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Effect of dietary protein to energy ratio, stocking density and feeding level on performance of Nile tilapia in pond aquaculture

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ABSTRACT

There is growing interest to understand the dietary P:E requirements for the supplemental feed used in tilapia pond culture where natural food contributes to production. In an on-farm trial, we tested the effect of lowering dietary P:E ratio on fish performance, pond nutrient utilization and economic benefit under two stocking densities and feeding levels. Forty ponds, (average size 234 ± 112 m^2), were assigned to test the effect of two diets, which differed in P:E ratio (18 vs 14 $g.MJ^{-1}$), two feeding levels (14 vs 18 $g.kg^{-0.8}.d^{-1}$) and two stocking densities (2 vs 3 $fish.m^{-2}$). Initial fish biomass was 45(± 21) vs 67(± 38) $g.m^{-2}$ at 2 vs 3 $fish.m^{-2}$, respectively. The experiment lasted 82 days. Decreasing P:E ratio enhanced tilapia production ($P < 0.05$; 459 vs 399 $g.m^{-2}$). Increasing stocking density of tilapia from 2 to 3 m^{-2} increased biomass gain 43% ($P < 0.001$; 354 vs 505 $g.m^{-2}$). Averaged over both diets and stocking densities, growth and feed conversion ratio increased with increasing feeding level ($P < 0.001$). Fish survival was unaffected by diet, stocking density and feeding level. Dissolved oxygen increased with increased stocking density with low P:E diet. The opposite happened for high P:E diet ($P < 0.05$). Increasing the feeding level also increased the DO concentration ($P < 0.001$). N retention efficiency was higher with the low P:E ratio diet ($P < 0.001$; 71 vs 52%) and decreased with increasing feeding level ($P < 0.001$). The data on N gain and N balance at the pond level suggest that the food web productivity was stimulated by reducing the dietary P:E ratio. The low P:E diet increased the gross margin by 95% ($P < 0.001$; 2076 vs 1067 $USD.ha^{-1}$) and benefit cost ratio by 22% ($P < 0.05$; 1.57 vs 1.29). The P:E ratio of the low P:E diet is less than the presently advised. Lowering the P:E ratio from 18 to 14 $g.MJ^{-1}$ in pond feeds for tilapia will increase the economic viability of pond aquaculture.

1. Introduction

In terms of production volume, tilapia is the second largest farmed fish group after carp, showing a fast growth in this sector, particularly in the last decade(FAO, 2018). It is grown across all the tropics, in > 100 countries, at different culture intensities and in diverse production systems (Wang and Lu, 2016). In Southeast Asia, where the majority of Nile tilapia is produced, it is mainly farmed in extensive to semi intensive ponds. The farm gate price of tilapia in Southeast Asia is low. Therefore, the economic viability of farming tilapia is challenged in many countries. The largest expenditure for tilapia farming is feed, constituting ~70% of total operating cost (Yuan et al., 2017; Ahmed, 2007). Therefore, making feed affordable and increasing efficiency of feed utilization can help ensure good economic benefits for the producers.

Feed cost depends largely on the crude protein content in the diet. Most of the commercial diets comply with the NRC (1993 and 2011) recommendation to have a dietary digestible protein to digestible energy (DP:DE) ratio of 18–23 $g.MJ^{-1}$. This NRC recommendation is based on studies done in tanks in absence of natural food. However, in ponds where additional feeding is applied, natural foods can still contribute up to 40–68% to the production (Anderson et al., 1987; Burford et al., 2002; Burford et al., 2004; Cam and Mariotti, 1991; Porchas-Cornejo et al., 2012). This contribution can be enhanced by increasing the C:N ratio of nutrient inputs (Asaduzzaman et al., 2010). Natural food availability depends on a well-functioning food web. Bacteria can mineralize waste and prevent ammonia accumulation form the base of the food web. To mineralize all the waste, bacteria need energy. The energy in pond aquaculture is often provided by administration of carbohydrates to raise the C:N ratio to 15–20 (Asaduzzaman et al.,

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2008; Avnimelech and Kochba, 2009; Crab et al., 2007). By increasing the carbon or energy availability in the pond, production can be increased. When the C:N ratio of the nutrient input raises above 10, heterotrophic bacteria become dominant (Boyd, 1996; Lancelot and Billen, 1985), contributing substantial amounts of bacterial biomass to the food web. Organic, but also inorganic nitrogen are taken up by heterotrophic bacteria, thus keeping ammonia and nitrite levels in the pond low (Avnimelech, 1999; Hari et al., 2004, 2006)). Heterotrophic bacteria, are a protein source, stimulating the food web and the production of fish grazing on natural foods (Asaduzzaman et al., 2008).

Kabir et al. (2019) demonstrated that the tilapia yield in semi-intensive culture system was better with a diet of P:E ratio 14 g.MJ^{-1} compared to a diet of P:E ratio 18 g.MJ^{-1}, while realizing FCR 0.88 vs 1.02 respectively. Application of this concept could substantially reduce the cost of feed and thus total production cost, allowing to increase economic profitability and long-term sustainability of tilapia culture in ponds.

However, the study of Kabir et al. (2019) was done in uniform experimental ponds. It remains to be verified if the same effects will also be obtained under less uniform rearing conditions typical for farmer ponds. The underlying mechanism of the better fish performance with low P:E diet was due to increased intake of natural food. This higher natural food intake is related to their higher prevalence in ponds, steered by the low P:E (or high C:N) ratio diet. However, enhancement of natural food in pond also depends on the quality of pond soil and water, and the availability of sunlight to stimulate the autotrophic food web. In addition, increased culture intensity (stocking density) and input of supplemental feed is believed to reduce the relative contribution of natural food to fish production. All these factors may vary among different farmer ponds.

Therefore, the current study was planned to test the effect of lowering dietary P:E ratio in an on farm trial with two feeding levels and two stocking densities. The diets were the same as in Kabir et al. (2019). The high P:E diet (C:N ratio 8) was comparable to a diet similar in P:E ratio of a standard commercial tilapia diet (NRC, 1993, 2011) and the low P:E diet (C:N ratio 11) was in the direction to the recommended P:E ratio for pond aquaculture by Asaduzzaman et al., 2008, 2010), and Hari et al. (2004, 2006). The feeding levels were comparable with a commercial feeding schedule. The high stocking density was selected considering the reported carrying capacity for tilapia in non-aerated ponds, 5800 kg/ha (Xu et al., 2011). Effect of diet, stocking density and feeding level on fish production; nitrogen retention; accumulation of nutrients in pond water and soil, and economic return were assessed.

