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Can a small change in the tilapia's on-going feeding strategy impair its growth?

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ABSTRACT. The aim of the present work was to answer the following question: can a small change in the tilapia's on-going feeding strategy impair its growth? In the initial phase of the study, fish were evenly distributed and maintained for two weeks in four tanks. In tanks 1 and 2, the ration was given in three equal meals at 8, 12 and 16h or at 9, 13 and 17h, respectively. In tanks 3 and 4, there was only one daily meal at 9 or 10 o'clock, respectively. In the second 8-week phase, fish from tank 1 were distributed into two groups in ten 100-L tanks: the 8-12-16 group, in which fish were always fed at 8, 12 and 16h; and the 8-12-16/9-13-17 group, in which fish were fed at 8, 12 and 16h in the first four weeks and at 9, 13 and 17h in the last four weeks. Fish from tank 2 were allocated into five 100-L tanks and their mealtimes were maintained unchanged. The same procedures were used in tanks 3 and 4. The delay of one hour in the allowance of the artificial diet has significantly reduced fish survival.

Keywords: feeding schedule, circadian rhythm, feeding management, fish culture.

Uma pequena alteração na estratégia alimentar em curso da tilápia pode prejudicar seu crescimento?

RESUMO. O objetivo do presente trabalho foi responder a seguinte questão: uma pequena alteração na estratégia alimentar em curso da tilápia pode prejudicar seu crescimento? Na fase inicial do estudo, os peixes foram igualmente distribuídos e mantidos por duas semanas em quatro tanques. Nos tanques nº 1 e 2, a ração foi fornecida aos peixes em três refeições iguais às 8, 12 e 16h ou às 9, 13 e 17h, respectivamente. Nos tanques nº 3 e 4, havia apenas uma refeição diária às 9 ou 10h, respectivamente. Na segunda fase (8 semanas), os peixes do tanque nº 1 foram distribuídos em dez tanques de 100 L em dois grupos: o grupo 8-12-16, os peixes foram sempre alimentados às 8, 12 e 16h; e o grupo 8-12-16/9-13-17, os peixes foram alimentados às 8, 12 e 16h, nas primeiras quatro semanas, e às 9, 13 e 17h, nas quatro últimas semanas. Os peixes do tanque nº 2 foram distribuídos em cinco tanques de 100 L e suas refeições foram mantidas inalteradas. Os mesmos procedimentos foram utilizados nos tanques nº 3 e 4. O atraso de 1 h no fornecimento de ração reduziu significativamente a sobrevivência dos peixes.

Palavras-chave: horário alimentar, ritmo circadiano, manejo alimentar, piscicultura.

Introduction

Endogenous daily rhythms in fish try to follow some cyclic patterns that happen in the environmental factors. These circadian rhythms in fish are strategies of survival that seek the best adjustment possible between the animal's physiology and some predictable events, such as sunrise (MADRID et al., 2001).

Among the cyclic environmental factors capable to create circadian rhythms in fish, such as temperature and light, stands out the availability of food (MARTINS et al., 2012). Fish will probably develop a food anticipatory activity (FAA) if some forecasting about the food allowance times is possible, as observed in aquaculture. FAA aims at getting the maximum use of the available food to fish. FAA in fish encompasses behavioral, physiological mainly digestive and also cellular modifications (LÓPEZ-OLMEDA et al., 2012). That way, fish would be capable to prepare itself beforehand to take the greatest advantage possible of the food available to it. In this case, there would be a synchronism between the fish's FAA and its mealtimes.

Tilapia is a strictly diurnal fish (TOGUYENI et al., 1997) but with loose daily feeding activity pattern (VERA et al., 2009). Therefore, tilapia presents flexibility in its circadian feeding rhythms enabling the best adaptation to unstable

environments. Notwithstanding that, the Brazilian producers of tilapia do not change the daily mealtimes of fish throughout the culture period. They believe that changing an already established feeding strategy would result in wastage of diet and, consequently, economical losses. As far as we know, that supposition has not been scientifically proven yet.

