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*Research Article*

# The growth performance and metabolic responses of juvenile spotted rose snapper (*Lutjanus guttatus*) fed diets with different precooked cornstarch to protein ratio

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**ABSTRACT.** The effect of diets with varying crude protein-to-precooked cornstarch (P:S) ratios on growth performance, feed efficiency, carbohydrase activity, and blood chemistry of juvenile *Lutjanus guttatus* was evaluated. Four isoenergetic (19.0 kJ $g^{-1}$) diets containing different P:S ratios (46:18, 38:30, 34:35, and 31:40) were formulated to feed fish for ten weeks in triplicate. Each diet was tested in triplicate in groups of 35 fish per tank (average body weight 19.4 ± 0.3 g) to apparent satiation three times a day. The weight gain, specific growth rate, and feed conversion ratio obtained with P38S30 and P34S35 were not significantly different *vs*. P46S18. Survival does not present significant differences among treatments. Biological indices (condition factor, hepatosomatic index, viscerosomatic index, and intraperitoneal fat rate) showed no significant differences among treatments. Body protein content decreased significantly with increasing precooked cornstarch concentration, while body lipid levels increased. Blood chemistry values were within those of healthy juveniles, except for glucose values. An increase in pancreatic α-amylase and intestinal α-glucosidase blood chemistry values were within those of healthy juveniles, except for glucose values. An increase in pancreatic α-amylase and intestinal α-glucosidase levels concomitant with increasing precooked cornstarch concentration showed the capacity of juvenile *L. gutattus* to digest high precooked cornstarch levels. Growth performance, biological indices, and hematological parameters showed that *L. guttatus* could digest and metabolize up to 35% of precooked cornstarch through increased plasma glucose levels and the α-amylase and α-glucosidase activities. Without negative effect on growth performance.

**Keywords:** *Lutjanus guttatus*; sparing effect; precooked cornstarch; amylase; pyruvate kinase; blood chemistry

## INTRODUCTION

Carbohydrates (CHOs) are non-essential nutrients but, being a low-cost source of dietary energy, they have become important ingredients in diets for fish and shrimp (NRC 2011, Zhou et al. 2013). Moreover, an optimal dietary inclusion of digestible CHOs reduces the catabolism of proteins and lipids for energy production (sparing effect) in fish (Castillo et al. 2018a). As well as enhance the catabolism of amino acids in the diet to glucose, preventing nitrogenous waste, improving water quality, and, at the same time,

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generating other environmental benefits (Palma et al. 2020). Digestible CHOs are used for key biological and metabolic functions; for instance, they act as metabolic intermediates to synthesize important biological compounds and improve the physical properties of pelleted feed (Wilson 1994). Polysaccharides such as starch and dextrin are more efficient as a dietary energy source utilized by several marine fish (Boonanuntanasarn et al. 2018, Schrama et al. 2018). Digestible CHOs have also been utilized in lipogenesis (Viegas et al. 2019). The physical treatment of starch, such as cooking, extrusion, vaporization, and expansion, alters starch's physical structure (Stone 2003, Booth et al. 2006, Zhou et al. 2015, Kamalam et al. 2017). The presence of water at temperatures between 60-80°C penetrates the starch granules until they swell and break. After the breakdown, amylose and amylopectin leave the granule and produce a gelatinous suspension (Enes et al. 2011, NRC, 2011). Gelatinization of starch leads to better digestion, assimilation, and utilization by fish (Honorato et al. 2010). Gelatinized starch feeding is more useful in carnivores such as trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), and sea bream (*Sparus aurata*), through maximized starch digestibility, energy supply, and resulting in protein savings (Bergot 1993, Peres & Oliva-Teles 2002, Gaylord et al. 2009, Hua & Bureau 2009, Kamalam et al. 2017).

*Lutjanus guttatus* is a carnivorous species, can be commercially farmed, and is an important fishery resource in Mexico and other Latin American countries (Benetti et al. 2002). In addition, it has been successfully reproduced in captivity (Alvarez-Lajonchère et al. 2012), obtaining larvae and juveniles to grow in floating cages to their commercial size (Hernández et al. 2016).

Our group carried out many dose-response studies aiming appropriate both animal or plant protein sources for *L. guttatus* feed (Silva-Carrillo et al. 2012, Hernández et al. 2014a,b,c, 2016, 2020a,b, Leyva-López et al. 2020). All these diets had carbohydrate inclusions within 129 to 255 g $kg^{-1}$.

This study on *L. guttatus* suggests that the formulation with carbohydrates may be included up to 35% and consequently provide substantial feed cost savings with probably no loss in growth performance and feed energy utilization. The potential use of precooked cornstarch as an energy source in feeds for *L. guttatus* has not been studied yet. Therefore, the objectives of the present study were (i) to evaluate the effect of dietary starch (precooked cornstarch) on growth performance for *L. guttatus* juvenile, and (ii) to evaluate the metabolic response to different carbohydrate to protein ratios at this stage to provide the information required for diet formulation and cost.

