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Effects of stocking density on growth, yield and profitability of farming Nile tilapia, *Oreochromis niloticus* L., fed *Azolla* diet, in earthen ponds

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Abstract

Two consecutive experiments were conducted to study the effects of stocking density on growth, food utilization, production and farming profitability of Nile tilapia (*Oreochromis niloticus*) fingerlings (initial mean weight: 16.2 ± 0.2 g) fed *Azolla*, as a main component in diet. In experiment 1, fish were hand-fed twice daily with three isonitrogenous (28.5% crude protein) and isocaloric (14.5 kJ g^{-1}) diets A30, A35 and A40 containing 30%, 35% and 40% *Azolla*, respectively, for 90 days. Diets were formulated by mixing *Azolla* with locally available by-products. No significant differences were found in growth parameters and production ($P > 0.05$). Total investment cost was significantly higher with A30 ($P < 0.05$), but same profitability values were obtained with all diets ($P > 0.05$). In experiment 2, three stocking densities, 1, 3 and 5 m^{-2}, were assigned to three treatments T_1, T_2 and T_3 respectively. Fish were hand-fed twice daily with diet A40. The final mean weight (89.53–115.12 g), the mean weight gain (0.81–1.10 g day^{-1}), the specific growth rate (1.90–2.20% day^{-1}) and the apparent food conversion ratio (1.29–1.58) were affected by stocking density, with significant difference ($P < 0.05$) at 5 m^{-2}, compared with the other densities. Stocking density did not affect survival rate ($P > 0.05$). Yield and annual production increased with increasing stocking density, ranging from 7.10 ± 0.90 to 25.01 ± 1.84 kg are^{-1} and 28.79 ± 3.66 to 101.42 ± 7.48 kg are^{-1} year^{-1}, respectively, with significant differences between all densities ($P < 0.05$). Higher stocking density resulted in higher gross return and lower cost of fish production, with significant variations ($P < 0.05$). The net return increased with increasing stocking density ($P < 0.05$). However, both densities of 3 and 5 m^{-2} produced the same profitability values. On the basis of growth values and economic return, it was concluded that Nile tilapia could be raised at a density of 3 fish m^{-2} with A40 to improve production and generate profit for nutritional security and poverty alleviation in rural areas.

Keywords: stocking density, growth, production, food utilization, profitability, *Azolla*, *Oreochromis niloticus*, earthen pond

Introduction

Fish is a main source of animal protein in Southern Bénin. In recent years, natural stocks have declined due to environmental perturbations and man-made changes through 'acadjas systems', dumping of agrochemical and industrial pollutants, and indiscriminate and destructive fishing practises in lakes and lagoons. These not only reduces abundance and fish size but also results in an increased price on the market. As expected, fish consumption *per capita* has dropped, to about 9.4 kg year^{-1} nowadays (FAO 2006). Therefore, further growth in aquaculture production is needed to maintain the present *per capita* supply of aquatic products in the future, given that the supply through traditional fisheries cannot grow any further (FAO 2000). Southern Bénin has a large area of wetlands of about 2000 km^2, which are considered to be suitable for fish farming and where, unfortunately, these activities practiced in rural areas have failed due to high-feed cost. Indeed, fish were fed with diets containing high-cost fish meal with fluctuating quality (Olvera-Novoa, Capos, Sabido & Martinez Palacios 1990). According to FAO (2002), the long-term sustainability of aquaculture may be threatened by its present over-dependance on fish meal and fish oil. Thereby, development of viable and low-cost technologies could help to promote aquaculture in the area, as well as increase yields and profitability. In that way, many efforts have concentrated on replacing animal protein sources such as fish meal with cheaper plant protein in fish diets (Ng & Wee 1989; Pouomogne, Takam & Pouemegne 1997; Fasakin, Balogun & Fasuru 1999; Fasakin, Balogun & Fagbenro 2001; Adebayo, Fagbenro & Jegede 2004). Recently, the successful use of the aquatic fern *Azolla* as a component of fish diets has been widely demonstrated in both aquaria and tanks (El-Sayed 1992, 1999; Leonard, Breyne & Michaj 1998; Fiogbé, Micha & Van Hove 2004). However, data on its use in semi-intensive systems are very scarce. Our previous results showed that *Azolla* could be incorporated in more than 20% in diet for Nile tilapia *Oreochromis niloticus* (Abou, Fiogbé & Micha, in press). Therefore, to minimize diet costs and enhance profit, high proportions of this fern could be used to formulate low-cost practical diets for *O. niloticus*. Nevertheless, Micha and Leonard (2001–2002) suggested a level of 50% in diet as an upper limit for maintaining growth of Nile tilapia.