The present study aimed to answer the following question: can a small change in the tilapia's on-going feeding strategy impair its growth?

Material and methods

Fish and acclimatization period

One thousand 1-g juvenile Nile tilapia, *Oreochromis niloticus*, were obtained from a commercial fish producer from Guaiúba, Ceará State, 60 km from the headquarters. Fish were transported by road to the laboratory facilities in one plastic bag filled with water (1/3) and pure oxygen (2/3). In the first week, fish were maintained in one 1000-L polyethylene tank supplied with dechlorinated tap water and provided with continuous mechanical aeration from one 2.5-hp blower. Over the acclimation week, fish were fed daily on a commercial 500 g kg^{-1} crude protein diet (Fri-Acqua Inicial, Nutreco Fri-Ribe Animal Nutrition, Eusébio, Brazil) at 10% of the stocked biomass split in three even meals at 9, 13 and 17h.

Culture system and experimental design

The experimental period was divided into two distinct phases. In the initial one, two hundred and forty fish were evenly allotted into four 500-L polyethylene indoor tanks (60 fish $tank^{-1}$). In two of the tanks, the ration was allowed to fish in three equal meals at 8, 12 or 16h (Tank 1) or at 9, 13 and 17h (Tank 2). In the other two tanks, there was only one daily meal at 9 (Tank 3) or 10 (Tank 4) o'clock (Figure 1). The objective of the first phase was to condition fish to those four feeding rhythms (meals at 8-12-16, 9-13-17, 9 or 10h) for two weeks. All fish received the same commercial diet used in the acclimation period allowed at 10% per day.

After the first phase, fish were transferred to thirty 100-L polyethylene indoor tanks and stocked at three fish per tank (Figure 1). The indoor culture system of the laboratory provides continuous mechanical aeration to the tanks and has no water recirculation (static system).

In the second 8-week experimental phase, fish from Tank 1 were distributed into two different groups in ten 100-L tanks: the 8-12-16 group, in which fish were fed at 8, 12 and 16h from the beginning to the end of the experiment; and the 8-12-16/9-13-17 group, in which fish were fed at 8, 12 and 16h in the first four weeks and at 9, 13 and 17h in the last four weeks. Fish from the Tank 2 were allotted into five 100-L tanks and their feeding schedule was maintained unchanged at 9, 13 and 17h. Fish from the Tank 3 was distributed into two groups in ten 100-L tanks: the 9 group, in which fish were fed always at 9 o'clock, and the 9/10 group in which fish were fed at 9 o'clock in the first four weeks and at 10 o'clock in the last four weeks. Finally, fish from Tank 4 were allotted into five 100-L tanks and always fed at 10 o'clock. Therefore, there were six experimental groups with five replicates each distributed into a completely randomized design (Figure 1). In the second experimental phase, fish received the same commercial diet used previously that was given at 10% of the stocked biomass per day.

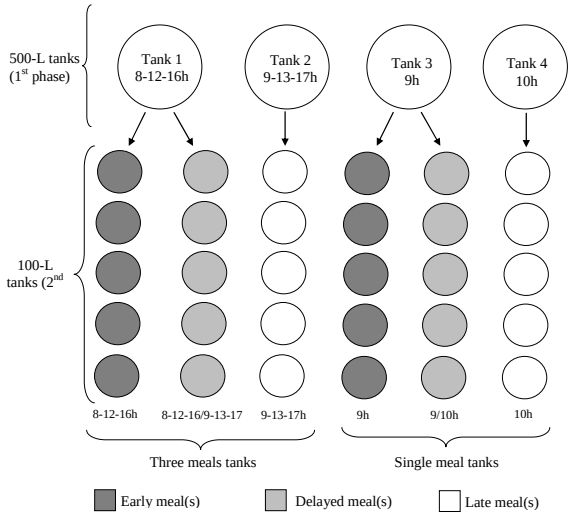

Figure 1. General layout of the present study.

