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Research Article

Effects of stocking densities in blood chemistry and biochemical body composition of Nile tilapia *Oreochromis niloticus* and the prawn *Macrobrachium americanum* in polyculture

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ABSTRACT. An assay on tilapia *Oreochromis niloticus* and freshwater prawn *Macrobrachium americanum* in polyculture was conducted during 112 days to study the effect on their blood chemistry and biochemical body composition to determine the feasibility of this polyculture in high density. Six densities were evaluated [$T_{4/5}$, $T_{9/5}$, $T_{14/5}$, $T_{0/5}$, $T_{9/0}$, and $T_{14/0}$; [$T_{tilapia/prawn\ (ind\ m^{-2})}$]. In tilapia were evaluated hemoglobin, red cells albumin, globulin, cholesterol, glucose, and triglycerides composition in blood. In *M. americanum* were evaluated hemocyanin, cholesterol, triglycerides, glucose and total protein in hemolymph. The total content of proteins, lipids, and carbohydrates in muscle and liver of tilapia, and muscle and hepatopancreas on prawns were also studied. Both animals presented significant differences ($P < 0.05$) in the glucose concentration, tilapia at $T_{9/5}$ density presented significantly lower concentration (26.25 ± 6.3 mg dL^{-1}) in blood, and prawn at $T_{4/5}$ presented significant higher concentration (12.2 ± 1.9 mg dL^{-1}). Tilapia monocultures $T_{14/0}$ displayed significant higher ($P < 0.05$) carbohydrate concentration in muscle. No significant differences ($P > 0.05$) appeared in the concentration of lipids and proteins in any tilapia group and proximal composition of muscle and hepatopancreas in prawn. The present study indicates that polyculture in a density $T_{14/5}$ is similar than monoculture with no effect in blood or hemolymph chemistry and proximal composition of tilapia and prawn. The blood glucose concentration can be considered an indicator of physiological changes for tilapia and prawn when cultured together.

Keywords: *Macrobrachium americanum*, *Oreochromis niloticus*, hematology, aquaculture.

INTRODUCTION

Production of tilapia in polyculture has been successful in Asia and America. Freshwater prawn *Macrobrachium rosenbergii* has been the main crustacean sharing the ponds with tilapia (Zimmermann & New, 2000), since both species may require similar culture conditions without interfering (New, 2000). In polyculture, animals could withstand physiological stress derived from their behavior and interaction (Papoutsoglou *et al*., 2001). Advantages of polyculture are based on animal ethology, which is commonly affected by several factors such as the age at initial stocking and the stocking density (Papoutsoglou *et al*., 1992). Tilapia-freshwater prawn polyculture may contribute to improve water quality and coprophagia, increase the total productivity of the pond, and produce organisms of high value at low prices (New, 2002).

Polyculture affects growth and lowering, or improvement of animal growth can be associated with variations of various chemicals (lipids, cholesterol, albumin, etc.) in blood and liver, indicating a reduction

in feed utilization (Papoutsoglou & Voutsinos, 1988; Shimeno *et al*., 1997). Thus, the blood chemistry and tissues biochemical composition could be used as indexes to explain possible differences in overall results culturing aquatic species together at different densities.

The purpose of this work was to analyze the feasibility of the polyculture of tilapia and the prawn *M. americanum* based on the effect of different stocking densities on their blood chemistry.

MATERIALS AND METHODS

The study was conducted at the Centro Interdisciplinario de Investigación y Desarrollo Integral Regional (CIIDIR-Sinaloa), IPN, Mexico. A stock of red tilapia *Oreochromi* sp. fry (sex-reversed males) was purchased from Aquatic Depot, S.A. de C.V., México. It was cultured in an outdoor system consisting of 1000 L circular plastic tanks. The fish were fed with floating extruded pellet of 32% crude protein (Purina®, Mexico). When desired weight was reached (6.5 ± 1.8 g), a total of 210 prawns were selected for the bioassay.

