



Effect of C/N ratio levels and stocking density of *Labeo victorinus* on pond environmental quality using maize flour as a carbon source

Esther Wairimu Magondu^{a,b,*}, Harrison Charo-Karisa^c, M.C.J. Verdegem^a

^a Wageningen University Fish Culture and Fisheries Group, Department of Animal Sciences, P.O. Box 338, 6700 AH Wageningen, The Netherlands

^b Kenya Marine and Fisheries Research Institute, P.O. Box 81651, Mombasa, Kenya

^c National Aquaculture Research Development and Training Centre, P.O. Box 26, Sagana, Kenya

ARTICLE INFO

Article history:

Received 14 March 2013

Received in revised form 17 June 2013

Accepted 24 June 2013

Available online 2 July 2013

Keywords:

C/N ratio

Stocking density

Labeo victorinus

Water quality

Sediment quality

ABSTRACT

The main obstacle in developing intensive fish culture is waste management which is detrimental to the environment. To mitigate environmental impacts associated with effluent discharge, measures should be put in place to avoid deterioration of the pond environment. The addition of carbon materials into culture facilities while manipulating the levels of carbon and nitrogen ratios is one of the best strategies of controlling ammonia and nitrite in ponds. This study was carried out in 18 hapas suspended in six, 150 m² earthen ponds to investigate the effects of C/N ratios (10 and 20) and stocking density (10, 15 and 25 fish m⁻²) on water quality, sediment quality and growth of *Labeo victorinus*. All treatments were carried out in triplicate during a time period of 72 days. A locally formulated and prepared feed containing 30% crude protein with a C/N ratio of 10 was applied. Maize flour was used as the carbohydrate source for manipulating C/N ratio and applied to the water column separately from the feed. Increasing C/N ratio from 10 to 20 reduced ($P < 0.001$) the total ammonia nitrogen (TAN), nitrite–nitrogen (NO₂-N) and nitrate–nitrogen (NO₃-N) in the water column and total nitrogen in the sediment ($P < 0.001$). It also raised sediment pH, organic matter and total phosphorus ($P < 0.001$). The lowest protein efficiency ratio (PER), specific growth rate (SGR) and the highest food conversion ratio for the feed were recorded with a C/N ratio of 10 ($P < 0.05$). Based on highest growth, survival, production and net benefits, C/N ratio of 20 and a stocking density of 25 fish m⁻² are optimal. Therefore, carbohydrate addition in *L. victorinus* culture is a promising option for sustainable aquaculture.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

As the aquaculture industry in East African region develops towards intensification and higher productivity, the demand for efficient culture systems and low cost of production is becoming crucial. Heightened intensification, which comes hand in hand with increase in culture densities and higher input levels, inevitably leads to greater environmental impacts. Appropriate stocking densities in intensive and semi intensive culture systems help in maintaining water quality balances which are vital for profitable pond harvests. In most cases the principal factor affecting water quality is the amount of feed supplied and subsequent release of metabolites in form of nutrients (Milstein, 1990). Good husbandry practices like stocking densities, nutrient ratios, aeration and water exchange are aimed at reducing the impacts of metabolites that would hamper water quality. Finding a right balance between nutrient ratios and carrying capacity in fish ponds while maintaining

favorable culture conditions is a common problem in aquaculture pond management. Discharges from aquaculture deteriorate the receiving environment with fish serving as reservoirs of nutrients generated from the culture environment. Fish may also transport nutrients between compartments within an ecosystem or transfer nutrients to other ecosystems (Tanner et al., 2000). Different measures have been used to remove nitrogen from water: use of biological filters, addition of substrate for periphyton development, use of bio-floc technology and C/N ratio control (Avnimelech, 2007).

Stocking density is a major factor affecting fish survival, growth, behavior, health, water quality, feeding and production (Backiel and LeCren, 1978; Rui et al., 2006). For the development of rearing techniques, appropriate stocking densities must be determined for each species passing through successive production stages to enable efficient management and maximize production benefits. Culture of fish in enclosures applying the right stocking density can effectively improve the yield per unit area. However, stocking density can also cause stress, lower the feed conversion efficiency, increase fish energy consumption and reduce feeding rate and digestibility (Gibtan et al., 2008; Islam et al., 2006; Rowland et al., 2006; Wallat et al., 2004).

Labeo victorinus (Boulenger, 1901), commonly known as Ningu, is a potamodromus fresh water fish native of Lake Victoria (Greenwood,

* Corresponding author at: Wageningen University Fish Culture and Fisheries Group, Department of Animal Sciences, P.O. Box 338, 6700 AH Wageningen, The Netherlands. Tel.: +31 254705175177.

E-mail address: nimms2003@yahoo.com (E.W. Magondu).

1966). The fish was once widely distributed in the Lake Victoria basin and supported the most important fishery of all the potamodromous species in the lake (Cadwalladr, 1965). The introduction of gill nets, set at the river mouths during spawning migrations, has been reported to be responsible for the rapid decline of the species since the 1950s (Ogutu-Ohwayo, 1990; Rutaisire, 2003; Seehausen, 1996). An alternative to ameliorate this problem is captive propagation. Although *L. victorinus* has not been cultured widely, it has potential where besides production of rich protein food, it would consequently reduce fishing pressure on the wild stocks. Members of the same genus rohu, *Labeo rohita* and *Labeo fimbriatus*, are widely cultivated in India (Halder et al., 1991; Jhingran and Pullin, 1985; Mridula et al., 2003) and *Labeo calbasu* in Bangladesh (Wahab et al., 1999). In Malawi two indigenous species *Labeo mesops* and *Labeo cylindricus* have been tested for small-scale aquaculture (Hiroyuki et al., 1999). The present study utilized *L. victorinus* as a test species as a step towards sustainable culture of the species.

