

## Performance of poly cultured fish species fed organic or commercial diets

### *Desempenho de peixes em policultivo alimentados com ração orgânica e comercial*

Betina Muelbert<sup>1</sup>, Marcos Weingartner<sup>1</sup>, Ricardo Yuji Sado<sup>2</sup>, Fernanda Ferrari<sup>2</sup>, Maude Regina de Borba<sup>1</sup>

<sup>1</sup>Professores Doutores da Universidade Federal da Fronteira Sul, Laranjeiras do Sul, Paraná. +55423635-8691. E-mails: [betina.muelbert@uffs.edu.br](mailto:betina.muelbert@uffs.edu.br); [marcos.weingartner@uffs.edu.br](mailto:marcos.weingartner@uffs.edu.br); [maude.borba@uffs.edu.br](mailto:maude.borba@uffs.edu.br). <sup>2</sup>Professores Doutores da Universidade Tecnológica Federal do Paraná. Dois Vizinhos, Paraná. E-mails: [ricardoysado@utfpr.edu.br](mailto:ricardoysado@utfpr.edu.br); [fermandaferrari@utfpr.edu.br](mailto:fermandaferrari@utfpr.edu.br).

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#### ABSTRACT

Polyculture with supplementary diets is considered a viable model for organic fish production, although is still a system on its infancy. The aim of the present study was to compare the performance of fishes in polyculture fed organic or commercial diets in an on-farm experiment in southern Brazil. In four farms, two dietary treatments were evaluated: pelleted Organic artisanal diet and extruded conventional Commercial diet. Polyculture was a combination of *Rhamdia quelen* (30%), *Cyprinus carpio* (20%), *Pterygoplichthys joselimaianus* (15%), *Prochilodus lineatus* (15%) *Hypophthalmichthys molitrix* (7,5%), *H. nobilis* (7,5%) and *Ctenopharyngodon idella* (5%). Juveniles were stocked in earthen ponds at a total density of 1.5 fish m<sup>-2</sup> and fed for 12 months. Final biomass was significantly higher in the Organic (4,877.8 kg ha<sup>-1</sup>) than in the Commercial treatment (3,067.1 kg ha<sup>-1</sup>). Mean final weight, specific growth rate, fillet composition and total survival of fishes did not differ among treatments. In conclusion, feeding organic diets recorded bigger production and water quality than commercial diets in poly culture system. We hope that these results will stimulate further research on organic fish farming.

#### RESUMO

Para a produção de peixes orgânicos o sistema de policultivo é indicado. O objetivo deste estudo foi verificar o desempenho de peixes criados em sistema de policultivo, alimentados com dieta orgânica ou comercial, em experimento de campo realizado no sul do Brasil. O policultivo compreendeu a combinação de espécies com diferentes hábitos alimentares: *Rhamdia quelen* (30%), *Cyprinus carpio* (20%), *Pterygoplichthys joselimaianus* (15%), *Prochilodus lineatus* (15%) *Hypophthalmichthys molitrix* (7,5%), *H. nobilis* (7,5%) e *Ctenopharyngodon idella* (5%), distribuídas (1,5 peixe m<sup>-2</sup>) em viveiros escavados de quatro propriedades rurais, em delineamento em blocos casualizados. Foram avaliados dois tratamentos alimentares, dieta orgânica artesanal peletizada e dieta convencional comercial extrusada, ao longo de 12 meses. Ao final do período de cultivo sobrevivência média, peso médio final, taxa de crescimento específico e composição do filé não diferiram entre os tratamentos dietéticos. Todavia, foi obtida produtividade média significativamente maior nos viveiros em que os peixes foram alimentados com a dieta orgânica (4.877,8kg ha<sup>-1</sup>) em comparação aos que receberam a dieta comercial (3.067,1 kg ha<sup>-1</sup>). Conclui-se que a suplementação alimentar com dietas orgânicas peletizadas registrou maior produção do que dieta comercial neste sistema de policultivo. Esperamos que estes resultados estimulem mais pesquisas sobre a piscicultura orgânica.

