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Stoichiometry of fish-phytoplankton interaction in fish ponds receiving local and industrial feed in the pre-growth phase

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Abstract

The use of agricultural by-products could modify the stoichiometry of carbon (C), nitrogen (N) and phosphorus (P), leading to changes in the composition of phytoplankton and affecting the structure of fish. The aim of this study is to determine the stoichiometry of phytoplankton and fish using industrial and local feeds. Samples of *Oreochromis niloticus* fish and phytoplankton were collected from six pre-growth ponds. Two ponds were treated with feed (industrial and local) and two other ponds that received no feed were considered as controls. C, N and P contents were measured and their ratios were analysed to characterise the stoichiometry of phytoplankton and fish. The respective stoichiometric C/P, C/N, N/P ratios of phytoplankton (274.38; 8.31; 33.33) in ponds receiving local feed and control ponds (358.04; 8.75; 39.97) were higher than those in ponds receiving industrial feed (161.50; 6.43; 22.42). The same trend was observed in fish, with respective stoichiometric C/P, C/N and N/P ratios of 62.56; 4.86; 20.50 for fish fed with local feed, 46.86; 3.84; 12.76 for those fed with industrial feed and 74.13; 3.94; 18.62 for control fish. Correlation analysis showed that the weight of fish fed the industrial feed was significantly correlated with phosphorus and nitrogen in those fed the local feed and those not fed. These results show that the fish have the same nutrient and carbon compositions as those present in their environment.

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Introduction

Self-sufficiency in fish products is a major challenge for developing countries, especially in Africa (Amoussou, 2017). Ivory Coast, in particular, has put a great deal of effort into aquaculture research, with fish farming being practiced throughout the country (Aboua, 2016).

Despite numerous investments, Ivorian fish production, estimated at 3,750 tonnes in 2016, only contributed 4.12% of its production (FAO, 2018). According to several authors (Agbohessi *et al.*, 2019; FAO, 2022), the cost of feed represents around 50% of the total cost of production, which hampers the emergence of fish farming. To remedy this situation, some fish farmers resort to using feed based on agri-food by-products available in rural areas at lower cost (Yao *et al.*, 2017). However, these feeds enrich the environment with nutrients which, through a combination of factors, stimulate plankton production (Anougbo *et al.*, 2019). The development of plankton, particularly phytoplankton, is influenced by the availability of nutrients, light conditions and water temperature. Nitrogen (N) and phosphorus (P) are the nutrients that control phytoplankton biomass in ponds (Burgess, 2018), while carbon (C) is an important source of energy for phytoplankton during photosynthesis (Groga, 2012). However, changes in these nutrients and carbons can lead to changes in phytoplankton composition. It is therefore important to understand how nutrients function in controlling phytoplankton biomass because changes in biomass at a trophic level can affect fish structure (Soudijn and Van de Wolfshaar, 2021).

Ecological stoichiometry appears to be an important tool for studying and understanding how nutrients accumulate in ecosystems. Indeed, stoichiometric ecology focuses mainly on three elements that are essential to life: carbon, nitrogen and phosphorus. In Ivory Coast, numerous studies, such as those by Yao *et al.* (2017), Gbai *et al.* (2018) and Brou *et al.* (2020), have been carried out in fish ponds, but no study has yet been conducted on the stoichiometric distribution of carbon, nitrogen and phosphorus in phytoplankton and fish in fish ponds. With a view to obtaining good fish production at lower cost, the objective of this study is (1) to evaluate the stoichiometry of phytoplankton and fish nutrients in fish ponds receiving exogenous feed (local and industrial) and (2) to explore the influence of nutrients on fish growth.

Materials and methods

Description of the study site

The study took place at the Bélier Ecological Farm (Fig. 1), a peri-urban fish farm located 3 km from Kacoubroukro and 14 km from Yamoussoukro (06°49 06°47 N and 05°16 05°15W). The site is supplied with water by the spillway of a dam that covers the water needs of the 10-hectare farm, which comprises 61 ponds with a surface area of 800 m² (20 × 40 m) and an average depth of 0.8 m, 40 of which are operational. Six of these ponds (E1, E2, E3, E4, E5, E6) were used, with the respective codes AG1, AG2, AP1, AP2, AN1 and AN2.

