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Effect of dietary carbohydrate to lipid ratio on performance of Nile tilapia and enhancement of natural food in pond aquaculture

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Abstract

This study tested the effect of two diets differing in carbohydrate to lipid (CHO:LIP) ratio (4.7 vs. 19.5 g/g) on the contribution of natural food and the total fish production in tilapia ponds. Eight ponds, each divided into three equally sized compartments, were assigned to one of the two diets, which differed in CHO:LIP ratio but had the same digestible protein to digestible energy (DP:DE) ratio (15.5 and 15.6 g/MJ). Ponds were fed equal amounts of crude protein. Three feeding levels (no, low and high) were nested in each pond in a split plot design. Average body weight of fish at stocking was 90 g, and the duration of the experiment was 42 days. Increasing the CHO:LIP ratio had no impact on tilapia production. However, the feeding level influenced both biomass gain, specific growth rate and survival. The apparent digestibility coefficient (ADC) for fat and carbohydrate was influenced by dietary CHO:LIP ratio but ADC for energy was unaffected. Proximate analysis of fish body composition showed no effect of diet except for levels of ash. Diet had no effect on the organic matter composition of the faeces, and the contribution of natural food to fish nitrogen gain. Therefore, we postulate that changing the dietary non-protein energy source from lipid to carbohydrate does not have any impact on tilapia culture in semi-intensive ponds.

KEYWORDS
CHO, LIP ratio, natural food, non-protein energy, pond

1 | INTRODUCTION

Fish production costs are increasing while fish price in the market is relatively stable (Rana, Siriwardena, & Hasan, 2009; Tacon, Hasan, & Metian, 2011; Tacon & Metian, 2015). Increased price of fish feed is one of the main reasons for increasing production cost as feed constitute 50%–70% of the operational cost in aquaculture. This creates pressure to develop new strategies to reduce the cost of feeding aquaculture-produced fish by improving efficiency and developing cheaper feeds. Formulating feeds with reduced digestible protein to digestible energy (DP:DE) ratios is a way to reduce feed costs (NRC, 2011), which has been practically demonstrated for tilapia (Kabir et al., 2019a; Kabir et al., 2019b). While reducing the protein to energy ratio, the non-protein energy in the diet increases. Non-protein energy can come either from lipid or carbohydrate. Fish oil and vegetable oil are major lipid sources but are more expensive than carbohydrates. Therefore, reducing lipid levels in the feed and partly replacement by carbohydrates

can make the feed more affordable. However, a minimum inclusion level (the specific amount depends on fish species) of lipid in the diet is required to cover the essential fatty acid (EFA) requirements and improve fat-soluble vitamin intake. For tilapia, limited research has been done on the carbohydrate: lipid (CHO: LIP) ratio of the diet.

Tilapia is an omnivorous fish that can utilize a wide range of plant-based ingredients. Previous studies indicated that a CHO:LIP ratio ranging between 2:1 and 6.5:1 provided best yields for tilapia (Ali & Al-Asgah, 2001; Coutinho et al., 2018; He et al., 2015; Xie et al., 2017). Keeping the DP:DE ratio constant and replacing lipid completely with starch leading to CHO:LIP ratio of 20:1 resulted in poor growth (Xie et al., 2017). This indicates that a minimum inclusion of lipid is required to ensure the presence of EFA in tilapia diets. The dietary CHO:LIP ratio also affects fish body composition. Decreasing the CHO:LIP ratio by increasing lipid content resulted in decreased moisture and crude protein (CP) contents whereas fat and ash contents increased (Ali & Al-Asgah, 2001; Haidar, Bleeker, Heinsbroek, & Schrama, 2018). Changing the CHO:LIP ratio can also have an impact on the apparent digestibility coefficient (ADC) of the nutrients, energy efficiency and waste composition (Amirkolaie, Verreth, & Schrama, 2006; Tran-Tu et al., 2018). Two factors (i.e. ADC of the nutrients, energy efficiency) have a direct impact on fish growth and the third factor (i.e. waste composition) can influence natural food availability in the pond. All the previous studies were carried out in either tanks or cages, with limited availability of natural food. The effect of the CHO:LIP ratio in ponds, where tilapia can resort to natural food, is yet to be evaluated.

Most tilapia production comes from ponds located in tropical and sub-tropical regions of the world (Gupta & Acosta, 2004). In semi-intensive ponds where the culture intensity is not too high (Tacon & De Silva, 1997), tilapia can obtain nutrients from natural food as well as from compound feeds. Manipulation of dietary non-protein energy by alteration of CHO:LIP ratio in diets can also influence faecal characteristics in tilapia which might influence the natural food in the pond (Schneider et al., 2004). Lack of adequate carbon in the faeces might lead to incomplete microbial digestion of the waste and thus less contribution of natural food and more sludge accumulation in the pond bottom. It has been shown that lowering the dietary DP:DE ratio in pond aquaculture enhances natural food availability, which when eaten increase fish production without raising the feed input (Kabir, Schrama, et al., 2019a). However, the impact on fish production and on natural food production when keeping the dietary DP:DE ratio constant and altering the CHO:LIP ratio is not known.

The aim of this study was to test the effect of using diets with different CHO:LIP ratios under three feeding levels on fish production and natural food enhancement in tilapia ponds while keeping the DP:DE ratio the same. The DP:DE ratio in this study was set as recommended for pond diets by Kabir, Schrama, et al. (2019a) to maximize the contribution of natural food to fish growth. The concept will be tested under culture intensity similar as the predominant farming system of Bangladesh, fed and no-aerated ponds—locally termed as semi-intensive farming.

Highlights

1. Replacing lipid with carbohydrate in tilapia diets did not impact fish growth in pond aquaculture
2. Body composition of tilapia was not affected by replacement of dietary lipid with carbohydrate
3. With equal dietary DP:DE (or C:N) ratios, the replacement of dietary lipid by carbohydrate did not affect the contribution of natural food to fish production, nor the composition of natural food in the pond.