This study therefore investigated the effect of C/N ratio levels and stocking density on pond environmental quality (water quality and sediment quality) in *L. victorinus* ponds using maize flour as a carbon source.

2. Materials and methods

2.1. Experimental fish

Juveniles of *L. victorinus* were collected by using of an electro fisher from Mara river Nyanza Province, Kenya. After collection, juveniles were transferred into inert polyethylene containers with river water and transported to the farm. From this stock, juveniles ranging from 5–10 g body weight and 5.0 to 8.5 cm total length were selected and were held at densities of 10, 15 and 25 fingerlings per m² (SD₁₀, SD₁₅ and SD₂₅), in white, 100 µm mesh size hapas. A sub-sample of juveniles in the size range of experimental individuals was taken from the stock and weighed using an electronic digital balance (make: Orion; precision: 1 mg) to determine the initial live body weight which varied from 4.75 to 9.45 g. Differences in total length and body weight of these juveniles were analyzed with a non-parametric analysis of variance (Kruskal and Wallis, 1952). No significant differences were found in the initial total length and body weight of the fish stocked in the different experimental units (Kruskal–Wallis; $H = 0.62, P = 0.73$).

2.2. Experimental design

The experiment was conducted in a 3 × 2 factorial design, with three levels of fish density in hapas as first factor and C/N ratios with two levels as second factor; C/N ratio 10 and 20. Six, 150 m², average depth 1 m earthen ponds were used in this study. Ponds were limed at 2000 kg ha⁻¹ with agricultural lime prior to filling. Eighteen hapas made from 100 µm mesh cloth, and 1 m³ volume (1 × 1 × 1 m) were attached to wooden poles in 6 ponds (3 hapas per pond) so that the hapa bottom was 10 cm above the pond bottom. The tops of the hapas were covered with hapa cloth to prevent predation and fish escape. The 100 µm mesh ensured that feed spillage was minimal. To replace water loss due to seepage and evaporation, water was added to the ponds on a weekly basis.

2.3. Experimental site and pond management

The experiment was carried out at National Aquaculture Research Development and Training Centre (NARDTC) Sagana for a period of 72 days. All hapas received a 30% crude protein diet with a C/N ratio of 10 at 3% body weight per day. Feed was administered twice daily at 09:00 and 03:00 o'clock. The C/N 20 ponds received additional locally purchased maize (*Zea mays*) flour, at 1.14 kg for each 1 kg of

30% protein feed. The pre-weighed maize flour starch was mixed in a beaker with hapa water and uniformly distributed over the hapa surface directly after the feed application at 09:00 h. The diet proximate composition is detailed in Table 1. The daily feed quantity was adjusted biweekly after sampling.

2.4. Growth analysis and estimation of yield parameters

Fish growth calculations involved computation of mean weight (g) and their standard deviations (±SD) for fish samples from each treatment and stocking density at each sampling occasion. Graphical plots of mean weights against time were used to visualize growth. At the end of the experiment all fish were harvested and weighed up to the nearest 0.1 g. Specific growth rate (% body weight day⁻¹) was calculated using the formula, $SGR = (\ln WT_F - \ln WT_I) * 100 / T$ where WT_F = average final fish weight (g), WT_I = average initial fish weight (g), T = duration of the experiment (days). Feed conversion ratio (FCR) and net yields were calculated as follows:

$FCR = \text{feed applied (dry weight) / live weight gain.}$

$\text{Net yield} = \text{Total biomass at harvest} - \text{total biomass at stocking.}$

Geometric mean body weight (Wg) was calculated to determine the estimate for the body weight of the fish at the middle of the experiment period. Calculated as $Wg = e^{((\ln WT_F + \ln WT_I) / 2)}$.

Metabolic growth (RGR_m) was calculated to determine the growth achieved by the fish after utilizing the available food to generate energy for metabolism during the experiment period. Calculated as; $RGR_m = (WT_F - WT_I) W_g^{0.8} / T$.

Metabolic feeding rate was calculated to determine the daily feeding rate of the experimental fish. Calculated as (g feed/day/kg^{0.8}).

2.5. Determination of water quality parameters

Water samples were collected using a horizontal water sampler from inside the hapa and from the pond. Samples were pooled before analysis. Water quality parameters, surface and bottom temperature (Celsius thermometer), surface and bottom dissolved oxygen (YSI digital DO meter, model 58) and pH (Corning 445 pH meter) were monitored *in situ* at sunrise (07:00 h) and sunset (18:00 h) on a weekly basis. Transparency (Secchi disk) was recorded weekly at 10:00 h. Before nutrient analysis, water samples were filtered through microfiber glass filter paper (Whatman GF/C), using a vacuum pressure air pump. Total alkalinity (titrimetric method) and NO₂-N, NO₃-N, NH₃-N and PO₄-P concentrations according to standard methods described in American Public Health Association (APHA, American Water Works Association and Water Pollution Control Federation, 1995) cross-referenced to Boyd and Tucker (1992). The filter paper was kept in a test tube containing 10 mL of 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 h. Later, chlorophyll-*a* was determined using a spectrophotometer (Milton Roy Spectronic,

Table 1

Proximate composition of the prepared feed and maize flour. The percentages are given on a wet weight basis.

Proximate composition %	Feed ingredients			Treatment diets	
	Rice bran	Cotton seed cake	Fresh water shrimps	Prepared feed	Maize flour
Crude protein	7	35	63.3	29.5	7.71
Crude lipid	4.2	10.5	1.3	7.2	4.42
Crude fiber	30.9	25	5	5.1	5.4
Ash content	22.9	63	22.8	13.2	1.52
Dry matter	92.3	89.4	87.7	87.4	88.3
Nitrogen free extracts	35	19.2	6.7	32.4	69.6

Table 2
Effects of C/N ratio, stocking density and sampling time on different water quality parameters based on two-way ANOVA.