In the 2nd phase of the study, the use of the single meal treatments, beside the three meals treatments, have aimed to strengthen the present work's experimental design as Chen and Purser (2001) have detected FAA in juveniles of sole, *Rhombosolea tapirina*, only in the one-daily-meal-fed groups. The water quality results were pooled as follows: the treatments 8-12-16 and 9 were referred as the early meal(s) treatments; the treatments 8-12-16/9-13-17 and 9/10 were referred as the delayed meal(s) treatments; and the treatments 9-13-17 and 10 were referred as the late meal(s) treatments.

Luminous triggers to induce food anticipatory activity

Although fish without photoperiodic information can also exhibit FAA (ARANDA et al., 2001), their most common way to associate FAA

with food allowance is through the environmental lighting conditions (REEBS; LAGUE, 2000; LÓPEZ-OLMEDA et al., 2009). Therefore, not knowing if the mealtimes alone would be sufficient to synchronize the tilapia's FAA in a roofed room with black-painted glass windows, two artificial luminous triggers were employed in the present study to induce the FAA establishment.

Firstly, the lamps of the experimental room were always turned on 30 minutes before the first daily meal and were turned off 30 minutes after the last one. Consequently, the photoperiod in the experimental room was 10h of light (7:30AM – 5:30PM) and 14h of darkness (5:30PM – 7:30AM). The room lighting was provided by fluorescent lamps installed on the roof. Secondly, a flashlight was always blinked twice near the tank surface water ten minutes before each mealtime. As a result, the diet was delivered to fish stocked in the 8-12-16/9-13-17 and 9/10 tanks with one hour of delay in the second phase of the study.

Tank management and experimental variables

No water exchange was carried out over the entire period. Except by a rapid removal of the tank debris in the onset of the 5th experimental week, new freshwater was added to the tanks just to compensate evaporation and water samplings. From the 4th experimental week to the end, weekly applications of NaCl at 2 g per tank were made in all tanks to prevent nitrite intoxication of fish.

Variables of water quality and growth performance were monitored in the present work. The water quality variables, their methods and frequency of monitoring were the followings: temperature, pH and electrical conductivity (Instrutherm digital thermometer, Marconi MA522 pH-meter and Lutron CD-4301 water conductivity meter, respectively), twice a week, at 9 – 10h; total alkalinity (standard sulfuric acid titration), total hardness (standard EDTA titration), concentrations of dissolved oxygen (Winkler method with azide modification), free CO_2 (standard sodium carbonate titration), total ammonia nitrogen (indophenol method), nitrite (diazotization and coupling method) and reactive phosphorus (ammonium molybdate method), fortnightly, at 9 – 10h. The analytical methods followed the guidelines presented by APHA (1999).

The growth performance variables performed in the present study were the following: fish survival, final body weight, specific growth rate (ln final BW - ln initial BW/days of culture x 100), tank yield and feed conversion ratio.

Statistical analyses

Water and growth performance variables were analyzed by one-way ANOVA. Tukey's test was used for pairwise comparisons of the means. The assumptions of normal distributions and homogeneity of variances were checked before analysis. Percentage and ratio data were analyzed using arcsine-transformed data. All ANOVA analyses were carried out at 5% level of significance using SigmaStat for Windows 2.0 (Jandel Statistics).

Results and discussion

Water temperature in the culture tanks has ranged between 26 and 28°C along the experimental period. On average, water temperature was 26.7 ± 0.6°C and no significant differences existed between tanks (Figure 2). The water temperature is within the comfort range for Nile tilapia optimum growth (AZAZA et al., 2008).