2 | METHODS

Two diets, with different carbohydrate to lipid ratios (CHO:LIP; 4.7:1 vs. 19.5:1), were tested on Nile tilapia (*Oreochromis niloticus*) in eight outdoor ponds for 42 days (four replicates per diet). Ponds were divided into three compartments to which three different feeding levels (high, medium and no) were assigned within each diet in a split plot design. In the tables, the feeding levels are expressed as FL0, FL1, FL2 for no, low and high feeding respectively.

2.1 | Diets

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-digestible carbohydrate sources). 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, which is below the recommended level of NRC (1993) and was done to enhance the natural food availability in the pond (Kabir, Schrama, et al., 2019a). An inert marker, yttrium oxide (Y_2O_3), was included in the diets to test 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*) of 14th generation WorldFish GIFT strain were collected from Asha Hatchery, Bagerhat, southern Bangladesh with average weight of 90 g. Eight, 30 m^2, outdoor ponds, in a field experimental station were used for

TABLE 1 Ingredient and analysed chemical composition of the experimental Nile tilapia diets differing in carbohydrate to lipid (CHO:LIP) ratio

	Diets	
	Low CHO:LIP ratio	High CHO:LIP ratio
Ingredients (%)		
Soybean meal	6.5	5
Wheat bran	5	7.5
Wheat	18	25.1
De-oiled rice bran (DORB)	5	7.5
Cassava	17.98	24.98
Rapeseed meal	6.5	5
Soy protein concentrate (fermented)	19	14
Meat and bone meal	6.5	5
Fish meal (CP > 68%)	3	2.5
Fish oil (salmon)	10	1
Vitamin and mineral premix[a]	1	1
Mono calcium phosphate (MCP)	1.2	1.15
DL-methionine	0.3	0.25
Yttrium oxide (Y_2O_3)	0.02	0.02
Analysed chemical composition		
Dry matter (DM) (g/kg)	932	892
Crude Protein (g kg^{-1} DM)	301	273
Fat (g kg^{-1} DM)	111	32
Ash (g kg^{-1} DM)	69	71
Phosphorus (g kg^{-1} DM)	10.7	11.1
Carbohydrate[b] (g kg^{-1} DM)	519	625
Starch (g kg^{-1} DM)	273	347
NSP (g kg^{-1} DM)	240	263
Gross energy (kj g^{-1} DM)	20.8	18.8
DP:DE ratio[c] (g MJ^{-1})	15.5	15.6
CHO:LIP ratio g/g	4.7	19.5
C:N ratio[d] g/g	9.9	10.6

[a]Commercial product manufactured by Vietnam Biomin Company, Ltd.

[b]This is calculated as follows carbohydrate = 1,000 − CP − Fat − Ash.

[c]Calculated based on the apparent digestibility coefficient obtained in this experiment.

[d]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 (Waal & Boersma, 2012).

this experiment. Each pond was divided into three equal compartments as described by Kabir, Schrama, et al. (2019a) by bamboo frames fitted with 1 mm mesh nets allowing mixing of nutrients and dissolved solids within the compartments but preventing pellets and fish from passing between the compartments. At the pond bottom, the compartments were separated by 105 cm high, 8 cm thick concrete walls, where 75 cm was buried below the pond bottom and 30 cm was above the pond bottom to prevent exchange of uneaten feed and benthos between the compartments. All the pond compartments (PC) were aerated to ensure adequate dissolved oxygen levels as well as mixing of dissolved nutrients within and between the compartments.

2.3 | Experimental procedures

Prior to the experiment, ponds were dried and 250 g of $CaCO_3$ was applied to the soil of each PC before water was filled. After the ponds had been filled with water from the reservoir pond, 40 g of dolomite ($CaMg(CO_3)_2$) was spread over the water surface of each compartment. Ten grams of urea (CH_4N_2O) and 20 g of triple super phosphate (TSP), $[Ca(H_2PO_4)_2{\cdot}H_2O]$, per PC (Rakocy & Mcginty, 1989) were applied 1 week after liming. Urea and TSP fertilizers were products of Bangladesh Chemical Industries Corporation, and dolomite was a product of Eon Agro Industries Limited, Bangladesh. Fish were stocked in the ponds 5 days after applying the fertilizer (i.e. 12 days after filling the ponds).

Forty juvenile tilapia (equivalent to four fish per m^{-2}) were stocked in each PC. Fish were fed daily at 8.00 and 16.00 hr according to their metabolic body weight. Within each pond, one of three feeding levels was applied per compartment, high (18 and 20.6 g $kg^{-0.8}$ day^{-1} for low CHO:Lip and high CHO:LIP diet respectively), low (9 and 10.3 g $kg^{-0.8}$ day^{-1} 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 CP 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.

2.3.1 | Water quality monitoring

Dissolved oxygen levels (DO), pH, total dissolved solids (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 hr, by using Lutron dissolved oxygen metre model PDO-519, Hanna instruments pocket tester HI98128-phep5, Lutron conductivity metre model PCD-431, Secchi disc, Hanna digital thermometer model HI98501 and Atago refractometer model MASTER-S28M instruments. NH_4^+, NO_2 and NO_3 were measured at day 1, 21 and 42 by colorimetric, Nessler method, with colour card and sliding comparator: 108025 | Nitrite Test, 111117 | Ammonium Test, 110022 | Nitrite Test; Merck KGaA, Darmstadt, Germany. Total suspended solids of pond water from each compartment was also measured at day 1, 21 and 42 following the procedure of APHA methods # 2540 D (APHA, 1995).

2.3.2 | Sampling and analysing soil and water nutrients

Sample collection, processing and preparation

Samples were collected at day 1, 21 and 42. Soil samples were collected from the top 20 cm layer of the pond bottom at three points in each PC and then mixed homogeneously. Approximately 1 kg of wet soil was collected from each PC, labelled and packed in tight plastic bags and transported to the laboratory. The collected samples were air-dried, crumbled and ground, and then preserved in labelled plastic containers until analysis. Water samples were collected with a depth sampler (10 cm width and 25 cm length) from each pond at the same five soil sampling locations, within 25 cm of the pond surface, transferred and sealed in airtight bottles and preserved at −20°C until analysis.