In Bénin rural areas, as in most developing countries, human population growth is outpacing animal production. To meet current needs for fish products, farmers resort to increase fish stocking density. There is no research literature on tilapia stocking density in Bénin wetlands. However, it is well established that overstocking can cause stress, which leads to enhanced energy requirements and causes reduced growth (Siddiqui, Howlader & Adam 1989) and food utilization (Leatherland & Cho 1985), but understocking will result in failure to maximize production.

Hence, the aims of this study are to determine the effects of stocking density on growth, production and farming profitability of Nile tilapia fed low-cost *Azolla*-diet. It is expected that both fish management practices sustain production and improve revenue for nutritional security and poverty alleviation.

Materials and methods

Study site and experimental design

The two experiments were carried out at the rural demonstration site (6°29′15.12″N 2°37′6.42″E) of the Research Unit on Wetlands of Abomey-Calavi University, in Porto-Novo suburb, Bénin, West Africa. They were conducted in succession in the same set of nine stagnant earthen ponds with an average surface area of 100 m^2 and a depth of 1 m. Ponds were filled continuously from the water table. They were randomly assigned to three treatments. In experiment 1, fish were stocked at 1 m^{-2} and each treatment receives one of the three diets A30, A35 and A40. The daily ration was calculated according to Mélard (1986) and adjusted fortnightly on the basis of fish biomass. Daily rations were divided into two parts, each was hand-distributed at 08:00 and 16:00 hours, respectively, for 90 days. In experiment 2, three stocking densities, 1, 3 and 5 m^{-2}, were assigned to three treatments T_1, T_2 and T_3 respectively. Fish were fed with diet A40, as used in experiment 1. Daily rations were divided into two parts, each hand distributed at 08:00 and 16:00 hours, respectively, for 90 days. Daily rations were calculated according to Mélard (1986) and adjusted fortnightly.

Diet formulation and methods for biochemical analysis

Three isonitrogenous (28.5% CP) and isocaloric (14.5 kJ kg^{-1}) diets were formulated containing 30% (A30), 35% (A35) and 40% (A40) *Azolla*, using locally available fish meal, maize bran, palmseed cake, cottonseed cake, brewery draff and the aquatic fern *Azolla*. The *Azolla* strain used is *Azolla filiculoides* Lam., which is the most suitable strain for fish and the more productive and easiest to grow under local conditions.

Proximate composition of ingredients, formulation and proximate composition of experimental diets are shown in Tables 1 and 2. One-half litre of warm water in which the binder was dissolved was added to 1 kg of each diet thus formulated and mixed. The dough obtained after 2 min of mixing was cut in paste and sun-dried at a temperature of 30–35 °C for about 2 days. After drying, the paste is manually broken into smaller particles of about 5 mm (passage through a 5 mm sieve). These were preserved in the refrigerator (-1 °C) until used for feeding fish.

Table 1 Proximate composition of ingredients (% dry matter basis) used in preparation of the diets for feeding *Oreochromis niloticus* in stagnant earthen ponds

Ingredients*	Fish meal	*Azolla* meal	Cottonseed cake	Maize bran	Brewery draff	Palmseed cake
Dry matter	91.51	90.03	90.70	90.48	91.16	89.66
Crude protein	40.18	28.51	36.43	11.50	22.47	18.00
Crude fat	13.41	3.97	8.96	7.99	5.43	4.75
Ash	31.78	12.67	6.31	3.97	3.01	3.82
Crude fibre†	1.00	9.60	14.00	12.30	16.00	15.10
NFE‡	5.14	35.28	25.00	54.72	44.25	47.99

*Available locally, supplied by Ovograin Feeds Depot (Abomey-Calavi, Bénin, West Africa).

†According to Ovograin Feeds Depot (Abomey-Calavi, Bénin, West Africa) and Leonard (1997) for ingredients and *Azolla filiculoides* respectively.

‡Nitrogen-Free Extract (NFE) calculated as: 100 − (%moisture+%protein+%lipid+%ash+%crude fibre).