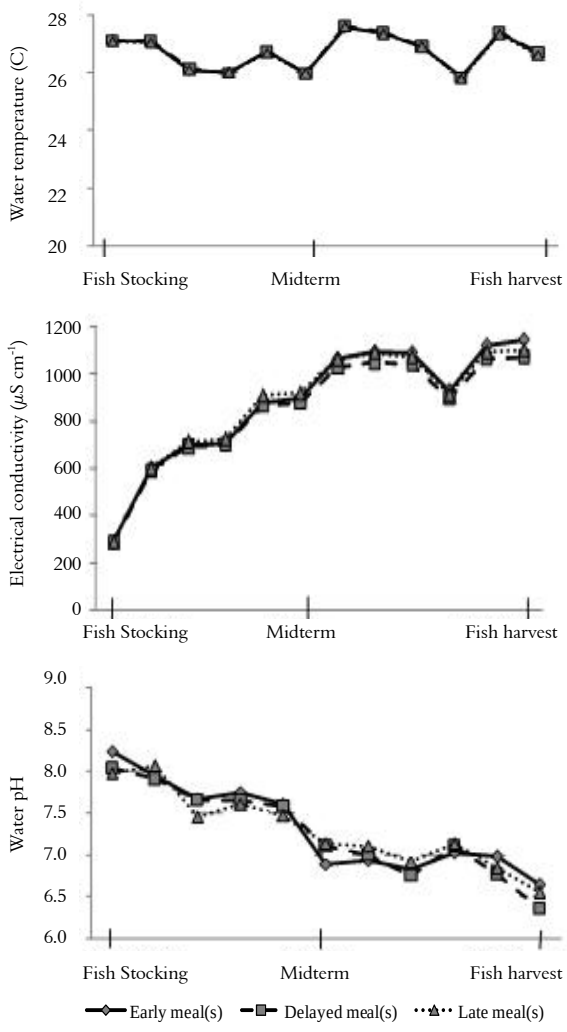

Figure 2. Water temperature, pH and electrical conductivity of 100-L experimental indoor tanks for tilapia subjected to different feeding rhythms for 8 weeks (n = 5).

The pH of water of the culture tanks has progressively decreased over the experimental period starting from 8.0 - 8.3 in the beginning and reaching 6.3 – 6.7 at the end. The water pH differences between the culture tanks were not significant. The average values of water pH at fish stocking, midterm and harvest were 8.1 ± 0.1, 7.0 ± 0.3 and 6.5 ± 0.3, respectively (Figure 2). Those values of water pH are within the proper range for Nile tilapia fish culture (PILLAY; KUTTY, 2005).

Since there was no water exchange over the entire study, the accumulation of organic matter (fish feces and non-ingested feed) on the bottom of the tanks has led to a progressive increase in CO_2 concentrations, acidifying the water (CAVALCANTE et al., 2010).

The electrical conductivity of water (EC) in the culture tanks has increased over the experimental period from near 300 μS cm^{-1} at the fish stocking up to 1200 μS cm^{-1} at the fish harvest. The differences between the tanks for EC were not significant (Figure 2). The absence of water exchange has allowed an increase in the ionic concentration of water in the culture tanks.

It is recommended that the EC of water for fish culture in tanks does not exceed 1000 μS cm^{-1} (BOYD; TUCKER, 1998). Therefore, the EC of water in the present work was above the maximum limit for aquaculture in the last half. Very high values of water EC mean that very high nutrient concentrations are present in the water. This situation can promote dangerous algal blooms under adequate environmental conditions, especially light incidence (AKKOYUNLU; AKINER, 2012). Since the culture tanks of the present work were located in a roofed room, no algal blooms were observed. Hence, the water EC unsuitability limit of 1000 μS cm^{-1} has had no effect in the present work's conditions.

The average total alkalinity (TA) of culture water was lower than 40 mg L^{-1} $CaCO_3$. Freshwater for fish culture should present TA ≥ 20 mg L^{-1} $CaCO_3$. TA below 20 mg L^{-1} $CaCO_3$ can impair fish growth due to a weak primary productivity (ANDRADE et al., 2007). Again, in the present study it had no consequence because fish culture was performed in a roofed room. However, the TAs of the culture tanks were not significantly different between each other (ANOVA $p > 0.05$; Figure 3), not complying with their free CO_2 results. Since TA and free CO_2 are inversely related, it would be expected a significantly lower TA in the early-meal tanks because these tanks showed a significantly higher concentration of free CO_2 in water (Figure 3). This discordance could be associated with differences in the two analytical methods and also in the technicians' skills. Anyway, the significantly lower concentration of free CO_2 in the delayed-meal tanks when compared to the early-meal tanks is interesting and requires a careful consideration.