Analysis of the soil samples

The organic carbon content of the soil was determined by Walkley and Black's wet oxidation method as described by Jackson (1973). Total nitrogen of the soil was determined using a micro-Kjeldahl method following H_2SO_4 acid digestion and alkali distillation procedures as suggested by Jackson (1962). Total phosphorus of the soil was determined colorimetrically by Vanadomolybdophosphoric yellow colour method in nitric acid system (Barton, 1948). The colour intensity was determined using a spectrophotometer at 470 nm wavelength (Jackson, 1958). The available potassium was determined after extraction of the soil samples with 1N NH_4OAc, pH-7.0 solution followed by measurement of extractable K^+ by a flame emission spectrophotometer (Model: Jenway, PEP-7) at 766 nm wavelength using a potassium filter, as outlined by Jackson (1973).

Analysis of the water samples

The organic carbon content of the water was determined by the method described by Tyrine (1965). As there were low levels of organic matter (OM), the samples were dried first. The total inorganic nitrogen concentration was determined by micro-Kjeldahl (Jones, 1991) and alkali distillation procedures as suggested by Jackson (1962). Available phosphorus was determined colorimetrically by molybdophosphoric blue colour method (Murphy & Riley, 1962). The available potassium levels in the water were determined by a flame analyzer at 589 nm wavelength (Jackson, 1967).

2.3.3 | Sampling and analysing plankton

Phytoplankton and zooplankton samples were collected at day 1, 21 and 42. Samples (15 L per sample) were collected from 9.00–11.00 hr from three equally spaced points on a diagonal line in each PC and passed through a 45 μm mesh plankton net, thus 45 L of water was sampled from each compartment.

The filtrates from the samples were preserved in small plastic bottles with 5% buffered formalin. Diversity (group/L) and abundance (ind/L) estimations of plankton were done using a Sedgewick-Rafter (S-R) cell containing 1,000 cells of $1mm^3$making a volume of 1 ml. A 1 ml sample was put in the S-R cell and left undisturbed for 15 min to allow the plankton to settle. The plankton in 10 randomly selected cells were identified (where possible to genus level) and counted using a binocular microscope (LABOMED America.inc; Lx 300). Plankton was identified using keys by Ward and Whipple (1959), Prescott (1962), Belcher and Swale (1976) and Bellinger (1992). Plankton abundance was calculated using the following formula:

$$N = (P \times C \times 100)/V.$$

N is the number of plankton cells or units per litre of pond water; P = the number of plankton counted in 10 fields of the S-R cell; C = the volume of final concentrate of the sample (ml); V = the volume of the pond water sample in litres.

2.3.4 | Sampling and analysing benthos

The benthic macroinvertebrate samples were also collected on day 1, 21 and 42 with an Ekman grab (area: 225 cm^2). In each PC, bottom mud samples were collected from three different locations, which were then combined into a composite sample. Benthic macroinvertebrates were collected after filtering sediments through four different mesh sieves and preserved in a plastic vial containing 10% buffered formalin. Identification keys used for benthic macroinvertebrates were Brinkhurst (1971), and Pinder and Reiss (1983). Benthic macroinvertebrate density was calculated using the formula:

$$N = Y \times 10{,}000/3A,$$

where N = the number of benthic organisms per square metre; Y = total number of benthic organisms counted in 3 samples and A = area of the Ekman dredge.

2.3.5 | Sampling and analysing bacteria

In order to isolate and quantify bacterial communities, samples from both water and soil sediments were collected at day 1, 21 and 42. All samples were collected from three different locations of each PC in sterile containers (15 ml tube, Falcon, USA), and mixed homogenously before being transported back to the Limnological Laboratory of the Environmental Science Discipline of Khulna University, Bangladesh. One ml of each water sample was transferred using a sterile pipette to a test tube containing 9 ml of phosphate-buffered saline (PBS) and the tube was shaken thoroughly, while 5 g of each sediment and water samples were weighed and transferred to a sterile conical flask and made up to 50 ml with PBS and the contents were mixed thoroughly to prepare a stock solution. Serial dilutions of up to 10^{-6} for water and 10^{-8} for sediment were prepared with PBS. Volumes (0.1 ml) of each dilution were spread over the surface of duplicate

plates of tryptone soya agar (TSA; Difco, Detroit, MI, USA) for incubation at 30°C for 24–48 hr. Plates with 30–300 colony forming units (CFU) were counted with a Leica Quebec Dark field Colony Counter (Leica, Inc.) and expressed as CFU/ml.

2.3.6 | Sampling and analysing Chlorophyll a

Water samples from the water column of three parts of the PC were collected, mixed and stored in 500 ml bottles. The samples were transferred to the laboratory within one hour for analysis where 250 ml of water was filtered through Whatman GF/C filter paper. The filter paper was torn into 5–6 pieces and inserted into a 50 ml centrifuge tube. Thereafter, 20 ml of methanol was added to each tube to cover the filter paper pieces, shaken and swirled until the filter paper disintegrated. After storage in a freezer overnight, they were centrifuged at 3,200 rpm (85.86 g-force) for 10 min. The supernatant was poured into a 1 cm cuvette and absorption measured at both 665 and 750 nm (zero with methanol). Chlorophyll a was calculated as Chl-a (μg/L) = ((Abs [665 nm] − Abs [750 nm]) × A × V_m)/V_f × L. Here, A = absorbance coefficient of chlorophyll a in methanol (12.63); V_m = volume of methanol used for extinction (ml); V_f = litres of water filtered; and L = path length of cuvette.

2.3.7 | 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 were euthanized by an overdose of a phenoxy-ethanol solution (1.0 ml/L) and stored at −20°C. Before chemical analysis, the fish were cut into small pieces, homogenized by grinding in a mincing machine twice through a 4.5 mm screen grinder and subsequently oven-dried. Chemical analyses were carried out in triplicate. Dry matter (DM) was determined gravimetrically after drying at 103°C for 4, and 24 hr until constant weight, respectively, for feed and fish samples (ISO 6496, 1983). Crude ash was determined after incineration at 550°C for 4 hr (ISO 5984, 1978). CP was determined by the Kjeldahl method (ISO 5983, 1979) and calculated by multiplying the measured N content by 6.25. Fat was quantified by petroleum–diethyl ether extraction (ISO 6492, 1999). Before fat analysis, feed samples were hydrolysed by boiling for 1 hr with 3 M HCl. Dietary energy content was measured by direct combustion in an adiabatic bomb calorimeter (IKA-C-7000; IKA analysentechnik). Starch including free sugars was enzymatically determined in feed and faecal samples by using amyloglucosidase without the ethanol extraction step and measuring glucose content as described by Goelema, Spreeuwenberg, Hof, Poel, and Tamminga (1998). Non-starch polysaccharides (NSP) content was calculated as total carbohydrates—'Starch + free sugars'. Yttrium oxide (Y_2O_3), phosphorus (P) and calcium (Ca) in feed and faeces were analysed using inductively coupled plasma mass spectrometry (ICP-OES) according to the standard method, NEN 15510 (2007).