M. americanum prawns were capture in the Sinaloa River (107°30'W, 25°00'N). Sixty prawns with an average weight of 25.9 ± 8.5 g were acclimated and maintained in the same tilapia culture system but in separated tanks during one month before the starting. Prawns were fed with fresh squid and shrimp commercial pellet (35% crude protein, Purina®, Mexico).

In previous experiences (García-Guerrero & Apún-Molina, 2008), the maximum density recommended for prawns in the systems is five per tank. Then, four testing densities of tilapia and one density of prawn were stocked [$T_{4/5}$, $T_{9/5}$, $T_{14/5}$, $T_{0/5}$, $T_{9/0}$, and $T_{14/0}$; $T_{\text{tilapia/prawn (ind m}^{-2})}$ with three replicates each. Eighteen plastic tanks filled with 800 L of filtered (5 μ) freshwater were utilized, maintained with constant aeration and covered with a shade net at ambient temperature. 75% of water volume was exchanged every week.

Water temperature and dissolved oxygen (DO) were monitored daily at 8:00 and 16:00 h (YSI 150 oximeter, Yellow Spring, Ohio). Every two weeks, all organisms were individually weighted to calculate feed rations as 3% of total weight day^{-1} in both species. Tilapia was fed (Purina® 32% protein) three times a day (7:00, 12:00 and 18:00 h), and prawns were fed (Purina® Shrimp Feed 35% protein) once a day (18:00 h) during the whole trial.

For biochemical testing of differences among groups, fifteen tilapia blood samples collected from the sinus venous and atrium using a 5 mL syringe previously filled with 0.5 mL of heparin as an anticoagulant (Ramesh *et al*., 2008). Hemoglobin (g dL^{-1}) and red blood cells (10^6 × mL), hematocrit (g%), globulin and albumin (mg mL^{-1}) were directly determined according to Van-Kampen & Zijlstra (1961). Then, blood was centrifuged (960 *g* × 5 min at 4ºC) to separate the plasma in where cholesterol, glucose, and triglycerides contents were quantified. Determinations were adapted to microplates using 20 μL samples and 200 μL enzyme chromogen reagent (Racotta & Palacios, 1998; Apún-Molina *et al.*, 2015).

Hemolymph samples of approximately 200 μL from 12 hard-shelled prawns per density group were extracted from the ventral sinus at the base of the first abdominal segment using a 3 mL syringe rinsed with cooled 5% potassium oxalate in distilled water as an anticoagulant solution (Apún-Molina *et al*., 2015). Hemolymph was centrifuged (800 *g* × 10 min at 4°C). Then, hemocyanin was detected by direct absorbance at 335 nm (Hagerman, 1983). The commercial kits CHOD-PAP, GOD-PA, and GPO-PAP reagents were used respectively to quantify cholesterol, glucose, and triglycerides concentration (mg dL^{-1}) (Randox, U.K.; Racotta & Palacios 1998; Apún-Molina *et al.*, 2015).

Total proteins, lipids and carbohydrates concentration (mg dL^{-1}) were quantified from 0.1 g of liver or muscle and 0.1 g of hepatopancreas or muscle in prawns. Each tissue was lyophilized for 24 h, weighed and homogenized in 1 mL of distilled water using a 1:10 w/v tissue-H_2O ratio. Total proteins (TP) were determined as in Bradford (1976); total lipids (TL) by the sulphophosvanillin method (Barnes & Blackstock, 1973), and total carbohydrates (TC) were quantified according to Roe (1955). Trichloroacetic acid was used for protein precipitation and anthrone for the colorimetric reaction content using the same kit as plasma. Absorbance was determined with a microplate reader (Multiskan Go, Thermo Scientific UV, United States) and concentrations were calculated from a standard solution of substrates.

Data were analyzed with analysis of variance after testing them for normality and homogeneity using Statistica® version 5.5. One way ANOVA was applied per variable among all densities ($\alpha = 0.05$). If significant differences among densities were observed, a Tukey's test was executed to detect significant groups (Zar, 2010).