Table 2 Formulation (%) and proximate composition (% dry matter) of experimental diets used for feeding *Oreochromis niloticus* in stagnant earthen ponds

	Experimental diets		
	A30	A35	A40
Ingredients			
Fish meal	15	10	10
Azolla	30	35	40
Maize bran	8	6	6
Cottonseed cake	22	30	27
Palmseed cake	8	8	4
Brewery draff	15	09	11
Binder*	1	1	1
Salt (NaCl)	1	1	1
Proximate composition			
Dry matter	90.36	90.07	90.20
Crud protein	28.62	28.46	28.50
Lipid	7.08	6.55	6.40
Ash	11.18	10.65	11.22
Crude fibre†	10.70	11.05	10.82
NFE‡	32.78	33.36	33.26
Gross energy§ (kJ g^{-1})	14.91	14.78	14.71
Cost of feed¶ (US$ kg^{-1})	0.39	0.34	0.33

*Cassava starch was used as binder.

†Calculated according to Ovograin Feeds Depot (Abomey-Calavi, Bénin, West Africa) and Leonard (1997) for ingredients and *Azolla filiculoides* respectively.

‡Nitrogen-Free Extract (NFE) calculated as: 100 − (%moisture+%protein+%lipid+%ash+%crude fibre).

§According to Tacon (1990).

¶Including handling and process (for comparison, US$ 1 = CFA 494.82, December 2006).

Ingredients and experimental diets analysis were performed using the nitrogen content method (N × 6.25, Kjeldahl method) for crude protein, and the Soxhlet apparatus method according to Folch, Lees and Sloane-stanley (1957) for lipid content. Total ash content was determined by samples incineration at 550 °C for 12 h. Moisture was measured by drying samples in an oven at 105 °C for 24 h (AOAC 1990). Gross energy was calculated using conversion factors of 23.0, 38.1 and 17.2kJ g^{-1} for protein, lipids and carbohydrates (Tacon 1990).

Water quality

During both experiments, water-quality parameters were monitored fortnightly in the ponds. Water temperature, dissolved oxygen (DO) and pH were measured at a depth of 10 cm at the following times: 08:00, 11:00, 14:00 and 17:00 hours, using an oxythermometer (WTW Oxi 197i, WTW, Weilheim, Germany, precision: ± 0.01 °C and ± 0.01 mg L^{-1}) and a pH metre (WTW pH 330i, precision: ± 0.01) respectively. Values for water transparency were obtained using a Secchi disc. Nitrate, nitrite, ammonium and orthophosphates levels were measured using spectrophotometric methods according to APHA (1992). Estimation of chlorophyll *a* concentration was carried out according to standard methods described in APHA (1992). To estimate zooplankton abundance in experimental ponds, 20 L of water were collected in three points of each pond and filtered through a 55 μm plankton net to obtain a concentrated sample. The samples were preserved immediately with 5% formalin. The absolute abundance of zooplankton was estimated by counting samples in a Dolfus cell under a binocular magnifying glass (× 40).

Estimation of growth, survival, production and feed utilization

For each experiment, about 40% of fish in ponds were sampled fortnightly and weighed to calculate the individual mean weight and to adjust the daily ration for the following 2 weeks. At the end of the trials, the fish were harvested using repeated netting, and counted. Growth in terms of final mean weight (FMW), daily weight gain (DWG), specific growth rate (SGR) and apparent food conversion ratio (AFCR) were estimated. Daily weight gain was calculated as total weight gain/experiment duration. Specific growth rate and AFCR were calculated as follows, according to Brown (1957) and Castell and Tiews (1980) respectively:

$$\text{SGR}\ (\%\ \text{day}^{-1}) = (\ln W_2 - \ln W_1) \times 100/(T_2 - T_1)$$

where W_1 is the initial live body weight (g) at time T_1 (day) and W_2 is the final live body weight (g) at time T_2 (day):

$$\text{AFCR} = \text{Feed fed (dry weight)/Live weight gain}$$

Survival rate, net yield and annual production were evaluated as described below

$$\text{Survival rate}\ (\%) = \text{Number of fish harvested} \times 100/\text{Number of fish stocked}$$

$$\text{Net yield}\ (\text{kg are}^{-1}) = \text{Biomass gain (kg)}/\text{surface area (are)}$$

$$\text{Production}\ (\text{kg are}^{-1}\ \text{year}^{-1}) = [\text{Biomass gain (kg)} \times 365]/[\text{surface area (are)} \times \text{time (days)}]$$