2.3.8 | Analysis of stomach contents

For stomach content analysis, fish were harvested from the outdoor pond experiment on day 43, 19 hr after the last feeding, to ensure that no pellets remained in the stomach. The fish were euthanized by an overdose of a phenoxy-ethanol solution (1.0 ml/L) and transported to the laboratory. In the laboratory, the fish were dissected to remove the stomachs which were preserved in 10% formalin. The total volume of the stomach and the number of food items were recorded. The volume of food items occupying the stomach in general and by each food group was visually estimated (Jude, 1973). The total weight of food was expressed as a percentage of weight of the stomach on a wet weight basis (Gibbons & Gee, 1972). The index of relative importance (IRI) of observed natural food groups was estimated by diet to understand the relative importance of each natural food group in the growth of the fish following the methods described by Pinkas, Oliphant, and Iverson (1971) and Prince (1975). IRI = (%G_n + %G_v) × %G_f; where, G_n is the percentage by group number, G_v is the volume of the group number and G_f is the frequency of occurrence by the group number.

2.3.9 | Faeces collection and preservation

Short indoor trial was conducted to determine ADC. For this purpose, at the end of the pond experiment, 180 tilapia of mean body weight 161 (±31) g were harvested from the fed compartments of the experimental ponds and restocked in the indoor concrete tanks. Ten fish were allocated in each of 18 tanks of 1,000 litre water holding capacity, filled with 700 L of water. All tanks were aerated. Both experimental diets were fed at 6, 9 and 12 g $kg^{-0.8}$ day^{-1} with three replicates per treatment. Fish collected from low CHO:LIP and high CHO:LIP diet-fed ponds respectively were assigned to receive the same diet in the tanks. Fish were fed daily at 7.00 and 15.00 hr. For the first seven days, fish were fed in the tank for acclimation to the tank environment. Starting from day 8, faeces were collected by siphoning 3 hr after each feeding for a total period of 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.

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. Specific growth rates

(SGR) were calculated as $SGR = ((\ln(IndBW_{42}) - \ln(IndBW_0))/42) \times 100$; where $IndBW_{42}$ and $IndBW_0$ were individual body weight at day 42 and day 0. Growth (g/d) was calculated as individual gain (g) divided by the duration of the experiment (d). Feed conversion ratio (FCR) was calculated per compartment using the feed given and weight gain. The survival of fish per compartment was calculated as $(N_f/N_i) \times 100$, where N_f is the final number of fish and N_i the initial number at PC level.

3.2 | ADC calculation

The ADC of nutrients was measured for each tank using yttrium oxide (Y_2O_3) as an inert marker. ADC of CP, crude fat, energy and carbohydrate in the diets was calculated by using the following formula:

$$\%ADC_{diet} = 100\% * \left(1 - \left[Y_{diet}/Y_{faeces}\right] * \left[N_{faeces}/N_{diet}\right]\right)$$

Here, Y_{diet} and Y_{faeces} are the content of the inert marker (Y_2O_3) in the diet and faeces, respectively (g kg^{-1} DM); and N_{faeces} and N_{diet} are the contents of the dietary components in the faeces and diets respectively (g kg^{-1} DM).

3.3 | Fish N gain

Nitrogen (N) gain in fish was calculated by the difference between the N_h and N_s. Here, N_h = amount of N in the harvested fish biomass and N_s = 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 of 40% (Azevedo, Leeson, Cho, & Bureau, 2004) for both 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 | 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). The effect of diet was tested between ponds while the effect of feeding level and diet and feeding level interaction were tested between compartments within the pond. Univariate analysis was carried out to determine 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 using univariate analysis by the procedure GLM. When a significant interaction effect was found, multiple comparisons of means using Tukey's multiple range test were performed.

4 | RESULTS

The average individual body weight (BW) at stocking was 91 (±5) g and did not vary by diet and feeding level. There were no effects of the dietary CHO:LIP ratio and the interaction of diet and feeding level on the measured indicators for fish performance (Table 2). Biomass harvested, biomass gain, individual gain, survival, SGR and daily growth rate increased with increasing feeding levels (all $p < .001$ significance level except for survival $p < .05$). The average overall FCR for fed compartments was 1.7 and was unaffected by feeding levels ($p < .10$).

The ADC for fat and carbohydrate was affected by the dietary CHO:LIP ratio. ADC for fat was higher (93% vs. 77%) with the low CHO:LIP diet (4.7 vs. 19.5 g/g; $p < .001$) while ADC for carbohydrate

TABLE 2 Effect of dietary carbohydrate to lipid ratio and feeding level on performance of tilapia

		Low CHO:LIP diet			High CHO:LIP diet				*p*-values		
Variables	Units	FL0	FL1	FL2	FL0	FL1	FL2	Pooled *SEM*	D	FL	D*FL
Initial individual BW	g	90	88	89	91	94	92	3	ns	ns	ns
Biomass stocked	g/comp	3,586	3,534	3,544	3,642	3,756	3,692	108	ns	ns	ns
Biomass harvested	g/comp	4,329	5,455	7,341	4,062	5,493	7,767	278	ns	***	ns
Individual BW Gain	g	44	67	100	40	63	110	9	ns	***	ns
Biomass gain	g/comp	743	1921	3,798	420	1737	4,075	232	ns	***	ns
Survival	%	82	88	98	81	88	96	6	ns	*	ns
FCR	g/g		1.54	1.77		1.81	1.79	0.19	ns	ns	ns
SGR	$\%.d^{-1}$	0.9	1.3	1.8	0.8	1.2	1.9	0.1	ns	***	ns
Growth Rate	g/d	1.0	1.6	2.4	0.9	1.5	2.6	0.2	ns	***	ns

Abbreviations: BW, body weight; CHO:LIP ratio, dietary carbohydrate to lipid ratio; Comp, compartment; D*FL, diet and feeding level interactions; d, day; D, diet; FCR, feed conversion ratio; FL, feeding level; FL0, no feeding; FL1, low feeding; FL2, high feeding; SGR, specific growth rate.