We hypothesised that:

1. With low P:E diet, more energy (carbon) will be available to enhance natural food in the pond;
2. Enhanced natural food will compensate for lowering the dietary P:E ratio for fish performance;
3. Increasing stocking density, will contribute to increased fish production; and.
4. If stocking density is not too high feed utilization efficiency will remain same.

2. Methods

2.1. Experimental design

Two diets contrasting in P:E ratio (18 vs 14 g.MJ^{-1}), 2 stocking densities (2 vs 3 m^{-2}) and 2 feeding levels (14 vs 18 g.kg$^{-0.8}$.d^{-1}) were tested in a 3-way full factorial design, with 5 replicates per treatment. All-male juvenile Nile tilapia were stocked and grown for 82 days. The feed input was gradually reduced assuming 80% survival at the end of the experiment.

Table 1
Ingredient and analysed chemical composition of the experimental Nile tilapia diets differing in protein to energy (P:E) ratio.

		Diets	
		High P:E ratio	Low P:E ratio
Ingredients (%)			
Maize		20	20
Soybean meal		12	6
Wheat bran			15
Wheat flour		20	20
Rice bran			12
Sunflower meal		12	6
Rapeseed meal		12	6
Meat & bone meal		15	8
Fish meal		5	3
Fish oil		2	2
Vitamin & Mineral premix[a]		1	1
Mono calcium phosphate (MCP)		0.7	0.8
DL Methionine		0.3	0.2
Chemical composition			
Dry matter (DM),	(g.kg^{-1})	893	889
Crude Protein (CP)	(g.kg^{-1} DM)	322	255
Fat	(g.kg^{-1} DM)	37	34
Ash	(g.kg^{-1} DM)	124	96
Phosphorus	(g.kg^{-1} DM)	15	13
Carbohydrate[b]	(g.kg^{-1} DM)	518	615
Gross energy	(kj.g^{-1} DM)	18	18
P:E ratio	(g.MJ^{-1})	17.5	14.1
C:N ratio[c]	(g.g^{-1} DM)	8	11

[a] commercial product made by ACI Godrej Agrovet Private Limited.
[b] This is calculated value where Carbohydrate = 1000-CP-Fat-Ash.
[c] 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 and Boersma, 2012).

2.2. Preparation of diets

Experimental diets were contrast in P:E ratio, extruded pellets of 3 mm size. The high P:Ediet was formulated to have a P:E ratio of 18 g.MJ^{-1}, which is at the lower range of the recommendation for tilapia (NRC, 1993). This high P:E diet had a C:N ratio of 8 and was comparable regarding nutrient content to currently used commercial diets in Southeast Asia (formulated in compliance with NRC, 1993). The low P:E was formulated to have a C:N ratio of 11. This was achieved by replacing protein ingredients (i.e., soybean meal, sunflower meal, rapeseed meal, meat and bone meal, and fish meal) with carbohydrate ingredients (i.e., wheat barn and rice bran). As a result, the high and low P:E diets contained 32 and 25% protein on a dry matter basis, respectively, and were isocaloric.

The inclusion of rice bran and wheat bran resulted in an increase of the non-starch polysaccharide content at the Low P:E diet compared to the High P:E diet. Both diets were identical in energy content. This was further confirmed by the chemical analysis of the feed (Table 1).

2.3. Study area, fish rearing and housing facilities

Forty outdoor ponds, average surface area 234 (± 112) m^2, in farmer fields in south-western Bangladesh (Fig. 1) were used for this experiment. Though the ponds belong to two different sub-district, they actually lie two sides of river Bhadra under the same agro-ecological zone of lower Ganges tidal flood plain. During the experiment, the ponds were exclusively dedicated for the experiment and the protocol was strictly followed by the project field research assistants. All male sex reversed 30 days old Nile tilapia fry, 14th generation WorldFish GIFT strain, were collected for this experiment from Asha Hatchery, a GIFT Tilapia Multiplication Center in Bangladesh.