Economical evaluation

For economical analyses of each treatment, the results of the experiments were extrapolated to 1 year, assuming that 1 man-day (8 h) of labour is US\$ 4.04 (for comparison, US\$ 1 = CFA 494.82, at December 2006). The cost of feed ingredients and the cost of fingerlings were based on the wholesale market price during the experimental periods. Values of total investment cost (*TC*) consisted of variable costs (*VC*) and fixed costs (*FC*). The gross return (*GR*) (value of total output) was based on the price of US\$ 2.43 kg $^{-1}$ of unprocessed fresh fish, as marketable-size tilapia sold on the national market. Then the following profitability indicators were calculated:

$$\text{Return above variable costs (RAVC)} = GR - VC$$

$$\text{Net return}\ (NR) = GR - TC$$

$$\text{Profitability} = NR/TC$$

Statistical analysis

The mean values for growth parameters, survival, yield, production, water quality, chlorophyll *a* and zooplankton abundance for each treatment in each experiment were tested using one-way analysis of variance (ANOVA 1) after verifying the homogeneity of variance using 'Hartley's test' (Hartley 1959). Before analysing survival rate, percentage data were arcsin transformed. Significant results from ANOVA 1 test were further analysed using Duncan's New Multiple Range Test (Duncan 1955) to detail any difference among treatments. All statistical analyses were performed with SPSS (SPSS, Chicago, Illinois, USA).

Results

Experiment 1

There were no significant differences in water quality among treatments (Table 3). Also, growth para-

Table 3 Mean values (± SD) of water-quality parameters in the ponds during both experiments

	Experiment 1 (*Azolla* level)			Experiment 2 (fish m $^{-2}$)		
Parameters	A30	A35	A40	1	3	5
Secchi disc (cm)	42.2 ± 8.8^{a}	40.4 ± 7.2^{a}	45.1 ± 2.1^{a}	35.0 ± 1.4^{a}	42.4 ± 5.6^{b}	51.3 ± 1.1^{c}
Temperature (°C)	28.8 ± 0.2^{a}	28.9 ± 0.1^{a}	29.0 ± 0.1^{a}	28.9 ± 1.7^{a}	28.7 ± 1.8^{a}	28.8 ± 1.7^{a}
pH	6.61 ± 0.11^{a}	6.40 ± 0.29^{a}	6.46 ± 0.20^{a}	6.56 ± 0.11^{a}	6.45 ± 0.29^{a}	6.42 ± 0.20^{a}
Dissolved oxygen (mg L $^{-1}$)	4.48 ± 0.24^{a}	4.66 ± 0.26^{a}	4.44 ± 0.10^{a}	5.15 ± 0.87^{a}	4.02 ± 0.63^{b}	3.20 ± 0.71^{c}
Nitrates (mg L $^{-1}$)	0.12 ± 0.01^{a}	0.10 ± 0.02^{a}	0.11 ± 0.02^{a}	0.24 ± 0.05^{a}	0.29 ± 0.01^{a}	0.30 ± 0.06^{a}
Nitrites (mg L $^{-1}$)	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}
Ammonium (mg L $^{-1}$)	0.46 ± 0.07^{a}	0.42 ± 0.01^{a}	0.48 ± 0.07^{a}	0.49 ± 0.03^{a}	0.74 ± 0.16^{b}	1.10 ± 0.07^{c}
Orthophosphates (μg L $^{-1}$)	2.22 ± 0.15^{a}	2.53 ± 0.41^{a}	2.32 ± 0.23^{a}	2.48 ± 0.69^{a}	2.80 ± 0.40^{a}	2.79 ± 0.78^{a}
Chlorophyll *a* (μg L $^{-1}$)	14.9 ± 2.4^{a}	14.5 ± 1.6^{a}	13.5 ± 1.9^{a}	17.38 ± 2.15^{a}	15.26 ± 1.40^{b}	11.55 ± 1.89^{c}
Zooplankton (number L $^{-1}$)	933 ± 102^{a}	848 ± 105^{a}	894 ± 174^{a}	906 ± 80^{a}	708 ± 71^{b}	561 ± 48^{c}

In each line for each experiment, means with the same letters as superscripts are not significantly different ($P > 0.05$).