Herein the main source of free CO_2 to tanks was the decomposition of fish feces. Therefore, the release of fish feces to water was probably greater in the early-meal tanks than in the delayed-meal tanks. This supposition would occur if the former tanks had a higher feeding allowance. In fact, fish stocked in the early-meal tanks received more artificial food than fish in the delayed-meal tanks because the feed allowances were a function of the stocked biomass. Since the feeding rate was set at 10% of the stocked biomass per day, more food was delivered to the early-meal tanks since their fish have grown more than those in the delayed-meal tanks (Table 1). A higher feeding allowance implies generally in a higher production of feces. In this case, the higher concentrations of free CO_2 in the early-meal tanks cannot be seen as a negative index of water quality. On the contrary, the higher concentrations of free CO_2 in the early-meal tanks indicate a higher degree of feed intake and growth by fish when compared to the delayed-meal tanks.

The total hardness (TH) of water has remained high throughout the experiment, always above 120 mg L^{-1} $CaCO_3$. No significant differences were detected between the tanks for TH (ANOVA $p > 0.05$; Figure 3). Freshwater fish succeed better in moderately high TH waters (60 – 150 mg L^{-1} $CaCO_3$) than in soft waters (BOYD, 1979). Therefore, the TH of culture water was within the adequate range for freshwater fish culture. Unlike TA, TH of water is a very conservative variable of water quality that is hardly affected by the fish feeding management.

In the present work, there was a continuous mechanical aeration to the culture tanks, allowing that suitable concentrations of dissolved oxygen (DO_2) were obtained in the culture water. On average, the DO_2 concentration has exceeded 6 mg L^{-1} and no significant differences were seen between the experimental tanks (Figure 3). It is assumed that DO_2 concentrations higher than 4 mg L^{-1} are adequate for warm water fish culture (BOYD; TUCKER, 1998). Therefore, the concentrations of DO_2 in the present work were suitable for the growth of juvenile Nile tilapia.