Water quality parameters	Means		Tukey test													
	C/N ratio (C/N)		Density (fish/m ²) (D)				Sample time				M & A		P interaction			
	10	20	10	15	25	Pond	18-08-11	01-09-11	15-09-11	29-09-11	13-10-11	Morning	Afternoon	C/N × D	C/N × ST	ST × D
Dissolved oxygen (mg L ⁻¹)	6.8 ^b	7.2 ^a	7 ^b	6.8 ^{bc}	6.7 ^c	7.6 ^a	6.6 ^b	6.9 ^c	7.1 ^{ab}	7 ^{ab}	7.5 ^a	5.2 ^b	8.9 ^a	NS	**	NS
Temperature (°C)	23.1 ^b	23.3 ^a	23.2 ^{ab}	23.1 ^b	23 ^b	23.3 ^a	21.7 ^b	24.6 ^{ab}	21.3 ^c	23.2 ^{ab}	24.7 ^{ab}	20.9 ^b	25.3 ^a	NS	NS	NS
pH	7.9 ^b	8.3 ^a	8 ^b	8.1 ^{ab}	8.1 ^{ab}	8.3 ^a	8.3 ^a	7.9 ^b	8 ^{ab}	8.2 ^{ab}	8.1 ^{ab}	7.8 ^b	8.5 ^a	NS	NS	NS
Transparency (cm)	28.4 ^a	26.2 ^b	27.5	27.3	27	22.7.4	35.8 ^a	26.5 ^b	24.9 ^{ab}	25.2 ^c	24.1 ^{ab}			NS	**	NS
Chlorophyll <i>a</i> (µg L ⁻¹)	138.4 ^b	188.6 ^a	165.1 ^{ab}	163.3 ^{ab}	160.3 ^b	165.3 ^a	133.1 ^c	150.3 ^d	162.7 ^c	179.1 ^b	192.7 ^a			NS	***	NS
Total alkalinity (mg L ⁻¹)	72.6 ^b	81.3 ^a	76.3	77	77.3	77.3	81.6 ^b	81.4 ^{ab}	69.6 ^c	74.9 ^b	77.9 ^b			NS	***	NS
SRP (mg L ⁻¹)	0.99 ^b	0.105 ^a	0.102 ^{ab}	0.097 ^b	0.099 ^{ab}	0.109 ^a	0.039 ^d	0.132 ^c	0.021 ^e	0.153 ^b	0.164 ^a			NS	***	NS
NO ₃ -N (mg L ⁻¹)	0.034 ^a	0.015 ^b	0.022 ^{ab}	0.028 ^{ab}	0.031 ^a	0.016 ^b	0.004 ^c	0.028 ^b	0.037 ^a	0.026 ^{ab}	0.027 ^{ab}			*	***	NS
TAN (mg L ⁻¹)	0.191 ^a	0.104 ^b	0.146 ^b	0.15 ^b	0.186 ^a	0.108 ^c	0.1 ^c	0.173 ^b	0.257 ^a	0.162 ^{ab}	0.135 ^{ab}			NS	***	NS
NO ₂ -N (mg L ⁻¹)	0.178 ^a	0.1 ^b	0.14 ^b	0.123 ^b	0.171 ^a	0.122 ^b	0.221 ^a	0.125 ^{ab}	0.129 ^{ab}	0.177 ^b	0.042 ^c			NS	***	NS

The mean values followed by the different superscript letter within factor indicate significant difference at (P < 0.05). If the effects were significant, ANOVA was followed by Tukey test. *P < 0.05; **P < 0.01; ***P < 0.001; NS, Not significant.

model 1001 plus) at 750- and 664-nm wavelength, following Boyd and Musig (1981).

2.6. Determination of sediment quality parameters

Sediment samples were collected from three locations of each pond using PVC pipes 4 cm in diameter and 10 cm in sampling depth. They were collected on biweekly basis at 1000 h. Samples were dried, ground and sieved with a 2 mm sieve. Soil pH was determined by directly reading a pH meter with soil water ratio 1:2.5 (McLean, 1982). Organic matter of sediment was determined by ignition method (Page et al., 1989). Total nitrogen of sediment was determined by micro-Kjedahl digestion method following Page et al. (1989). Total phosphorus of sediment samples was determined by acid digestion method (Jones and Case, 1990; Watson and Isaac, 1990).

2.7. Data analysis

Sediment quality and water quality were compared by repeated measures ANOVA with stocking density and C/N ratio as the main factors and time as sub-factor. Growth and yield parameters (growth, yield, FCR, SGR, PER and survival) were analyzed by two way ANOVA with stocking density and C/N ratio as the main factors. Prior to analysis, the data was checked for normality; and data in form of percentages were arcsine transformed. Raw data were expressed as mean ± S.E. or ± S.D. and statistically analyzed by two way ANOVA (Gomez and Gomez, 1984). All ANOVA were performed using SPSS (Statistical Package for Social Science) version 18. If the main effect was found significant, the ANOVA was followed by a Tukey's test at P < 0.05 level of significance.