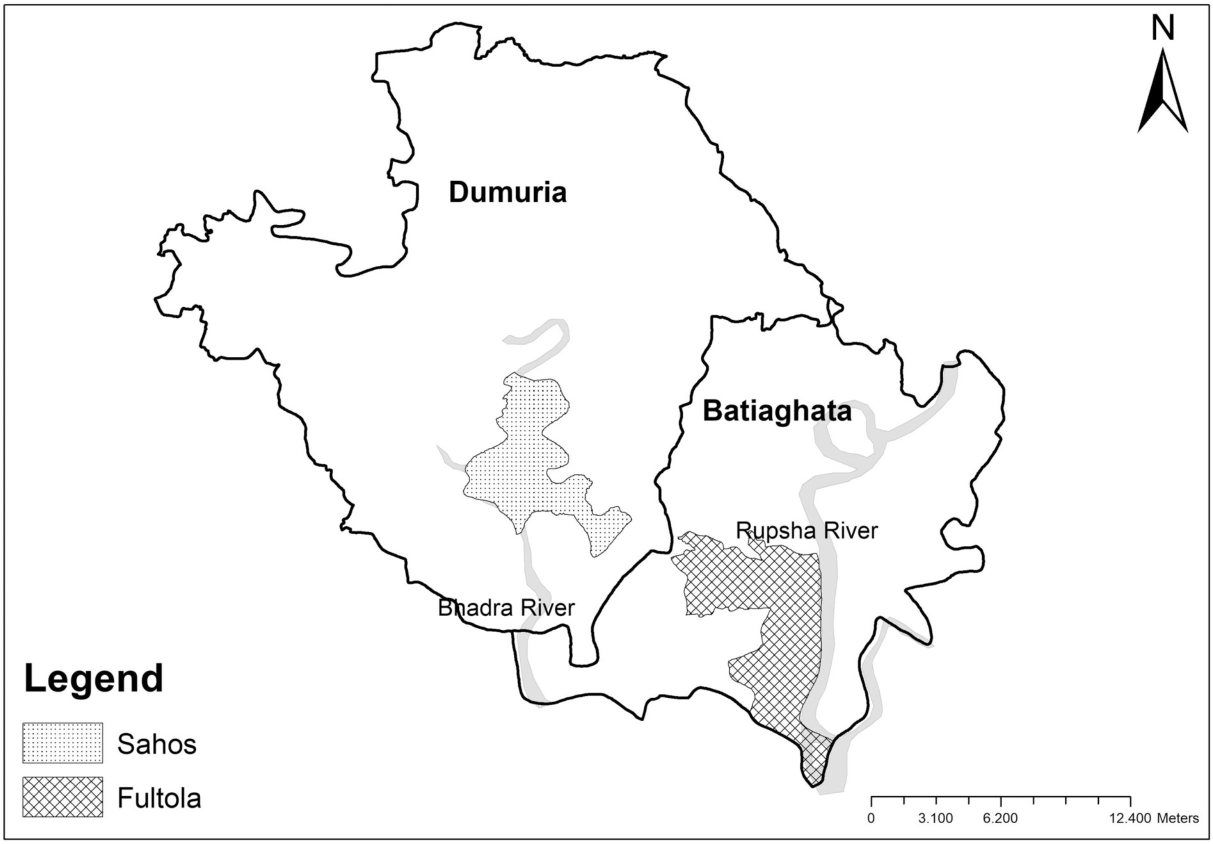

Fig. 1. Map of the study areas; 20 ponds in each location – in *Batiaghata* and *Dumuria* sub-district lying on opposite side of *Bhadra* river of Khulna District, Bangladesh.

2.4. Experimental procedure

2.4.1. Pond preparation

Ponds were dried by pumping out the water. Twenty-five g.m^{-2} $CaCO_3$ was applied at the bottom soil of each pond before water filling. After water filling, 4 g.m^{-2} dolomite ($CaMg(CO_3)_2$) was spread over the water surface of each pond. One g.m^{-2} urea (CH_4N_2O) and 2 g.m^{-2} triple super phosphate (TSP), [$Ca(H_2PO_4)_2.H_2O$], per pond (Rakocy and Mcginty, 1989) were applied 1 week after liming. Fish fries were stocked in a small pen, 2.25 m^{-2} frame covered with 1 mm mesh sized nylon net, in each pond, 5 days after fertilization. Fish fries (0.03 g) in the pen were fed a commercial nursery diet until the mean body weight was 23 (± 12) g, sufficient to eat 3 mm pellet.

Fish were fed daily at 8.00 and 16.00 h. Fish were fed according to their metabolic body weight. Two feeding levels were applied, high (18 g.kg$^{-0.8}$.d^{-1}) and low (14 g.kg$^{-0.8}$.d^{-1}). The feed rations were adjusted every two weeks based on body weight sampling in all ponds. Cast nets were used for sampling. Fish were harvested from four corners and center of the pond by single through of the cast net. 5–15% fish were sampled during each sampling. The amount of feed given was based on the measured DM content of the diet. Therefore, the crude protein input in the ponds at each diet was different. This experiment mimicked low intensity semi-intensive production in non-aerated ponds.

2.4.2. In-situ water quality monitoring

Dissolved oxygen (DO), pH, total dissolved solid (TDS), transparency, temperature and salinity of each pond were measured daily between 9.00 and 12.00 h; by using Lutron dissolved oxygen meter model PDO-519, Hanna instruments pocket tester HI98128-phep5, Lutron conductivity meter model PCD-431, Secchi disc, Hanna digital thermometer model HI98501 and Atago refractometer model MASTER-S28 M instruments.

2.4.3. Sampling and analysing soil and water nutrients

2.4.3.1. Sample collection, processing and preparation. Soil samples were collected from the top 20 cm layer of pond bottom at five points of each pond and then mixed homogeneously. Approximately 1 kg wet soil was collected from each pond, labelled and packed in tight plastic bags, and transported to the laboratory. The collected samples were air dried, crumbled and sieved through a 2 mm sieve to separate the coarse (> 2 mm) and fine (< 2 mm) fractions. The sieved fractions were then preserved in labelled plastic containers until analysis. Water samples were collected, with a depth sampler of 10 cm width and 25 cm length, from each pond at the same 5 soil sampling locations, within 25 cm of pond surface, transferred and sealed in airtight bottles, and preserved at −20 °C until analysed. These samples were collected at day 1, 41 and 82 of the experiment. Accumulation of nutrients over time in the culture pond were calculated by deducting the observation od day 1 from day 82.

2.4.3.2. Analysis of the soil samples. 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 by Micro-Kjeldahl's method following H_2SO_4 acid digestion and alkali distillation procedures as suggested by Jackson (1962). Total phosphorus of soil was determined colorimetrically by Vanado-molybdophosphoric yellow colour method in nitric acid system (Barton, 1948). The colour intensity was determined by the spectrophotometer at 470 nm light wavelength (Jackson, 1958). The available potassium was determined after extraction the soil samples with 1 N NH_4OAc, pH -7.0 solution followed by the measurement of extractable K+ by Flame emission spectrophotometer (Model: Jenway, PEP-7) at 766 nm wave length using Potassium filter, as outlined by

Jackson (1973).

2.4.3.3. Analysis of the water samples. The organic carbon content of the water was determined by Tyrine's method as water commonly contains relatively smaller amounts of organic matter. As under dilute conditions Tyrine's method does not function well, the sample was dried first (Tyrine's's, 1965). The total inorganic nitrogen concentration was determined by the Micro-Kjeldahl method (Jones Jr, 1991) and alkali distillation procedures as suggested by Jackson (1962). Available phosphorus was determined colorimetrically by molybdophosphoric blue colour method (Murphy and Riley, 1962). The available potassium of water was determined by a flame analyzer at 589 nm wavelength (Jackson, 1967).