## MATERIALS AND METHODS

### Experimental diets

Four isoenergetic diets (19.0 kJ $g^{-1}$) were formulated to contain varying crude protein and precooked cornstarch (P:S) ratios, a basal diet (P46S18) containing 464 g of protein (P) and 180 g of precooked cornstarch (S) per kilogram, (Hernández et al. 2014a) and three experimental diets, P38S30: 387 g P $kg^{-1}$ and 300 g S $kg^{-1}$, P34S35: 344 g P $kg^{-1}$ and 350 g S $kg^{-1}$, and P31S40: 317 g P $kg^{-1}$ and 400 g S $kg^{-1}$.

The experimental diets were manufactured as described in Hernández et al. (2014c). However, in this experiment, the cornstarch was heated to 70°C in water for 10 min to increase its digestibility. A sample of each diet was used for proximate composition analysis. The formulation and proximate composition of the diets are shown (Table 1).

### Fish and experimental design

*Lutjanus guttatus* juveniles were raised at the Laboratory of Marine Fish Reproduction (Laboratorio de Reproducción y Cultivo de Peces Marinos) and Culture pilot plant of the Centro de Investigación en Alimentación y Desarrollo-Mazatlán (CIAD, by its acronym in Spanish) following standard protocols for spawning and larval rearing (Ibarra-Castro et al. 2020).

All protocols were performed according to the Official Mexican Standard NOM-033-SAG/ZOO-2014 "Methods for killing domestics and wild animals", following international guidelines for using animals in research.

The fish were acclimatized at the CIAD finfish hatchery for 15 days before the beginning of the experiment. The feeding trial was conducted on a completely randomized design with three replicates per treatment. Four hundred twenty fishes with an average initial body weight of 19.4 ± 0.3 g were allocated randomly to one of 12 white-colored, 3000 L circular fiberglass tanks. Each tank was supplied continuously with seawater at a flow rate of 12 L $min^{-1}$; seawater was pumped from the seashore and filtered according to Alvarez-Lajonchère et al. (2007). Each tank was equipped with three aeration stones and a 3-inch central drain covered with a 0.5 cm mesh net to prevent the fish from escaping and cleaning the tank. Water quality was monitored daily using a YSI® 85-12FT multiparameter oximeter (YSI Inc., Yellow Springs, OH, USA). Dissolved oxygen, water temperature, and salinity were kept at 5.46 ± 0.55 mg $L^{-1}$, 27.4 ± 2.5°C, and 31.35 ± 0.94, respectively, throughout the experiment, under natural photoperiod.

Thirty-five juveniles were placed on each tank, and three tanks (replicates) were assigned randomly to each experimental diet. The fish were manually fed three

**Table 1.** Ingredients and proximate composition (g $kg^{-1}$ dry weight) of the experimental diets for spotted rose snapper *Lutjanus guttatus*. [1]Premium grade fishmeal was purchased from Selecta de Guaymas, S.A. de C.V. Guaymas, Sonora, Mexico. [2]Marine Protein and Agricultural, S.A. of C.V., Guadalajara, Jalisco, Mexico. [3]PROAQUA, S.A. de C.V. Mazatlán, Sinaloa, Mexico. [4]Drogueria Cosmopolita, S.A. de C.V. México, D.F., Mexico. [5]Sigma-Aldrich Chemical, S.A. de C.V. Toluca, Estado de México, Mexico. [6]Trouw Nutrition Mexico S.A. de C.V. (by courtesy). [a]Vitamin premix composition: vitamin A, 10,000,000 IU or mg $g^{-1}$; vitamin D3, 2,000,000 IU; vitamin E, 100.00 g; vitamin K3, 4.00 g; thiamine B1, 8.00 g; riboflavin B2, 8.70 g; pyridoxine B6, 7.30 g; vitamin B12, 20.00 mg; niacin, 50.00 g; pantothenic acid, 22.20 g; inositol, 153.80 g; nicotinic acid, 160.00 g; folic acid, 4.00 g; vitamin B12, 80 mg; biotin, 500 mg; vitamin C, 100.00 g; choline 300.00 g; excipient c.b.p. 2000.00 g. [b]Mineral premix composition: manganese, 100 g; magnesium, 45.00 g; zinc, 160 g; iron, 200 g; copper, 20 g; iodine, 5 g; selenium, 400.00 mg; cobalt 600.00 mg; excipient q.s. 1500.00 g. [7]DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, Mexico. [8]Values are means of three determinations. NFE: nitrogen-free extract.