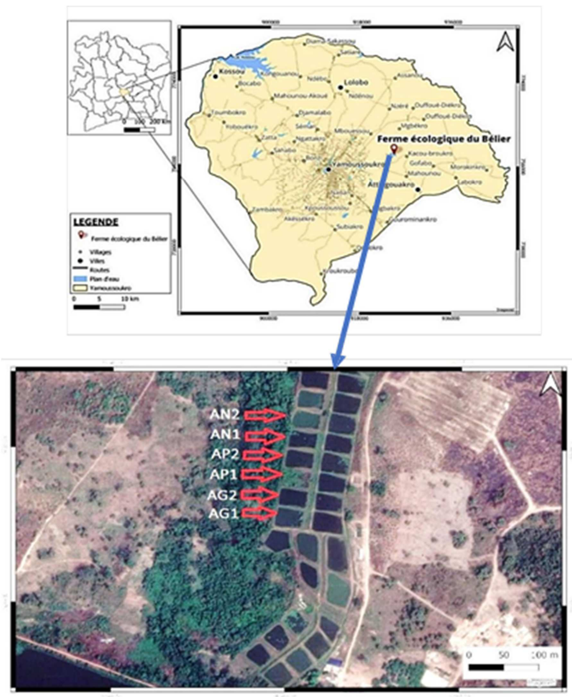

Fig. 1. Location of the Bélier ecological farm

Experimental feed

During the trials, two exogenous feeds (local diet and industrial diet) were used throughout the pre-growth phase. The raw materials used in the local diet consisted of low-grade rice flour, wheat bran, soya meal, vitamins, cooking salt and a mineral-vitamin complex. The industrial diet contained soya flour, wheat, rice bran, fish meal, poultry meal, salmon oil and a vitamin and mineral premix.

Sampling and breeding monitoring techniques

This study was carried out in six ponds. The weights of 20 individuals, taken at random from each pond, were determined individually. Sampling was carried out once a month for three months on *Oreochromis niloticus* using two seines with 20 mm and 60 mm mesh. Fish with an average weight of 5 g were used at a density of 1.25 ind./m², i.e.1000 fish per pound. These fish were fed at ratios of 5% and 4% of their body weight. The fry was fed every 2 h between 8 a.m. and 4 p.m., i.e.5 meals per day. The fish in ponds AG1 and AG2 were fed an industrial feed (extruded granules), those in ponds AP1 and AP2 were fed a local feed (powder) and the fish in control ponds AN1 and AN2 were not fed an exogenous feed in order to estimate the natural contribution of the rearing environment. Each experiment was replicated.

Determination of the concentration of C, N, P in phytoplankton and fish

For phytoplankton samples, 5-10 L of water were filtered through an 83 µm mesh (particulate matter assumed to be dominated by phytoplankton) and collected. Fish were collected at random from each pond, 5 healthy fish were arbitrarily selected and after 24 hours of starvation, were sacrificed. The samples were immediately placed on ice and transported to the laboratory where they were measured, eviscerated and dried to a constant weight. The whole fish was ground to a homogeneous mixture using an ultracentrifugal grinder (Restch ZM 100). The organic carbon (C) and nitrogen (N) of the seston and fish were measured using an analyzer, and the phosphorus (P) samples were analyzed after digestion with HCl using the molybdenum blue method with an auto-analyzer (Stainton *et al.*, 1977; Higgins *et al.*, 2006). Phytoplankton stoichiometry is linked to local nutrient concentrations (Prater *et al.*, 2017). Element contents and fish ratios were expressed as molar ratios of the elements. The stoichiometric ratios obtained were compared with those of Elser *et al.* (2000), Stener *et al.* (2008) and McIntyre and Flecker (2010).

Statistical analysis

Normality test

To process the results obtained, the normality of the data distribution was verified for each parameter studied. The Shapiro-wilk test was used to test the normality of the data distribution. Parameters with a normal distribution were subjected to analysis of variance (one-factor ANOVA). Parameters with an abnormal distribution, on the other hand, were subjected to the non-parametric Kruskal-Wallis test followed by the Mann- Whitney test for multiple comparisons. These different tests were used at a significance level of p value of 95% ($p < 0.05$) and were carried out using R 3.4.3 software with an RStudio interface.

Principal component analysis

The aim of this descriptive factorial statistics method is to present, in graphical form, the maximum amount of information contained in a large data table (Philippeau, 1992). The principal components are classified in descending order of the amount of information they explain and are obtained by diagonalizing a matrix which, depending on the nature of the initial variables, is either the correlation matrix or the covariance matrix (Legendre and Legendre, 1979). For the purposes of this study, the correlation matrix was used with the focused principal component analysis (FPCA).

Results

Stoichiometric analysis of biochemical elements (C, N, P) and their ratios in phytoplankton and fish

Table 1 and 2 show the stoichiometry of carbons, nutrients and their ratios in phytoplankton and fish. During pre-growth, the concentrations of C, N and P in the phytoplankton varied from one pond to another, with C varying between 63.52 mol and 97.36 mol. N ranged from7.68 mol to 16.48 mol and P from 0.17 mol to 0.61 mol (Table 1).

