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Effects of dietary protein and lipid levels on growth and body composition of the Gulf corvina, *Cynoscion othonopterus*

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Abstract: A 56–day 3 x 3 factorial experiment was conducted to evaluate the effects of three levels of dietary crude protein (40, 45, and 50%) and three levels of crude fat (8, 12, and 16%) on the performance of *Cynoscion othonopterus* juveniles (initial mean body weight of 102.6±14.1 g). The levels of dietary crude protein and fat tested, or their interaction, did not influence significantly the growth response, as evaluated by final weight, weight gain, percent weight gain, thermal growth coefficient, feed conversion ratio, or survival. Apparent digestibility coefficients of dry matter of experimental feeds ranged from 74.1 to 80.2%, without significant differences among treatments. In muscle tissue, increased crude fat deposition was observed in response to increasing levels of this nutrient. However, the content of crude protein and ash decreased significantly with both dietary crude protein and fat. In turn, moisture content increased significantly with dietary crude protein, from 71.2±2.5% (at 40% crude protein), to 75.9±1.4% (at 50% crude protein), but it was not affected by dietary crude fat. It appeared that 40% dietary crude protein was sufficient to promote adequate growth and survival for this species. Further research is warranted to evaluate if dietary protein requirements of *C. othonopterus* are below 40%, and to further elucidate the extent of the observed protein-sparing effect of dietary lipid.

Key Words: Protein, Lipid, *Cynoscion othonopterus*

Introduction

To a large extent stimulated by the near-downfall of semi-intensive commercial shrimp farming in Northwest Mexico (COSAES, 2014) caused by diseases, collective efforts of aquaculturists, researchers, feed manufacturers and governmental agencies have focused on the

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development of marine finfish farming. Marine fish species native to this region that have recently been examined as possible candidates for aquaculture include the Cortez flounder (*Paralichthys aestuarius*) (González-Félix *et al*., 2014) and two members of the family Scianidae, the totoaba (*Totoaba macdonaldi*) (Rueda-López *et al*., 2011; Minjarez-Osorio *et al*., 2012), and the Gulf corvina (*Cynoscion othonopterus*) (González-Félix *et al*., 2013). All three species inhabit the Gulf of California and have promising features for aquaculture. The Gulf corvina, the species under study, supports a commercial fishery that surpassed 3,700 MT in 2010, making it the second most abundant finfish fishery in the Gulf of California (SAGARPA, 2012). Studies of reproduction in captivity and growout of this species in sea cages are currently underway. However, the lack of species-specific feeds has been identified as a major bottleneck for the development of commercial farming. Hence, knowledge of the nutritional requirements of this species is needed in order to overcome this.

Protein is the major, as well as the most expensive component of aquafeeds (Thoman *et al*., 1999; Tacon *et al*., 2011). This is an important factor to consider, especially for carnivorous fish species, such as the Gulf corvina and other sciaenids, since they are known to have high dietary protein requirements and retain nitrogen (N) more efficiently (N-gained/N-consumed) than omnivorous fish species (Pirozzi *et al*., 2010; Rueda-López *et al*., 2011; National Research Council, 2011). It has long been established that dietary protein and lipid can be readily used as energy sources by fish (Kleiber, 1975), which implies that when fed in excess, protein catabolism as a fuel source represents a wasteful use of this costly nutrient. Some sciaenids and other fish have been shown to ingest food until their energy needs are satisfied, which implies that all dietary nutrients, including protein, must be balanced to dietary energy to achieve adequate growth. For example, a diet with very high levels of dietary energy will be consumed in limited amounts, which will, in turn, restrict growth (Shiau and Lan, 1996; McGoogan and Gatlin, 1999; Rueda-López *et al*., 2011). Ideally, an optimal ratio between these nutrients in the feed should be attained to maximize the use of protein for growth, while meeting energy needs mainly through dietary lipid. Optimal dietary protein and lipid levels for the Gulf corvina are not known. Thus, the aim of the present study was to evaluate the effect of dietary protein and lipid level on growth, survival and body composition of *C. othonopterus*.