3. Results

3.1. Water quality parameters

Mean values of water quality parameters and outcomes of ANOVA are presented in Table 2. Increasing C/N ratio from 10 to 20 increased the dissolved oxygen, temperature, pH, chlorophyll-*a* and total alkalinity in water (P < 0.05). It also reduced the water transparency (P < 0.05). The stocking density of *L. victorinus* influenced water quality parameters. Dissolved oxygen, temperature, pH and transparency were higher in open pond water as compared to hapas but not significantly different among the stocking densities (P > 0.05). Higher stocking density of *L. victorinus* coincided with low chlorophyll-*a* concentrations inside the hapas. Transparency and total alkalinity were not influenced by stocking density, but changed with C/N ratio. The ANOVA results showed that increasing C/N ratio significantly reduced nitrate-N, TAN and nitrite-N in the pond water column, while phosphorus availability increased as shown by soluble reactive phosphorous (SRP). Levels of nitrate-N, TAN and nitrite-N did not change between the first and second densities in hapas, but the highest density showed a significant increase of the three parameters. Nevertheless, the concentration of TAN was lower in the open pond water than in the hapas, while the opposite was the case for SRP. C/N ratio and stocking density did not have a significant interaction effect apart from nitrate-nitrogen at (P = 0.04). There was a significant interaction between C/N ratio and sampling time for all the parameters apart from temperature and pH. The *in situ* parameters, dissolved oxygen, temperature and pH showed a significant difference between the time of the day that the sampling was done (P < 0.05) and the highest mean for all the parameters recorded in the afternoon. For the same parameters, there was no significant interaction between sampling time and density. The different values obtained represent dietary effects that are somewhat lower than those expected in whole ponds treated with given diets. This is because the 100 µm hapas only slowed down water and solute exchange between inside and outside but did not prevent it.

Table 3
Effects of C/N ratio on different sediment quality parameters based on two-way ANOVA.

Variable	Means Tukey test							Interaction CN × T
	C/N ratio (CN)		Sample time (T)					
	10	20	19-08-11	02-09-11	16-09-11	30-09-11	14-10-2011	
pH	7.01 ^b	7.82 ^a	7.2 ^c	7.43 ^b	7.41 ^{bc}	7.29 ^{bc}	7.74 ^a	***
Organic matter (%)	2.3 ^b	2.8 ^a	2.5 ^b	2.4 ^{bc}	2.6 ^a	2.6 ^d	2.6 ^a	***
Total nitrogen (%)	19.8 ^a	13.6 ^b	15.8 ^c	15.3 ^c	17.2 ^b	16.9 ^d	18.4 ^a	***
Total phosphorus (mg L ⁻¹)	14.6 ^b	15.1 ^a	15.8 ^{cd}	15.2 ^d	16.4 ^{bc}	16.8 ^b	17.8 ^a	***

The mean values followed by the different superscript letter in each factor indicate significant difference at ($P < 0.05$). If the effects were significant, ANOVA was followed by Tukey test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not significant.

3.2. Sediment quality

The sediment quality parameters are summarized in Table 3. The addition of carbohydrates for increasing C/N ratio increased the organic matter content in the sediment, pH and total phosphorus. Total nitrogen concentration in the sediment was reduced by increasing C/N ratio. The ANOVA results showed that sampling time influenced the means of all the parameters with the highest values recorded at the end of the experiment. There was also significant interaction between C/N ratio and sampling time. The Pearson correlation analysis showed a significant positive relationship among sediment pH and plankton biovolume and total heterotrophic bacteria count ($P < 0.05$) (Figs. 1 and 2).

3.3. Effects on growth, survival and yield parameters of *L. victorinus*

Growth and yield parameters of *L. victorinus* and their combined performances under different treatments are shown in Table 4. The ANOVA results showed that increasing C/N ratio increased the individual *L. victorinus* weight at harvest ($P < 0.05$) (Fig. 3). Considering the formulated diet, the food conversion ratio (FCR) decreased by increasing C/N ratio. Protein efficiency ratio (PER) increased with increasing C/N ratio. Individual weight gain, survival percentage and specific growth rate (SGR) were higher in the high C/N ratio treatment. Geometric mean body weight, metabolic growth, metabolic feed rate, gross and net yield were higher with the high C/N ratio treatment. Stocking density influenced the growth and yield parameters differently. The highest average individual weight at harvest and the highest individual weight gain were obtained with the intermediate stocking density (Fig. 4). FCR for both diets was lower in the medium density as compared to the low and high densities. PER, individual weight gain, SGR, geometric mean body weight, metabolic growth and FCR were highest with a stocking density of 15 fish per hapa. With increasing stocking density from 10 to 25 fish per m² the combined gross and net yield of *L. victorinus* increased by 62% and 58% respectively. The interaction of C/N ratio and stocking density was not significant for all the growth parameters.

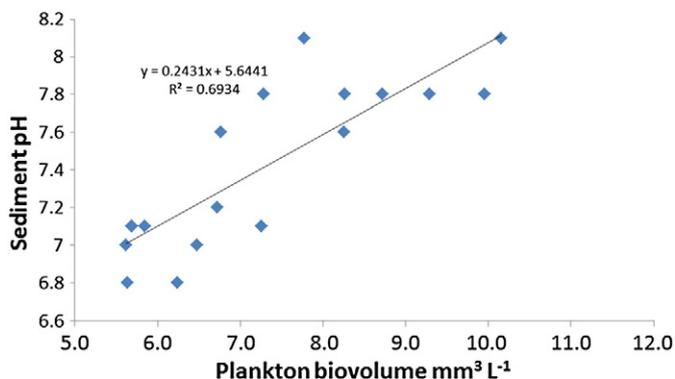


Fig. 1. Relationship between sediment pH and plankton biovolume.