Table 1. Stoichiometry of biochemical elements (C, N, P) of phytoplankton and their ratios during the pre-growth phase

Stations	Statistics	Chemical element content			Stoichiometric ratios		
		C (mol)	P (mol)	N (mol)	C:P	C:N	N:P
AP1	MIN	68,73	0,27	9,21	194,32	7,05	27,55
	MAX	91,64	0,35	10,59	345,45	8,71	39,92
	MOY	80,18	0,30	9,85	274,38	8,14	33,33
	ECAR	11,45	0,05	0,70	75,97	0,94	6,22
AP2	MIN	76,02	0,27	8,73	143,29	7,55	18,98
	MAX	91,64	0,53	11,31	345,45	9,78	42,65
	MOY	84,35	0,36	10,04	266,75	8,47	31,17
	ECAR	7,86	0,15	1,29	108,26	1,16	11,85
AG1	MIN	68,73	0,47	11,64	129,55	5,90	21,94
	MAX	97,36	0,62	13,62	207,76	7,49	27,73
	MOY	85,74	0,54	12,75	161,51	6,70	23,89
	ECAR	15,06	0,08	1,01	41,02	0,79	3,33
AG2	MIN	63,52	0,53	11,64	119,45	5,46	19,59
	MAX	91,12	0,62	13,19	147,21	6,91	21,94
	MOY	76,19	0,59	12,32	128,80	6,15	20,95
	ECAR	13,94	0,05	0,79	15,95	0,73	1,22
AN1	MIN	68,21	0,18	8,24	257,13	7,33	35,09
	MAX	91,64	0,27	9,31	414,55	11,12	47,57
	MOY	77,01	0,22	8,65	358,05	8,97	39,98
	ECAR	12,76	0,04	0,57	87,61	1,95	6,67
AN2	MIN	64,56	0,21	8,00	200,21	5,90	26,32
	MAX	96,84	0,35	10,95	456,35	12,10	37,70
	MOY	77,40	0,29	9,42	288,39	8,54	33,13
	ECAR	17,12	0,07	1,48	145,52	3,21	6,02

Table 2. Stoichiometry of biochemical elements (C, N, P) and their ratios in fish during the pre-growth phase

Stations	Statistics	Chemical element content			Stoichiometric ratios		
		%P	%N	%C	C/P	C/N	N/P
AP1	MIN	0,63	7,80	35,75	56,35	3,84	10,81
	MAX	0,98	14,78	59,36	73,00	6,75	15,08
	MOY	0,78	10,64	49,26	63,30	4,87	13,53
	ECAR	0,18	3,67	12,17	8,66	1,63	2,36
AP2	MIN	0,64	7,75	33,30	51,86	3,80	10,81
	MAX	0,98	14,65	60,11	72,32	6,69	14,95
	MOY	0,78	10,39	48,43	61,83	4,86	13,14
	ECAR	0,18	3,72	13,73	10,24	1,59	2,12
AG1	MIN	0,51	2,92	12,23	17,73	3,31	4,23
	MAX	0,69	12,24	40,48	68,25	4,19	20,63
	MOY	0,60	7,28	26,45	46,00	3,83	12,64
	ECAR	0,09	4,69	14,13	25,79	0,46	8,21
AG2	MIN	0,51	2,93	12,21	17,76	3,29	4,26
	MAX	0,69	11,36	37,34	63,50	4,17	19,32
	MOY	0,60	7,34	27,07	47,68	3,85	12,89
	ECAR	0,09	4,23	13,17	25,93	0,49	7,77
AN1	MIN	0,42	4,22	15,90	37,76	3,77	10,02
	MAX	0,68	18,59	75,64	110,96	4,07	27,27
	MOY	0,55	10,97	43,88	74,68	3,94	18,76
	ECAR	0,13	7,23	30,05	36,60	0,15	8,63
AN2	MIN	0,43	4,05	15,34	35,64	3,79	9,41
	MAX	0,68	17,97	73,40	107,79	4,08	26,39
	MOY	0,55	10,90	43,57	73,57	3,93	18,49
	ECAR	0,13	6,96	29,06	36,22	0,15	8,56