Table 4 Growth performance, survival, production and feed utilization of Nile tilapia (*Oreochromis niloticus*) fed *Azolla* diets (experiment 1) and reared at three stocking densities (experiment 2) for 90 days in stagnant earthen ponds

	Experiment 1 (*Azolla* level)			Experiment 2 (fish m^{-2})		
Parameters	**A30**	**A35**	**A40**	**1**	**3**	**5**
Initial weight (g)	16.53 ± 0.25^{a}	16.33 ± 0.32^{a}	16.40 ± 0.26^{a}	15.87 ± 0.40^{a}	16.10 ± 0.46^{a}	16.20 ± 0.36^{a}
Final weight (g)	116.71 ± 3.41^{a}	111.90 ± 5.43^{a}	109.36 ± 7.98^{a}	115.12 ± 6.00^{a}	108.25 ± 6.30^{a}	89.53 ± 4.50^{b}
Weight gain (g day^{-1})	1.11 ± 0.04^{a}	1.06 ± 0.06^{a}	1.03 ± 0.09^{a}	1.10 ± 0.07^{a}	1.02 ± 0.07^{a}	0.81 ± 0.05^{b}
SGR (% day^{-1})	2.17 ± 0.03^{a}	2.14 ± 0.03^{a}	2.11 ± 0.09^{a}	2.20 ± 0.09^{a}	2.12 ± 0.07^{a}	1.90 ± 0.05^{b}
Survival (%)	74.67 ± 2.52^{a}	75.33 ± 4.04^{a}	74.33 ± 0.58^{a}	75.33 ± 3.79^{a}	78.89 ± 3.72^{a}	74.00 ± 3.34^{a}
Yield (kg are^{-1})	7.06 ± 0.37^{a}	6.78 ± 0.17^{a}	6.49 ± 0.55^{a}	7.10 ± 0.90^{a}	20.82 ± 2.37^{b}	25.01 ± 1.84^{c}
Production (kg are^{-1} year^{-1})	28.63 ± 1.48^{a}	27.51 ± 0.71^{a}	26.31 ± 2.21^{a}	28.79 ± 3.66^{a}	84.42 ± 9.62^{b}	101.42 ± 7.48^{c}
AFCR	1.19 ± 0.05^{a}	1.22 ± 0.03^{a}	1.23 ± 0.10^{a}	1.29 ± 0.08^{a}	1.34 ± 0.07^{a}	1.58 ± 0.07^{b}

In each line for each experiment, means with the same letters as superscripts are not significantly different ($P > 0.05$).
Data are means (± SD) of three replicates.

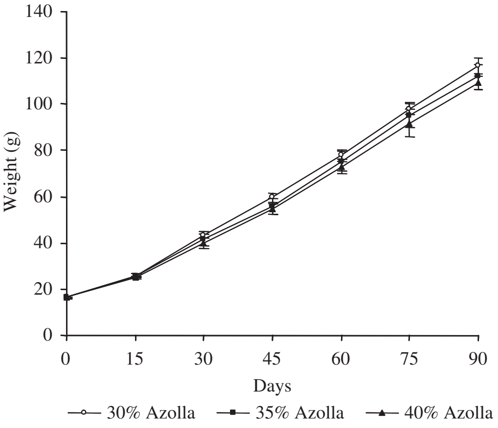

Figure 1 Fortnightly mean individual weight of Nile tilapia fed in earthen ponds for 90 days with diets containing three levels of *Azolla filiculoides*.

meters, yield and production were not significantly different (Table 4, Fig. 1, $P > 0.05$). The investment cost decreased with increasing fish density. This cost is significantly higher with diet A30 ($P < 0.05$), compared with those for A35 and A40 who did not differ ($P > 0.05$). However, the profitability index showed the same values (Table 5, $P > 0.05$).

Experiment 2

Water quality and plankton assessment

Water quality parameters in experiment 2 are given in Table 3. Dissolved oxygen and zooplankton abundance were significantly different among the treatments ($P < 0.05$). Decreasing values with increasing fish density were observed. Secchi disc transparency and ammonium concentrations increased with increasing stocking density ($P < 0.05$). Temperature, pH, nitrates, nitrites and orthophosphates did not differ significantly among treatments ($P > 0.05$). Similar chlorophyll *a* concentrations were obtained at 1 and 3 fish m^{-2}, and were significantly higher than that obtained at 5 fish m^{-2} ($P < 0.05$).

Growth, production and feed utilization

As shown in Table 4 and Fig. 2, stocking density has significant effects on growth rates. Final mean weight, DWG and SGR decreased with increasing density, with significant difference between treatment notably among the highest density (5 fish m^{-2}) and both others ($P < 0.05$) who did not differ significantly ($P > 0.05$). Fish survival was acceptable at all stocking densities, and was not affected by stocking density ($P > 0.05$). Net fish yields and annual production showed a positive relationship with stocking density, and the differences were significant ($P < 0.05$) between the three densities. The lowest yield (7.10 ± 0.90 kg are^{-1}) and production (28.79 ± 3.66 kg are^{-1}year^{-1}) were recorded at the lowest stocking density, and the highest yield (25.01 ± 1.84 kg are^{-1}) and production (101.42 ± 7.48 kg are^{-1}year^{-1}) at the highest stocking density.