4. Discussion

4.1. Effect on water quality parameters

Water quality management is of imperative importance in aquaculture. It is strongly influenced by stocking densities, culture species combinations, quality and quantity of nutrient inputs and the culture system to be used (Diana et al., 1997). In the present study, water temperature, dissolved oxygen and pH were within the suitable range for tropical fish culture (Boyd, 2002). Low temperatures at the start of the experiment were due to the colder weather condition during the season of the year. This might have negatively impacted on the growth of *L. victorinus*, which does best at optimal temperature conditions of about 24–26 °C (Rutaisire, 2003). The observed decreasing trend of dissolved oxygen concentration, chlorophyll-*a* and transparency across the stocking densities (Table 2) might have been as a result of increase in fish biomass that increased pond turbidity reducing photosynthesis and hence low primary production. Chlorophyll-*a* concentration was however observed to increase with time which might have been as an effect of high pH observed in all the treatments that stimulated phytoplankton proliferation. Total alkalinity was within a suitable range of 70–150 mg L⁻¹ recommended for tropical fish culture (Boyd, 2002). This provided a well buffered environment suitable for growth of the fish and pond primary productivity. Soluble reactive phosphorus (SRP) was the same in the three different stocking densities but significantly different during the sampling periods and high in the high C/N ratio treatment. This could be attributed to high bacterial production and decomposition with addition of carbohydrates. The relatively high SRP levels during the culture period could be the result of fertilizer application in addition to carbohydrates. Both soluble organic phosphorus and soluble reactive phosphorus are the main end-products of bacterial activity on organic matter (Elnady et al., 2010). Among the inorganic nitrogen species concentrations, manipulation of C/N ratio by addition of carbohydrate maintained good water quality conditions and significantly reduced inorganic nitrogen concentration in the water column. These findings are in agreement with Avnimelech (1999) and Hari et al. (2004) who reported that the addition of carbohydrate to the production systems will reduce the TAN concentration

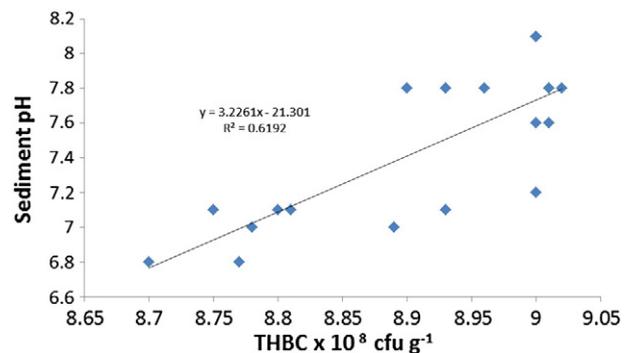


Fig. 2. Relationship between sediment pH and total bacteria count.

Table 4
Effects of C/N ratio and stocking density on growth and yield parameters of *Labeo victorinus* based on two-way ANOVA.

Growth and yield parameters	Means Turkey test					P interaction C/N × D
	C/N ratio (CN)		Density (fish/m ²) (D)			
	10	20	SD ₁₀	SD ₁₅	SD ₂₅	
Individual stocking weight (g)	6.7	7.0	6.95	6.97	6.59	NS
Individual harvest weight (g)	17.7 ^b	19.8 ^a	17.2 ^b	20.8 ^a	18.2 ^b	NS
Food conversion ratio diet 1	3.8	3.1	3.95	2.83	3.6	NS
Food conversion ratio diet 1 and 2	3.8 ^b	4.7 ^a	4.8 ^a	3.5 ^b	4.5 ^{ab}	NS
Protein efficiency ratio diet 1	0.8	1.0	0.8 ^b	1.1 ^a	0.9 ^{ab}	NS
Protein efficiency ratio diet 1 and 2	0.8	0.9	0.7 ^b	1.0 ^a	0.8 ^{ab}	NS
Individual weight gain (g)	11.0 ^b	12.7 ^a	10.23 ^b	13.8 ^a	11.58 ^b	NS
Survival (%)	90	91.7	95	92.1	86	NS
Specific growth rate (%bw day ⁻¹)	1.3	1.4	1.25	1.51	1.41	NS
Geometric mean body weight (g)	10.8 ^b	11.7 ^a	10.9 ^b	12 ^a	10.91 ^b	NS
Metabolic growth (g bw/day/kg ^{0.8})	5.7	6.2	5.28 ^b	6.58 ^a	5.98 ^{ab}	NS
Metabolic feeding rate (g feed/day/kg ^{0.8})	23 ^b	33.4 ^a	28.4 ^{ab}	26.4 ^b	30.4 ^a	NS
Conversion Efficiency (%)	25 ^a	18.6 ^b	19.05 ^b	25.6 ^a	20.1 ^b	NS
Gross yield (kg ha ⁻¹ 72 days ⁻¹)	2633.1	2982.1	1638.8 ^c	2867.3 ^b	3916.6 ^a	NS
Net yield (kg ha ⁻¹ 72 days ⁻¹)	1534.4	1821.6	943.6 ^b	1821.3 ^a	2269.1 ^a	NS

The mean values followed by the different superscript letter in each factor indicate significant difference ($P < 0.05$). If the effects were significant, ANOVA was followed by Tukey test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not significant.

through immobilization by bacterial biomass. The very low $\text{NO}_3\text{-N}$, TAN and $\text{NO}_2\text{-N}$ in the treatments compared to other studies using cyprinids (Azim et al., 2001) could be attributed to carbohydrate addition to maintain a high C/N ratio during the experimental period. Among the three stocking densities, concentration of the nitrogenous compounds was higher in the high stocking density hapas as a result of high fish biomass. Acosta-Nassar et al. (1994), Gross et al. (2000) and Davenport et al. (2003) reported that fish in a pond assimilate only 15–30% of the nitrogen added in the feed. The remainder is lost to the system as ammonia and organic nitrogen in feces and feed residue, which undergoes decomposition and eventually produces ammonia. The results of this study show that higher dietary protein levels resulted in significantly higher TAN and $\text{NO}_2\text{-N}$ concentrations in the water column. Li and Lovell (1992) reported that the ammonia concentration increased with increasing dietary protein concentration and protein feeding rate.

4.2. Effect on sediment quality

In this experiment there was no direct contact of fish with the sediment since they were grown in hapas. Hence, exogenous factors contributed to the sediment quality of the experimental ponds. Nevertheless all the analyzed sediment quality parameters were within the acceptable pH range (6.5 to 7.5), organic carbon (0.5 to 2.5%), total

nitrogen (15 to 20%) and total phosphorus (15–18 mg L⁻¹) Banerjea (1967). Manipulation of C/N ratio by carbohydrate addition in the high C/N ratio ponds significantly reduced total nitrogen in sediment. The significant increase in pH and availability of total organic matter and total phosphorus with a high C/N ratio contributed to total pond productivity. Boyd and Musig (1981) reported that phytoplankton uptake and nitrification are considered the principle sinks of ammonia whereas pond soils can be considered a source of sink for phosphorous and other biologically important materials such as carbon, nitrogen and sulfur.