P-values: ns (not significant, $p > .1$), * ($p < .05$), *** ($p < .001$).

was higher (68% vs. 60%) with the high CHO:LIP diet (19.5 vs. 4.7 g/g; $p < .05$). There was no effect of feeding level and the interaction of diet and feeding level on the ADC for any of the nutrients (Table 3).

The dietary CHO:LIP ratio affected only the ash content (50 vs. 46g/kg; $p < .05$) in the final fish body composition which increased at the lower CHO:LIP ratio (19.5 vs. 4.7 g/g). DM, protein and fat content increased with increasing feeding level ($p < .001$), but there was no interaction effect on final fish body composition (Table 4).

Irrespective of diet, potassium in both soil and the water column of the ponds were depleted during the experiment. OM in the pond soil and total inorganic nitrogen in the pond water column for half of the treatments were also depleted during the experiment (Table 5). The negative accumulation of OM (or carbon) and nitrogen from the pond water and soil reduced with increasing feeding level ($p < .05$). Over the duration of the experiment, all nutrients in the pond environment showed cyclical variations (Figure S1) in relation to sampling time points ($p < .001$).

The dietary CHO:LIP ratio influenced phytoplankton diversity in the ponds, which was higher ($p < .05$) with the low CHO:LIP diet. The interaction of diet and feeding level influenced the abundance of zooplankton. With the low CHO:LIP diet, it decreased ($p < .05$) with increasing feeding level. For the high CHO:LIP diet, the opposite happened (Table 6). Except for these two components, there was no effect of diet, feeding level and their interactions on the measured parameters of natural food in the pond (Table 6). Over the duration of the experiment, except for benthos diversity, all measured components of natural food increased over time (Table S1).

There was no effect of diet on the presence of natural food in the stomach of tilapia. Both the volume of natural food compared with the volume of the fish stomach, and the weight of natural food compared with the weight of the stomach content decreased at increased feeding levels ($p < .05$). Also, there was no interaction effect of diet and feeding level on the measured parameters (Table 7). The Index of relevance importance (IRI) is a measure of the dominant natural food groups consumed by the fish. IRI from the stomach content observations for both the diets was the same. The IRI of food groups in the fish stomach was zooplankton, phytoplankton, crustaceans and molluscs respectively. All the measured physical parameters of pond water were unaffected by the dietary CHO:LIP ratio and were within the optimum levels for pond aquaculture (Table 8).

5 | DISCUSSION

We investigated the effect of dietary non-protein energy (i.e. CHO:LIP ratio) on performance of tilapia in pond aquaculture, as well as its role in enhancing natural food in the pond as an indirect means of contributing to fish growth. Changing the dietary CHO:LIP ratio from 4.7 to 19.5 g/g by increasing the carbohydrate level did not affect fish growth. Also, at a fixed DP:DE (or C:N) ratio, the change in dietary energy source seemed to have had no impact on fish growth rates from natural food.

In the current study, the difference in CHO:LIP ratio in diets did not affect the tilapia performance. Very few studies have been

TABLE 3 Effect of dietary carbohydrate to lipid ratio and feeding level on apparent digestibility coefficient (ADC) of tilapia

	Units	Low CHO:LIP diet	High CHO:LIP diet	Pooled *SEM*	*p*-values		
					D	FL	D*FL
Crude protein	%	75	73	2.4	ns	ns	ns
Crude fat	%	93	77	3.1	***	ns	ns
Energy	%	70	67	33	ns	ns	ns
Carbohydrate	%	60	68	3.6	*	ns	ns
Organic matter (OM)	%	68	69	2.0	ns	ns	ns

Abbreviations: D, diet; FL, feeding level; DFL, diet and feeding level interactions.
p-values: ns (not significant, $p > .1$), * ($p < .05$), *** ($p < .001$).

TABLE 4 Effect of dietary carbohydrate to lipid (CHO:LIP) ratio and feeding level on body composition of tilapia

	Units	Low CHO:LIP diet			High CHO:LIP diet			Pooled *SEM*	*p*-values		
		FL0	FL1	FL2	FL0	FL1	FL2		D	FL	D*FL
DM	g/kg	261	274	287	260	262	280	4	ns	***	ns
Protein	g/kg	150	155	160	149	151	155	2	ns	***	ns
Fat	g/kg	45	53	53	48	49	52	2	ns	***	ns
Ash	g/kg	44	52	55	45	46	47	2	*	*	ns

Abbreviations: CHO:LIP, dietary carbohydrate to lipid ratio; D, diet; FL0, no feeding; FL1, low feeding; FL2, high feeding; FL, feeding level; D*FL, diet and feeding level interactions.
P-values: ns (not significant, $p > .1$), * ($p < .05$), *** ($p < .001$).

TABLE 5 Effect of dietary carbohydrate to lipid ratio and feeding level on accumulation of soil and water nutrients

		Low CHO:LIP diet			High CHO:LIP diet				*p*-values		
	Units	FL0	FL1	FL2	FL0	FL1	FL2	Pooled *SEM*	D	FL	D*FL
Phosphorus in soil	mg/L	78	141	92	10	29	63	268	ns	ns	ns
Phosphorus in water	mg/L	3	4	3	6	3	2	2	ns	ns	ns
Organic matter in soil	mg/L	−983	83	11	433	−417	−83	504	ns	ns	ns
Organic matter in water	mg/L	−34	−5	11	−110	−61	−83	59	ns	*	ns
Nitrogen in soil	mg/L	−58	12	23	23	0	35	34	ns	ns	ns
Nitrogen in water	mg/L	−2	0	1	−6	−3	−4	3	ns	*	ns
Potassium in soil	mg/L	−48	−96	−32	−159	−144	−128	49	ns	ns	ns
Potassium in water	mg/L	−8	−6	−8	−10	−10	−8	4	ns	ns	ns

Abbreviations: CHO:LIP, dietary carbohydrate to lipid ratio; D, diet; FL0, no feeding; FL1, low feeding; FL2, high feeding; FL, feeding level; D*FL, diet and feeding level interactions.