#### INTRODUCTION

Fish farming generates income and is a valuable source of highly nutritious animal protein (FAO, 2016). The recent increase in fish consumption and market share of organic foods have raised attention to organic aquaculture. Especially for small-scale fish farming operations, organic aquaculture can become a development model (PAUL; VOGL, 2013; BEG et

al., 2016). Organic production systems must follow principles and guidelines that are based on national regulations. The Brazilian organic aquaculture regulation is the IN 28/2011 and establishes, amongst many recommendations, the use of polyculture, prioritizing native species (BRASIL, 2011).

Fish polyculture benefits species synergistically and enhances nutrient cycling in environment. The combination of fishes with different feeding habits (omnivorous, herbivorous

and filter feeders) ensures the consumption of all kinds of natural organisms in the pond. Consequently, it is possible to obtain elevated growth rates and biomass due to these complementary interactions among fish species (HAO-REN, 1982).

Fish polyculture systems in southern Brazil are predominately based around small-scale farming units. Usually is a mixture of common carp *Cyprinus carpio*, silver carp *Hypophthalmichthys molitrix*, bighead carp *H. nobilis* and grass carp *Ctenopharyngodon idella*. Some studies have been conducted to introduce other species such as silver catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*) (SILVA et al., 2008; BARCELOS et al., 2012) and on farm inputs and commercial diets as supplementary feeding in polyculture (HERNANDEZ et al., 2014).

Although not essential in low-density systems supplementary feeding is crucial in more intensive polyculture systems as natural food production in the pond becomes insufficient to enhance production. With this in mind, artificial feed supplementation in an organic system must be produced in accordance to standards, i.e. manufactured with organic ingredients. Moreover, fishmeal should not be refined and the use of synthetic and genetically modified ingredients in feedstuffs are not allowed (BRASIL, 2011). Some authors have studied performances of fish fed organic diets (DIETERICH et al., 2012; BOSCOLO et al., 2013), but there is a lack of studies in a polyculture system.

Small-scale farmers dominate the agriculture landscape throughout Brazil, particularly in the south region. For a small fish farmer to convert to organic system many elements need to be accomplished and a range of different organic management models can be defined. The aim of this study is to contribute to determine one model by verifying the performance of fishes fed with organic artisanal and conventional commercial diets in a polyculture system.

## MATERIAL E METHODS

This research was conducted from November 2014 to November 2015 (352 days) in four small-scale producers located in the state of Paraná, southern Brazil. In each small farm, two earthen ponds (size varying from 200 to 500 m<sup>2</sup>) were managed following the Brazilian organic aquaculture legislation IN 28/2011 (BRASIL, 2011). Agricultural limestone and organic fertilizer in the form of chicken dung were spread in dry form on the bottom of the fishponds at the rate of 100g m<sup>-2</sup> one week prior filling up to 1.5 m depth. Subsequently, the water level was maintained through fortnight compensation of about 4–6 cm of seepage and evaporation loss. Considering an organic model, three neotropical native species were combine with cyprinid fishes in order to established the following experimental polyculture system: silver catfish *R. quelen* (30% of the individuals; 0.23 g initial weight), common carp *C. carpio* (20%; 0.55 g), armored catfish *Pterygoplichthys joselimaianus* (15%; 1.04 g), curimba *Prochilodus lineatus* (15%; 5.19 g), silver carp *H. molitrix* (7,5%; 1.10 g), bighead carp *H. nobilis* (7,5%; 14.57 g) and grass carp *C. idella* (5%; 0.59 g). Fingerlings were purchased from a commercial hatchery and stocked at a total density of 1.5 fish m<sup>-2</sup>.