4.3. Effects on growth, survival and yield parameters of *L. victorinus*

Yield parameters of *L. victorinus* varied with stocking density. C/N ratio also had a significant effect on growth and yield parameters. The treatment with a C/N ratio of 20 and 15 fish/hapa had the highest growth of all treatments ($P < 0.05$). The net weight gain of an individual fish with a C/N ratio of 20 was higher (12.7 g) than with a C/N ratio of 10 (11.0 g) agreeing with the hypotheses that C/N ratio has an effect on *L. victorinus* production in hapas. The intermediate density of 15 fish/hapa resulted in the highest individual mean weight gain at harvest (13.8 g) followed by stocking density 25 fish/hapa (11.58 g) and 10 fish/hapa (10.2 g). The estimated pond production with a pond area of 150 m² and a maximum of 6 hapas in each pond would be 612 g, 1242 g and 1737 g from the least to the highest density respectively. Zhu et al. (2011) growing sturgeon for 75 days in cages found that stocking density of 15 fish per m² though not achieving the highest production, had the best cost benefit ratio

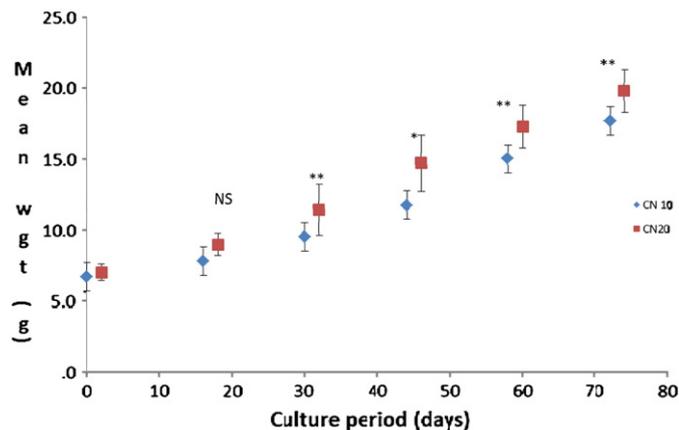


Fig. 3. Mean weight of *Labeo victorinus* for different C/N ratios during the experimental period. Values are means (\pm S.D.) of nine hapas in each treatment per sampling date. C/N 10 and C/N 20 are the C/N ratios in the different treatments. Significance level; NS, Not significant * $P < 0.05$; ** $P < 0.01$.

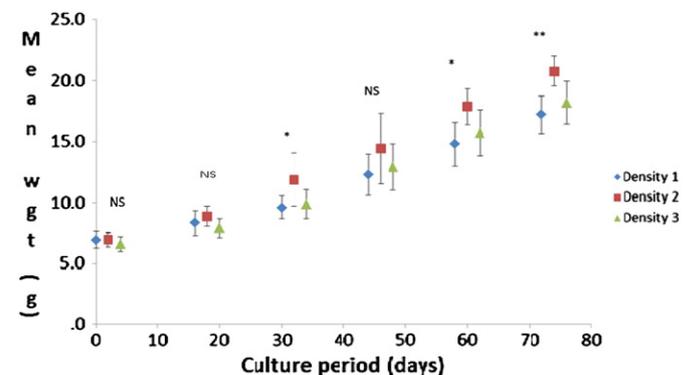


Fig. 4. Mean weight of *Labeo victorinus* in the different stocking densities during the experimental period. Values are means (\pm S.D.) of three replicates in each stocking density per sampling date. Significance level; NS, Not significant * $P < 0.05$; ** $P < 0.01$.

compared to densities of 30, 45, and 60 fish per m². The lowest stocking density provided more space, food and less competition as reported by Haque et al. (1984) and Narejo et al. (2005). The survival percentage was not significantly different in the different treatments and stocking densities indicating that C/N ratio control did not influence the survival of the fish. Survival was also not influenced by the stocking density of the fish (Table 4) only that the highest density had a bit lower mean survival which might have been a result of high competition for food and space among fishes. The highest net and gross yield of *L. victorinus* were recorded in the high C/N ratio treatment. The net yield increased by 15% when increasing C/N ratio from 10 to 20. The highest net production was obtained stocking 25 individuals per hapa (2269.1 kg ha⁻¹ 72 days⁻¹). The results agree with findings of Barua (1990) and Narejo et al. (2005) and further show that the hypotheses that a higher C/N ratio allows for higher stocking density was actually achieved. PER was higher and SGR was a little bit higher with a C/N ratio of 20. However metabolic growth and geometric mean body weight were higher with a C/N ratio of 20. It can therefore be considered that maize flour can benefit *L. victorinus* fish farming through reducing toxic inorganic nitrogenous content, increasing heterotrophic bacteria and algal abundance, improving productivity and enhancing overall sustainability. In previous studies, different carbohydrate sources like tapioca starch and molasses were used in shrimp and fish farming to improve water quality and productivity of ponds (Burford et al., 2004; Hari et al., 2004). In pond ecological and growth studies, Asaduzzaman et al. (2010) reported that maize flour can be a good source of organic carbon to maintain high C/N ratio in ponds. In that study realistic economic analysis indicated that use of maize flour in C/N system reduced carbohydrate cost thereby improving economic benefits. Economic analyses were not done for the present study considering the short culture period leaving the fish no time to reach market size.