P-values: ns (not significant, $p > .1$), *$p < .05$).

TABLE 6 Effect of dietary carbohydrate to lipid ratio and feeding level on the mean (average of three sampling times) natural food of the pond (by compartment)

		Low CHO:LIP diet			High CHO:LIP diet				*p*-values		
	Units	FL0	FL1	FL2	FL0	FL1	FL2	Pooled *SEM*	D	FL	D*FL
Chlorophyll a	µg/L	0.011	0.008	0.01	0.007	0.01	0.01	0.003	ns	ns	ns
Phytoplankton abundance	Ind/L	22,125	21,806	21,319	19,875	16,861	25,333	2,443	ns	ns	ns
Phytoplankton diversity	genus/L	8.1	8.4	8.9	7.2	6.8	8.1	0.66	*	ns	ns
Zooplankton abundance	ind/L	8,958	8,875	7,083	7,875	9,000	10,375	799	ns	ns	*
Zooplankton diversity	genus/L	5.167	5.278	4.833	4.778	5.056	5.278	0.40	ns	ns	ns
Benthos abundance	ind/m^2	7,742	8,533	6,617	10,075	10,383	7,867	2,907	ns	ns	ns
Benthos diversity	group/m^2	3	3	2	3	3	3	0.26	ns	ns	ns
Water bacteria	CFU/ml	2,804	2,588	2,656	2,652	2,799	3,106	237	ns	ns	ns
Soil bacteria	CFU/ml	2,283	2,463	2,368	2,394	2,492	2,481	115	ns	ns	ns

Abbreviations: CHO:LIP, dietary carbohydrate to lipid ratio; FL0, no feeding; FL1, low feeding; FL2, high feeding; D, diet; FL, feeding level; D*FL, diet and feeding level interactions.

p-values: ns (not significant, $p > .1$), * ($p < .05$).

conducted with a specific focus on dietary CHO:LIP ratio on tilapia. Out of those studies on the performance of tilapia in response to different levels of dietary carbohydrate and lipid report contradictory results. Increasing lipid as energy source at a fixed DP:DE ratio increased fish performance in some studies (Haidar et al., 2018; Saravanan et al., 2012), while the opposite has been observed also, for example, by Amirkolaie et al. (2006) and Tran-Duy, Smit, van Dam, and Schrama, (2008). In the above studies, all tested diets were within the CHO:LIP ratio between 1.5 and 7.0 g/g. Xie et al. (2017) tested an extreme diet with a CHO:LIP ratio of 20, entirely excluding lipid and noticed very poor performance. So, in addition to diet composition what else is impacting fish growth?

In the studies of Haidar et al. (2018) and Saravanan et al. (2012), fish were fed to satiation, while Amirkolaie et al. (2006) and Tran-Duy et al. (2008) restricted feeding. This means that fish performance showed a similar response when the feeding level was similar and comparable in dietary nutrient compositions. The extreme CHO:LIP ratio (i.e. 19.5 g/g) in the current study did not affect fish performance, which is contradictory to the findings of Xie et al. (2017). This may be due to two factors:

1. Xie et al. (2017) completely eliminated oil from the diet. The overall lipid content was only 27 g per kg of feed. Perhaps, a shortage of dietary EFA has impacted the growth performance with the high CHO:LIP diet in this study (Xie et al., 2017). In

TABLE 7 Effect of dietary carbohydrate to lipid ratio and feeding level on the natural food observed in the stomach content of tilapia

		Low CHO:LIP diet			High CHO:LIP diet				*p*-values		
	Units	FL0	FL1	FL2	FL0	FL1	FL2	Pooled *SEM*	D	FL	D*FL
Volumetric occurrence of natural food	%	34	34	24	34	32	18	4.3	ns	*	ns
Gravimetric occurrence of natural food	%	59	64	42	61	48	35	6.7	ns	*	ns

Abbreviations: CHO:LIP, dietary carbohydrate to lipid ratio; FL0, no feeding; FL1, low feeding; FL2, high feeding; D, diet; FL, feeding level; D*FL = diet and feeding level interactions.

p-values: ns (not significant, $p > .1$), * ($p < .05$).

TABLE 8 Effect of dietary carbohydrate to lipid ratio and feeding level on pond water quality

	Units	Low CHO:LIP diet	High CHO:LIP diet	Pooled *SEM*	*p*-values for diet
Dissolved oxygen (DO)	mg/L	3.4	3.5	0.1	ns
Temp	°C	33	34	0.1	ns
pH	–	8	8	8.0	ns
Transparency	Inch	9	9	0.1	ns
Water depth	Inch	35	34	2.0	ns
Salinity	ppt	3	3	0.3	ns
TSS	mg/L	286	276	14.6	ns
NO_2	mg/L	0.011	0.012	0.004	ns
NH_4	mg/L	0.19	0.17	0.053	ns

Abbreviation: CHO:LIP, dietary carbohydrate to lipid ratio; TSS, total suspended solids.

p value: ns (not significant, $p > .1$).

contrast, the high CHO:LIP diet in the current study still contained 10 g of fish oil and overall 32 g of lipid per kg feed.

