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Carbon/nitrogen ratio as a control element in aquaculture systems

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Abstract

Controlling the inorganic nitrogen by manipulating the carbon/nitrogen ratios is a potential control method for aquaculture systems. This approach seems to be a practical and inexpensive means of reducing the accumulation of inorganic nitrogen in the pond. Nitrogen control is induced by feeding bacteria with carbohydrates, and through the subsequent uptake of nitrogen from the water, by the synthesis of microbial proteins. The relationship among the addition of carbohydrates, the reduction of ammonium and the production of microbial proteins depends on the microbial conversion coefficient, the C/N ratio in the microbial biomass, and the carbon contents of the added material. The addition of carbonaceous substrate was found to reduce inorganic nitrogen in shrimp experimental tanks and in tilapia commercial-scale ponds. It was found in tilapia ponds that the produced microbial proteins are taken up by the fish. Thus, part of the feed protein is replaced and feeding costs are reduced. The addition of carbohydrates, or the equivalent reduction of proteins in the feed, can be quantitatively calculated and optimised, as shown here. Approximate parameters were used in this work. Additional research in this field should be directed at gathering the precise data needed for the exact planning of feed composition.

Keywords: C/N; Ammonium; Inorganic nitrogen; Microbial proteins; Feeding

1. Introduction

1.1. General

One of the major water quality problems in intensive aquaculture systems is the accumulation of toxic inorganic nitrogenous species ($NH4^+$ and NO_2^-) in the water

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(Colt and Armstrong, 1981). Aquatic animals, such as fish and shrimp, excrete ammonium, which may accumulate in the pond. A major source of ammonium is the typically protein-rich feed. Aquatic animals need a high concentration of protein in the feed, because their energy production pathway depends, to a large extent, on the oxidation and catabolism of proteins (Hepher, 1988). In highly aerated ponds, ammonium is oxidised by bacteria to nitrite and nitrate species. Unlike carbon dioxide which is released to the air by diffusion or forced aeration, there is no effective mechanism to release the nitrogenous metabolites out of the pond. Thus, intensification of aquaculture systems is inherently associated with the enrichment of the water with respect to ammonium and other inorganic nitrogenous species. The management of such systems depends on the developing methods to remove these compounds from the pond.

One of the common solutions used to remove the excessive nitrogen is to frequently exchange and replace the pond water. This approach is limited for three reasons:

(a) Environmental regulations prohibit the release of the nutrient rich water into the environment;

(b) The danger of introducing pathogens into the external water;

(c) The high expense of pumping huge amounts of water.

Another approach is based upon means to encourage and enhance nitrification of the ammonium and nitrites to the relatively inert nitrate species. This is often done by employing biofilters, essentially immobile surfaces serving as substrates to the nitrifying bacteria. A high surface area with immobilised nitrifying biomass enables a high nitrifying capacity in a controlled environment. One problem associated with biofiltration is the high cost involved and the need to treat and digest a large mass of feed residues. Effectively, about 50% of the feed material added to the pond needs to be digested.

An additional strategy that is presently getting more attention is the removal of ammonium from the water through its assimilation into microbial proteins by the addition of carbonaceous materials to the system. If properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation. A further important aspect of this process is the potential utilization of microbial protein as a source of feed protein for fish or shrimp.

Utilization of microbial protein depends upon the ability of the animal to harvest the bacteria and its ability to digest and utilise the microbial protein. Neither are trivial. One obvious problem is determined by the minimal size of particles that can be taken up by the fish. Schroeder (1978) reported that carp can filter out particles larger than 20–50 μm. Odum (1968) reported that *Mugil cephalus* take up particles as small as 10 μm. An interesting observation was made by Taghon (1982), who found that benthic invertebrates were able to take up microscopic glass beads when they were coated with proteins. This demonstrates that the chemical nature of the particle may favor its harvesting by fish. The fact that relatively large microbial cell clusters are formed due to flocculation of the cells, alone or in combination with clay or feed particles (Harris and Mitchell, 1973; Avnimelech et al., 1982), additionally favors cell uptake by fish.

The adjustment of the C/N ratio in the feed, as a means to control the pond water quality, is presently under active research in many research centers (e.g., presentations in the last WAS meeting: McGoogan and Galtin, 1998; Rudacille and Kohler, 1998;

Conquest et al., 1998). The objectives of this paper are to formulate the basic reactions and mechanisms affecting this process; to demonstrate its potential; to develop the quantitative means needed to adjust the C/N ratio; and to control inorganic nitrogen accumulation in ponds.