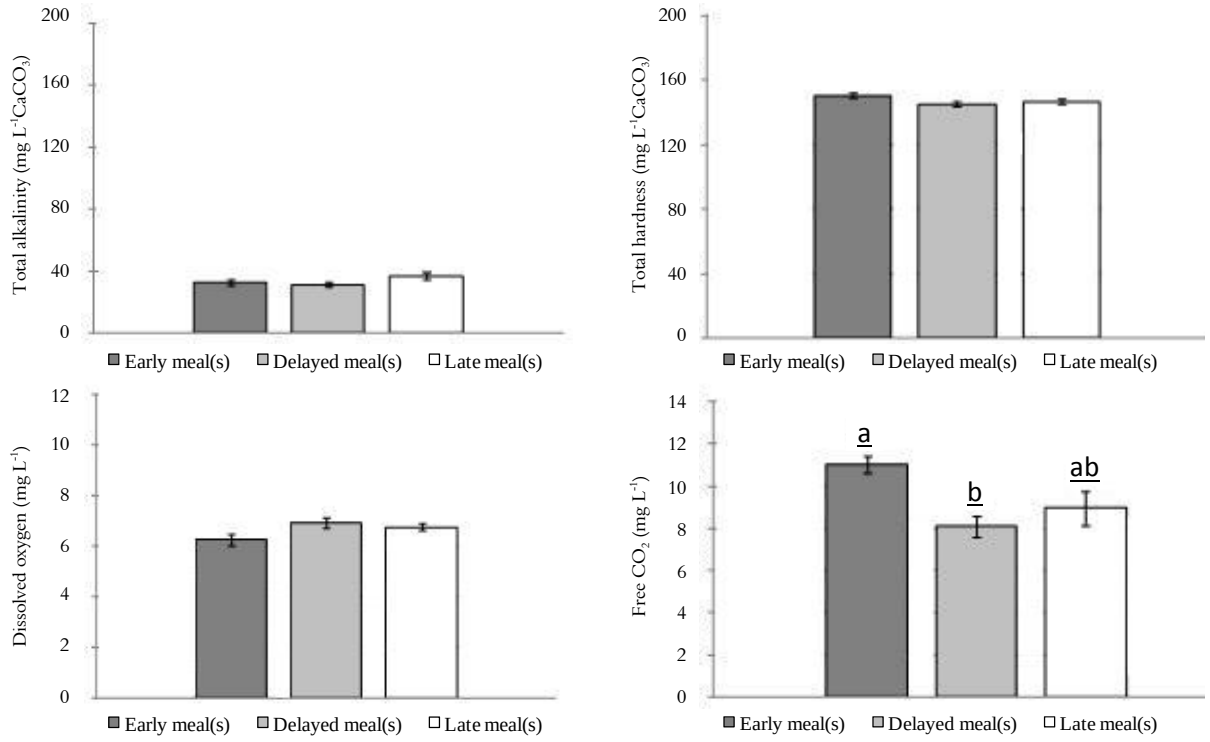

Figure 3. Total alkalinity, total hardness and concentrations of dissolved oxygen and free CO_2 in 100-L experimental indoor tanks for tilapia subjected to different feeding rhythms for 8 weeks (mean ± SE; n = 5). Columns not sharing a same letter represent means significantly different by the Tukey's test (ANOVA $p < 0.05$).

The total ammonia nitrogen (TAN) and nitrite concentrations in all tanks were high and very similar between the different treatments. Regardless the treatment, the average concentration of TAN was close to 0.8 mg L^{-1} (Figure 4). The toxicity of TAN to fish is mainly related to its un-ionized form (NH_3) which depends on the water pH and temperature. Greater proportions of NH_3 are found in alkaline (pH ≥ 9) and warm waters (DIANA et al., 1997). In the present work, there were probably very low and harmless concentrations of NH_3 because the maximum water pH observed was below 9 and has decreased over time. On the other hand, the risk of nitrite toxicity was real because its average concentration was near 0.8 mg L^{-1} (Figure 4) and stressful levels for fish are 0.3 mg L^{-1} or above (WEIRICH; RICHE, 2006a and b). Common salt (NaCl) was then added to all tanks to prevent fish mortalities by nitrite in the present study.

Significant differences for reactive phosphorus in water were observed in the culture tanks (Figure 4; ANOVA $p < 0.05$). The reactive phosphorus in the delayed-meal(s) tanks, i.e., the 8-12-16/9-13-17 and 9/10 tanks, was significantly higher than in the early-meal and late-meal tanks. The reactive phosphorus in water was not significantly different between the early-meal and the late-meal tanks. In the present study, the main source of phosphorus to the culture water was the artificial diet delivered to fish. Remains of non-ingested diet have probably released phosphorus to the water after their physical and biological processing. Besides, and mainly, a major part of the ingested diet was lost to the water as fish feces after its incomplete digestion. It is known that fish feces are phosphorus-rich (GUO et al., 2009). Thus, it is suggested that the fish stocked in the delayed-meal(s) tanks have presented a lower feed intake and/or a higher phosphorus wastage to water via feces than fish allotted into the other tanks. Maybe the disturbance in the feeding schedule of the former tanks, caused by the delay of one hour in the dietary allowance, has impaired both feeding intake and phosphorus absorption by fish.