RESULTS

Blood concentration of various components of tilapia and prawns are shown in Tables 1 and 2, respectively. Significant differences ($P < 0.05$) in glucose concentration were observed.

Tilapia at $T_{9/5}$ density presented significantly lower concentrations (26.25 ± 6.3 mg dL^{-1}) in blood and prawns at $T_{4/5}$ presented significant higher concentrations (12.2 ± 1.9 mg dL^{-1}) when compared with all

Table 1. Concentration of blood biochemical components in tilapia *O. niloticus* reared in polyculture with the prawn *M. americanum* ($T_{tilapia/prawn\ (ind\ m^{-2})}$, mean ± SD), hemoglobin (g dL^{-1}), cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}), glucose mg (dL^{-1}), total protein (mg dL^{-1}), red blood cell ($10^6 \times mL^{-1}$), hematocrit (g%), globulin (mg mL^{-1}), albumin (mg mL^{-1}). Lines with different letter differ significantly ($P < 0.05$).

Treatment	$T_{4/5}$	$T_{9/5}$	$T_{14/5}$	$T_{9/0}$	$T_{14/0}$
Hemoglobin (g dL^{-1})	9.1 ± 0.3	10.22 ± 0.2	9.9 ± 0.4	10.3 ± 0.2	10.0 ± 0.5
Cholesterol (mg dL^{-1})	22.6 ± 1.8	18.75 ± 1.6	23.1 ± 2.1	24.3 ± 9.4	24.0 ± 1.7
Triglycerides (mg dL^{-1})	26.9 ± 6.1	20.18 ± 3.1	30.8 ± 8.4	28.1 ± 5.6	24.2 ± 2.2
Glucose (mg dL^{-1})	45.2 ± 3.9[a]	26.25 ± 6.3[b]	36.9 ± 3.7[a]	48.7 ± 6.5[a]	48.5 ± 2.1[a]
Total protein (mg dL^{-1})	39.4 ± 0.2	40.20 ± 0.2	42.5 ± 0.2	45.0 ± 0.3	45.0 ± 0.3
Red blood (cell $10^6 \times mL^{-1}$)	3.0 ± 0.2	3.47 ± 0.3	3.3 ± 0.2	3.4 ± 0.1	3.4 ± 0.5
Hematocrit (g%)	27.6 ± 1.8	31.08 ± 0.5	30.7 ± 1.9	32.0 ± 1.4	32.0 ± 4.2
Globulin (mg mL^{-1})	11.1 ± 1.9	12.4 ± 2.1	11.6 ± 1.3	11.8 ± 1.3	11.8 ± 1.5
Albumin (mg mL^{-1})	22. 6 ± 2.8	21.70 ± 5.7	20.85 ± 1.4	27.25 ± 5.0	27.5 ± 5.0

Table 2. Concentration biochemical components in hemolymph in prawn *M. americanum* reared in polyculture with the tilapia *O. niloticus* ($T_{tilapia/prawn\ (ind\ m^{-2})}$, mean ± SD). Cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}), glucose (mg dL^{-1}), total protein (mg dL^{-1}). Lines with different letter differ significantly ($P < 0.05$).

Treatment	$T_{4/5}$	$T_{9/5}$	$T_{14/5}$	$T_{0/5}$
Hemocyanin (g dL^{-1})	56.5 ± 0.8	48.5 ± 1.4	62.0 ± 0.7	58.6 ± 1.0
Cholesterol (mg dL^{-1})	8.6 ± 1.4	8.7 ± 2.0	8.9 ± 1.3	9.1 ± 0.8
Triglycerides (mg dL^{-1})	10.7 ± 1.2	11.6 ± 2.9	15.1 ± 2.0	12.9 ± 1.5
Glucose (mg dL^{-1})	12.2 ± 1.9[a]	6.0 ± 1.2[b]	8.9 ± 1.3[b]	6.3 ± 0.4[b]
Total protein (mg mL^{-1})	133 ± 2.1	146 ± 0.3	137 ± 1.3	168 ± 1.7

