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Research Article

Effect of short-term starvation on hematological and blood biochemical parameters in juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869)

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ABSTRACT. This study evaluated the effect of short-term starvation on biological condition indices, hematological parameters and blood biochemical composition of reared spotted rose snapper *Lutjanus guttatus*. Triplicate groups of juvenile fish were subjected to different feeding treatments: fed for 14 days (Group A or control); fed for seven days followed by seven days of starvation (Group B) and starved for 14 days (Group C). At the end of the experiment, body, liver, and viscera were randomly sampled for proximate composition, and biological condition indices were assessed. Blood was also collected and analyzed by standard clinical methods. Body composition, liver lipid content, hepatosomatic index, condition factor, and relative intestine length were significantly ($P < 0.05$) reduced in starved compared to fed fish. A statistically significant ($P < 0.05$) reduction in hematocrit (12%), haemoglobin (33%), glucose (18%) and calcium (9%) levels were assessed in the blood of starved fish for 14 days, while triacylglyceride levels decreased (36%) in fish starved for 7 days, subsequently returning to values similar to control by the end of the trial. No statistically significant differences were observed in total protein, inorganic phosphorus and magnesium levels in starved fish. These hematological blood and biometric parameters seem to have potential as a predictive tool for establishing the nutritional status or physiological condition during short-term starvation of *L. guttatus*, which may be useful to manage and monitor feeding practices during culture of this species.

Keywords: *Lutjanus guttatus*; triacylglyceride; blood chemistry; nutrition; starvation; aquaculture

INTRODUCTION

The rose snapper (*Lutjanus guttatus*) is an important species for marine aquaculture in Mexico and Central American countries. This fish species has performed well in floating marine cages since it readily accepts pelleted diets and tolerates captive conditions, its intensive cage culture is envisioned as an economical alternative for coastal fishermen (Hernández *et al.*, 2016; Martínez-Cordero *et al.*, 2017). In addition to improving rose snapper cultivation, it is necessary to develop methods that accurately assess nutritional status and health condition under intensive farming conditions.

Several physiological aspects are affected by feeding, particularly growth and survival of fish. In wild populations, fish are commonly deprived of food, *e.g.*, less availability of food, seasonal changes in temperature, migration, reproduction, and spawning, etc. In farm-raising practices, starvation and welfare of fish could occur by an inadequate diet or feeding protocols (López-Olmeda *et al.*, 2012), but feed restriction could also be intentionally performed to evaluate specific effects of starvation on the digestive process in fish (Krogdahl & Bakke-McKellep, 2005). Each fish species exhibits different physiological conditions and digestive capabilities to absorb and transform the nutrients contained in the food. Different

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biotic (*i.e.*, food composition, health state condition of fish, age, sex, etc.) and abiotic (*i.e.*, temperature, salinity, etc.) factors during cultivation may influence the physiological state of the fish subsequently affecting digestion and chemical transformation of nutrients (Furné *et al.*, 2005).

The measurement of hematological and biochemical blood parameters are reliable diagnostic tools to determine the health condition of fish. These parameters are sensitive enough to evaluate the effect of physical (*i.e.*, temperature, salinity) and chemical (*i.e.*, metallic or organic compounds) stressors (Roche & Boge, 1996; Del Río-Zaragoza *et al.*, 2008; Öner *et al.*, 2008; Prakoso *et al.*, 2016). In addition, blood parameters have been used for evaluating short- and long-term starving periods in freshwater (Kondera *et al.*, 2017) and marine (Jia *et al.*, 2018) fish species, fish density during rearing conditions (Tan *et al.*, 2018) and packing densities for fish transport (Pereira-Cardona *et al.*, 2016). Moreover, blood parameters are commonly used as a suitable tool for clinical diagnosis, particularly in assessing the health and nutritional status of species for aquaculture purposes (Tavares-Dias & Moraes, 2007; Caruso *et al.*, 2011; Peres *et al.*, 2014a,b). It should be noted that reference values of blood data are defined by upper and lower limits that cover most of the parameters that can be obtained from healthy individuals; therefore, blood data should not be used as a representative model for all fish species (Fazio *et al.*, 2013).