1.2. Theory

The control of inorganic nitrogen accumulation in ponds is based upon carbon metabolism and nitrogen-immobilizing microbial processes. Bacteria and other microorganisms use carbohydrates (sugars, starch and cellulose) as a food, to generate energy and to grow, i.e., to produce proteins and new cells:

$$\text{organic C} \rightarrow CO_2 + \text{energy} + \text{C assimilated in microbial cells}. \quad (1)$$

The percentage of the assimilated carbon with respect to the metabolised feed carbon, is defined as the microbial conversion efficiency (E) and is in the range of 40–60% (Paul and van Veen, 1978; Gaudy and Gaudy, 1980). Nitrogen is also required since the major component of the new cell material is protein. Thus, microbial utilization of carbohydrate (or any other low nitrogen feed) is accompanied by the immobilization of inorganic nitrogen. This process is a basic microbial process and practically every microbial assemblage performs it.

The addition of carbohydrates is a potential means to reduce the concentration of inorganic nitrogen in intensive aquaculture systems. The amount of carbohydrate addition (ΔCH) needed to reduce the ammonium can easily be evaluated.

According to Eq. (1) and to the definition of the microbial conversion coefficient, E, the potential amount of microbial carbon assimilation, when a given amount of carbohydrate is metabolised (ΔCH), is:

$$\Delta C_{mic} = \Delta CH \times \%C \times E, \quad (2)$$

where ΔC_{mic} is the amount of carbon assimilated by microorganisms and %C is the carbon content of the added carbohydrate (roughly 50% for most substrates).

The amount of nitrogen needed for the production of new cell material (ΔN) depends on the C/N ratio in the microbial biomass which is about 4 (Gaudy and Gaudy, 1980):

$$\Delta N = \Delta C_{mic}/[C/N]_{mic} = \Delta CH \times \%C \times E/[C/N]_{mic}, \quad (3)$$

and (using approximate values of %C, E and $[C/N]_{mic}$ as 0.5, 0.4 and 4, respectively):

$$\Delta CH = \Delta N/(0.5 \times 0.4/4) = \Delta N/0.05. \quad (4)$$

According to Eq. (4), and assuming that the added carbohydrate contains 50% C, the CH addition needed to reduce total ammonia nitrogen (TAN) concentration by 1 ppm N (i.e., 1 g N/m^3) is 20 g/m^3.

A different approach is to estimate the amount of carbohydrate that has to be added in order to immobilise the ammonium excreted by the fish or shrimp. It was found that fish or shrimp in a pond (Avnimelech and Lacher, 1979; Boyd, 1985; Muthuwani and Lin, 1996) assimilate only about 25% of the nitrogen added in the feed. The rest is excreted as NH_4 or as organic N in feces or feed residue. It can be assumed that the ammonium

flux into the water, ΔNH_4, directly by excretion or indirectly by microbial degradation of the organic N residues, is roughly 50% of the feed nitrogen flux:

$$\Delta N = \text{feed} \times \%N\,\text{feed} \times \%N\,\text{excretion}. \quad (5)$$

A partial water exchange or removal of sludge reduces the ammonium flux in a manner that can be calculated or estimated. In zero exchange ponds, all the ammonium remain in the pond. The amount of carbohydrate addition needed to assimilate the ammonium flux into microbial proteins is calculated using Eqs. (4) and (5):

$$\Delta CH = \text{feed} \times \%N\,\text{feed} \times \%N\,\text{excretion}/0.05. \quad (6)$$

The C/N ratio, or the equivalent protein concentration of the feed, can be calculated using the derived Eq. (6). Assuming 30% protein feed pellets (4.65% N) and 50% of the feed nitrogen are excreted (%N excretion), we get:

$$\Delta CH = \text{feed} \times 0.0465 \times 0.5/0.05 = 0.465 \times \text{feed}. \quad (7)$$

According to Eq. (7), the feed having 30% protein should be amended by an additional portion of 46.5% made of carbohydrates with no protein. The corrected protein percentage should accordingly be:

$$\text{corrected protein percentage} = 30\%/1.465 = 20.48\%, \quad (8)$$

and the original C/N ratio (10.75 in the 30% protein feed) should be raised to 15.75.