5. Conclusion

The present study demonstrates that increasing the C/N ratio by addition of carbohydrate benefited *L. victorinus* farming at a stocking density of 25 fish/m². The C/N ratio control improved water quality and sediment quality through reducing toxic inorganic nitrogen contents like ammonia and nitrite, improving nutrient utilization efficiency and reducing nutrient discharge. Maize flour, which is locally produced and utilized as an animal feed ingredient by farmers, is inexpensive and therefore can be used as on-farm carbohydrate source to culture *L. victorinus*. Nevertheless, maize flour in Kenya is also human food, hence potential human-animal user conflicts should be taken into account. This opens a scope for further improvement of economic sustainability of this technology by comparing the potential of other cheap carbohydrate sources such as sugarcane wastes, molasses and native starch like potato or cassava. More so, sensitizing farmers and training them on adoption of this technology at the farm levels through direct participation would be of great benefit due to the high cost of protein rich feeds. In this research, *L. victorinus* was used in relatively low stocking densities which might have underutilized the available pond communities. Therefore further research with much higher densities and in combination with a column and bottom grazing fish species like tilapia might enhance production further.

Acknowledgment

This research was financially supported by the Kenya Agricultural Productivity and Agribusiness Program KAPAP-CGS/FP 2011 and the Netherlands Fellowship Programme. The authors are grateful to the field staff and technicians of National Aquaculture Research Development and Training Centre, Sagana Kenya for their kind help and contribution to this research.

References

- Acosta-Nassar, M.V., Morell, J.M., Corredor, J.E., 1994. The nitrogen budget of a tropical semi-intensive fresh water fish culture pond. *Journal of the World Aquaculture Society* 25 (2), 261–270.
- APHA (American Water Works Association and Water Pollution Control Federation), 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th edn. American Public Health Association, Washington, DC.
- Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Adhikary, R.K., Rahman, S.M.S., Azim, M.E., Verreth, J.A.J., 2010. Effects of carbohydrate source for maintaining a high C/N ratio and fish driven re-suspension on pond ecology and production in periphyton-based fresh water prawn culture systems. *Aquaculture* 301, 37–46.
- Avnimelech, Y., 1999. C/N ratio as a control element in aquaculture systems. *Aquaculture* 176, 227–235.
- Avnimelech, Y., 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture* 264, 140–147.
- Azim, M.E., Wahab, M.A., DamVan, A.A., Beveridge, M.C.M., Huisman, E.A., Verdegem, M.C.J., 2001. Optimization of Stocking Ratios of Two Indian Major Carps Rohu (*Labeo rohita* Ham.) and Catla (*Catla catla* Ham.) in a Periphyton-Based Aquaculture System.
- Backiel, T., LeCren, E.D., 1978. Some density relationship for the population parameters. In: Gerking, S.D. (Ed.), *Ecology of freshwater fish production*. Blackwell Scientific Publications, Oxford, pp. 279–302.
- Banerjee, S.M., 1967. Water quality and soil condition of fish ponds in some states of India in relation to fish production. *Indian Journal of Fisheries* 14, 113–144.
- Barua, G., 1990. *Gonadal Development And Fry Rearing of Clarias Batrachus*. (Ph.D. thesis submitted to the Department of Fisheries Biology and Limnology) Bangladesh Agricultural University, Mymensingh 310.
- Boulenger, G.A., 1901. *Catalogue of the freshwater fishes of Africa*. Longmans, London pp. 322–323.
- Boyd, C.E., 2002. *Water quality. An Introduction*. Kluwer Academic Publishers, Boston, Dordrecht, London (325 pp.).
- Boyd, C.E., Musig, Y., 1981. Orthophosphate uptake by phytoplankton and sediment. *Aquaculture* 22, 165–173.
- Boyd, C.E., Tucker, C.S., 1992. *Water Quality and Pond Soil Analysis for Aquaculture*, Alabama Agricultural Experimentation Station Alabama. 183.
- Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture* 232, 525–537.
- Cadwalladr, D.A., 1965. The decline in the *Labeo victorinus* (Boulenger) (Pisces: Cyprinidae) fishery of Lake Victoria and an associated deterioration in some indigenous fishing methods in the Nzoia river, Kenya. *East African Agricultural and Forestry Journal* 30, 249–256.
- Davenport, J., Black, K., Burnell, G., Cross, T., Culloty, S., Ekaratne, S., Furness, B., Mulcahy, M., Thetmeyer, H., 2003. *Aquaculture. The Ecological Issues*. Blackwell Publishing, Oxford.
- Diana, J.S., Szyper, J.P., Batterson, T.R., Boyd, C.E., Piedrahita, R.H., 1997. *Water Quality in Ponds*. In: Egna, H.S., Boyd, C.E. (Eds.), *Dynamics of pond aquaculture*. CRC Press LLC, pp. 53–72.
- Elnady, M.A., Alkobabay, A.I., Salem, M.A., Abdel-Salam, M., Asran, B.M., 2010. Effect of fertilization and low quality feed on water quality dynamics and growth performance of Nile Tilapia (*Oreochromis niloticus*). *Journal of American Science* 6 (10), 1044–1054.
- Gibtan, A., Getahun, A., Mengistou, S., 2008. Effect of stocking density on the growth performance and yield of Nile tilapia (*Oreochromis niloticus* (L., 1758)) in a hapa culture system in Lake Kuriutu, Ethiopia. *Aquaculture Research* 39, 1450–1460.
- Gomez, K.A., Gomez, A.A., 1984. *Statistical Procedures for Agricultural Research*, 2nd edition. John Wiley and Sons, New York (680 pp.).
- Greenwood, P.H., 1966. *The Fishes of Uganda Family Cyprinidae, the Geneva Labeo III*. 50–58.
- Gross, A., Boyd, C.E., Wood, C.W., 2000. Nitrogen transformations and balance in channel catfish ponds. *Aquacultural Engineering* 24, 1–14.
- Halder, S., Sen, S., Bhattacharya, S., Ray, A.K., Ghosh, A., Jhingran, A.G., 1991. Induced spawning of Indian major carps and maturation of a perch and a catfish by murrel gonadotropin releasing hormone, pimozone and calcium. *Aquaculture* 97, 373–382.
- Haque, M.M., Islam, M.A., Ahmed, G.U., Haq, M.S., 1984. Intensive culture of java tilapia (*Oreochromis mossumbica*) in floating pond at different stocking density. *Bangladesh Journal of Fisheries* 7, 55–59.
- Hari, B., Kurup, M.B., Varghese, J.T., Sharma, J.W., Verdegem, M.C.J., 2004. Effects of carbohydrate addition on production in extensive shrimp culture systems. *Aquaculture* 241, 179–194.
- Hiroyuki, C., Kamanga, G., Mususa, W., Kataya, P.B., 1999. *Technical report and instruction of aquaculture in Malawi on seed production no. 1*. Ministry of Forestry, Fisheries and Environmental affairs and Japan international Cooperation Agency, pp. 9–19.
- Islam, M.S., Rahman, M.M., Tanaka, M., 2006. Stocking density positively influences the yield and farm profitability in cage aquaculture of sutchi catfish, *Pangasius sutchi*. *Journal of Applied Ichthyology* 22, 441–445.
- Jhingran, V.G., Pullin, R.S.V., 1985. *A Hatchery Manual for the Common, Chinese and Indian Major Carps*. Asian Development Bank, Manila, Philippines 13–15.
- Jones Jr., J.B., Case, V.W., 1990. Sampling, handling and analyzing plant tissue samples. In: Westerman, W.L. (Ed.), *Soil Testing and Plant Analysis*, 3rd edition. SSSA Book series, vol. 3. Soil Science Society of America, Madison, WI, pp. 389–427.
- Kruskal, W.H., Wallis, W.A., 1952. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* 47, 583–621.