To our knowledge, blood parameters in *L. guttatus* have only been assessed in healthy and naturally infected fish by a common ectoparasite (Del Río-Zaragoza *et al.*, 2011), and, additionally, in diets replacing fish meal with poultry by-product (Hernández *et al.*, 2014a,b) and soybean meals (Silva-Carrillo *et al.*, 2012). Nevertheless, this species has been identified as a candidate for large-scale commercial culture (Hernández *et al.*, 2016).

Thus, this study aimed to determine the most sensitive biological condition indices, and hematological and blood biochemical parameters, which provides a direct assessment of the nutritional condition of reared juvenile spotted rose snapper, as well as understand the physiological strategies of the organism when exposed to short-term starvation.

MATERIALS AND METHODS

Growth trial

Spotted rose snapper juveniles were obtained from a single spawning batch, in the Reproduction and Marine Laboratory (CIAD) of Mazatlán, Sinaloa, México, as described by Alvarez-Lajonchère *et al.*, 2012. Fish were allowed to acclimatize for two weeks, where healthy fish (without signs of disease, skin and fin lesions, feeding behaviour or swimming problems) were selected, which constituted the reference population. Subsequently, fish fasted for 24 h before nine homogeneous groups of 10 juvenile spotted rose snapper (170.7 ± 21.4 g mean initial weight) were randomly distributed into each fiberglass tank (volume 300 L). The fish density selected in this study was intended to avoid any detrimental effects on the parameters under investigation resulting from overstocking; moreover, the fish density was within a range (6-50 fish m^{-3}) commonly used for groupers, snappers and others marine fishes by sea farmers in Asia (Kongkeo *et al.*, 2010). Each tank had a 0.5 cm mesh net in addition to a central 50 mm drain cover to clean tank water while preventing fish from escaping, and each tank received supplemental aeration and a continuous flow of seawater at a rate of 1.5 L min^{-1} under natural lighting conditions. Seawater was pumped from the seashore, passed through two parallel sand filters and delivered to four 25 m^3 high-density polyethylene head/sedimentation tanks. From the head tanks, the sea water was pumped through a double parallel filtration system consisting of a pressured sand filter and cartridge filter (four 9.3 m^2 cartridge filters, 16 µm relative particle retention).

Triplicate groups of fish were then fed three times a day (09:00, 12:00 and 17:00 h) until apparent satiety, for 14 days (Group A; control); fed for 7 days followed by 7 days of starvation (Group B); or starved for 14 days (Group C). The water temperature was 31.4 ± 0.5°C, the dissolved oxygen level was maintained at 6.40 ± 0.51 mg L^{-1}, and salinity was 32.8 ± 0.87 g L^{-1}. The levels of total ammonia (NH_3^+-NH_4^+) (0.05-0.2 mg L^{-1}), NO_2^- (0.016 mg L^{-1}) and NO_3^- (0.21 mg L^{-1}) were recorded weekly according to the methods reported by Spotte (1979).

During both acclimatisation and the experimental period, fish were fed with a practical diet that was manufactured *in situ* using a single screw extruder (Insta Pro 2000; Insta-Pro International, Urbandale, IA, USA), to provide 450 g kg^{-1} crude protein and 140 g kg^{-1} crude lipid (Silva-Carrillo *et al.*, 2012), these values were based on the species requirements reported by Abdo *et al.* (2010) (Table 1).

At the end of the experimental period, fish fasted for 24 h and were anesthetized with clove oil (0.2 mL L^{-1}). Each fish was weighed, and their lengths were measured to determine the final body weight, Daily growth rate (DGI) and Condition factor (CF). Then three fish per treatment (nine fish in total) were sacrificed; each fish was dissected to obtain viscera,

liver, mesenteric fat weight, and intestine length to determine the Viscerosomatic index (VSI), Hepatosomatic index (HSI), Mesenteric fat index (MFI) and Relative intestine length (RIL) as follow:

DGI = (final weight⅓ - initial weight⅓) / (time in days) × 100 CF = [body weight (g) / body length 3 (cm)] × 100