2. Materials and methods

Several experimental results are presented, in order to demonstrate and substantiate the theoretical approach developed.

The basic process of microbial ammonium immobilization was demonstrated in a laboratory experiment where pond sediment suspension was enriched with ammonium salt. Twenty-gram samples of pond bottom (clay soil, from commercial tilapia pond) were shaken for 12 h with 1000 ml tap-water enriched with $(NH_4)_2SO_4$, at an initial concentration of about 10 mg/l, and 200 mg/l glucose. Samples were taken periodically and filtered. Ammonium concentrations were determined according to standard methods using an auto-analyzer (EPA, 1974).

The effects of carbohydrates addition on ammonium accumulation in a dense shrimp culture were tested in 25 m^2 indoor tanks stocked with 0.8 kg/m^2 *Penaeus monodon*. Shrimp were fed with pellets containing 40% protein at a daily rate of 2% body weight (i.e., 16 g feed, 6.4 g protein or daily 0.96 g N/m^2. It was assumed that 33% of the feed nitrogen is excreted. Sugar (glucose) or cassava meal were added at a rate of seven times the expected ammonium excretion, i.e., daily 2.2 g/m^2. The experiment was conducted in triplicates.

The effects of changing the C/N ratio in the feed on the growth and feed utilization in tilapia are shown in data adapted in part from previous research. In the first experiment (Avnimelech et al., 1989), tilapia grown in tanks were fed by either:

(I) Conventional feed pellets with 30% protein;

(II) Pellets made of wheat meal (10% protein); and

(III) Feeding with 10% protein pellets at one-half the daily ratios as compared to treatment (II), amended by daily additions of cellulose powder and $(NH_4)_2SO_4$.

Protein, fat and stable carbon isotopes were determined in fish tissue at the end of the experimental period.

The second experiment (Avnimelech et al., 1994) was a pond experiment where tilapia were grown in circular 50 m^2 ponds at a density of about 10 kg/m^2. Fish were fed using conventional 30% protein pellets ($C/N = 11.1$) or a tested formulation of low protein diet of 20% protein ($C/N = 16.7$). The daily feed addition was 2% of body weight for the conventional feed and 2.6%, to include carbohydrates needed for the microbial ammonium conversion, with the low protein pellets. The results presented here, partially recalculated from the original report, summarise two triplicated experiments.

3. Results and discussion

The effect of addition of carbohydrates on the immobilization of TAN was demonstrated in a laboratory experiment consisting of a sediment suspension amended with ammonium (about 10 mg/l) and glucose at a concentration 20 times higher than that of the TAN. It was found (Fig. 1) that almost all the added ammonium disappeared over a period of about 2 h, following a short lag period, with no concomitant production of NO_2^- or NO_3^- (not shown).

The addition of carbohydrates to control nitrogen concentrations in shrimp ponds was tested in tanks containing dense (0.8 kg/m^2) shrimp biomass. Sugar (glucose) and cassava meal were added to reduce TAN accumulation. The carbonaceous substrates additions were calculated assuming an ammonium excretion equivalent to 33% of the feed (a significant underestimation of ammonium excretion). The carbonaceous sub-

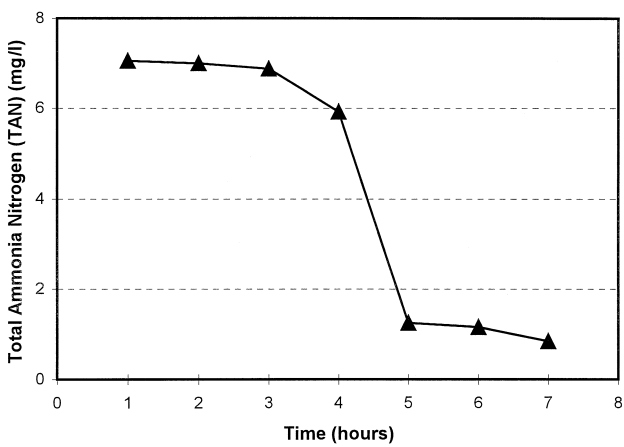

Fig. 1. Changes in TAN concentration in a suspension of pond bottom soil (2% dry soil) following the addition of glucose (TAN/glucose ratio of 1/20).