2. Husbandry conditions might have impacted the outcome as well. The study of Xie et al. (2017) was in flow-through tanks, with a daily water exchange of 50% of the tank volume and the fish had no access to natural food. The current study was conducted in outdoor ponds where fish had access to natural food in addition to the formulated feed which can stimulate fish performance (Porchas-Cornejo, Martínez-Porchas, Luis, Ramos-Trujillo, & Barraza, 2012; Pucher & Focken, 2017; Rahman, Nagelkerke, Verdegem, Wahab, & Verreth, 2008; Roy, Allen Davis, & Whitis, 2012). Algae can be a potential source of EFA for tilapia (Mizambwa, 2017; Patil, Källqvist, Olsen, Vogt, & Gislerød, 2007; Teuling, Schrama, Gruppen, & Wierenga, 2017). Based on the stomach content analysis, phytoplankton was the second most important natural food in the diet of tilapia, and thus most likely gave tilapia access to additional EFA. Moreover, the utilization efficiency of a sub-optimal supplementary diet can be influenced by the amount and composition of natural food present in the pond (Tacon & De Silva, 1997). In addition to the nutritional factors, the stocking density in the current study was mimicked with the non-aerated fed ponds, the predominant tilapia farming in Bangladesh, which was lower than that of Xie et al. (2017). Difference in the culture intensity might have impact on the outcome as well.

Therefore, in addition to the dietary CHO:LIP ratio, feeding level and husbandry conditions (i.e. pond or tank due to presence of natural food) may determine fish performance.

The different feed ingredients which provide the dietary macronutrients in fish feed influence the ADC of the nutrients and thus ultimately also fish performance (Teuling et al., 2017; Tran-Ngoc et al., 2017). In the current study, increasing the CHO:LIP ratio decreased the ADC of lipid and increased the ADC of carbohydrate. Fish oil was the main lipid ingredient source in the low CHO:LIP diet (Table 1), which is more digestible compared with plant-based lipid. The observed difference in carbohydrate ADC between both diets is most likely related to the changes in carbohydrate composition. In the high CHO:LIP diet, the starch content was higher by 74 g/kg (347 vs. 273 g/kg) and the NSP content only by 23 g/kg (263 vs. 240 g/kg). Compared with starch, NSP is poorly digestible in Nile tilapia (Amirkolaie et al., 2006; Haidar, Petie, Heinsbroek, Verreth, & Schrama, 2016). This might explain the higher carbohydrate ADC when using the high CHO:LIP diet. The higher fat ADC with low CHO:LIP diet and the higher carbohydrate ADC with high CHO:LIP diet might have resulted into an equal energy ADC for both diets, contributing to the fact that no difference in fish growth was observed. However, comparing both experimental diets, the composition of the digestible energy coming from fats and carbohydrates differed strongly between both experimental diets. It has been shown recently in tilapia that digested fat is

used more efficiently than digested starch (Schrama, Haidar, Geurden, Heinsbroek, & Kaushik, 2018). However, in the current study this was not reflected in growth differences, which also might be due to differences in husbandry conditions between experiments. The calculated N gain at pond level in the current study indicated that about 40% of growth was from the natural food of the pond (Figure 1), which may have masked the effects of differences in digestible energy composition. Numerically with low CHO:LIP diet, the contribution of feed is higher (not statistically significant) compared with the high CHO:LIP diet which has been levelled by increased natural food, indicating that the minor (not statistically significant) growth difference due to difference in ADC was probably compensated by the natural food of the pond (Figure 2).

In pond aquaculture, natural food contributes between 40% and 65% of total fish growth (Anderson, Parker, & Lawrence, 1987; Burford, Preston, Glibert, & Dennison, 2002; Burford et al., 2004; Cam & Mariotti, 1991; Porchas-Cornejo et al., 2012) depending on the culture intensity amount of feed supplied. The level of contribution of natural food depends on the enhancement effect of the dietary nutrient inputs. Increasing the dietary C:N ratio to ~15:1, either by addition of carbohydrate in addition to a conventional feed (Asaduzzaman et al., 2010; Avnimelech, 1999) or by lowering the dietary protein to energy ratio (Kabir, Schrama, et al., 2019a), can greatly increase this natural food contribution. In this study dietary, the C:N ratio was ~10:1 and hence the contribution of natural food (i.e. ~52%) is comparable with the lower range of observations in previous studies (Kabir, Schrama, et al., 2019a). As the dietary C:N ratios were comparable (9.9:1 vs. 10.6:1), the contribution of natural food to fish growth remained the same between the two diets, so the opportunity for natural food enhancement through increasing the dietary C:N ratio was missed. Mo et al. (2014) demonstrated that difference in CHO:LIP ratio influences the planktonic food web in aquaculture ponds due to higher inclusion of cereals (i.e. rice bran, rice grain, soybean meal and spaghetti), which might have containing high level of NSPs and have likely impact on ADC and OM composition as well. Unlike the ingredient composition (Mo et al., 2014) in the current study, the cereals were same with slight difference in their inclusion level. The contrast in CHO:LIP ratio was created by additional inclusion of fish oil (10% vs. 1%) not by changing cereals which might have minimized the effect of CHO:LIP ratio on planktonic community in the pond. Though, in this study the composition of C was different between the two feeds (Table 1), which could impact the composition of OM in the faeces and thus the availability of nutrients in the pond for enhancement of natural food. In the current study, the ADC of OM was unaffected by the dietary CHO:LIP ratio (Table 3). This could be because the difference in the carbohydrate content is mainly for starch which is highly digestible and the NSPs level was comparable (Table 1). Moreover, the macronutrient composition in the OM of the faeces (as measured for the ADC determination) did not change between the diets (Figure 3). In next paragraph, we will explore what has been reported about the impact of CHO:LIP ratio on the composition of OM in the faeces.

We calculated the macronutrient concentrations in the OM of the faeces in relation to different dietary CHO:LIP ratios in tilapia in previous studies (Schrama et al., 2012; Teuling et al., 2017; Trần Ngọc, 2017) which were comparable with the current study (Figure 3). In addition to the CHO:LIP ratio, the composition of carbohydrate (starch vs. NSP) can alter the ADC as well as the faeces composition (Haidar et al., 2016). In this study, NSP content of the diets was comparable (240.2 vs. 262.5 g/kg). So, this did not impact the faeces composition.