| Ingredient | Diet (g $kg^{-1}$ diet) | | | |
|---|---|---|---|---|
| | P46S18 | P38S30 | P34S35 | P31S40 |
| Fishmeal[1] | 478.8 | 356.2 | 304.0 | 253.3 |
| Squid meal[2] | 60.0 | 60.0 | 60.0 | 60.0 |
| Krill meal[3] | 75.9 | 75.9 | 75.9 | 75.9 |
| Fish oil[4] | 11.9 | 26.7 | 22.9 | 34.0 |
| Alginate[4] | 40.0 | 40.0 | 50.0 | 45.0 |
| Cornstarch[4] | 180.0 | 300.0 | 350.0 | 400.0 |
| Cellulose[5] | 107.8 | 95.6 | 91.6 | 86.2 |
| Wheat gluten[5] | 20.0 | 20.0 | 20.0 | 20.0 |
| Vitamin premix[6a] | 6.0 | 6.0 | 6.0 | 6.0 |
| Minerals premix[6b] | 2.3 | 2.3 | 2.3 | 2.3 |
| Carotenoids[7] | 0.8 | 0.8 | 0.8 | 0.8 |
| Antioxidants[7] | 0.5 | 0.5 | 0.5 | 0.5 |
| Soy bean lecithin[7] | 15.0 | 15.0 | 15.0 | 15.0 |
| Vitamin C[7] | 1.0 | 1.0 | 1.0 | 1.0 |
| Proximate composition[8] | | | | |
| Dry matter | 937.4 | 939.4 | 924.6 | 924.5 |
| Crude protein | 464 | 387 | 344 | 317 |
| Crude lipid | 94 | 99 | 90 | 94 |
| Ash | 94 | 79 | 74 | 67 |
| NFE | 216 | 327 | 379 | 424 |
| Gross energy (kJ $g^{-1}$) | 19.3 | 19.4 | 19.0 | 19.0 |

times a day (08:00, 12:00, and 16:00 h) until apparent satiation for 10 weeks. The amount of feed consumed was recorded, and uneaten pellets were siphoned out and oven-dried at 60°C to determine feed consumption. With a siphon, feces were removed daily 2 h after feeding. Mortality in each tank was recorded to estimate the feed conversion ratio (FCR).

## Growth performance, feed utilization, and biological indices

Every 15 days from the beginning and until the end of the experiment, all the fish were caught, anesthetized with a clove oil solution (0.3 mL $L^{-1}$ in seawater) for 10 min (Neiffer & Stamper 2009), and individually weighed to check the fish development.

Growth performance and feed utilization by the fish were evaluated in terms of survival rate (SR), weight gain (WG), feed intake (FI), feed conversion ratio (FCR) specific growth rate (SGR), and protein efficiency ratio (PER). The following formulas were used (Hernández et al. 2020a):

SR (%) = 100 × [final count / initial count]

WG (g) = mean final weight (g) - mean initial weight (g)

FI (g $fish^{-1}$ $d^{-1}$) = [total feed consumption (g) / (number of fish / days of experiment)]

FCR = FI / weight gain (g)

SGR (% $d^{-1}$) = 100 × [((ln final weight) - (ln initial weight)) / time (days of experiment)]

PER = weight gain (g) / protein intake (g)

Three fishes from each tank (nine fishes for each treatment) were collected at the end of the experiment; fish were fasted for 24 h and euthanized using an

overdose of clove oil, and their total length, body weight, visceral mass, liver, and visceral fat were measured to compute the following biological indices (Benitez-Hernández et al. 2018): hepatosomatic index (HSI), viscerosomatic index (VSI), intraperitoneal fat rate (IFR), and condition factor (CF). The following equations were used:

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

VSI (%) = 100 × [viscera weight (g) / body weight (g)]

IFR (%) = 100 × [intraperitoneal fat weight (g) / body weight (g)]

CF = 100 × [body weight (g) / length$^3$ (cm$^3$)]

**Chemical analyses**

Nine fish randomly chosen from the initial population were fasted for 24 h to determine initial body composition. At the end of the feeding trial, six fish from each treatment (two per tank) were selected, fasted for 24 h, weighed, and used to analyze final body composition. The fishes were euthanized using an overdose of clove oil.

The fish, ingredients, and diet samples were analyzed to determine moisture, crude fat, and ash contents using standard methods of the Association of Official Analytical Chemists (AOAC, 2011). Moisture was determined by gravimetry using an oven, as per method 4.1.06. Crude protein content was determined with the Dumas combustion method (Ebling, 1968) using a Flash 2000 Organic Elemental Analyzer (Thermo Scientific, Italy). Crude fat content was determined as per method 4.5.05 using a micro Soxhlet Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec, Hoganäs, Sweden). Ash content (method 32.1.05) was analyzed by calcinating the samples in a muffle furnace (Fisher Scientific International, Pittsburgh, PA, USA) at 550°C.

Nitrogen-free extract (NFE) was determined by nutrient difference as follows: nitrogen-free extract (including fiber) = 100% – (% moisture + % protein + % lipid + % ash) (Hossain & Alam 2015). Gross energy was measured using an adiabatic calorimeter (Parr Instruments, model 1261, Moline, IL, USA).

**Hematological parameters**

Nine fish were selected randomly from the initial batch and six fish from each treatment at the end of the experiment. The fish were fasted for 24 h and anesthetized for 10 min with a clove oil solution (0.3 mL L$^{-1}$ in seawater) (Neiffer & Stamper 2009). From each fish, a 400 µL blood sample was extracted from the caudal vein using a 1 mL insulin syringe. The blood sample was divided into 200 µL subsamples and placed into two tubes. The first subsample was added with $K_2$EDTA (BD Microtainer, Franklin Lakes, NJ, USA) and used to measure hematocrit and hemoglobin concentration, and the second subsample was centrifuged to obtain serum and determine glucose, total protein, and triglycerides. The hematological parameters were determined using commercial kits from Randox™ and Biosystem Co™. Mean corpuscular hemoglobin concentration (MCHC) was determined using the standard formula from hemoglobin concentration and microhematocrit percentage values (Del Río-Zaragoza et al. 2008).

