefore, it can be hypothesised that the type of dietary NSP might also shift the place where faecal nutrients (C and N) end up in the pond: dissolved in the water column versus settled at the bottom as solids. The “PecHem-Diet” containing most likely more soluble NSP, may have created less stable faeces, which was probably emitting from the system more rapidly instead of being available to the biota of the pond for a prolonged time. Organic matter levels were not different between the ponds fed different diets, which supports this statement. On the other hand, faeces with low soluble NSPs (most likely at the “LigCel-Diet) are usually solid (Amirkolaie et al., 2005) and therefore can reach the pond sediment. We do not have data on the consequence of the faeces reaching the pond bottom. The possible explanation might be that microbes integrate faecal nutrients both in the benthic and in the pelagic food web in the pond. This may have resulted in a high natural food production in the pond fed with “LigCel-Diet”. However, further research should elucidate how the type of NSP is altering the contribution of natural foods to pond production.

## 6. Conclusion

The “LigCel-Diet” enhanced natural food and increased its contribution to fish growth in pond culture of tilapia while both the diets had comparable C:N ratios. Therefore, not only the amount of C contributing to the C:N ratio, but also the composition of carbon is important for food web enhancement. The current study showed that the type of dietary NSP determines pond food web productivity.

## Authors statement

Kazi Ahmed Kabir: Conducted experiment, data collection, analysis, visualization and original draft preparation.

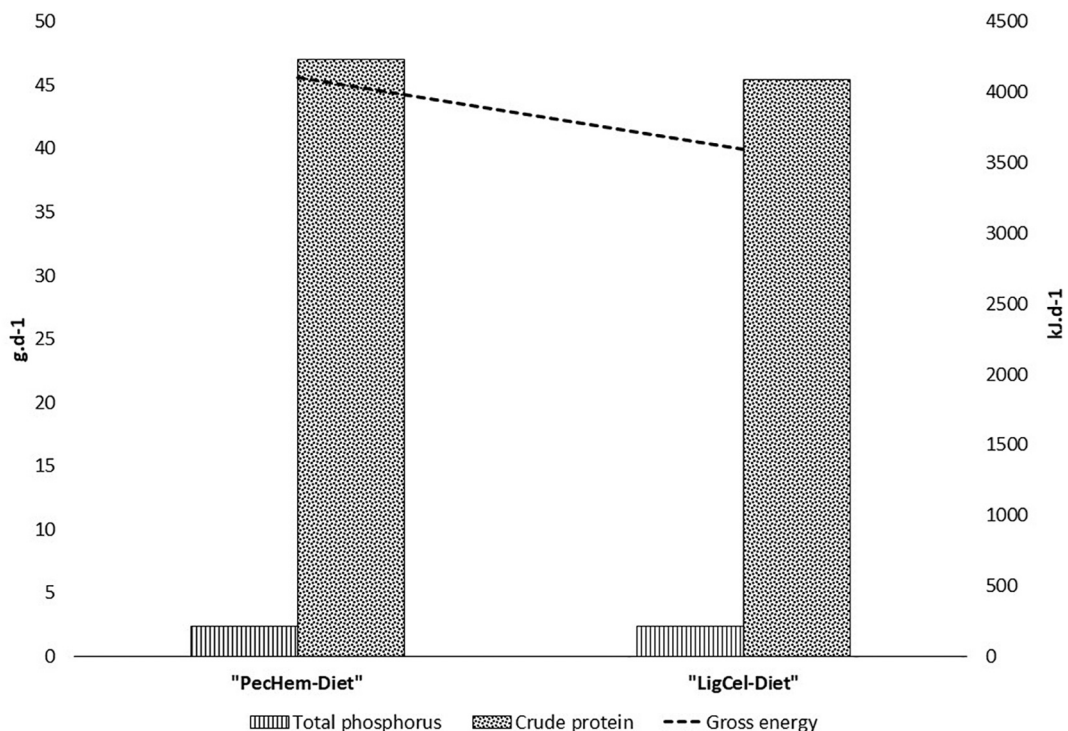


Fig. 2. Daily input of dietary crude protein, gross energy and total phosphorus in each pond. "PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP.

Marc Verdegem: Supervision, conceptualization, methodology, data analysis, writing- reviewing and editing.

Johan Verreth: Supervision, conceptualization, methodology, reviewing and editing.