The mean C/P and N/P ratios of phytoplankton (Table 2) present in ponds receiving powdered feed (AP1: 274.38; 33.33 and AP2: 266.75; 31.17) and those receiving no exogenous feed (AN: 358.05; 39.98 and AN2: 288.39; 33.13) are significantly high, on the other hand the C/P and N/P ratios of phytoplankton from ponds receiving granulated feed (AG: 161.51; 21.97 and AG2: 128.80; 23.42) are low. Concerning the average C/N ratios, all the ponds AP1: 8.14; AP2: 8.47; AN: 8.97; AN2: 8.54 and AG1: 6.69; AG2: 6.15 are substantially similar. For fish, C concentrations ranged from 12.17 to 75.64. N ranged from 2.92 to 18.59 and P from 0.09 mol to 0.98 (Table 2). The mean C/P ratios of the fish (Table 2) were significantly low for both fed and unfed fish (AP1: 63.30; AP: 61.83; AG1: 45.99; AG2: 47.68; AN1: 74.68; AN2: 73.57). However, fish fed with local feed had a lower C/P ratio. The C/N ratios of all the fish were also low (AP1: 4.87; AP2: 4.86; AG1: 3.83; AG2: 3.85; AN1: 3.94; AN2: 3.93). With regard to the mean N/P ratios, it was the fish receiving the powdered feed (20.85; 20.14) and not fed (18.76; 18.49) that were significantly higher than those receiving the granulated feed (13.52; 13.13). The Kruskal-Wallis test on the mean C/P (p=0.0003486), C/N (p=0.0006748) and N/P (p=0.0006748) ratios of phytoplankton (Fig. 2) and fish (Fig. 3) indicated that the concentrations of these ratios varied significantly within the ponds (P <0.005).

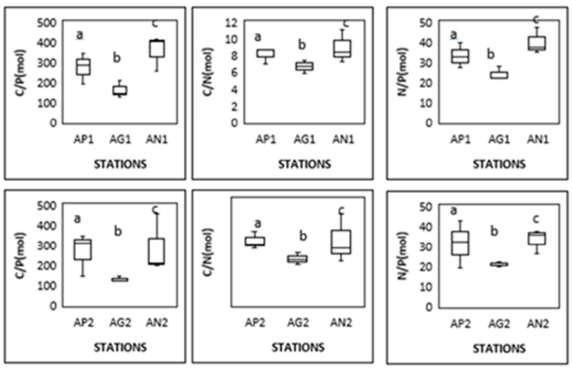

Fig. 2. C/P, C/N, and N/P Ratios of phytoplankton during the pre-growth phase

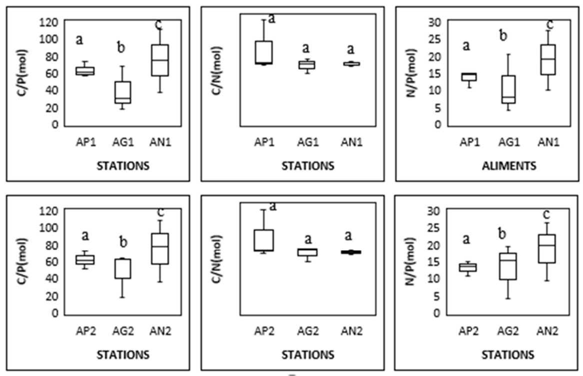

Fig. 3. C/P, C/N and N/P ratios of fish during the pre-growth phase

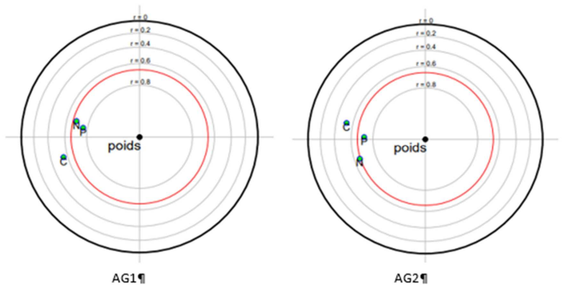

Fig. 4. Focused principal component analysis of nitrogen, phosphorus, carbon and weight of fish fed pelleted feed in the two trials AG1 (trial 1) and AG2 (trial 2)

Influence of nutrients on the weight of pre-pregnancy fish

The average weights (Pm) achieved by fish fed with powdered feed in the first trial (AP1) ranged from 23.5±4.36 g to 74.71±3.58 g and those in the second trial (AP2) from 30.6±5.67 g to 80±3.86 g. The mean weights of the unfed fish (AN1 and AN2) ranged from 15.6±2.05 g to 41.94±8.74 g and from 18.8±2.47 g to 56.45±11.77 g respectively. However, the mean weights of the fish fed the pelleted feed (AG1: 35.6±1.2 g - 87,21±5.28 g) and (AG2: 37.4±1.3 g - 95,46±6.09 g) were higher than those of the fish fed the powdered feed and those not fed. Focused principal component analysis (FPCA) between fish weight and chemical elements showed the influence of nutrients (N, P) on the weight of fish. Weight was positively and significantly correlated with phosphorus in ponds fed pelleted feed (Fig. 4). The weight of fish fed powdered feed was positively and significantly correlated with nitrogen (Fig. 5). For fish not fed, weight was positively and significantly correlated with all elements (Fig. 6).