2.4.4. Sampling and analysing proximate composition of fish and feed

The initial body composition was determined on 200 fingerlings with 23 (± 12) g mean body weight. Five fish fingerlings were collected from each pond at the start day of the feeding trial. They were euthanized by an overdose of a phenoxy-ethanol solution (1.0 ml. L^{-1}) and stored at −20 °C. For final body composition, 5 fish were randomly selected from each pond at the end of the experiment. Fish, which were used for body composition analysis, were euthanized by an overdose of a phenoxy-ethanol solution (1.0 ml.L^{-1}) and stored at −20 °C. Before chemical analysis, the sampled fish were cut into small pieces, homogenised by passing them twice through a 4·5 mm screen grinder and subsequently oven-dried. Chemical analyses were done in triplicate. Dry matter was determined gravimetrically after drying at 103 °C for 4 and 24 h for feed and fish samples respectively (ISO 6496, 1983). Crude ash was determined after incineration at 550 °C for 4 h (ISO 5984, 1978). Crude protein (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 h with 3 M-HCl. Dietary energy content was measured by direct combustion in an adiabatic bomb calorimeter (IKA-C-7000; IKA analysentechnik, Weitersheim, Germany).

2.5. Data analysis

2.5.1. Performance

Biomass gain (g.m^{-2}) was calculated as the difference between the biomass stocked and biomass harvested (in g.m^{-2}). The specific growth rate (SGR) was calculated as SGR = ((ln($IndBW_f$)-ln($IndBW_i$))/82) × 100; where, $IndBW_f$ and $IndBW_i$ means individual body weight at harvest (day 82) and at stocking (day 0) of the experimental feeding. Feed conversion ratio (FCR) was calculated as FCR = weight of the total feed applied/fish produced (wet weight basis). The survival of fish per pond was calculated as (F_f/F_i) × 100, where F_f is the number of fish harvested and F_i is the number of fish stocked.

2.5.2. Nitrogen (N) retention

N gain in fish was calculated by the difference between the N_f and N_i. Here, N_f = amount of N in the harvested fish biomass and N_i = amount of N in the fish biomass stocked. N feed was calculated by total feed input per square meter multiplying the N content in feed; N balance was calculated by deducting N gain in fish from the feed N input based on proximate composition. N retained from natural food was calculated by deducting N retention from feed from the total N gain in fish. In this study it was not possible to measure the apparent digestibility as the experiment was in ponds. So, the calculation of N retention resulting from direct feed consumption was based on a 90% apparent digestibility coefficient (ADC) for N (Azevedo et al., 2004; Kaushik et al., 1995) and N retention efficiency (RE) of 40% (Azevedo et al., 2004). The difference between total N retention in fish biomass gain and the N retention based on feed is considered as N retention from natural food.

The ADC and RE of N may vary based on the concentration of CP and feeding levels. However, the simulation with different ADC and RE from Kabir et al. (2019) indicates that those changes does not make major changes in the contribution of natural food and supplementary feed on the N gain in fish. As this study is a validation of the proof of concept of the previous study of Kabir et al. (2019). Therefore, we kept the analysis simple and used the most common level of ADC and RE in this study. There might be variation in the reality. However, this analysis is intended to give the reader an impression of importance of natural food to fish production in ponds.

2.5.3. Calculation of economic benefit

The total cost was estimated by the sum of the depreciated pond construction cost considering the pond life 20 years, rent of the land area of the pond for a cycle (half of a year), fish seed, feed, labour and other inputs and contingency cost. Rent of the land and pond construction cost was based on the local context of the research area for the year of the study. Return is the sell value of the total harvested fish. The price of the fish was set based on the wholesale price of tilapia in the local auction center on the day of harvest. Gross margin was calculated by deducting the total cost from the total return. Benefit cost ratio (BCR) was calculated by dividing the return with the total cost.

2.5.4. Statistical analysis

All parameters were analysed for the effects of diet and stocking density. Though we designed the experiment with two feeding levels (14 vs 18 g.$kg^{-0.8}$.d^{-1}), the actual feed ration varied due to difference in biomass at stocking and adjustment of the feed ration based fortnightly body weight sampling. Therefore, instead of using feeding level as a fixed factor, feed ration (g.$fish^{-1}$.m^{-2}.d^{-1}) was used as a continuous variable in a covariate analysis in applying univariate ANOVA using the procedure general linear model (GLM). The value of the covariate for feed ration has been presented as beta in tables showing results representing the slope of the regression. Before using feed ration as covariate, the effect of diet, stocking density and their interaction on feed ration (g.$fish^{-1}$.m^{-2}.d^{-1}) was tested by two way ANOVA. None of these factors were significant, this indicates that the feed ration (g.$fish^{-1}$.m^{-2}.d^{-1}) can be considered as an independent (continuous) variable, making a similar effect as feeding level. When significant interaction found multiple comparisons of means using Tukey's multiple range test were performed. Deviation from the mean has been expressed as standard error throughout the analysis.

As we had varying pond environment, we wanted to see how pond environment explains the variation in fish growth in different ponds. For this analysis dissolved oxygen, pH, temperature, water transparency, pond water depth, and organic matter, total nitrogen, total phosphorus and available potassium of pond water and bottom soil were included as environmental explanatory variable. Individual weight gain, biomass gain per square meter, fish survival, feed conversion ratio and specific growth rate were grouped as response variables. These data were fitted into a multivariate distance based linear model (DistLM) using BEST procedure in Primer 6 and Permanova$^+$.

3. Results

In the design of the study, it was intended to have 2 distinct feeding levels (14 vs 18 g.$kg^{-0.8}$.d^{-1}). The feeding rations applied were calculated based on the measured initial BW and were adjusted based on body weight sampling every two weeks. The initial individual BW of tilapia was 23 (± 12) g. Thus, differences in mean BW per pond, concurred with differences in feed ration between ponds, ranging from 1 to 3.8 g.$fish^{-1}$.m^{-2}.d^{-1}. The effect of feed ration is presented by the beta variable in Table 2 through Table 6, which summarize the experimental results. The feed ration was unaffected by diet and stocking density, confirming that the fixed effects and the covariate in the statistical model used were independent of each other. Moreover,

Table 2
Effect of dietary protein to energy ratio (P:E), stocking densities and feed input on performance of tilapia.