A complete randomized block experimental design was used, in which each of four small farmers individually was considered a block with two ponds, where fish was fed pelleted Organic artisanal diet (Organic) and extruded conventional Commercial diet (Commercial). Each pond was considered a replicate (n=4). Crude protein concentration (CP) and particle size of the diets and feeding rates were the same for both Organic and Commercial treatments and varied during the culture period (Table 1). Feeding rates (% of biomass) were adjusted considering *R. quelen* and common carp growth measured through intermittent sampling every 60-90 days.

**Table 1.** Crude protein (%) and particle size of Organic and Commercial diets and feeding rate for 352 days experimental growth period.

Period of growth	Number of days	Feed and Feeding Regime		
		Crude protein (%)	Particle size	Feeding Rate (% of biomass)*
November 2014 – January 2015	90	40	Powder	10
February – August 2015	210	36	3 mm	3
September - November 2015	52	32	5 mm	2

\*Considering only the biomass of the two main species (*R. quelen* and *C. carpio*).

Organic diets were prepared with fishmeal, mineral/vitamin premix and certified organic ingredients and three levels of crude protein (Table 2). Ingredients were weighted, homogenized, mixed, moistened and pelleted. Then, each diet was dried using a forced ventilation oven at 45°C for 24h and packed to use in each producer. Fish were fed once daily six days per week.

The commercial fish diets were purchased at a local store. The levels of guarantee from the manufacturer are presented in table 3.

Water quality was monitored every month during experimental period. The abiotic variables water temperature (°C), pH, dissolved oxygen (mg L<sup>-1</sup>), electric conductivity (µS

cm<sup>-1</sup>) and water transparency (cm) were measured in the field using standard electrodes of multiparameter probe (Horiba U-50, Kyoto, Japan) and, in the latter case, a Secchi disk. Total alkalinity (mg CaCO<sub>3</sub> L<sup>-1</sup>) and total hardness (mg CaCO<sub>3</sub> L<sup>-1</sup>) were performed with colorimetric tests using an Aquaculture Kit from February to October 2015. Samples were obtained at a depth of 30 cm in ponds on the same sampling day and transported under refrigeration to the laboratory, where the analyses of nitrogenous and phosphorus compounds (mg L<sup>-1</sup>) were conducted. Total N (TN) and total P (TP) were measured according to Valderrama (1981), from unfiltered water samples. Ammonium (N-NH<sub>4</sub><sup>+</sup>) was determined from filtered water samples, following Solorzano (1969).

**Table 2.** Ingredients and composition of the Organic diets (% dry matter).

Ingredients	Diets (% Crude Protein)		
	40	36	32
Fish meal	24	20	17
Soybean meal	44	36.6	33
Wheat flour	6	15.5	8.6
Maize	21	25.2	36.9
Soybean oil	3	1.2	1
Salt	0.5	0.5	0.5
Premix <sup>1</sup>	1.5	1	2
Dicalcium phosphate	0	0	1
Proximate composition			
Dry matter (%)	90	90	90
Crude fat (%)	11.0	8.7	8.1
Ash (%)	10.2	9.0	9.4
Fiber (%)	3.6	4.0	3.4
NFE (%) <sup>2</sup>	35.0	42.3	46.5
Digestible energy (kcal/kg) <sup>3</sup>	3610	3400	3230

<sup>1</sup>Composition – units/kg of premix: antioxidant 0.6 g; folic acid 250 mg; pantothenic acid 5.000 mg; biotin 125 mg; niacin 5,000 mg; vitamin A 1,000,000 IU; thiamine 1.250 mg; cyanocobalamin 3.750 mg; riboflavin 2.500 mg; pyridoxine 2.485 mg; ascorbic acid 42.000 mg; vitamin D3 500,000 IU; vitamin E 20,000 IU; vitamin K3 500 mg; cobalt 25 mg; copper 2.000 mg; iron 13,820 mg; iodine 100 mg; manganese 50 mg; selenium 75 mg and zinc 17.500 mg. <sup>2</sup>Nitrogen free extract (Digestible carbohydrates) = 100 – (ash + fat + crude protein + fiber). <sup>3</sup>Digestible energy = (5.64 cal g<sup>-1</sup> x %CP x 0.9) + (9.51 cal g<sup>-1</sup> x %fat x 0.85) + (4.11 cal g<sup>-1</sup> x % carbohydrates x 0.5) (JOBLING, 1983).