**Enzyme activity**

Six fish per treatment were randomly selected at the end of the experiment, fasted for 24 h, euthanized using an overdose of clove oil, and dissected at 0-4°C. Pyloric caeca, pancreatic tissue, liver, and intestine were excised, frozen in liquid nitrogen, and stored separately at -80°C until analysis. Enzymes were extracted by first homogenizing the tissues in cold distilled water (1:5 w:v), using a homogenizers Model T18 (Ultra-Turrax IKA, North Chase, Wilmington); the homogenized tissues were centrifuged at 16170 *g* for 20 min at -4°C (Galaviz et al. 2012), and the supernatant recovered and stored at -80°C until analysis.

The α-amylase activity was measured using 2% starch dissolved in 100 mM L$^{-1}$ citrate-phosphate buffer as substrate, with a 50 mM L$^{-1}$ NaCl solution at pH 7.5 (Clark et al. 1984). The α-glucosidase activity was read at 415 nm with 4-nitrophenyl β-D-glucopyranoside (3 mg mL$^{-1}$ buffer) as substrate in sodium phosphate buffer (pH 7.6) (Clark et al. 1984).

The pyruvate kinase (PK, EC 2.7.1.40) activity was evaluated in liver tissue was homogenized in 50 mM Tris-HCl with 4 mM EDTA at pH 7.5, 50 mM sodium fluoride, 0.5 mM phenylmethylsulfonyl fluoride, 500 mM 1,4-dithiothreitol, and 250 mM sucrose, and centrifuged at 16170 *g* for 30 min at 4°C in a homogenizers Model T18 (Ultra-Turrax IKA, North Chase, Wilmington) (position 4, 10 s). After centrifugation at 16170 g for 40 min at 4°C, the supernatants were separated on a Sephadex G-25 column. PK activity was assayed in crude liver extracts as per method EC 2.7.1.40 using a microplate reader Model 550 (Bio-Rad, Hercules, CA, USA) (Bonamusa et al. 1992).

All enzymatic assays were carried out at 30°C and read at 340 nm (Castillo et al. 2018a). Protein concentration (Bradford 1976) was determined using a standard sigma protein assay kit with bovine serum albumin. Enzyme activity was expressed as units per mg of soluble protein. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the hydrolysis of 1 µmol of substrate per minute at the temperature of the assay.

### Statistical analyses

Before statistical analysis, data were tested for normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's test). Percent variables (SGR, SR, biological indices, proximate composition, and hematocrit) were arcsine-transformed, but the results are reported as percentages. A one-way ANOVA test for differences among diets followed Tukey's multiple comparison test when significant differences were detected (Zar 1984). A significance level $P < 0.05$ was used for all statistical tests. All statistical analyses were carried out using NCSS 2007 (Kaysville, UT, USA). Data were expressed as mean with standard deviation (SD) and *P*-value.

## RESULTS

### Survival, feed intake, growth performance, feed efficiency, and biological indices

Survival was above 97% in all cases, with no significant differences between treatments (Table 2). Feed intake, PER, and biological indices such as CF, HSI, VSI, and IFR, did not show significant differences between treatments (Table 2). FBW, WG, SGR, and FCR, were not significantly different among the P46S18, P38S30, and P34S35 diets.

### Whole-body composition

The whole-body composition analysis of the *L. guttatus* fed the P46S18 basal diet showed the highest moisture, protein, and ash contents, compared to the rest of the diets. In addition, animals fed the lowest protein (P31S40) had the highest lipids and NFE values than animals on the P46S18 (Table 3).

### Hematological parameters

Hematocrit and hemoglobin values showed no significant differences between treatments (Table 4). Fish fed the P31S40 diet showed a significantly lower total protein content, but no significant differences were found in the rest of the treatments (Table 4). Fish fed P34S35, and P31S40 diets had the highest triglycerides values, while the highest glucose value was recorded in fish fed P34S35 diet (Table 4).

### The activity of digestive and metabolic enzymes

Table 5 shows the activity values of α-amylase, α-glucosidase, and PK. The highest α-amylase activity was recorded in the pancreatic tissue of fish fed the P31S40 diet. The activity of this enzyme in the pyloric caeca, proximal intestine, and distal intestine decreased with increasing protein levels in the diet.

No significant differences between diets were found in α-glucosidase activity in pancreatic tissue. However, the activity of this enzyme in crude extracts from pyloric caeca and proximal and distal intestinal tissues increased with increasing dietary starch levels.

The lowest PK activity in the liver was recorded in fish fed the P31S40 diet, and no significant differences were found in the rest of the treatments.

## DISCUSSION

Precooked cornstarch can be used up to 35% and reduce the protein content (until 34%) into the diet for juvenile *Lutjanus guttatus* without adversely affecting growth performance and feed efficiency.