Variables		Units	Low P:E Diet		High P:E Diet		Pooled SEM	P-values			Beta for Feed ration (g.fish^{-1}.m^{-2}.d^{-1})	P value of Beta
			SD2	SD3	SD2	SD3		Diet (D)	Stocking density (SD)	D*SD		
Individual performance	Initial body weight	g	22	22	24	23	3.8	ns	ns	ns	–	–
	Final body weight	g	265	273	254	235	14	#	ns	ns	72 (± 10)	***
	Weight gain	g	243	252	230	213	14	#	ns	ns	60 (± 10)	***
	Growth	g.d^{-1}	2.958	3.09	2.799	2.602	0.175	#	ns	ns	0.7 (± 0.1)	***
Pond level performance	Biomass stocked	g.m^{-2}	43	64	47	69	9.9	ns	*	ns	–	–
	Biomass harvested	g. m^{-2}	393	607	384	499	29	#	***	#	150 (± 20)	***
	Biomass gain	g.m^{-2}	360	559	347	451	28	*	***	#	126(± 19)	***
	Survival	%	74	76	76	71	2.5	ns	ns	ns	2.9(± 1.8)	ns
	FCR	g.g^{-1}	0.99	0.95	0.99	1.12	0.064	ns	ns	ns	0.16(± 0.04)	***

D = Diet and SD = Stocking density, D*SD = Diet and stocking density interactions, *P* values: ns (not significant, P > 0.1), # (P < 0.1), * (P < 0.05), *** (P < 0.001); SEM = standard error of the mean; Beta for feed ration represents the estimated regression coefficient of the feeding ration (expressed in g.fish^{-1}.m^{-2}.d^{-1}) on the respective depend parameter (e.g., biomass gain, FCR etc.).

preliminary analysis showed that no interaction effects were present between feeding ration and both fixed effects (diet and stocking density) for any of the parameters related to fish performance. This implies that the effect of feeding ration (if present) was similar for both diets and also for both stocking densities.

3.1. Fish performance

At stocking, the average BW was 23 (± 12) g and was unaffected by diet and stocking density. The final BW and individual fish growth were not influenced by stocking density and tended to be higher with the low P:E diet ($P < 0.10$). Feeding ration strongly affected final BW and individual BW gain ($P < 0.001$). Increasing the feeding ration with 1 g.fish^{-1}.m^{-2}.d^{-1} increased the final BW with 72 g. Averaged over all treatments, the survival rate was 74% and equal between treatments ($p > 0.1$) (Table 2).

As expected, the stocked biomass per pond (in g.m^{-2}) was only affected by the stocking density. Increasing stocking density from 2 to 3 fish.m^{-2} increased the harvested biomass with 42% and biomass gain with 43% ($P < 0.001$). Lowering the dietary P:E ratio from 18 to 14 g.MJ^{-1} increased weight gain of tilapia from 399 to 459 g.m^{-2} ($P < 0.05$; Table 2). This diet effect on biomass gain tended to depend on stocking density, being reflected by the interaction effect between diet and stocking density ($P < 0.10$). The impact of dietary P:E ratio on biomass gain tended to be higher at the high stocking density (Table 2). Similar to individual BW gain, performance at pond level strongly increased with increased feeding ration. Increasing the feeding ration with 1 g.fish^{-1}.m^{-2}.d^{-1} increased the biomass gain with 120 g.m^{-2}.

Average over all treatments, the FCR was 1.01. Diet and stocking density did not affect FCR. Increasing the feeding ration increased the FCR; per 1 g.fish^{-1}.m^{-2}.d^{-1} the FCR at the pond level increased by 0.16 g.g^{-1} (Table 2).

3.2. Fish body composition

At stocking, dry matter (DM), crude protein (CP), crude fat (CFat), and ash content of the tilapia were 293, 132, 27, and 68 g.kg^{-1}, respectively. Lowering the dietary P:E ratio from 18 to 14 g.MJ^{-1} decreased the DM and ash content, while feeding ration, stocking density, and the interaction of diet and stocking density had no influence on the final body composition of the fish (Table 3).

3.3. Feed nitrogen (N) input and output

The nitrogen balance at pond level is given in Table 4. Averaged over all treatments, 18 g of N.m^{-2} was added to the pond via the feed during the 82 days of the experiment. This N input via feed was higher at the high P:E diet ($P < 0.001$); was higher at the high stocking density ($P < 0.001$); and increased with feeding level ($P < 0.001$). The interaction effect between stocking density and dietary P:E ratio influenced the amount of N retained in fish harvested per m^2. At the low stocking density (2 fish.m^{-2}), N gain in fish did not differ between both diets, but at high stocking density (3 fish.m^{-2}), the N gain in fish was higher with the low P:E ratio diet (14 g.MJ^{-1}) ($P \leq 0.05$). Feed N retention efficiency with the low P:E diet was 71% compared to 51% with the high P:E diet (P 〈001); but did not differ between stocking densities ($P > 0.1$). With the low P:E diet, the N input into the pond was less than with the high P:E diet, but the amount of N gained in fish was equal or greater. This was due to an increased gain of N originating from the food web. Similar to total N gain in fish, the amount of N gain from the food web was affected by the interaction effect between diet and stocking density ($P < 0.05$). The increased N gain from the food web with the low P:E diet was stronger at high stocking density than at low stocking density. The feed input on the last day of the experiment was on average 134 kg.ha^{-1} in ponds fed the high feeding level (18 g.kg$^{-0.8}$.d^{-1}) and applying the high stocking density (3 fish.m^{-2}).

Table 3
Effect of dietary protein to energy ratio (P:E), stocking densities and feed input on the final body composition of tilapia.