HSI = [liver weight (g) / body weight (g)] × 100

VSI = [viscera weight (g) / body weight (g)] × 100 MFI = [mesenteric fat weight (g) / body weight (g)] × 100

RIL = [intestine length (cm) / body length (cm)]

Chemical analysis

Fish were dissected and fresh samples and diets were homogenized and dried at 105°C for 24 h before the chemical analyses. Crude protein, crude lipid and ash levels in fish (*i.e*., eviscerated body and liver) and diet were determined in triplicate samples according to standard procedures (AOAC, 1990): crude protein content was determined by the Dumas combustion method and the use of a Leco FP-528 nitrogen analyser. Crude lipid concentration was determined by a micro Foss Soxtec Avanti 2050 extraction with petroleum ether. Ash content was measured by burning samples in a muffle furnace at 550ºC for 24 h.

Blood chemistry parameters

Finally (24 h after the last meal) four fish per tank (12 fish per treatment) were carefully handled to minimize stress. Fish were anesthetized with 0.2 mL L^{-1} of clove oil in <3 min. Blood samples were taken from each fish by puncturing the caudal vein using 1 mL tuberculin syringes. The hematocrit was evaluated according to Del Río-Zaragoza *et al.* (2008). The hemoglobin concentration was determined using a colorimetric kit (Randox), based on the cyanmethemoglobin method. The total protein and triacylglyceride contents were determined from blood plasma. Total protein was determined by the Biuret method using a colorimetric kit (BioSystems). The triacylglycerides were measured according to the specifications stated in the commercial kit (MexLab Group), and the readings were performed in a spectrophotometer at 545 and 520 nm, respectively (Silva-Carrillo *et al*., 2012; Hernández *et al*., 2014a).

Statistical analysis

One-way analysis of variance (ANOVA) was applied to growth performance, biological condition indices and blood parameters obtained from fish for each feeding treatment, followed by *post-hoc* Tukey test to assess significant differences ($P < 0.05$) between means. Normality and homoscedasticity were tested using the Kolmogorov–Smirnov test and Levene test, respectively. Percentage values were transformed to arcsine before analyses (Zar, 1999), but results are expressed as untransformed means. All data are expressed as the mean ± standard error (SE) and were analyzed with Statistica v. 13 (Statsoft, Inc. Tulsa, OK).

RESULTS

During the experiment, no mortality or disease symptoms were observed in fish. Final body weight, growth performance, Hepatosomatic index (HIS), Viscerosomatic index (VSI), Condition factor (CF), and Relative intestine length (RIL) significantly decreased with the duration of starvation, while the lipid content increased in the liver. The protein content assessed on the eviscerated fish carcass significantly decreased in fish starved for 14 days (Group C) (Table 2).

Fish starved for 14 days (Group C) showed significant decreases in hematocrit, hemoglobin, plasma glucose, and calcium levels compared to fish fed for 14 days (Group A or control), while protein, cholesterol, inorganic phosphorous and magnesium showed no significant differences. Triacylglyceride levels in plasma decreased during the first week of starvation (Group B), but levels recovered significantly during the second week of starvation (Group C) (Table 3).

DISCUSSION

During the experiment, different feeding protocols were implemented to assess the effect of short-term starvation on juvenile rose snapper. This study indicated that during short-term starvation, this fish species exhibited reduced plasma glucose levels and decreased hepatosomatic index. It may be due to a depletion in glycogen reserves that results in the use of endogenous protein as a primary energy source, increased lipid reserves in the liver and in the mesentery of fish which suggests a physiological adaptation in preparation for a more extended starvation period.