Precooked cornstarch was used as the major carbohydrate source in the diets tested in this study. We tested different proportions of precooked cornstarch to make more efficient use of dietary protein (sparing effect) (Kamalam et al. 2017). FCR and PER values were comparable to those reported by Booth et al. (2013) for juvenile yellowtail kingfish *Seriola lalandi* fed similar starch levels (10-40%) and higher than those reported for yellowfin seabream *Sparus latus* fed starch levels (18-27%) (Hu et al. 2007) and golden pompano *Trachinotus ovatus* fed starch levels (17-28%) (Zhou et al. 2015).

Fish fed the P38S30, and P34S35 diets attained a growth similar to fish fed the P46S18 diet; this may be due to *L. gutattus*' ability to digest, absorb and metabolize dietary starch. Similar results have been reported in other species such as the European seabass *Dicentrarchus labrax* (Peres & Oliva-Teles 2002), the silver perch *Bidyanus bidyanus* (Stone et al. 2003), and the sea bream *S. latus* (Wu et al. 2007).

Abdo de la Parra et al. (2010) reported that about 45-50% of the dietary protein inclusion into the feed for *L guttatus* is recommendable for optimum growth of the species. However, this study aimed to use low dietary protein content and high starch content (protein-sparing) to decrease the feed cost. In this study, the dietary protein inclusions of 31% reduced the growth of *L. guttatus* (WG and SGR), which is attributed to a low dietary protein content since the organism did not strictly use starch as the main source of energy. Both et al. (2013) reported that *S. lalandi* fed a diet with less than 40% protein and more than 31% starch reduced its growth attributed to a low protein content and the deficiency of amino acids related to growth; mainly methionine (Palma et al. 2020). Similarly, in our case, because *L. guttatus* was fed with 38 and 34% dietary protein, less than the recommended optimal level of protein, it did not affect growth (WG and SGR). There-

**Table 2.** Growth, feed efficiency, and biological indices of spotted rose snapper *Lutjanus guttatus* fed experimental diets containing varying protein levels and precooked cornstarch for 10 weeks. Values in the same row with different superscripts are significantly different ($P < 0.05$). Mean ± standard deviation, number of determinations for growth and feed efficiency = 9, number of determinations for biological indices = 9. IBW: initial body weight, FBW: final body weight, WG: weight gain, SGR: specific growth rate, FI: feed intake, FCR: feed conversion ratio, PER: protein efficiency ratio, SR: survival rate, CF: condition factor, HSI: hepatosomatic index, VSI: viscerosomatic index, IFR: intraperitoneal fat index.

| Variable | Diet | | | | |
|---|---|---|---|---|---|
| | P46S18 | P38S30 | P34S35 | P31S40 | *P*-value |
| IBW (g) | 19.4 ± 2.2 | 19.4 ± 2.3 | 19.4 ± 2.2 | 19.4 ± 2.2 | 0.441 |
| FBW (g) | 57.8 ± 2.4$^{a}$ | 53.5 ± 3.4$^{ab}$ | 52.7 ± 3.5$^{ab}$ | 49.2 ± 0.6$^{b}$ | 0.030 |
| WG (g) | 38.4 ± 2.4$^{a}$ | 34.1 ± 3.4$^{ab}$ | 33.3 ± 3.5$^{ab}$ | 29.9 ± 0.6$^{b}$ | 0.031 |
| SGR (% $d^{-1}$) | 1.56 ± 0.1$^{a}$ | 1.45 ± 0.1$^{ab}$ | 1.43 ± 0.1$^{ab}$ | 1.33 ± 0.0$^{b}$ | 0.035 |
| FI (g $fish^{-1}$ $d^{-1}$) | 50.9 ± 2.7 | 52.3 ± 5.2 | 53.5 ± 2.4 | 53.0 ± 5.4 | 0.882 |
| FCR | 1.29 ± 0.1$^{a}$ | 1.50 ± 0.1$^{ab}$ | 1.58 ± 0.1$^{ab}$ | 1.74 ± 0.2$^{b}$ | 0.009 |
| PER | 1.68 ± 0.2 | 1.72 ± 0.1 | 1.84 ± 0.1 | 1.83 ± 0.2 | 0.444 |
| SR (%) | 98.1 ± 3.3 | 99.0 ± 1.7 | 99.0 ± 1.7 | 97.1 ± 2.9 | 0.752 |
| Biological indices | | | | | |
| CF | 1.33 ± 0.1 | 1.29 ± 0.0 | 1.31 ± 0.0 | 1.32 ± 0.1 | 0.510 |
| HSI (%) | 0.92 ± 0.1 | 0.97 ± 0.2 | 0.85 ± 0.0 | 0.90 ± 0.1 | 0.219 |
| VSI (%) | 6.91 ± 1.0 | 6.49 ±1.6 | 7.31 ± 0.4 | 7.01 ± 0.6 | 0.401 |
| IFR (%) | 1.86 ± 0.4 | 2.03 ± 1.2 | 1.81 ± 0.4 | 1.87 ± 0 .5 | 0.851 |

**Table 3.** Whole-body composition (%) (wet weight) of juvenile spotted rose snapper *Lutjanus guttatus* fed experimental diets for 10 weeks. Mean ± standard deviation, number of determinations for initial analysis = 9, number of determinations per diet = 6. Values in the same row with different superscripts are significantly different ($P < 0.05$). NFE: nitrogen-free extract.