Variables	Units	Low P:E Diet		High P:E Diet		Pooled SEM	P-values			Beta for Feed ration ($g.fish^{-1}.m^{-2}.d^{-1}$)	P value of Beta
		SD2	SD3	SD2	SD3		Diet (D)	Stocking density (SD)	D*SD		
Dry matter (DM)	$g.kg^{-1}$	288	294	302	302	5.4	*	ns	ns	0.3(± 3.8)	ns
Crude protein (CP)	$g.kg^{-1}$	154	155	158	154	1.9	ns	ns	ns	0.96(± 1.3)	ns
Crude fat (CFat)	$g.kg^{-1}$	51	53	54	52	1.4	ns	ns	ns	0.4(± 1)	ns
Ash	$g.kg^{-1}$	55	59	62	61	1.5	*	ns	ns	1.4(± 1.1)	ns

D = Diet and SD = Stocking density, D*SD = Diet and stocking density interactions, P values: ns (not significant, $P > 0.1$), * ($P < 0.05$); SEM = standard error of the mean; Beta for feed ration represents the estimated regression coefficient of the feeding ration (expressed in $g.fish^{-1}.m^{-2}.d^{-1}$) on the respective depend parameter (e.g., biomass gain, FCR etc.).

Results of this experiment suggest that with the high P:E diet the natural food contribution to production in ponds with a low stocking density (2 $fish.m^{-2}$) was greater than in ponds with a high stocking density (3 $fish.m^{-2}$), which was opposite for the low P:E diet (Fig. 2). Comparing high and low P:E diets at higher culture intensities than in the present experiment will be necessary to determine at which stocking density the contribution of natural food to fish production will also start to decrease with the low P:E diet.

Increasing the feeding ration increased the N input via feed into the ponds ($P < 0.001$), which directly related to an increased N gain in fish ($P < 0.001$). The increased N gain in fish concurred with the increased N gain from feed ($P < 0.001$) because increasing the feeding ration did not increase the N gain in fish from food web ($P > 0.1$; Table 4).

3.4. Accumulation of soil and water nutrients

Organic matter content in water increased with increasing feed input ($P \leq 0.05$). Except for this, there was no effect of diet, stocking density and feeding ration on accumulation of nutrients in pond soil and water during the 82 days of culture period ($P > 0.05$). Nutrient accumulation was evaluated 3 times during the culture period, sampling organic matter, total nitrogen, total phosphorous and total potassium in both water and soil. The observed differences were large and highly variable, showing no statistical differences between the treatments.

3.5. Economics of fish production

Lowering the dietary P:E ratio from 18 to 14 $g.MJ^{-1}$ increased gross margin ($P < 0.05$) and benefit cost ratio ($P < 0.05$). Gross margin was higher ($P < 0.05$) at high stocking density and there was a tend towards interaction between dietary P:E ratio and stocking density ($P < 0.07$). Stocking density did not affect the benefit cost ratio (BCR). Also, feeding ration did not influence gross margin and BCR ($P > 0.1$) (Table 5).

3.6. Pond water quality

There was an interaction ($P < 0.05$) between diet and stoking density on dissolved oxygen (DO) concentration. Overall, this concurs with an increased DO concentration at the high feeding level ($P < 0.001$). The highest DO concentration was found with the low P:E diet – high stocking density treatment. This concurs with a tendency for lower transparency with higher stocking density ($P < 0.1$) (Table 6). Stocking density also influenced ($P < 0.05$) the NH_4 and NO_3 concentrations in the water and there was a tendency ($P < 0.1$) of increased NO_2 concentration with increased stocking density. There was no effect of diet, stocking density and their interaction on pond water quality. Only the organic matter content in the water column increased with increasing feed ration (data not shown).

4. Discussion

In this on-farm trial we confirmed that the effects of lowering the dietary P:E ratio in pond diets on production performance observed in the experimental ponds remains same. Biomass gain, food web contribution to fish growth, nitrogen retention efficiency and economic benefit of tilapia aquaculture in the farmer ponds increased with low P:E diet (14 $g.MJ^{-1}$) compared to the high P:E diet (18 $g.MJ^{-1}$).

Higher biomass gain found with the low P:E diet (14 $g.MJ^{-1}$) confirms the findings of Kabir et al. (2019). A P:E ratio of 14 $g.MJ^{-1}$ is less than the recommended dietary P:E ratio range (18–23 $g.MJ^{-1}$) for tilapia (El-Sayed and Teshima, 1992; Kaushik et al., 1995; NRC, 1993). Several recent studies (Abdel-tawwab, 2012; Abdel-Tawwab et al., 2010; Fernandes et al., 2016; Liu et al., 2018) observed better

Table 4
Effect of dietary protein to energy ratio (P:E), stocking densities and feed input on feed N input and gain in fish.

Variables	Units	Low P:E Diet		High P:E Diet		Pooled SEM	P-values			Beta for feed ration ($g.fish^{-1}.m^{-2}.d^{-1}$)	*P* value of Beta
		SD2	SD3	SD2	SD3		Diet (D)	Stocking density (SD)	D*SD		
N input via feed	$g.m^{-2}$	12.5	19.3	16.3	23.9	0.47	***	***	NS	9(± 0.3)	***
N gain in fish	$g.m^{-2}$	8.7	13.5	8.7	10.8	0.67	*	***	*	3(± 0.5)	***
N gain in fish from feed[a]	$g.m^{-2}$	4.5	6.9	5.9	8.6	0.17	***	***	NS	3.2(± 0.12)	***
N gain in fish from food web[a]	$g.m^{-2}$	4.2	6.6	2.9	2.2	0.68	***	NS	*	(−0.26(± 0.48)	NS
Total N recovery	%	70.3	72.3	55.9	47.5	4.50	***	NS	NS	(−11(± 3))	***
N loss (not retained in fish)	$g.m^{-2}$	3.9	5.8	7.6	13.1	0.78	***	***	*	6(± 0.5)	***

D = Diet and SD = Stocking density, D*SD = Diet and stocking density interactions, P values: ns (not significant), # ($P < 0.1$), * ($P < 0.05$), *** ($P < 0.001$); SEM = standard error of the mean; Beta for feed ration represents the estimated regression coefficient of the feeding ration (expressed in $g.fish^{-1}.m^{-2}.d^{-1}$) on the respective depend parameter (e.g., biomass gain, FCR etc.).

[a] Calculated values based on an ADC of CP of 90% and a retention efficiency of 40% for all diets, stocking densities and feeding levels.