other groups. Tilapia monocultures ($T_{14/0}$) displayed significant higher ($P < 0.05$) carbohydrate concentration in muscle. No significant differences ($P > 0.05$) were observed in hemoglobin, red cells albumin, globulin, cholesterol, glucose, and triglycerides concentration in blood among Tilapia groups. The same trend occurred for the concentrations of TP and TL among treatments ($P > 0.05$). However, the TC concentration in tilapia $T_{14/0}$ group muscle was significantly higher ($P < 0.05$). No significances were detected in the concentration of hemocyanin, cholesterol, triglycerides, glucose or total protein in hemolymph. Besides, no significant differences ($P > 0.05$) in concentrations of TP, TL, and TC in tilapia liver were found (Table 3) or in the proximal composition of muscle and hepatopancreas in prawn (Table 4).

There were neither significant differences in the concentrations of TP, and TL among treatments ($P > 0.05$). The TC concentration in tilapia muscle was significantly higher ($P < 0.05$) for the $T_{14/0}$ group. No significant differences ($P > 0.05$) in concentrations of TP, TL, and TC for the tilapia liver were found (Table 3). Concentrations of TP, TL, and TC in muscle and hepatopancreas of *M. americanum* had no significant differences among treatments ($P > 0.05$) (Table 4).

DISCUSSION

Polyculture may affect or improve animal development and fitness. Papoutsoglou *et al*. (2001) mention that physiological changes, growth delay or low survival of scaled carp and blue tilapia under mono and polyculture seem to be a stress response related to fish behavior. Blood chemistry and hematology of tilapia and other fishes are influenced by the interactions with other species in cultured together Fry *et al*. (1971). Papoutsoglou *et al*. (2001) observed an increase in the triglycerides concentration, and cholesterol in tilapia's plasma reared in monoculture as compared with polyculture. However, in the present study, the content of hemoglobin, hemolymph, red cells, albumin, globulin, hemocyanin, cholesterol, and triglycerides in the blood, as well as the total content of proteins, lipids in muscle, liver of tilapia, and muscle and hepatopancreas of prawn, in all tested densities, were better at polyculture.

In this study, a significant difference was observed in the glucose concentration in the hemolymph of $T_{4/5}$, and in tilapia blood in the group $T_{9/5,}$ in which higher concentrations of total carbohydrates in hepatopancreas was observed that. This combination may produce better carbohydrate metabolism. Thus maintaining a

Table 3. Body biochemical components TP (total protein, mg g^{-1}), TL (total lipids, mg g^{-1}) and TC (total carbohydrates, mg g^{-1}) of muscle and liver of red tilapia *O. niloticus* reared in polyculture with *M. americanum* ($T_{tilapia/prawn\ (ind\ m^{-2})}$, mean ± SD). Lines with different letter differ significantly ($P < 0.05$).

Treatment	$T_{4/5}$	$T_{9/5}$	$T_{14/5}$	$T_{9/0}$	$T_{14/0}$
Muscle					
TP (mg g^{-1})	404.9 ± 255.8	505.1 ± 325.4	534.3 ± 125.5	594.5 ± 191.7	435.8 ± 97.4
TL (mg g^{-1})	28.8 ± 7.5	25.3 ± 13.1	26.0 ± 7.2	36.6 ± 1.5	32.3 ± 8.6
TC (mg g^{-1})	23.3 ± 11.3^{b}	36.6 ± 4.2^{b}	29.9 ± 14.3^{b}	32.9 ± 9.71^{b}	79.0 ± 100.2^{a}
Liver					
TP (mg g^{-1})	55.5 ± 4.3	58.7 ± 3.4	69.6 ± 1.5	81.4 ± 3.8	91.4 ± 6.9
TL (mg g^{-1})	33.1 ± 7.7	33.6 ± 6.6	35.0 ± 3.9	33.7 ± 1.6	41.9 ± 2.1
TC (mg g^{-1})	30.7 ± 22.6^{a}	22.5 ± 14.6^{a}	16.1 ± 6.2^{a}	19.1 ± 8.6^{a}	20.5 ± 7.8^{a}

Table 4. Body biochemical components TP (total protein mg g^{-1}), TL (total lipids mg g^{-1}), and TC (total carbohydrates, mg g^{-1}) of muscle and hepatopancreas of the prawn *M. americanum* reared in polyculture with *O. niloticus*. ($T_{tilapia/prawn\ (ind\ m^{-2})}$, mean ± SD). Lines with different letter differ significantly ($P < 0.05$).