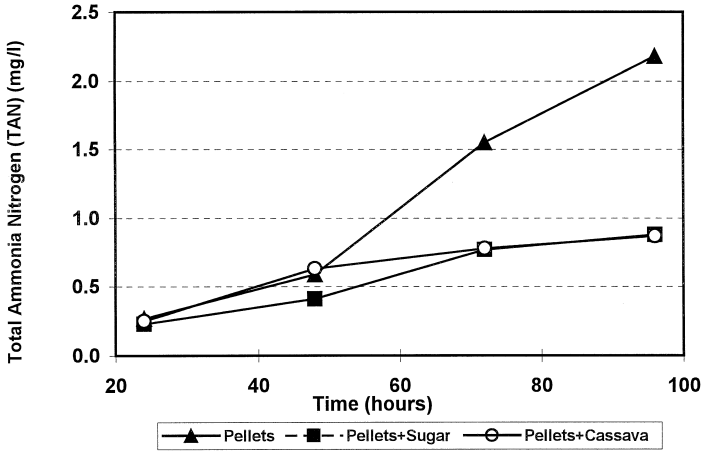

Fig. 2. Changes with time of TAN concentration in shrimp tank experiment. The shrimp biomass was 0.8 kg/m^2. The control tank was supplied with 40% protein pellets. Carbohydrates (glucose, cassava meal) were added daily at a rate of seven times the assumed TAN excretion by the shrimps.

strates addition led to a significant reduction in the accumulation of ammonium in the tanks (Fig. 2). Nitrates and nitrites were also reduced, from 1.97 mg $(NO_3 + NO_2) - N/l$ in the control treatment to 1.13 mg $(NO_3 + NO_2) - N/l$ in the treatment tank.

Results of tank experiment comparing growth and body composition of tilapia fed with (I) conventional pellets, (II) control, 10% protein pellets, made from wheat flour, and (III) 10% protein pellets + cellulose powder + $(NH_4)_2SO_4$ are given in Table 1. Detailed results were published elsewhere (Avnimelech and Mokady, 1988; Avnimelech et al., 1989). Bacterial flocculation was observed, probably supporting filtering out by the fish, and thus supplying protein that was available and suitable to fish nutrition. Though tilapia do not digest cellulose, it was found that the addition of cellulose supported fish growth, obviously through the ingestion and digestion of bacteria growing on the cellulose. Daily growth of tilapia fingerlings was 0.5, 0.12 and 0.33% for diets I,

Table 1
Tilapia feeding with microbial protein

		Treatment		
		I. Conventional pellets	II. Control pellets	III. Test pellets
Daily growth	%	0.5	0.12	0.33
Protein	% in muscle	15.5	14.2	15.9
Fat	% in muscle	4.2	4.3	2.6
$\delta^{13}C$ in feed	%	−13.8	−14.8	−23.5 (cellulose)
$\delta^{13}C$ in fish muscle	%	−20.8	−20.5	−23.0

Growth and feed utilization data for fish fed with I. conventional 30% protein pellets; II. 10% protein pellets; and III. test treatments of 10% protein pellets + cellulose powder + $(NH_4)_2SO_4$, as a substrate for the production of microbial proteins.

II and III, respectively. Protein content of the fish in the control treatment (II) was low, yet that in fish fed with microbial protein (III) was as high as with conventional feeding (II). Carbon isotopes distribution in the feed materials and fish tissues indicated that the fish digested and ingested carbon derived from the cellulose, most probably by uptake of the microbial proteins.

Following the pilot scale work, a series of pond scale experiments was conducted. (Results presented here are adapted from Avnimelech et al., 1992, 1994.) It was found that the addition of carbohydrates, essentially changing the 30% protein feed material to 20% protein feed, led to:

(a) a significant reduction of inorganic nitrogen accumulation;

(b) increased utilization of protein feed;

(c) a significant reduction of feed expenditure.