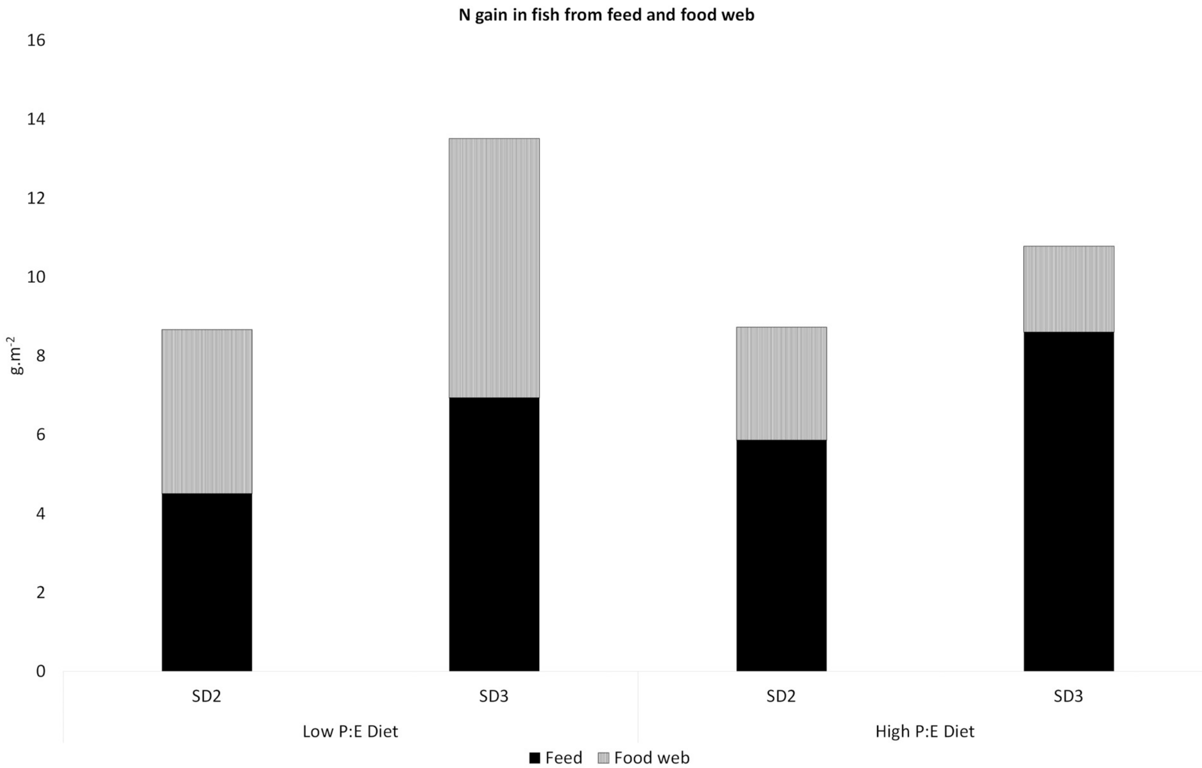

Fig. 2. Effect of dietary P:E ratio and stocking density on the estimated contribution of food web to fish N gain. (SD2 = stocking density 2 fish.m^{-2} and SD3 = stocking density 3 fish.m^{-2}). (These are calculated values based on ADC of CP 90% and RE 40% for all diets, stocking densities and feeding levels).

Table 5
Effect of dietary protein to energy ratio (P:E), stocking densities and feed input on the production economics of Tilapia in pond aquaculture.

Variables	Units	Low P:E Diet		High P:E Diet		Pooled SEM	P-values			Beta for feed ration (g.fish^{-1}.m^{-2}.d^{-1})	P value of Beta
		SD2	SD3	SD2	SD3		Diet (D)	Stocking density (SD)	D*SD		
Feed cost	USD.ha^{-1}	1902	2834	2112	3173	63	***	***	ns	1240(± 44)	***
Total cost	USD.ha^{-1}	3018	4035	3228	4374	63	***	***	ns	1240(± 44)	***
Return	USD.ha^{-1}	4391	6814	4236	5500	337	*	***	#	1542(± 236)	***
Gross margin	USD.ha^{-1}	1373	2779	1008	1126	340	*	*	#	301 (± 237)	ns
BCR	USD.ha^{-1}	1.4	1.7	1.3	1.3	0.1	*	ns	ns	(−0.02(± 0.07))	ns

D = Diet and SD = Stocking density, D*SD = Diet and stocking density interactions, BCR = Benefit cost ratio (Return/Total Cost), Gross Margin = Return-Total Cost, Return = Total sale value. P values: ns (not significant, P > 0.1), # (P < 0.1), * (P < 0.05), *** (P < 0.001); SEM = standard error of the mean; Beta for feed ration represents the estimated regression coefficient of the feeding ration (expressed in g.fish^{-1}·m^{-2}.d^{-1}) on the respective depend parameter (e.g., biomass gain, FCR etc.).

Table 6
Effect of dietary protein to energy ratio (P:E), stocking densities and feed input on the pond water quality.

Variables	Units	Low P:E Diet		High P:E Diet		Pooled SEM	P-values			Beta for feed ration (g.fish^{-1}.m^{-2}.d^{-1})	P value of Beta
		SD2	SD3	SD2	SD3		Diet (D)	Stocking density (SD)	D*SD		
Dissolved oxygen (DO)	mg.L^{-1}	2.7	3.5	3.0	2.9	0.21	ns	#	*	0.7(± 0.1)	***
pH		8.1	8.3	8.2	8.1	0.10	ns	ns	ns	0.002(± 0.07)	ns
Transparency	cm	32.6	25.7	34.1	27.1	3.76	ns	#	ns	(−0.5(± 2.6))	ns
NH_4	mg.L^{-1}	0.86	0.95	0.85	0.92	0.03	ns	*	ns	0.025(± 0.02)	ns
NO_2	mg.L^{-1}	1.92	2.02	1.84	1.99	0.07	ns	#	ns	0.046(± 0.05)	ns
NO_3	mg.L^{-1}	3.84	3.67	4.51	3.66	0.24	ns	*	ns	0.106(± 0.17)	ns

D = Diet and SD = Stocking density, D*SD = Diet and stocking density interactions, P values: ns (not significant, P > 0.1), # (P < 0.1), * (P < 0.05), *** (P < 0.001); SEM = standard error of the mean; Beta for feed ration represents the estimated regression coefficient of the feeding ration (expressed in g.fish^{-1}·m^{-2}.d^{-1}) on the respective depend parameter (e.g., biomass gain, FCR etc.)