The different feeding rhythms employed in the present study have significantly affected the growth performance of fish (Table 1; ANOVA $p < 0.05$). The most remarkable case was the final survival in the 8-12-16/9-13-17 tanks. The delay of one hour in the allowance of artificial diet to

those tanks has significantly lowered fish survival to 60%, on average. The survival of fish stocked in the 8-12-16 tanks (unchanged mealtimes) was 100%. However, the same result was not seen in the 9/10 tanks. The culture tanks with unchanged feeding schedules, i.e., the 9 and 8-12-16 tanks (early-meal tanks), and the 10 and 9-13-17 tanks (late-meal tanks), have presented significantly superior growth performance results when compared to the tanks in which the feeding schedules underwent a one-hour delay, i.e., the 9/10 and 8-12-16/9-13/17 tanks (delayed-meal tanks). The most striking result was observed for tank yield. The tank yields for the unchanged-feeding-schedule tanks (9, 10, 8-12-16 and 9-13-17) were significantly higher than for the delayed-feeding schedule tanks (9/10 and 8-12-16/9-13-17; Table 1). The probably greater wastage of diet in the delayed-meal tanks, as non-ingested food as well as fish feces, has led fish to malnutrition and, consequently, to growth performance impairment. It is supposed that the fish's FAA has been lost or at least misused in the delayed-meal tanks when compared to the on-time meal tanks.

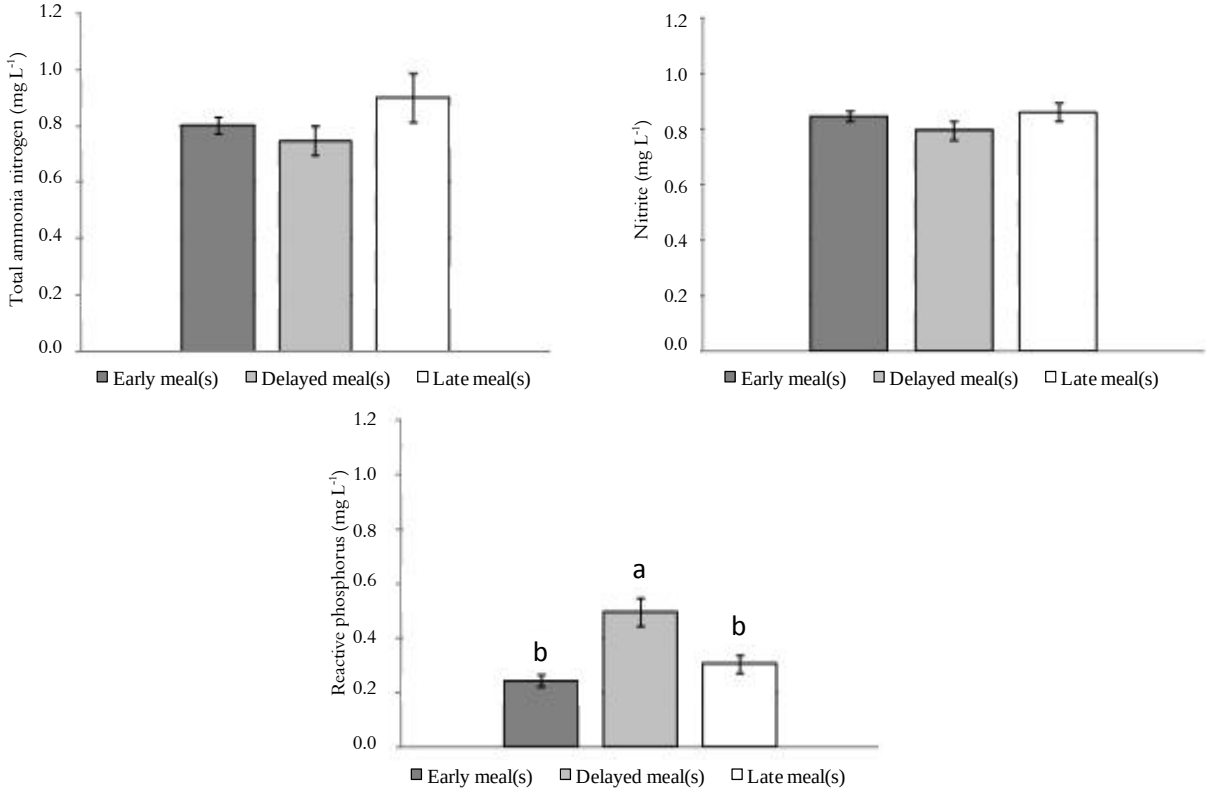

Figure 4. Concentrations of total ammonia nitrogen, nitrite and reactive phosphorus in 100-L experimental indoor tanks for tilapia subjected to different feeding rhythms for 8 weeks (mean ± SE; n = 5). Columns not sharing a same letter represent means significantly different by the Tukey's test (ANOVA $p < 0.05$).