| Chemical composition | Diet | | | | |
|---|---|---|---|---|---|
| | P46S18 | P38S30 | P34S35 | P31S40 | *P*-value |
| Moisture | 70.7 ± 0.1$^{a}$ | 69.2 ± 0.4$^{bc}$ | 69.9 ± 0.1$^{b}$ | 68.9 ± 0.1$^{c}$ | 0.000 |
| Protein | 18.2 ± 0.1$^{a}$ | 17.4 ± 0.2$^{b}$ | 17.4 ± 0.4$^{b}$ | 16.6 ± 0.3$^{c}$ | 0.000 |
| Lipids | 4.9 ± 0.0$^{b}$ | 6.9 ± 0.1$^{ab}$ | 6.4 ± 0.4$^{ab}$ | 7.8 ± 0.2$^{a}$ | 0.000 |
| Ash | 5.1 ± 0.0$^{a}$ | 5.0 ± 0.1$^{a}$ | 4.9 ± 0.1$^{b}$ | 4.9 ± 0.0$^{b}$ | 0.000 |
| NFE | 1.1 ± 0.1$^{b}$ | 1.3 ± 0.2$^{ab}$ | 1.4 ± 0.2$^{ab}$ | 1.8 ± 0.4$^{a}$ | 0.000 |

fore, the decrease observed in *L. guttatus* fed P31S40 can be attributed to the low protein content, the deficiency in amino acids, and the inefficiency of this species to efficiently use starch as an energy source. FCR values increased concomitantly with increasing precooked cornstarch content. These results are related to the weight reduction experienced by fish due to nutritional restriction, specifically protein restriction (low protein content). As a result, the fish demand more feeds to meet their nutritional and energy needs, as occurred in research with *Lates calcarifer*, using a feed with a 31% starch content (Glencross et al. 2014).

The whole-body protein content of fish fed P46S18 was higher than the other diets. With a tendency to decrease as the amount of starch increased. Protein reduction probably resulted in insufficient amino acids for better growth than organisms fed the P46S18 diet. In this situation, *L. guttatus* consumed more feed than *L. calcarifer*, fed high in pregelatinized starch (Glencross et al. 2017). However, the ingested amino acids could be involved in energy production and not in growth (Palma et al. 2020). Such results suggest that *L. guttatus* has a low capacity to use starch carbohydrates for energy (Palma et al. 2020), indicating that digestible starch converted to glucose is redirected to lipogenesis (Viegas et al. 2019).

The observed increase in lipids of *L. guttatus* fed a high content of precooked corn starch (40%) could be because glucose derived from starch is underutilized as an energy source. Therefore, it is used for the synthesis of lipid *de novo*, which has been previously reported by Glencross et al. (2014), where *L. calcarifer* fed a diet rich in starch uses glucose to convert it to lipids *de novo* through lipogenesis, instead of using it as an energy source. Fish fed digestible starch have been reported to retain their adaptation to these diets so that excess glucose is transformed into fat (Harmon et al. 1991, Castillo et al. 2018a). However, the ability of different

**Table 4.** Hematological parameters of spotted rose snapper *Lutjanus guttatus* fed experimental diets for 10 weeks. Mean ± standard deviation, number of determinations for initial analysis = 9, number of determinations per diet = 6. Values in the same row with different superscripts are significantly different ($P < 0.05$).

| Hematological parameter | Diet | | | | |
|---|---|---|---|---|---|
| | P46S18 | P38S30 | P34S35 | P31S40 | *P* |
| Hematocrit (%) | 43.7 ± 5.8 | 46.2 ± 0.5 | 42.4 ± 2.7 | 41.7 ± 4.3 | 0.210 |
| Hemoglobin (g $dL^{-1}$) | 11.5 ± 1.6 | 9.6 ± 2.9 | 9.9 ± 1.9 | 8.3 ± 1.9 | 0.121 |
| Total protein (g $dL^{-1}$) | 5.1 ± 0.3$^{ab}$ | 5.5 ± 0.1$^{a}$ | 5.2 ± 0.2$^{ab}$ | 4.5 ± 0.1$^{b}$ | 0.000 |
| Triglycerides (mg $dL^{-1}$) | 232.3 ± 20.9$^{b}$ | 269.5 ± 21.1$^{ab}$ | 283.2 ± 25.6$^{a}$ | 292.9 ± 55.7$^{a}$ | 0.030 |
| Glucose (mg $dL^{-1}$) | 213.4 ± 5.5$^{bc}$ | 230.7 ± 12.2$^{ab}$ | 237.0 ± 12.4$^{a}$ | 207.0 ± 18.4$^{c}$ | 0.001 |

**Table 5.** The digestive and metabolic enzymes (U $mg^{-1}$ protein) activity in tissues of juvenile spotted rose snapper *Lutjanus guttatus*. Values in the same row with different superscripts are significantly different ($P < 0.05$). Mean ± standard deviation, number of determinations per diet = 6. PAT: pancreatic tissue. PC: pyloric caeca. PI: proximal intestine. DI: distal intestine.