Treatment	$T_{4/5}$	$T_{9/5}$	$T_{14/5}$	$T_{0/5}$
Muscle				
TP (mg g^{-1})	504.0 ± 48.0^{a}	351.7 ± 120.3^{b}	404.1 ± 63.9^{a}	391.1 ± 124.4^{a}
TL (mg g^{-1})	86.0 ± 0.5^{a}	62.0 ± 0.6^{b}	46.0 ± 1.6^{b}	52.0 ± 1.6^{b}
TC (mg g^{-1})	24.7 ± 10.1^{b}	25.9 ± 8.2^{b}	44.1 ± 3.1^{a}	27.9 ± 7.2^{b}
Hepatopancreas				
TP (mg g^{-1})	59.2 ± 6.3	60.7 ± 20.3	46.3 ± 5.0	60.8 ± 37.8
TL (mg g^{-1})	6.1 ± 0.5^{b}	20.8 ± 1.5^{a}	18.7 ± 3.8^{a}	12.1 ± 1.2^{a}
TC (mg g^{-1})	15.3 ± 3.8^{a}	5.4 ± 1.8^{b}	10.4 ± 3.9^{ab}	7.6 ± 1.9^{b}

better nutritional condition, since the concentration of 12.2 ± 1.9 does not show the glucose amounts (it has 45 mg dL^{-1}), commonly observed in stressed cultured specimens (Racotta & Palacios, 1998; Mercier *et al.*, 2009). Glucose can be an indicator of the metabolic strategy utilized which is an index of stress, in both, fish and crustacean (Keller & Sedlmeier 1988; Rosas *et al.*, 1993; Pascual *et al.*, 2003; Mercier *et al.*, 2009). It is also, an indicator of nutritional condition (Pascual *et al.*, 2003). According to Ruane *et al.* (1999) and Rotlant *et al.* (2000), the low levels of glucose in plasma may indicate low stress or stress recovery. However, for tilapia in present work, the glucose concentration among treatments varied without a clear glucose-stress tendency and the same trend was observed for the glucose level in prawn. Balm *et al.* (1994) suggested that variations in blood and liver parameters of tilapia compared with other fish species are smaller since cichlids can quickly regulate down the hormonal activity during stress.

In this study, it was assumed that dietary lipids and proteins were efficiently incorporated into muscle and liver in tilapia, and into muscle and hepatopancreas in prawns. Only the carbohydrates content was significantly higher in the $T_{14/0}$ muscle tilapia group since it was almost the double of all other groups. It was not clear if the increase in carbohydrates was structural or energetic since the method used for determining them, does not allows establishing this difference. However, glucose in blood was similar, suggesting that available energy was enough for metabolic demand in all groups. Considering that *M. americanum* had similar glucose concentration at all densities and in control group, it seems that the metabolic energy demand was satisfied and animals from the $T_{4/5}$ density had enough glucose from food without interfering with crustaceans. The mean TL concentrations obtained for tilapia in the present work is in agreement with previous reports, showing low lipid amount in muscle. Huss (1998) concluded that tilapia store lipids mostly in the liver than in fresh muscle where its concentration is a low but relatively stable ant that is supported by present work.

Glucose concentration in blood of tilapia and prawn can be considered as a sensitive indicator of physiological changes in polyculture, but differences in glucose among treatments needs to be analyzed based on more specific indicators of stress physiology, immune response capacity, and energy balance. It can be suggested that, since no adverse effects were

produced by the polyculture of these species, this combination could be a good option and research on the topic must continue to determine the real potential of the polyculture of tilapia *O. niloticus* with con *M. americanum* prawns.

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