**Table 3.** Composition of the Commercial diets (% dry matter), levels of guarantee from the manufacturer.

Proximate composition	Diets (% Crude Protein)		
	40	36	32
Dry matter (%)	90	90	90
Crude fat (%) minimum	4	4	4
Ash (%) maximum	10	10	10
Fiber (%) maximum	5	5	5

**Table 4.** Final biomass, survival and feed conversion ratio for Organic and Commercial treatments, at the end of 352 days experimental growth period.

Treatments	Final biomass (kg ha <sup>-1</sup> )	Survival (%)	FCR ( <i>R. quelen</i> + <i>C. carpio</i> )
Organic	4,877.8 ± 1,044.9 <sup>a</sup>	63.2 ± 10.9	1.2 ± 0.1 <sup>a</sup>
Commercial	3,067.12 ± 483.5 <sup>b</sup>	57.4 ± 2.9	1.6 ± 0.1 <sup>b</sup>
<i>P</i> value Student t test	0.020	0.730	0.009

Different superscript letters in the same column indicate statistical difference between treatments ( $P < 0.05$ ); values are expressed as mean ± SD (n=4).

Final biomass can be considered high for both treatments even though the survival rates were relatively low. In a similar polyculture system, where fishes were fed commercial diet, fresh grass and ground corn for seven months, final biomass of 3,198 kg ha<sup>-1</sup> and survival rates above 83% were obtained (BARCELOS et al., 2012). In the present study, final biomass was 59% superior in fish fed with Organic diet treatment in comparison to Commercial treatment. Despite total survival rates were not different, individually *C. carpio* fed organic diet presented higher rate and as a main specie in the polyculture, representing 20% of the species, could explain the higher biomass in organic feed treatment. In addition, the common carp bottom dwelling habit (MATSUZAKI et al., 2009) also

favoured sinking (organic) feed intake. Numerically, the main species (*C. carpio* and *R. quelen*) had a higher final weight, although no effects were observed among treatments. Similar results also was observed for another silver catfish species *Rhamdia voulezi* fed organic certified diet when compared to conventional feeding practices, but in net-cages system for 78 days (FEIDEN et al., 2010). The survival of both treatments can be considered less than within the desired range of 75–85% for earthen ponds in semi intensive systems, and it was clearly influenced by the low survival of the *P. joselimaianus* (30%). There is a lack of studies focusing on fish fed with organic diets. The scarce literature is comprised of studies carried out over a short period