The metabolic strategy used to supply energy differs between fish species and relies on the environmental conditions and the physiological state of the fish, as well as other less significant factors, such as the use of nutrients including carbohydrates, lipids, and proteins from different body organs (Bandeen & Leatherland, 1997). Therefore, while some fish species use muscle protein as the main energy source, others use lipids following the depletion of liver glycogen reserves which exist as the primary energy source (Alliot *et al.*, 1974; Barcellos *et al.*, 2010; McCue, 2010). The results

of the present study indicate that short-term starvation causes a significant decrease in the growth of fish, which coincides with the lower protein body content (Table 1). The MFI was not affected by starvation, while, VSI, HSI, CF, and RIL were significantly decreased by feed deprivation, indicating that those physiological factors could be useful parameters of fish health condition for juvenile snapper. Similar results were found for red porgy (*Pagrus pagrus*), juvenile olive flounder (*Paralichthys olivaceus*) and Senegalese sole (*Solea senegalensis*), where body weight, CF, or HSI decreased during fish starvation (Rueda *et al.*, 1998; Cho *et al.*, 2006; Peres *et al.*, 2014a). The decrease in the body protein content, HSI, VSI, and CF, is probably the result of the utilization of energy for basal metabolism, where energy requirements were met by liver glycogen reserves, followed by the use of protein contained in fish muscle. Interestingly, mesenteric lipid reserves were preserved, while lipid content in the liver increased, probably due to increasing lipogenesis activity where amino acids from muscle were used as a substrate (Tocher, 2003).

The energy requirements of starved fish are obtained from carbohydrates stored in the liver as glycogen, where it is metabolized and transported to the extrahepatic tissues as glucose (Barcellos *et al.*, 2010). Therefore, in numerous fish species, glycaemic maintenance during food deprivation has a direct relationship with the capacity to mobilize glycogen from the liver, noticeable at the beginning of starvation, with a concomitant reduction in the weight of the liver (Pérez-Jiménez *et al.*, 2007). In this study, liver glycogen levels were not measured. Nevertheless, a decrease in the glucose level of the plasma, in addition to a reduction in HSI, indicates a depletion of available glycogen reserves. This physiological response has been previously reported for gilthead seabream (*Sparus aurata*) (Peres *et al.*, 2012), while species such as blackspot sea bream (*Pagellus bogaraveo*) and European seabass (*Dicentrarchus labrax*) have shown a significant capacity to regulate plasma glucose levels during starvation periods (Caruso *et al.*, 2011; Peres *et al.*, 2014a). Basal blood glucose levels are known to differ considerably between fish species (Polakof *et al.*, 2012). In the present study, average glycaemic levels range from 97.5 mg dL^{-1} in fish fed for two weeks to 79.4 mg dL^{-1} in fish starved for two weeks, which was significant level changes, indicating potential as a health indicator for this species. Previous studies of this species, which replaced fishmeal with food grade poultry by-products, have reported lower plasma glucose levels than observed in the present study, ranging between 65.9 and 39.8 mg dL^{-1} for fish fed experimental diets (Hernández *et al.*, 2014a).

Table 1. Formulation and proximate composition of the experimental diet.

Ingredients	(% dry weight)
Fish meal[a]	52.6
Squid meal[b]	6.0
Krill meal[c]	75.9
Fish oil[d]	8.78
Dextrin[d]	17.47
Wheat gluten[d]	2.0
Carotenoid[e]	0.08
Soybean lecithin (70%)[g]	1.5
Vitamin premix[f]	0.6
Mineral premix[f]	0.23
Vitamin C[f]	0.1
Alginate[g]	3.0
Antioxidants	0.05
Proximate composition (g kg^{-1} dry diet)	
Dry matter	929.1
Crude protein	463.4
Crude fat	138.6
Ash	140.1
NFE[h]	236.5
Gross energy (kJ g^{-1})[i]	19.3