| Digestive enzyme | Tissue | Diet | | | | |
|---|---|---|---|---|---|---|
| | | P46S18 | P38S30 | P34S35 | P31S40 | *P* |
| α-amylase | PAT | 1849.9 ± 355.8$^{c}$ | 2398.7 ± 303.7$^{c}$ | 7536.1 ± 1744.4$^{b}$ | 27760.3 ± 580.4$^{a}$ | 0.000 |
| | PC | 1610.2 ± 412.9$^{a}$ | 1426.3 ± 190.1$^{a}$ | 683.8 ± 268.5$^{ab}$ | 452.7 ± 137.9$^{b}$ | 0.000 |
| | PI | 522.2 ± 135.3$^{a}$ | 413.1 ± 68.8$^{ab}$ | 380.1 ± 108.7$^{b}$ | 288.8 ± 92.4$^{b}$ | 0.008 |
| | DI | 2041.3 ± 306.1$^{a}$ | 1999.5 ± 492.7$^{a}$ | 1613.0 ± 321.2$^{ab}$ | 1431.7 ± 189.1$^{b}$ | 0.014 |
| α-glucosidase | PAT | 14.7 ± 4.7 | 17.3 ± 2.2 | 20.9 ± 11.5 | 25.1 ± 8.8 | 0.138 |
| | PC | 14.7 ± 4.2$^{b}$ | 15.3 ± 2.3$^{ab}$ | 20.6 ± 2.4$^{a}$ | 22.4 ± 2.0$^{a}$ | 0.000 |
| | PI | 14.4 ± 0.6$^{b}$ | 16.1 ± 1.2$^{b}$ | 20.7 ± 1.8$^{a}$ | 23.6 ± 1.6$^{a}$ | 0.000 |
| | DI | 19.9 ± 0.8$^{b}$ | 22.0 ± 1.9$^{ab}$ | 30.5 ± 6.1$^{ab}$ | 35.2 ± 2.0$^{a}$ | 0.000 |
| Metabolic enzyme | | | | | | |
| Pyruvate kinase | Liver | 1.5 ± 0.2$^{a}$ | 1.5 ± 0.1$^{a}$ | 1.2 ± 0.1$^{a}$ | 0.7 ± 0.1$^{b}$ | 0.000 |

species to use CHOs may depend on their ability to oxidize glucose and store excess levels as lipids (Guo et al. 2006, Xiao et al. 2014); these digestive and absorptive capacities play a central role in fish growth (Hakim et al. 2006).

Measuring hematological parameters is one of the tools used to assess fish's health and nutritional status as such parameters are sensitive to physical and chemical factors (Del Río-Zaragoza et al. 2008, Peres et al. 2014b, Prakoso et al. 2016). Hematocrit values of *L. guttatus* ranged from 41.6 to 46.2%, similar to values reported by other studies on the same species (Hernández et al. 2014b,c, Hernández et al. 2019). The hemoglobin values recorded in this study are comparable to those reported for *L. guttatus* fed soybean meal (Silva-Carrillo et al. 2012), poultry byproducts (Hernández et al. 2014b), corn gluten (Hernández et al. 2020a), or canola flour (Hernández et al. 2020b). Both results, hematocrit and hemoglobin, are within the range reported for healthy *L. guttatus* (Del Río-Zaragoza et al. 2011).

Fish's physiological and nutritional status is often related to protein concentrations in blood plasma (De Pedro et al. 2005). Although the high consumption of carbohydrates in the feed can negatively affect the health of the organisms, the total protein content recorded in the present study are consistent with those (4.6 and 5.5 g $dL^{-1}$) reported for *L. guttatus* fed diets formulated with poultry byproducts (Hernández et al. 2014b,c) or conventional diets (Hernández et al. 2019, 2020a,b), and are also similar to values reported for other species including the European seabass (Peres et al. 2014a) and Senegalese sole (*Solea senegalensis*) (Peres et al. 2014b, Andrade et al. 2015). These results show that stable total protein levels are frequently associated with well-nourished fish's nutritional and physiological status (Coeurdacier et al. 2011), in which amino acid oxidation or peripheral proteolysis are not disrupted (Di Marco et al. 2008).

The plasma triglyceride values recorded in this study are within the range (189-243 mg $dL^{-1}$) reported for healthy juvenile *L. guttatus* (Hernández et al. 2014b).

Some authors regard teleost fish as diabetic animals because hyperglycemia has been recorded after ingesting high CHO concentrations, which could be partly the result of low secretion of endogenous insulin (Wilson 1994) or variations in feeding habits and type

of feed, type of nutrients, and the amount consumed by fish (Moon 2001). The plasma glucose levels recorded in this study (113.1-237.0 mg $dL^{-1}$) after 24 h fastening are higher than those previously reported for marine fish: 104.5-108.1 mg $dL^{-1}$ (Enes et al. 2010) and 98.4-113.3 mg $dL^{-1}$ (Zhou et al. 2015). Moreover, plasma glucose levels in juvenile *L. guttatus* increased significantly as precooked cornstarch levels increased, indicating that glucose transport was stimulated in response to a higher dietary starch level (Ren et al. 2011, Zhou et al. 2015, 2016). Blood glucose levels may have likely decreased during the fastening period, thus improving endogenous glycolysis but decreasing gluconeogenesis (Zhou et al. 2015). However, the plasma glucose level decreased with a 40% inclusion of precooked cornstarch; this can be attributed to having exceeded the ability of *L. guttatus* to digest high amounts of starch (Liu et al. 2012, Booth et al. 2013).