Table 1. Growth performance of juvenile Nile tilapia, *Oreochromis niloticus* (initial body weight = 3.4 ± 0.1 g) subjected to different feeding rhythms for 8 weeks (mean ± SE; n = 5).

Daily meal time (h)	Survival (%)	Final BW[4] (g fish^{-1})	SGR[5] (% BW day^{-1})	Yield (g m^{-3})	FCR[6]
9	100.0 ± 0.0	28.8 ± 2.0 a	3.53 ± 0.11 a	852 ± 60 a	1.75 ± 0.11
10	86.7 ± 13.3	33.7 ± 1.7 a	3.49 ± 0.08 a	828 ± 164 a	1.88 ± 0.10
9/10[1]	93.3 ± 6.7	21.9 ± 1.4 b	3.03 ± 0.14 b	651 ± 43 b	2.03 ± 0.08
ANOVA P	ns[2]	< 0.05	< 0.05	< 0.05	ns
8-12-16	100.0 ± 0.0 a[3]	29.7 ± 1.3	3.91 ± 0.05	892 ± 38 a	1.64 ± 0.02 a
9-13-17	80.0 ± 13.3 ab	31.5 ± 4.0	3.86 ± 0.19	831 ± 59 a	1.71 ± 0.11 a
8-12-16/9-13-17[1]	60.0 ± 12.5 b	23.2 ± 2.7	3.39 ± 0.24	509 ± 31 b	2.39 ± 0.12 b
ANOVA P	< 0.05	ns	ns	< 0.05	< 0.05

[1]There was a delay of one hour in the delivery of each meal to fish after the initial four weeks; [2] Non-significant (ANOVA $p > 0.05$); [3]For each feeding rhythm (one daily meal or multiple daily meals), means not sharing the same letter in a column are significantly different by the Tukey's test (ANOVA $p < 0.05$); [4]Final body weight; [5] Specific growth rate = (ln final BW - ln initial BW)/days of culture x 100.

These results clearly indicated that even a minor change in a previously established feeding strategy, specifically the daily mealtimes, can negatively affect the tilapia's growth performance. Therefore, it is recommended do not change the daily mealtimes over the entire production cycle in order to avoid fish growth impairment.

Our results are in agreement with Sánchez-Vázquez and Tabata (1998), Azzaydi et al. (1999) and Spieler (2001) who concluded that the meal timing is important to obtain better growth performance in fish. The meal timing can be understood as the matching between the dietary allowances to fish and its FAA behavior. On the other hand, our results diverged from Vera et al. (2009) who have observed flexibility in the tilapia feeding behavior to variable environments. Fish had only artificial feed for its nutrition in the present work. Perhaps the fish growth would not have decreased in the second experimental period (after mealtime delay) if there was sufficient natural food (plankton, periphyton) in the tanks.

Several studies on self-feeding or demand-feeding in fish have been carried out so far (ANDREW et al., 2002; BENHAIM et al., 2011; FIGUEIREDO-SILVA et al., 2010; FORTES-SILVA et al., 2010; LEAL et al., 2011). In general, those works have concluded that the best feeding strategy that maximizes feed intake and digestibility by fish is to allow the own animals to choose their mealtimes. However, as the large-scale use of demand feeders is a hindrance, the adoption of mealtimes bound to the food anticipatory activity of fish is crucial to achieve successful feeding management in aquaculture.

Conclusion

Concluding, even a minor modification in an already established feeding strategy, specifically daily mealtimes, can negatively affect the tilapia's growth performance.

Acknowledgements

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