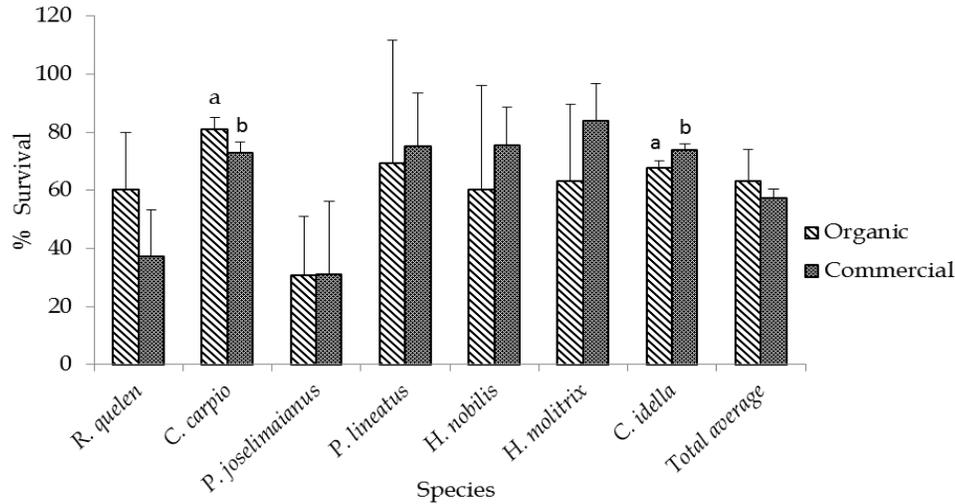
## RESULTS AND DISCUSSION

At the end of the experiment, fishes were harvested by repeated netting and finally by total draining of ponds for complete fish removal. Each species was individually counted to record biometrical measures and calculate survival rates and growth parameters – Survival (%); Final Weight (g); Specific Growth Rate – SGR (% day<sup>-1</sup>) = 100\*(Ln final weight – Ln initial weight)/days of culture), Condition Factor – K (g cm<sup>-3</sup>) = 100\*(wet weight/length<sup>3</sup>) and Final Biomass – FB (kg ha<sup>-1</sup>). The feed conversion ratio – FCR was calculated considering the sum of the feed weight provided to each system and the biomass production of the two main species (i.e. *R. quelen* and *C. carpio*). As a matter of fact, the weight gain of those main species were the only ones considered for the feeding rate. In addition, at the end of the experiment, samples of three *R. quelen* and three *C. carpio* were randomly collected from each pond, euthanized with anaesthetic overdose (clove oil, 400 mg L<sup>-1</sup>) and stored at -20 °C until analysis to determine the fillet composition. The fillets obtained from the three sampled fish of each species per replicate (n=12) were minced and homogenized for analysis following standard procedures (AOAC, 2000). Dry matter was obtained through drying samples at 105 °C, ash by incineration at 550 °C, fat by ether extraction and crude protein by Kjeldahl method (N x 6.25). Data from the feeding trial, fish file composition and water quality parameters were compared by using Student's t test.

The results of final biomass, survival for all species and feed conversion ratio of *R. quelen* and *C. carpio* are shown in Table 4. Final biomass was significantly higher ( $P < 0.05$ ) in ponds fed with Organic diet (4,877.8 kg ha<sup>-1</sup>) than in the Commercial diet treatment (3,067.1 kg ha<sup>-1</sup>). Also, feed conversion ratio for both main species (*R. quelen* + *C. carpio*) was significantly better for Organic treatment. On the other hand, total final survival did not differ between treatments.

of time and under controlled experimental conditions, which makes it difficult to compare with our results.

**Figure 1.** Survival rates for individual species fed Organic and Commercial diet, at the end of 352 days experimental period.



Different letter above columns for the same specie indicates statistical difference between treatments ( $P < 0.05$ ; Student t test). Values are expressed as mean  $\pm$  SD (n=4).

The survival rate of *C. carpio* and *C. idella* when analysed individually, was affected ( $P < 0.05$ ) by treatment (Figure 1). *C. carpio* fed organic diet presented higher survival rate when compared to those fed Commercial diet. In contrast, *C. idella* fed Organic diet presented lower survival rate when compared to fish fed Commercial diet, but as the percentage of this specie was very low in the polyculture (5%) it did not affect the final biomass results. The variability of survival for individual species ranged from 30.7% for *P. joselimaianus* to

81% for *C. carpio* in Organic treatment and from 31.3% for *P. joselimaianus* to 84% for *H. molitrix* in Commercial treatment.

The initial weight of each fish species used in the polyculture system was similar and, after the 352 days feeding trial, there was no significant difference between treatments for final weight, specific growth rate and condition factor as shown in Table 5.

*R. quelen* and common carp fillet composition are summarized in Table 6. Moisture, protein, fat and ash were not influenced ( $P > 0.05$ ) by the type of diet.