The same increasing trend effects of stocking density ($P < 0.05$) were obtained with the feed utilization efficiency, expressed as AFCR. Values ranged from 1.29 ± 0.08 at the lowest stocking density to 1.34 ± 0.07 and 1.58 ± 0.07 at the densities of 3 and 5 fish m^{-2} respectively.

Economic analysis

Total investment cost, gross return and net return were directly related to stocking density. The highest

Table 5 Costs, returns and profitability of feeding Nile tilapia with three *Azolla* diets, and rearing at three stocking densities

	Experiment 1 (*Azolla* level)			**Experiment 2 (fish m^{-2})**		
Parameters	**A30**	**A35**	**A40**	**1**	**3**	**5**
Fixed costs (US$)						
Feeding	10.2	10.2	10.2	10.2	10.2	10.2
Pond management	14.4	14.4	14.4	14.4	14.4	14.4
Harvest procedures	3.1	3.1	3.1	3.1	3.1	3.1
Variable costs (US$)						
Feed cost	4.8 ± 0.3	4.0 ± 0.1	3.8 ± 0.0	4.3 ± 0. 1	12.4 ± 0.2	19.5 ± 0.6
Fingerlings cost	5.05	5.05	5.05	5.05	15.16	25.26
Investment cost (US$)	37.5 ± 0.3^{a}	36.8 ± 0.1^{b}	36.6 ± 0.0^{b}	37.0 ± 0.1^{a}	55.2 ± 0.2^{b}	72.4 ± 0.6^{c}
Fish production (US$ kg^{-1})	1.1 ± 0.0^{a}	1.1 ± 0.0^{a}	1.1 ± 0.1^{a}	1.1 ± 0.1^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}
Gross return (US$))	85.7 ± 3.4^{a}	82.8 ± 1.7^{a}	79.9 ± 5.3^{a}	85.4 ± 8.5^{a}	252.2 ± 24.0^{b}	325.6 ± 17.1^{c}
RAVC (US$))	75.9 ± 3.6^{a}	73.7 ± 1.7^{a}	71.1 ± 5.3^{a}	76.1 ± 8.5^{a}	224.7 ± 23.8^{b}	280.9 ± 16.5^{c}
Net return (US$)	48.2 ± 3.6^{a}	46.0 ± 1.7^{a}	43.4 ± 5.3^{a}	48.4 ± 8.5^{a}	197.0 ± 23.8^{b}	253.2 ± 16.5^{c}
Profitability (%)	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}	1.2 ± 0.1^{a}	1.3 ± 0.2^{a}	3.6 ± 0.4^{b}	3.5 ± 0.2^{b}

In each line for each experiment, means with the same letters as superscripts are not significantly different ($P < 0.05$).
Data are means (± SD) of three replicates.
RAVC, return above variable costs.

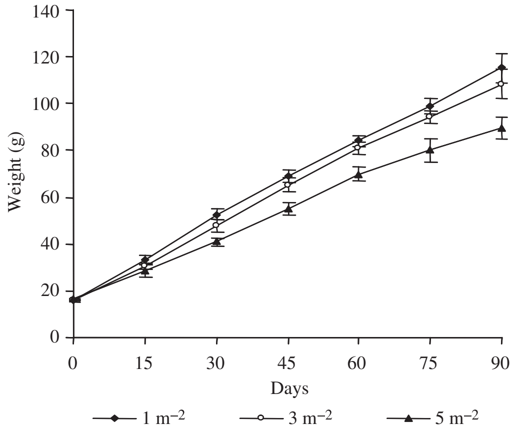

Figure 2 Fortnightly mean individual weight of Nile tilapia reared in earthen ponds for 90 days at three stocking densities (1, 3 and 5 fish m^{-2}) and fed with the same diet containing 40% of *Azolla filiculoides*.

density required the highest total investment cost and also provided the highest revenue (Table 5). Densities of 3 and 5 fish m^{-2} provided the lowest cost of fish production and the highest farming profitability. Both these two densities showed the same cost of fish production and farming profitability.