As the nutrient inputs through the diets were similar, the ADC of CP and energy was similar and the nutrients in the OM of the faeces entering in the pond were also similar, we did not notice differences

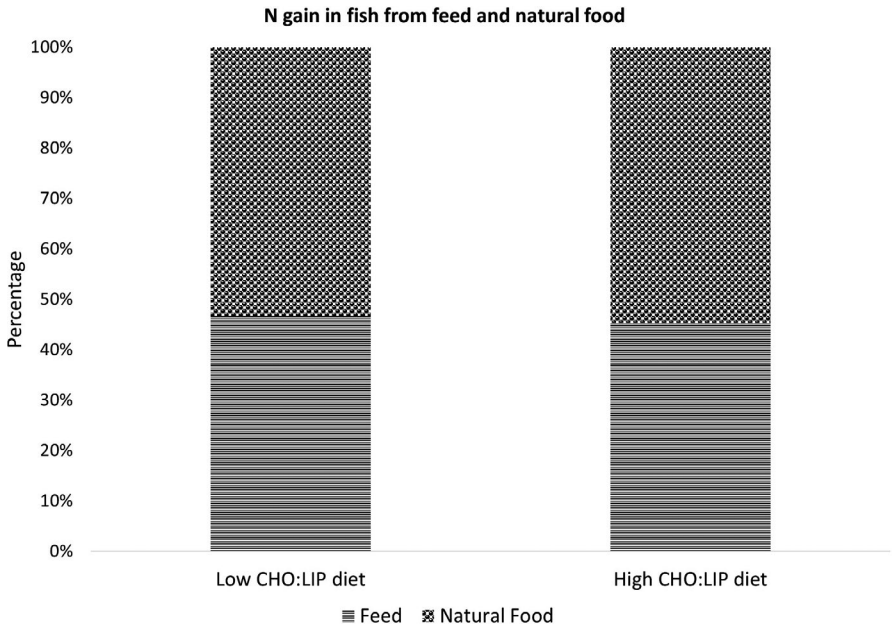

FIGURE 1 Effect of dietary CHO:LIP ratio on the relative contribution of feed and natural food to N gain in fish

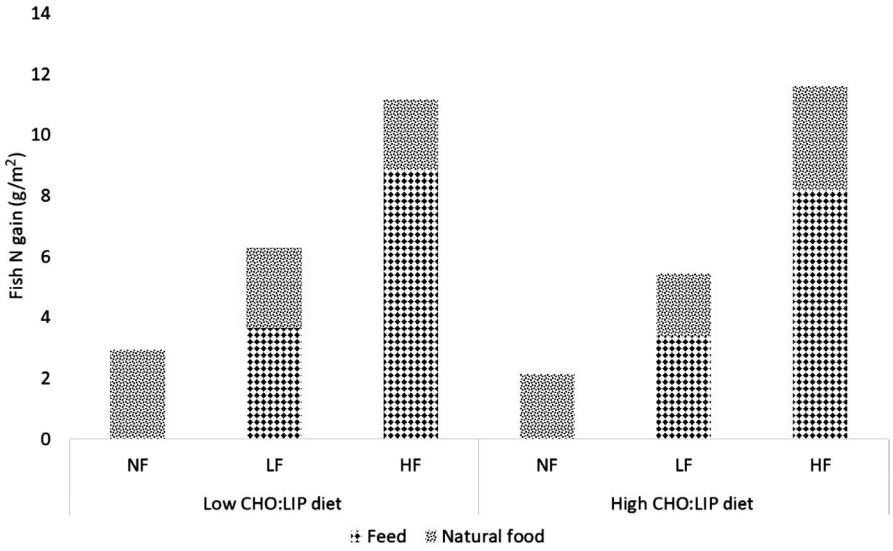

FIGURE 2 Effect of dietary CHO:LIP ratio and feeding level on the contribution of feed and natural food to N gain in fish

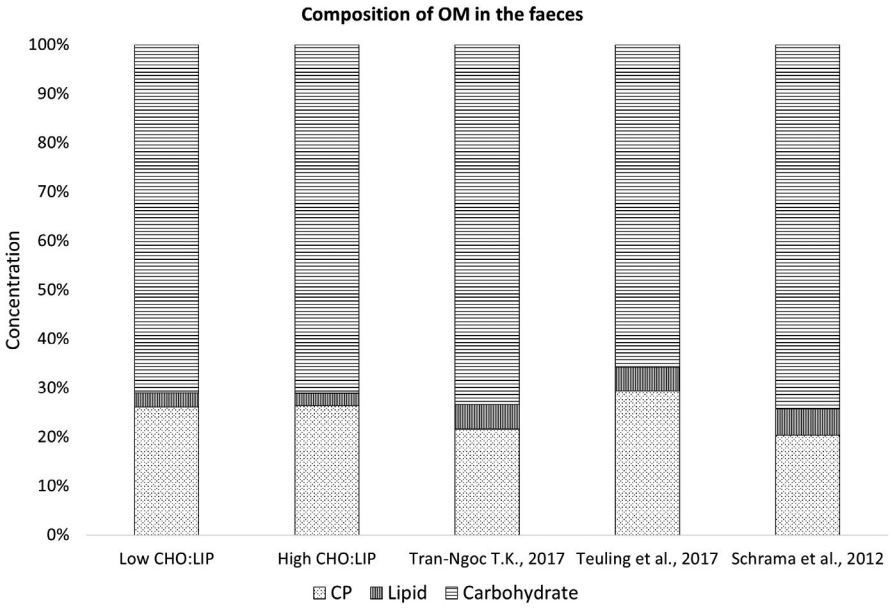

FIGURE 3 The concentration of macronutrients in the composition of organic matter (OM) of the faeces. 'Low' and 'high' CHO:LIP are from the current study; the other three are calculated values from the referred studies. For the referred studies, OM composition of faeces was based on the calculated mean value of all the experimental diets in these studies, where different sources of dietary non-protein energy were tested. CP = crude protein

in the growth of the fish (Table 2) and in the enhancement of natural food contribution to tilapia production (Figure 1).

6 | CONCLUSION

Changing the CHO:LIP ratio within the tested range (4.7 vs. 19.5 g/g) did not affect the production of tilapia in pond culture fed diets with similar, low DP:DE ratios. This study suggests that lipid can be replaced by carbohydrate as a source of non-protein energy in tilapia pond culture without compromising growth performance. This finding could reduce tilapia feed costs in pond aquaculture as carbohydrates are usually cheaper than lipids.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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