performance with high P:E ratio diets compared to the low P:E diets. However, all these studies were done in clearwater tanks. We therefore hypothesize that in ponds, the presence of natural food makes the difference. Nitrogen gain based on direct feed consumption was higher with the high P:E diet (7.2 $g.m^{-2}$ vs 5.7 $g.m^{-2}$). However, the total N gain in fish was 9.7 $g.m^{-2}$ with the high P:E ratio diet, which is less than the 11.0 $g.m^{-2}$ with the low P:E ratio diet. The influence of the feed N was superseded by the stronger contribution of N coming from the natural food (Table 4, Fig. 2). Overall depletion of inorganic N from the pond environment with the low P:E diet (Table 4) also indicates that this N was used to compensate for the lower dietary N inclusion. Similar observations were also reported by several pond studies where dietary C:N ratio was increased by adding carbohydrate besides the regular diet (Anderson et al., 1987; Burford et al., 2002, 2004; Cam and Mariotti, 1991; Porchas-Cornejo et al., 2012) to enhance effect of natural food. In the high P:E dietary treatment, the proportion of feed N that was not deposited in the fish was high. On the other hand, reducing the dietary P:E ratio (i.e. increasing dietary C:N ratio in this study), demonstrated a higher N retention efficiency in fish biomass. This corroborates the results of Kabir et al. (2019). However, how a low P:E ratio diet will affect pond production when stocking density and feeding level increases needs further research.

In this experiment, increasing the stocking density from 2 to 3 $tilapia.m^{-2}$ also increased fish biomass gain. Such an effect of stocking density was also observed by Wu et al. (2018) and Abdel-Tawwab et al. (2014). However, this gain was mainly derived by the increased feed input in response to the higher stocking density (Table 4) as increasing stocking density did not influence the contribution of natural food to fish N gain or on feed N retention efficiency. The rate of increase of biomass gain in relation to stocking density with the low P:E diet was 25% greater than with the high P:E ratio diet. This large increase in biomass gain due to the interaction effect between diet and stocking density was probably influenced by the 23% difference in the C:N ratio of the pond soil observed between the low and high P:E ratio diets at high stocking density (59.9 vs 48.7). The 607 $g.m^{-2}$ harvested tilapia biomass with the low P:E diet is in the same range as the 588 $g.m^{-2}$ carrying capacity for tilapia in non-aerated ponds reported by Xu et al. (2011). Whether application of low P:E diets will positively affect the pond's carrying capacity needs further research.

In this experiment, increasing the feed ration increased fish growth ($g.d^{-1}$), biomass gain ($g.m^{-2}$) and FCR ($g.g^{-1}$). Literature shows contradictory results regarding the relation between FCR and feed ration. Liu et al. (2018) and El-Sayed (2002) reported the same outcome as we observed, while Haidar et al. (2018) and Deyab and Hussein (2015) observed that FCR first decreased with increasing feed ration before starting to increase when further increasing of feed ration. In the current experiment, growth increased with increasing feed ration, but also FCR increased. The N balance parameters at pond level (Table 4) demonstrated that increasing the feeding level (i.e. increasing N feed input) resulted in a higher N gain. This increase in N gain was not related to an increased N intake from the natural food but fully due to a higher N intake by the fish. The estimated beta for feed ration for the N gain originating from the food web was almost zero (slightly negative; Table 4). This suggests that in the current range of production intensity, feeding level has no stimulating impact on the productivity of the natural food web. This may explain why increasing the feeding level by 1 $g.fish^{-1}.m^{-2}.d^{-1}$ only slightly increased the gross margin by 301 $\$US.ha^{-1}$ and that is was not statically significant (Table 5). However, economically on a yearly basis (2 production cycles) and for the Bangladesh setting it is still a relevant increase in income. Moreover, from a local perspective of food security, increasing the feed level by 1 $g.fish^{-1}.m^{-2}.d^{-1}$ still can increase 1260 $kg.ha^{-1}$ biomass gain (i.e., yield; Table 2). Although, advising for increasing the feeding level should be handled cautiously, because the above conclusion is only valid within the range of feed ration applied in the current study. Increasing the feeding level beyond the maximal level in the current study, might even lead to collapse of production of the natural food web and deterioration of water quality. As a consequence fish yield might decrease as well. Additionally, a much larger field experiment, involving large numbers of farmers, is needed to confirm the economic benefit of applying increased feed ration in non-aerated ponds.

The economic benefit of applying low P:E ratio diet was mainly due to low the feed cost (as well as reduced total production cost) while increasing the yield (and return) at the same time. In aquaculture, feed cost is the main factor determining economic return (Hebicha et al., 2013; Yuan et al., 2017). On the other hand, Yuan et al. (2017) also mentioned that fish price also plays an important role in the economic profitability as the profit margin is low and fish price varies due to season and location. In the present study calculation of economic return was based on the wholesale price of tilapia at the day of harvest. Price fluctuation was not considered. Our calculations were only intended to give an idea of economic benefit that can be achieved by using the low P:E diet for tilapia aquaculture in ponds.

5. Conclusion

Lowering the P:E ratio from 18 to 14 $g.MJ^{-1}$, concurring with a reduction of the dietary protein level from 32 to 25% DM in formulated feeds, improved tilapia production in farmer ponds due to the enhanced contribution of natural food to total fish production. Similar results obtained in experimental ponds (Kabir et al., 2019) and in farmer ponds indicate that the P:E ratio in supplemental diets applied to tilapia ponds should be less than recommended (NRC, 1993).

Apparently, fish consumed more natural foods to compensate for the smaller input through the formulated feed. Better yield with high stocking density without compromising the feed efficiency indicates possibility of further intensification even in non-aerated ponds. Increasing feeding level increased growth and yield but also created more nutrients accumulating or discharged from aquaculture ponds (in terms of feed N not retained in fish). The economic assessment indicates that using low P:E diet will increase farmers economic benefit and will increase economic viability of tilapia farming in areas of low profit margin. Additional research to test the performance at higher intensity will help to understand effect of this concept on more commercial implication as well as on improving carrying capacity of the pond system.

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