Discussion

In experiment 1, water quality, growth parameters and production values were quite similar among fish fed with the three diets. The SGRs obtained were very similar to those reported by Fasakin *et al.* (1999) for Nile tilapia-fed diets (30% CP) containing 20% and 30% of duckweed *Spirodella polyrrhiza* L. Schleiden. The growth and production values obtained here were slightly lower than those reported previously when feeding Nile tilapia with diets containing 0%, 10% and 20% *Azolla* in the same ponds (Abou *et al.*, in press). However, these values were acceptable for Nile tilapia fed in tropical wetland ponds, indicating that A30, A35 and A40 could be used for rearing this fish in rural areas. Diet A30 showed significantly higher investment cost and the lowest cost is associated with A40. In rural areas of Bénin, poor people lack capital and *Azolla* will not be a limitant, as the fern spontaneously grow with high productivity in Bénin marshlands. As the goal of this experiment is to develop suitable approach for low-cost investment in practical fish feed, diet A40 appears cost effective to boost revenue.

In experiment 2, water transparency was consistently higher at high densities, possibly due to the reduction in phytoplankton by fish feeding pressure, as shown by the lower chlorophyll *a* concentration in these ponds. The reduction in zooplankton abundance at high densities could be also due to high predation by fish. Dissolved oxygen significantly decreased with increasing stocking density. Similar results were observed by Ahmed (1993) and Rahman and Rahman (2003) who also reported lower DO in fertilized ponds with supplementary feed for carp fingerling. The lower values for DO might be primarily attributed to lower photosynthetic activity and high oxygen consumption by fish. This could explain the accumulation of ammonium at high densities. Despite these variations in water quality, their values are within the tolerable range for survival and production of Tilapia in tropical waters (Beveridge 1996; Mélard 1999).

Final mean weight, DWG and SGR were negatively correlated ($P < 0.05$) with stocking density. However, no significant differences in growth parameters ($P > 0.05$) were found between the densities of 1 and 3 m^{-2}. The explanations for the effects of stocking density on fish growth are very complex, as many interdependent factors are concerned. Published research literature on the effects of stocking density on growth in *O. niloticus* in stagnant earthen ponds in the tropics is particularly scarce. Stocking density is already cited as an inhibitory factor for fish growth (Helser & Almeida 1997; Irwin, O'Halloran & FitzGerald 1999), due to competition for food (Helser & Almeida 1997; Irwin *et al.* 1999; Islam 2002), space limitation (Ewing, Sheahan, Lewis & Palmisano 1998) and low DO (Yi, Lin & Diana 1996). Huang and Chiu (1997) argued that tilapia is a territorial and aggressive fish, so that the density effects on growth might be explainable by their competition for territories, as well as by the permanent stress caused by crowding. This last observation has been reported by Ruane, Carballo and Komen (2002) and Ruane and Komen (2003) in common carp *Cyprinus carpio* (L.). This chronic stress leads to impaired fish growth, probably due to the mobilization of dietary energy by the physiological alterations elicited by stress responses (Kebus, Collins, Brownfield, Amundson, Kayes & Malison 1992). Increasing stocking density may also result in a deterioration of water quality (Pankhurst & Van der Kraak 1997). Hence, the lower growth performances obtained in our study at 3 and 5 fish m^{-2} could be due to lower DO values observed in these treatments.

In the present study, AFCR is density dependent; i.e. higher fish density resulted in higher AFCR. Similar observations have been made in caged farming by Cruz and Ridha (1989) and Watanabe, Clark, Dunham, Wicklund and Olla (1990) with *O. niloticus* and Florida Red tilapia, respectively, as well as with the catfish *Pangacius sutchi* (Almazán-Rueda 2004). In the literature, inverse relationships between stocking density and feed efficiency have been signalled by some authors as the result of decreasing efficiency in search for food (Vijayan & Leatherland 1988) or water quality (Soderberg, Meade & Redell 1993). In this study, the amount of feed given in all ponds was on the basis of the fish stocked and the differences between the amounts of feed provided *per capita* was quite insignificant. Hence, the lower growth at high density could be due to a reduced availability of natural food in the ponds, as Rahman, Mazid, Rahman, Khan, Hossain and Hussain (2005) found. These statements, combined with the lower DO, could result in increasing AFCR. Diana, Lin and Schneeberger (1991) indicate that there is an adequate protein level from natural food that can sustain growth, until a critical biomass of fish is reached. This may mean that fish biomass at 5 m^{-2} is greater than the natural food capacity of ponds.