**Table 5.** Final weight, specific growth rate (SGR) and condition factor (K) for species under Organic and Commercial diet treatments at the end of the 352 days experimental growth period<sup>1</sup>.

	Species <sup>2</sup>	Diets		P value Student t test
		Organic	Commercial	
Final weight (g)	<i>R. quelen</i>	489.08 $\pm$ 118.46	341.21 $\pm$ 83.63	0.088
	<i>C. carpio</i>	784.77 $\pm$ 306.52	660.28 $\pm$ 205.90	0.525
	<i>P. joselimaianus</i>	248.71 $\pm$ 205.70	124.24 $\pm$ 25.44	0.699
	<i>P. lineatus</i>	269.31 $\pm$ 60.83	247.32 $\pm$ 89.53	0.699
	<i>H. molitrix</i>	320.08 $\pm$ 95.40	207.42 $\pm$ 36.53	0.070
	<i>H. nobilis</i>	414.16 $\pm$ 166.74	244.11 $\pm$ 103.85	0.134
	<i>C. idella</i>	669.94 $\pm$ 188.88	483.37 $\pm$ 234.72	0.262
	SGR (% day <sup>-1</sup> )	<i>R. quelen</i>	2.18 $\pm$ 0.08	2.02 $\pm$ 0.07
<i>C. carpio</i>		2.06 $\pm$ 0.10	2.02 $\pm$ 0.10	0.524
<i>P. joselimaianus</i>		1.48 $\pm$ 0.27	1.36 $\pm$ 0.06	0.449
<i>P. lineatus</i>		1.12 $\pm$ 0.07	1.09 $\pm$ 0.10	0.616
<i>H. molitrix</i>		1.61 $\pm$ 0.10	1.49 $\pm$ 0.05	0.079
<i>H. nobilis</i>		0.94 $\pm$ 0.10	0.79 $\pm$ 0.11	0.088
<i>C. idella</i>		2.00 $\pm$ 0.07	1.88 $\pm$ 0.16	0.239
K		<i>R. quelen</i>	1.31 $\pm$ 0.10	1.25 $\pm$ 0.09
	<i>C. carpio</i>	2.18 $\pm$ 0.25	2.21 $\pm$ 0.38	0.490

<sup>1</sup>Values are expressed as mean  $\pm$  SD (n=4). <sup>2</sup>*Rhamdia quelen*, *Cyprinus carpio*, *Pterygoplichthys joselimaianus*, *Prochilodus lineatus*, *Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis*, *Ctenopharyngodon Idella*

**Table 6.** Fillet proximal composition (wet basis) of *Rhamdia quelen* and *Cyprinus carpio* fed Organic and Commercial diet, at the end of the 352 days experimental growth period.

Species	Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
<i>R. quelen</i>	Organic	73.22 ± 1.88	17.95 ± 0.86	10.22 ± 1.56	1.07 ± 0.02
	Commercial	73.80 ± 2.89	18.10 ± 1.12	8.19 ± 2.77	1.06 ± 0.06
<i>C. carpio</i>	Organic	73.57 ± 2.36	17.41 ± 1.22	8.98 ± 3.20	1.03 ± 0.04
	Commercial	74.93 ± 1.43	18.51 ± 1.32	7.91 ± 1.12	0.93 ± 0.04

Values are expressed as mean±SD. Means of pooled samples of three fish from each of four replicate groups.

Few data on the quality of organically reared fish meat are available. Nutritional characteristics of fish meat are dependent on chemical composition and can be influenced by the food consumed (GRIGORAKIS, 2007; CORRÊA et al., 2013). In the present study, proximate analysis showed that fillet composition was similar for fish fed with Organic and Commercial diets. In the same way, other fish species such as European sea bass

(*Dicentrarchus labrax*) (TROCINO et al., 2012); *Rhamdia voulezi*, Nile tilapia (*Oreochromis niloticus*) and *Piaractus mesopotamicus* (BOSCOLO et al., 2013) also presented no differences for moisture, protein, fat and ash levels in the fillet of fishes fed organic or commercial diets.