Michael Phillips: Supervision, conceptualization, methodology, reviewing and editing.

Johan Schrama: Supervision, conceptualization, methodology, data analysis and visualization, writing- reviewing and editing.

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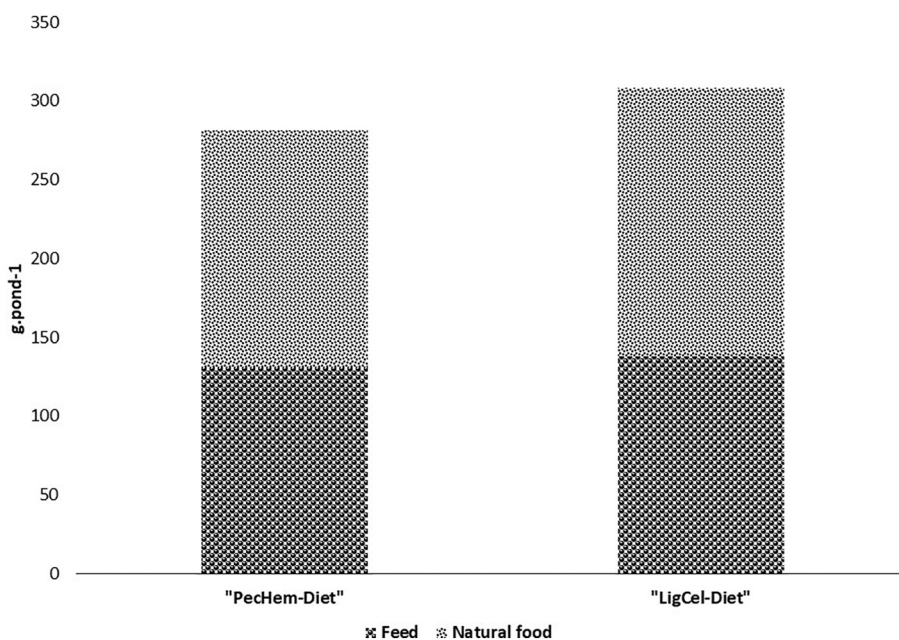


Fig. 3. Effect of types of dietary NSPs on the total N gain in fish over the experimental period originating from feed and from natural food . "PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP.



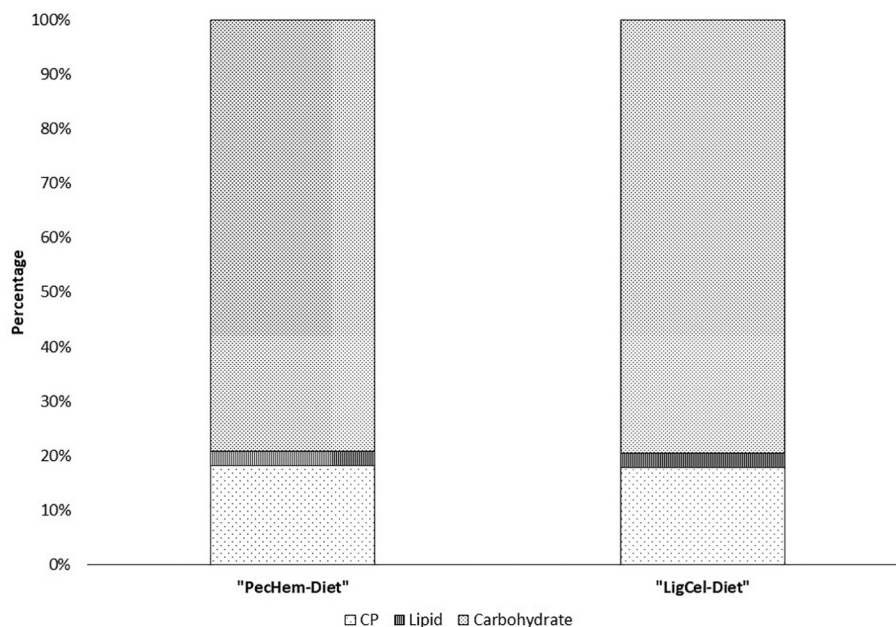


Fig. 4. Effect of types of dietary NSPs on organic matter (OM) composition in faeces. "PecHem-Diet", a diet with quick/easy bio-degradable (fermentable) NSP, "LigCel-Diet", a diet with slow bio-degradable (fermentable) NSP,

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2020.735506>.

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