Fish growth and feed utilization data in two triplicated pond experiments (50 m^2 ponds stocked with tilapia hybrids at a density of 80 fish/m^2) are given in Table 2. It can be seen that fish growth was better in the 20% protein treatment, most likely due to the lower concentrations of toxic inorganic nitrogen species. In addition to a lower feed conversion ratio (FCR), the protein conversion ratio (PCR) was markedly reduced in the 20% protein treatment. The PCR in the conventional 30% protein feed treatment was 4.35–4.38, meaning that only 23% of the feed protein was recovered by the fish. The

Table 2
Fish growth and yield coefficients of tilapia fed with conventional pellets (30% protein, C/N = 11.1) and low protein pellets (20% protein, C/N = 16.6) in two pond experiments

	Treatment	
	Conventional feeding (30% protein)	C-enriched (20% protein)
Experiment no. 1: 51 days, average of three replicates		
Feed C/N ratio	11.1	16.6
Fish weight (g/fish)		
Initial weight	112	112
Final weight	193	218
Daily gain*	1.59[a]	2.0[b]
Mortality (%)	14.6	10.3
Feed conversion coefficient	2.62	2.17
Protein conversion coefficient	4.38	2.42
Feed cost coefficient (US$/kg fish)	0.848	0.583
Experiment no. 2: 30 days, average of three replicates		
Fish weight (g/fish)		
Initial weight	205	205
Final weight	254	272
Daily gain*	1.63[a]	2.22[b]
Mortality (%)	3.4	0
Feed conversion coefficient	2.62	2.02
Protein conversion coefficient	4.35	2.18
Feed cost coefficient (US$/kg fish)	0.848	0.543

* Values not sharing a common letter differ significantly ($p < 0.05$).

PCR in the tested treatment was 2.2–2.4, i.e., protein utilization was twice as high. The increased protein utilization is due to its recycling by the microorganisms. It may be said that the proteins are eaten by the fish twice, first in the feed and then harvested again as microbial proteins. It is possible that protein recycling and utilization can be further increased.

Due to the fact that proteins are the expensive component of the feed, its reduction was reflected in the feed price which decreased from US$0.85/kg fish to about US$0.55/kg (Table 2). Similar results were obtained recently in the desert aquaculture farms that are operated following principles presented here (Avnimelech et al., unpublished data).

4. Conclusions

Controlling the inorganic nitrogen by manipulating the carbon/nitrogen ratios is a potential control method for aquaculture systems. This approach seems to be a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in the pond. Such a strategy can be practiced as an emergency response, i.e., addition of a carbonaceous substrate in case of increased ammonium concentration. It is possible to add cheap sources of carbohydrates (e.g., cassava meal, flor) in cases such as a series of cloudy days slowing down algae growth, or severe algae crash. However, additional pond aeration may be required to compensate for the additional oxygen consumption. The conventional control means for ponds are to intensively exchange the water, a strategy that is not always practical, and to stop feeding to slow down TAN build up. The proposed method enables keeping a high biomass and to have a corrective means in case of a failure of conventional controls.

A more advanced approach is to adjust the protein level in the feed so as to avoid the build up of inorganic nitrogen in the water. This approach was tested and proven successful in intensive ponds that are continually mixed and aerated. The intensive culture of fish in these ponds is based on a system that is similar to biotechnological reactors. Such systems are amendable to a set of controls similar to biotechnological controls (Avnimelech, 1998). The ability to control inorganic nitrogen concentrations through the manipulation of C/N ratios in the system is one example of such a control. The addition of carbohydrates was done as a part of the feeding scheme. In this case, the addition of carbonaceous substrate leads to the recycling and increased utilization of proteins through the utilization of the microbial proteins. Production and utilization of microbial proteins (SCP, single-cell protein) have been studied extensively during the last few decades (e.g., Tannenbaum and Wang, 1975). The major problem involved in economically sound utilization of SCP cultures is the harvesting, dehydration and packaging of the material. In contrast, for in situ microbial protein culturing in the pond, all of these expensive processing stages are not needed since harvesting is done by the fish, as part of the system.

The applicability of the same approach in earthen stagnant ponds is not trivial and has to be further studied in conventional fish and shrimp ponds. The addition of carbohydrates to the feed may result in an accelerated sedimentation of organic matter to the

pond bottom, where the microbial biomass will not be utilised by the fish and will increase the organic load in the pond.

The addition of carbohydrates, or the equivalent reduction of proteins in the feed, can be quantitatively calculated and optimised, as shown in Eqs. (6)–(8). However, approximate parameters were used in this work. Additional research in this field should be directed at gathering precise data needed for the exact planning of feed composition and feeding rate.

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