On the other hand, growth of Nile tilapia is primarily dependant on the absolute protein intake (g protein $\mathrm{kg}^{-1}\,\mathrm{day}^{-1}$) (Tacon & Cowey 1985; Bowen 1987) rather than on the dietary protein level (Yakupitiyage 1989). Considering the fact that density-induced stressors reduce fish appetite for feed, which could result in feed not being utilized (Alanärä 1996; Alanärä & Brännäs 1996), one could explain the increase in AFCR at the density of 5 m^{-2} by the loss of an amount of feed that simply ends up in sediment. Despite the higher values observed with increasing stocking density, the lower AFCR values obtained in our study indicate a better food utilization efficiency. Our values are close to those reported by Yi *et al.* (1996) in Nile tilapia cultured for 90 days in ponds stocked at 2 m^{-3} and containing a 4 m^3 cage stocked at either 60 or 70 fish m^{-3}.

Survival rates were not affected by stocking density, which is consistent with Daungsawasdi, Chomchei, Yamorbsin and Kertkomut (1986) who reported that mortality in Nile tilapia raised in cages was not dependent on stocking density. Other reports on catfish support these findings (Haylor 1992; Islam, Rahman & Tanaka 2006). However, care should be taken with that result, as the effects of density on survival rate will be entirely dependent on the range of stock densities.

There was a strong trend for net yield and annual production to increase with increasing stocking density. These findings are in agreement with those reported by Cruz and Ridha (1989) and Watanabe *et al.* (1990) for tilapias. Similar production scenarios were also obtained with many other species such as catfish (Engle & Valderrama 2001; Islam *et al.* 2006) and silver perch (Rowland, Allan, Hollis & Pontifex 2004). The positive relationship between stocking density and yield has been described in culture-based fisheries in reservoirs (Phan & De Silva 2000; Sugunan & Katiha 2004; Nguyen, Bui, Nguyen, Truong, Le,

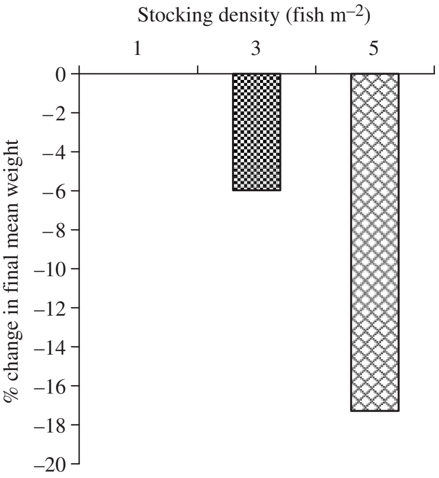

Figure 3 Percentage (%) change in final mean individual weight of Nile tilapia fed with same *Azolla* diet at three stocking densities. Percentage at each density was obtained by comparing the final individual mean weight at this density with that of the previous density.

Abery & De Silva 2005). Culture of all these species in cages or in reservoirs showed that the highest stocking density led to the highest biomass.

Production estimation, which are based on biomass estimates adjusted for mortality and corrected for growth, is the basis for estimating the economic revenue from fish culture operations. Obviously, feed and fingerling costs increase with increasing stocking density, resulting in higher total investment costs at high densities. The increasing pattern of production with increasing stocking density resulted in a similarly increasing pattern of gross return. For that reason, the cost of fish produced decreased at high densities, as the amount of feed given per fish was closely the same in the study. Also, net return increased with increasing stocking density. As a result, the profitability index was higher at the high densities.

Fish growth is lower at 5 than at 3 fish m^{-2}, but similar values for profitability were obtained with both culture practices. Surely, the percentage reduction in FMWs between the two densities (Fig. 3) is sufficient to influence the marketable price over time. However, because market size was not reached, we did not consider the consumer's choice for fish size in our profitability analysis, as it proved difficult to introduce this factor in numerical terms in simple cost balancing. When this can be carried out, fish produced at 5 m^{-2} will be obviously priced much lower. Hence, net return and, as a consequence, profitability will be low. Finally, based on its growth performance, low-cost investment and profitability value, the density of 3 m^{-2} appears to be the most cost effective.

Stocking density had negative effects on both growth and feed utilization efficiency, but positively sustains fish production. This study clearly indicates that feeding Nile tilapia with A40 at 1 and 3 m^{-2} resulted in rapid growth and better food utilization. High densities improve yield, production and profitability. It was concluded that diet A40 and a stocking density of 3 m^{-2} could be used to minimize fish production cost and improve farm profitability of Nile tilapia in stagnant earthen ponds.

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