[a]Steam dried fish meal from Selecta de Guaymas, S.A. de C.V. Guaymas, Sonora, México. [b]Proteínas marinas y Agropecuarias, S.A. of C.V., Guadalajara, Jalisco, México. [c]PROAQUA, S.A. de C.V. Mazatlán, Sinaloa, México. [d]Droguería Cosmopolita, S.A. de C.V. México, D.F., México [e]DSM Nutritional Products México S.A. de C.V., El Salto, Jalisco, México. [f]Vitamin Premix provided the following per kg diet: Vitamin A, 30,000 IU; Vitamin D3, 6,000 IU; Vitamin E, 300 IU; Vitamin K3, 12 mg; Thiamine B1, 24 mg; Riboflavin B2, 26.1 mg; Pyridoxine B6, 21.9 mg; Vitamin B12, 0.06 mg; Niacin, 150 mg; Pantothenic Acid, 66.6 g; Inositol, 461.4 mg; Folic Acid, 15 mg, Biotin, 1.5 mg; Vitamin C, 300 mg; and, Choline, 900 mg. Manufactured by Trouw Nutrition International. [f]Mineral premix provided the following per kg diet: Manganese, 306.7 mg; Magnesium, 138 mg; Zinc, 490.9 mg; Iron, 613.3 mg; Copper, 61.3 mg; Iodine, 15.5 mg; Selenium,1.2 mg; Cobalt 1.8 mg; and, Excipient c.b.p. 750 g. Manufactured by Trouw Nutrition International. [g]Sigma-Aldrich Chemical, S.A. de C.V. Toluca, México State, México. [h]NFE (nitrogen-free extract) with fiber included = 100-(% moisture+% crude protein+% crude lipids+% ash). [i]The gross energy was calculated according to the physiological fuel values of protein; 20.93 kJ g^{-1}; lipids, 37.68 kJ g^{-1}; and, nitrogen-free extract, 16.75 kJ g^{-1} (Shiau & Chou, 1991). Nevertheless, blood glucose results in this study are within ranges observed in other fish species, such as adult jundiá (*Rhamdia quelen*) (Barcellos *et al.*, 2010), European seabass, red porgy (*Pagrus pagrus*) and blackspot sea bream (Caruso *et al.*, 2011, 2012; Peres *et al.*, 2014a), but were higher than those reported for Senegalese sole (*Solea senegalensis*) (Peres *et al.*, 2014b).

Table 2. Survival, growth, body composition and biometric parameters of *Lutjanus guttatus* reared with different feeding treatments. Group A (control): feed for two weeks; Group B: feed one week and starved one week; Group C: starved for two weeks. Data are expressed as mean ± SE ($P < 0.05$), different letters indicate significant differences between means, n = 10. CF: Condition factor, HIS: Hepatosomatic index, VSI: Viscerosomatic index, MFI: Mesenteric fat index, RIL: Relative intestine length.

Parameters	Treatment			
	Initial	A	B	C
Survival (%)	–	100	100	100
Initial body weight (g)	–	171.1 ± 4.6	170.2 ± 4.8	170.6 ± 5.0
Final body weight (g)	–	181 ± 0.01	162 ± 0.00	153 ± 0.00
Daily growth index	–	0.81 ± 0.09a	-0.12 ± 0.07b	-1.6 ± 0.86c
Eviscerated body composition (% wet weight)				
Moisture	57.7 ± 0.1c	60.2 ± 0.1b	60.4 ± 0.2b	62.6 ± 0.2a
Protein	26.2 ± 0.2a	25.5 ± 0.3ab	25.1 ± 0.1b	23.4 ± 0.1c
Lipids	15.5 ± 0.1	16.0 ± 0.9	16.0 ± 0.7	14.1 ± 0.1
Ash	5.4 ± 0.1a	4.3 ± 0.1c	4.9 ± 0.1b	5.1 ± 0.0ab
Visceral composition (% body weight)				
Liver lipid	66.3 ± 3.2bc	66.5 ± 2.0c	74.4 ± 1.1ab	79.8 ± 2.2a
CF	–	1.88 ± 0.04a	1.75 ± 0.05b	1.61 ± 0.03c
HSI	–	2.4 ± 0.2a	1.4 ± 0.2b	1.2 ± 0.1b
VSI	–	1.4 ± 0.1a	1.1 ± 0.1b	1.1 ± 0.0b
MFI	–	7.8 ± 0.9	7.8 ± 0.7	7.5 ± 0.6
RIL	–	0.9 ± 0.0a	0.7 ± 0.0b	0.7 ± 0.0b