- Li, M., Lovell, R.T., 1992. Effect of dietary protein concentration on nitrogenous waste in intensively fed catfish ponds. *Journal of the World Aquaculture Society* 23, 122–127.
- McLean, E.O., 1982. Soil pH and lime requirement. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*. American Society of Agronomy Inc., Madison, WI, USA, pp. 199–224.
- Milstein, A., 1990. Water quality in an intensive outdoor commercial fish culture system with mechanically stirred ponds. *Israeli Journal of Aquaculture-BAMIDGEH* 42, 99–109.
- Mridula, R.M., Maniserry, J.K., Keshavanath, P., Shankar, K.M., Nandeesh, M.C., Rajesh, K.M., 2003. Water quality, biofilm production and growth of fringe-lipped carp (*Labeo fimbriatus*) in tanks provided with two solid substrates. *Bioresource Technology* 87, 263–267.
- Narejo, N.T., Salam, M.A., Sabur, M.A., Rahmatullah, S.M., 2005. Effect of Stocking density on growth and survival of indigenous catfish, *Heteropneustes fossilis* (Bloch) reared in cemented cisterns fed on formulated feed. *Pakistan Journal of Zoology* 37 (1), 49–52.
- Ogutu-Ohwayo, R., 1990. The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch *Lates niloticus* and the Nile tilapia *Oreochromis niloticus*. *Environmental Biology of Fishes* 27, 81–96.
- Page, A.L., Miller, R.H., Keeney, D.R., 1989. *Methods of Soil Analysis. Part-2*, 2nd ed. American Society of Agronomy, Inc., Madison, Wisconsin, USA (1159 pp.).
- Rowland, S.J., Mifsud, C., Nixon, M., Boyd, P., 2006. Effects of stocking density on the performance of the Australian freshwater silver perch (*Bidyanus bidyanus*) in hapas. *Aquaculture* 253, 301–308.
- Rui, L., Youjun, O., Xiaowei, G., 2006. A review: influence of stocking density on fish welfare mortality, growth, feeding and stress response. *South China Fisheries Science* 2, 76–80 (in Chinese).
- Rutaisire, J., 2003. *The Reproductive Biology and Artificial Breeding of Ningu Labeo victorianus* (Pisces: Cyprinidae). (PhD Thesis) Rhodes University, Grahams town, South Africa.
- Seehausen, O., 1996. Lake Victoria Rock Cichlids. Verdyun cichlids, The Netherlands 263–264.
- Tanner, D.K., Brazner, J.C., Brandy, V.J., 2000. Factors influencing carbon, nitrogen and phosphorus content of fish from Lake Superior coastal wetland. *Canadian Journal of Fisheries and Aquatic Sciences* 57, 1243–1251.
- Wahab, M.A., Azim, M.E., Ali, M.H., Beveridge, M.C.M., Khan, S., 1999. The potential of periphyton-based culture of the native major carp calbasu, *Labeo calbasu* (Hamilton). *Aquaculture Research* 30, 409–419.
- Wallat, G.K., Tiu, L.G., Rapp, J.D., Moore, R., 2004. Effect of stocking density on growth, yield, and costs of producing rainbow trout, *Oncorhynchus mykiss*, in hapas. *Journal of Applied Aquaculture* 15, 73–82.
- Watson, M.E., Isaac, R.A., 1990. Analytical instruments for soil and plant analysis. In: Westerman, W.L. (Ed.), *Soil Testing and Plant Analysis*, 3rd edition. SSSA Book series, vol. 3. Soil Science Society of America, Madison, WI, pp. 691–740.
- Zhu, Y.J., Yang, D.G., Chen, J.W., Yi, J.G., Liu, W.C., Zhao, J.H., 2011. An evaluation of stocking density in the cage culture efficiency of Amur sturgeon *Acipenser schrenckii*. *Journal of Applied Ichthyology* 27 (2011), 545–549.