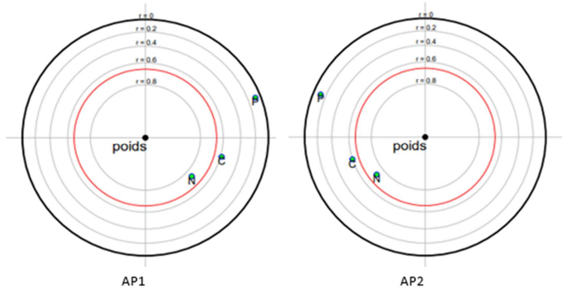

Fig. 5. Focused principal component analysis of nitrogen, phosphorus, carbon and weight of fish fed powdered feed in the two trials AP1 (trial 1) and AP2 (trial

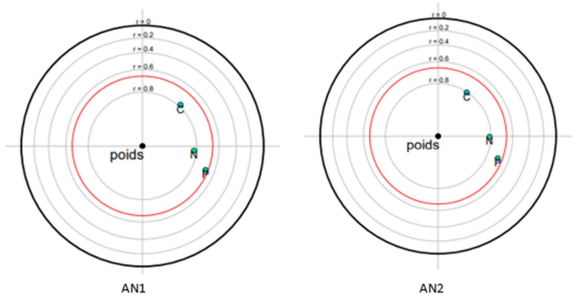

Fig. 6. Focused principal component analysis of nitrogen, phosphorus, carbon and weight of unfed fish in the two trials AN1 (trial 1) and AN2 (trial 2)

Discussion

Carbon and nutrient stoichiometry showed that during pre-growth the mean C/P (274.38; 358.04) and N/P (33.3; 39.97) ratios of phytoplankton present in ponds receiving powdered feed and ponds not fed were high. These results are in line with the findings of Elser *et al.* (2000) who obtained a high average C/P ratio of 307 and N/P of 30.2 in freshwater. This indicates a certain richness of carbon and nitrogen in relation to phosphorus in these environments. According to Tanioka *et al.* (2020), low phosphorus inputs can lead to high C/P ratios in phytoplankton.

However, the phytoplankton present in ponds receiving granulated feed are rich in phosphorus because the C/P and N/P ratios (161.50; 22.42) are low. The average C/N ratios (AG: 6.43; AP: 8.31; AN: 8 .75) of all the ponds are low, which shows that nitrogen is more important than carbon in the various ponds. Indeed, the C/N ratio of phytoplankton decreases with increasing nutrient levels (Galbraith and Martiny, 2015). On the other hand, the average C/P ratios (AG: 46.86; AP: 62 .56; AN: 74.13) and C/N (AG: 3.84; AP: 4.86; AN: 3.94) of the fish are all low, so they are richer in nitrogen and phosphorus than in carbon. However, the average N/P ratios are only low in fish receiving pelleted feed (12.76) and high in fish receiving powder feed (20.50) and fish receiving no external feed (18.62). The average C/P, C/N and N/P ratios show that the fish fed the pelleted feed are rich in phosphorus, whereas those fed the powder feed and those fed no feed are richer in nitrogen. These results corroborate those of Guo *et al.* (2018) and could be due to muscle growth and bone tissue growth in relation to greater lipid synthesis. Indeed, according to Guo *et al.* (2018), lipids are the essential reservoir of carbon storage, in addition, muscle tissue is the reservoir of nitrogen while RNA and bone tissue are the main reservoir of phosphorus (Boros *et al.*, 2015). The average C/P, C/N and N/P ratios show that fish fed with the industrial feed are richer in phosphorus, those fed with the powder feed and those not fed with exogenous feed are richer in nitrogen. This information corroborates the correlation observed between the weight of fish fed with granulated feed and phosphorus, and between that of nitrogen and fish fed with powdered feed and fish not fed.

Conclusion

Our stoichiometric examination reveals that during pre-growth, ponds receiving pelleted feed have a high phosphorus content, which translates into the presence of more phosphorus in the flesh of the fish. On the other hand, the ponds receiving the local feed and the ponds with no exogenous feed contained more nitrogen, reflecting the richness of nitrogen in the flesh of these fish. In nature, fish eat other organisms whose biochemical composition is fairly similar to their own.

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