The nutritional and physiological state of fish is often associated with plasma protein levels (De Pedro *et al.*, 2005; Rehulka *et al.*, 2005). Proteins are an energy resource that is typically the last resort whereby the physiological switch from lipid-dominated to protein-dominated catabolism occurs when lipid levels of animals reach a critical threshold (McCue, 2010). However, in the present study, the plasma protein levels were unchanged, instead body protein content and CF decreased, suggesting that energy was liberated from the catabolism of protein rather than lipids (*i.e.*, lipids); this corroborates the carnivorous nature of the species, as previously reported for other fish species (*i.e.*, red porgy) (Rueda *et al.*, 1998). Indeed, catabolism of lipids as a first energy source during fish starvation has been reported in species such as barramundi (*Lates calcarifer)* and hybrid tilapia (*Oreochromis mossambicus* x *O. niloticus)* (Wang *et al.*, 2000; Tian & Qin, 2003). In comparison, rose spotted snapper *L. guttatus* has exhibited a constant level of plasma protein content (between 5.5 to 4.6 g dL^{-1}), without variation between feeding treatments in food grade poultry by-product based-diets (Hernández *et al.*, 2014a,b). Moreover, protein plasma levels found in this species were in the range observed in other marine fish species such as wedge sole (*Dicologoglossa cuneate*) (Herrera *et al.*, 2009), gilthead seabream (Peres *et al.*, 2012), European seabass (Peres *et al.*, 2014a) and Senegalese sole (Costas *et al.*, 2011; Peres *et al.*, 2014b; Andrade *et al.*, 2015).

In this work, plasma triacylglycerides decreased (36%) during the first starvation week, which recovered during the second starvation week to values comparable to those observed in the control treatment (Table 3). A similar trend was observed in juvenile turbot (*Scophthalmus maximus*), where triacylglycerides significantly decreased within 72 h of starvation (Jia *et al.*, 2018), while in juveniles of *Sparus aurata* these values decreased after one week of starvation, later recovering to similar values to those observed in fish starved for 24 h during refeeding (Peres *et al.*, 2012). It is possible that juveniles of *L. guttatus* exhausted their triacylglyceride reserves in the blood plasma during the first days of starvation as a response to the depletion of glucose stores (Table 3); restored lipid levels, following the starvation period, probably resulted from an increase in lipogenesis. The lipid content increased in the liver, suggesting that plasma levels were not restored by the mesenteric fat reserves, despite the body lipid content decreasing with starvation (Table 2). In previous studies of this species (Hernández *et al.*, 2014a), triacylglyceride plasma levels ranged between 189 and 243 mg dL^{-1}, which is lower than the values obtained in the present study. Triacylglyceride levels are within the range of reported values reported for sea bass (Pérez-Jiménez *et al.*, 2007; Chatzifotis *et al.*, 2011; Peres *et al.*, 2014b) and gilthead seabream (Peres *et al.*, 1999; 2012), but lower than those reported for Senegalese sole (Peres *et al.*, 2014a).

Table 3. Hematocrit and blood parameters of *Lutjanus guttatus* reared with different feeding treatments. Group A (control): fed for two weeks; Group B: fed one week and starved one week; Group C: starved two weeks. Data in parentheses correspond to the range for each parameter.

	Treatments		
	A	B	C
Hematocrit (%)	59.4 ± 2.0a (52.0-68.0)	52.8 ± 2.0b (42.0-60.0)	47.8 ± 2.7b (34.0-58.0)
Protein (g dL^{-1})	5.4 ± 0.1 (5.1-6.0)	5.1 ± 0.2 (4.4-5.9)	5.1 ± 0.2 (4.2-5.8)
Haemoglobin (g dL^{-1})	6.0 ± 0.5a (4.0-8.2)	3.8 ± 0.3b (1.9-4.8)	4.4 ± 0.5b (2.6-6.5)
Glucose (mg dL^{-1})	97.5 ± 5.5a (77.2-124.0)	85.4 ± 3.0ab (70.0-99.0)	79.4 ± 2.4b (72.0-92.0)
Cholesterol (mg dL^{-1})	371.4 ± 21.5 (295.7-494.7)	371.1 ± 24.1 (306.4-470.2)	326.6 ± 16.3 (268.1-375.5)
Triacylglycerides (mg dL^{-1})	421.5 ± 25.0a (353.8-544.5)	271.8 ± 30.1b (182.1-435.7)	400.9 ± 31.3a (277.3-495.9)
Inorganic phosphorus (mg dL^{-1})	9.0 ± 0.6 (6.9-10.9)	9.3 ± 0.4 (7.6-10.8)	8.5 ± 0.9 (4.2-11.3)
Calcium (mg dL^{-1})	10.7 ± 0.1a (10.1-11.3)	9.7 ± 0.3b (8.5-10.8)	9.7 ± 0.4b (7.3-10.9)
Magnesium (mg dL^{-1})	2.5 ± 0.0 (2.3-2.7)	2.4 ± 0.0 (2.1-2.6)	2.4 ± 0.0 (2.1-2.6)