Since endogenous metabolic enzymes are good indicators of the ability of fish to use dietary carbohydrates as an energy source (Stone et al. 2003), determining their activity level could help formulate diets with higher but still suitable levels of carbohydrates. This latter would reduce the use of proteins as an energy source, thereby avoiding the catabolism of amino acids in the diet to synthesize glucose and reduce the unnecessary loss of nitrogen (Palma et al. 2020). Furthermore, fish have enzymes capable of metabolizing carbohydrates. Thus, carbohydrates as an energy source to reduce protein should be feasible. The α-amylase activity was recorded in all the digestive tissues examined in this species. Moreover, pancreatic tissues showed the highest α-amylase activity levels, as exocrine pancreatic cells are the primary source of α-amylase in fish (Krogdahl et al. 2005).

Furthermore, the increase of α-amylase activity in pancreatic cells was concomitant with the increasing inclusion of CHOs in the diet. These results show the ability of juvenile *L. guttatus* to accommodate their amylolytic capacity according to the amount of CHOs in the diet. Also, a similar positive relationship between amylase activity and starch inclusion in the diet was reported in other carnivorous fish species such as cobia *Rachycentron canadum* (Ren et al. 2011), golden pompano *T. ovatus* (Zhou et al. 2015), large yellow croaker *Larmichthys crocea* (Zhou et al. 2016), and others. However, golden pompano is less able to tolerate dietary carbohydrates (11.2-16.8% of the diet) (Zhou et al. 2015) and, thus, the increase in amylase activity with higher inclusion of CHOs in the diet was limited to some extent. However, the amylase activity in PC, PI, and DI of fish fed the P31S40 diet was lower than in fish fed the P45S18 diet; this can be explained, to a certain extent, by the reduction in the apparent digestibility coefficient of starch when it was included at 40% (Ren et al. 2011).

The hydrolase α-glucosidase catalyzes the digestion of oligosaccharides and disaccharides into monosaccharides, which are finally taken up by intestinal enterocytes. Thus, α-glucosidase activity improves the digestibility of carbohydrates in teleost fish (Papoutsoglou & Lyndon 2006). Furthermore, the high levels of α-glucosidase activity recorded in the distal intestine show the importance of this intestinal region for disaccharide digestion and carbohydrate absorption, as previously reported for other carnivorous fish (Papoutsoglou & Lyndon 2006, Zhang et al. 2020). Unfortunately, the digestibility of the diets was not evaluated in this study. Nevertheless, the hypothesis is that the increasing precooked cornstarch levels stimulated the α-amylase secretion in pancreatic tissue and α-glucosidase in pyloric ceca and intestinal tissues from juvenile *L. guttatus*. Some authors have stated that the intestinal α-amylase activity of fish is correlated with different levels of CHOs in their diet and feeding frequency (Aderolu & Sahu 2015). However, the amount secreted is likely insufficient to digest high amounts of CHOs in the diet, and, as a result, the body is unable to break them down, so they pass through the digestive system without being fully utilized.

Regarding PK activity, it was detected in the liver of juvenile *L. guttatus*; this means that, although *L. guttatus* is a carnivorous species, it shows intermediate metabolism for CHOs. PK catalyzes the last stage of glycolysis and participates in the conversion of phosphoenolpyruvate to pyruvate, which is then transformed into ATP to provide energy to the chemical reactions of the organism (Fan et al. 2019). PK also acts as the third checkpoint in glycolysis (Castillo et al. 2018b). This study presents findings in which PK activity in *L. guttatus* decreased when fed with high carbohydrate levels and low protein, consistent with what was observed with *Erythroculter ilishaeformis* and *Ancherythroculter nigrocauda* showed that the mRNA expression of PK was induced fed diets with a low proportion of digestible carbohydrates (dextrine) and a high proportion of protein (Tian et al. 2020).

Therefore, the present study's PK activity results show that dietary protein content savings can be achieved by including up to 35% CHOs in diets for juvenile *L. guttatus*. Moreover, it would be even possible to determine the percentage of precooked cornstarch that can be optimally included in the *L. guttatus* diet aiming to reduce fishmeal and yet meet the body energy demands for its metabolic processes without compromising its health which is possible

through the carbohydrases secretion and the ability of *L. guttatus* to tolerate high contents of CHOs in diets.

## CONCLUSION

The results in the present study show that precooked cornstarch inclusion levels of up to 35% can be used without adversely affecting growth or feed utilization. Juvenile *L. guttatus* can digest, absorb, and metabolize high dietary carbohydrate levels (up to 35%). In addition, high serum glucose levels caused changes in liver condition, forcing the liver to convert excess glucose to triglycerides through lipogenesis.

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