Mean values and ranges of water quality parameters measured during trial are presented in Table 7.

**Table 7.** Water quality parameters for Organic and Commercial diet treatments, during the 352 days experimental growth period.

Water quality parameters	Treatment		P value Student t test
	Organic	Commercial	
Water temperature (°C)	22.2 ± 3.1	22.3 ± 3.2	0.852
Transparency (cm)	30.9 ± 32.7	32.5 ± 27.1	0.809
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	26.8 ± 8.7	30.3 ± 9.1	0.176
Total hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	30.8 ± 9.3	30.4 ± 9.2	0.878
pH	6.7 ± 0.9	6.7 ± 1.0	0.821
Electric conductivity (mS cm <sup>-1</sup> )	0.07 ± 0.04	0.06 ± 0.04	0.648
Dissolved oxygen – DO (mg L <sup>-1</sup> )	6.2 ± 2.1	5.5 ± 2.5	0.133
Total Nitrogen - TN (mg L <sup>-1</sup> )	0.275 ± 0.189 <sup>a</sup>	0.368 ± 0.25 <sup>b</sup>	0.055
Total Phosphorus - TP (mg L <sup>-1</sup> )	0.071 ± 0.055	0.081 ± 0.092	0.546
Ammonium - NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	0.060 ± 0.036	0.082 ± 0.085	0.121

Values are expressed as mean±SD. Different superscript letters in the same line indicate statistical difference between treatments ( $P < 0.05$ )

The mean values of alkalinity and total hardness were similar between treatments as well as to water temperature, water transparency, pH and electric conductivity. However, both in Organic and Commercial treatments, lower temperatures were recorded during the dry season months (April to August), transparency of water presented higher values at the beginning of the experiment (November and December), and the pH decreased towards final months of experimental period, which also occurred with electric conductivity. Dissolved oxygen did not differ statistically between treatments and was within acceptable limits. Total nitrogen was affected by treatment ( $P < 0.05$ ) and was higher in treatment with commercial diet in all experimental period.

Water quality parameters were within acceptable values for water ponds used in fish culture, despite alkalinity levels were considered low, but not enough to affect the primary production of the pond (BRASIL, 2005; BOYD; TUCKER, 1998; KUBITZA, 20118). The results of the nitrogen compounds, especially Total nitrogen, suggest that pelleted organic diet was superior to extruded commercial diet in maintaining a good water quality. Total phosphorus also pointed out better trophic conditions in organic system due to the tendency to reduce concentrations at the end of experiment when compared with the commercial system. Values of Ammonium and Total phosphorus in this experiment were lower than those reported by Dieterich et al. (2012) of 0,33

mg.L<sup>-1</sup> e 0,27 mg.L<sup>-1</sup> respectively, in tilapia culture system fed diets with organic ingredients.

In fish farming, nutrient ions are mainly from feeding and can trigger eutrophication process that negatively affects the water quality and consequently the growth and well-being of fishes (ABU-ELALA al., 2016; CHEZHIAN et al. 2012). In this experiment, the lower final nitrogen and phosphorus concentrations in water indicates that the organic diet probably allows better utilization of the feed nutrients by fishes decreasing possible remains in the cultivation environment and greater recycle of these nutrients. Thus, in addition to the direct benefits to fish production, the organic system can contribute to the maintenance of better water quality, with less susceptibility to occurrence of problems arising from eutrophication, such as blooms of toxic cyanobacteria and anoxia, therefore more compatibility with the bases of sustainable aquaculture.

## CONCLUSIONS

The poly cultured fish species fed organic diets has higher performance when comparing to commercial diets. This study contributed to determine a model of organic fish farming in a polyculture system, using omnivorous silver catfish, bottom feeder common carp, grass and plankton-feeders carps.

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