The cholesterol plasma levels did not show variation during the starvation period, which is an important component of cell membranes and functions as a precursor in the synthesis of steroids and stress hormones, either promoting gluconeogenesis or prolonging the longevity of fish (McDonald & Milligan, 1992; Godavarthy *et al.*, 2012). The response of snapper juveniles to starvation was similar to that of rainbow trout (*Oncorhynchus mykiss*) (Black & Skinner, 1986) and striped bass (*Morone saxatilis*) (McFarlane *et al.*, 1992), but contrary to observations of *Sparus aurata* (Peres *et al.*, 2012) and *Scophthalmus maximus* juveniles, in which cholesterol plasma values decreased with short-term starvation. Moreover, in this study, fish were in the juvenile stage, where reproductive maturation is a determinant physiological factor for the mobilization of cholesterol since this biochemical compound is strongly related to spermiation and ovulation in male and female fish respectively (García-Garrido *et al.*, 1990).

Starvation causes a significant decrease in hematocrit and hemoglobin levels (Table 3). Several responses have been described for the hematocrit value in fish because of starvation (Caruso *et al.*, 2010, 2011, 2012), where the decrease in both blood parameters have been addressed with a depression in erythropoiesis (McCue, 2010). Haemoglobin values assessed in this work were lower than those reported for the same species fed by poultry by-products (Hernández *et al.*, 2014a,b) and soybean meals (Silva-Carrillo *et al.*, 2012), but were in the range for other fish species such as Senegalese sole (Peres *et al.*, 2014b). Despite the observation of decreasing hematocrit levels (48 to 59%, Table 3) with starvation in juvenile *L. guttatus*, values were in the range (52.3 ± 9.6%) reported for the same species in healthy fish (Del Río-Zaragoza *et al.*, 2011), and those obtained (51 to 54%) from fish fed with soybean (Silva-Carrillo *et al.*, 2012), and slightly higher than those (48 to 44%) obtained in fish fed with poultry by-products (Hernández *et al.*, 2014a,b).

Growth, fat accumulation, bone deformities, and increased mortality rate could be affected by an irregular concentration of plasma calcium, phosphorus, and magnesium (Kousoulaki *et al.*, 2010; Peres *et al.*, 2014b). Among inorganic nutrients, only calcium showed a significant difference in starved fish, as has been observed in juveniles of *Sparus aurata* (Peres *et al.*, 2012), confirming the essential nature of this mineral in marine fish (Lall, 2002). The calcium values registered for spotted rose snapper are in the range reported for other marine fish such as for sea bass (Peres *et al.*, 2014a) and Senegalese sole (Peres *et al.*, 2014b).

In conclusion, the most sensitive physiological parameters during short-term starvation of rose snapper were the hepatosomatic index, viscerosomatic index, condition factor, relative intestine length, and liver lipid content; while the most sensitive recordings in the blood included the levels of hematocrit, hemoglobin, glucose, and calcium. Results demonstrated that these parameters could be useful as references to establish general health nutritional status of juvenile *L. guttatus*. The physiological strategy of this species for facing short-term starvation (<14 days) occurred as follows: firstly, plasma glucose levels and the hepatosomatic index decreased, is probably as a reflection of glycogen reserve depletion. Then endogenous protein was used as a primary energy source to meet metabolic energy requirements; and finally, lipid reserves increased in the liver, but were constant in the mesentery of fish, indicating a physiological preparation for a longer starvation period. Future studies are needed to assess the effects of longer starvation periods and refeeding, to assess the physiological recovery capacity of this species.

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