Materials and methods

Experimental fish

Gulf corvina (*C. othonopterus*) juveniles from the same cohort, obtained from wild spawners and reared in raceways at the "Center for

Reproduction of Marine Species of the State of Sonora", located at Kino Bay, Sonora, Mexico, were transported to the Wet Laboratory of Aquaculture Nutrition of Kino Bay Experiment Station (KBES), University of Sonora at Kino Bay, Sonora, Mexico. Fish were placed directly into experimental tanks (see description ahead in *Experimental culture system*) at a density of 5-6 fish/tank. Fish were fed a commercial feed for marine fish (Alimentos Balanceados Super S.A. de C.V., Guadalajara, Jalisco, México) with dietary crude protein and fat contents of 38.6 and 7.0%, respectively, and were allowed to acclimate to the experimental conditions for 15 days prior to initiation of the experiment.

Experimental culture system

The experimental culture system consisted of two identical modules, each composed of 24 polyethylene circular tanks (71 cm diameter, 0.4 m^2 bottom area, and 250 L capacity, but filled with 200 L of water), a 1.5-HP pump (Jacuzzi, Model 150MF-T, Little Rock, Arkansas, USA) that recirculated water through a 1,100-L sump tank, a biofilter, a sand filter (Jacuzzi, Model L-190-7, Little Rock, Arkansas, USA), a 120-Watt UV light chamber (Rainbow Lifeguard, Model UV97, El Monte, California, USA), a 1,500-Watt in-line heater (Aquatic Ecosystems, Model DE-6115, Apopka, Florida, USA), and a 1-HP in-line chiller (Aquatic Ecosystems, Model AE62B, Apopka, Florida, USA). The modules were interconnected with each other to share the same water quality. Water flowed into each tank at a rate of 1.5 L/min for a complete water turnover every 133 min. Approximately 80% of the water volume was replaced daily with new, filtered seawater. Aeration was supplied to individual tanks using a 1.0-HP blower (Fuji, Model VFC40, Saddle Brook, New Jersey, USA) and submerged airstones.

Experimental treatments

A 56–day 3 x 3 factorial experiment was carried out to test three levels of dietary crude protein (40, 45, and 50%) and three levels of crude fat (8, 12, and 16%), for a total of 9 experimental feeds (Table 1), in a completely randomized design. Feeds were prepared by cold extrusion using a Hobart grinder (Hobart Corporation, Model A-200, Troy, Ohio, USA) and dried overnight at 40°C. They were ground to appropriate size and kept frozen until used. In addition, the commercial feed used during acclimation was included as an external reference, but it was not included in the statistical analysis. Each experimental treatment was assigned to 5 replicate tanks, except for the external reference diet, which was assigned to 3 replicate tanks. Proximate composition of experimental feeds and fish muscle tissue, in terms of moisture, crude protein, crude fat, and ash was evaluated following the procedures 930.15, 976.05, 2003.05, and 942.05, respectively, of the Association of Official Analytical Chemists (2005). Gross energy of the

experimental feeds was analyzed with an isoperibol oxygen-bomb calorimeter (Model 1261, Parr Instrument Co., Moline, Illinois, USA) (Tab. 1).

Stocking and maintenance of fish

Individuals that were too big or too small were removed from tanks to establish a stocking density of three fish per tank for initiation of the experiment, with an overall individual mean (± standard deviation, s.d.) wet body weight of 102.6±14.1 g. Daily measurements of dissolved oxygen, temperature, and salinity of culture water were performed with a multi-function oxygen meter (YSI, Model Y85, Yellow Springs, Ohio, USA), while pH was measured with a hand-held pH meter (Oakton®, Model Double Junction pHTestr 1, Vernon Hills, Illinois, EUA). Weekly measurements of the concentrations of total ammonia nitrogen and nitrite were performed using a Thermo Scientific Orion ISE meter (4-Star pH/ISE Meter, Beverly, Maryland, USA), equipped with, respectively, an ammonia combination electrode (9512BNWP) or a nitrogen oxide combination electrode (9546BN). Mean values (± S.D.) for temperature, salinity, pH, and the concentration of dissolved oxygen, total ammonia nitrogen, and nitrite in the culture water were 27.8±1.2 °C, 37.3±0.5‰, 7.4±0.1, 6.2±0.2 mg/l, 0.17±0.11 mg/l, and 0.02±0.01 mg/l, respectively.

Feeding, digestibility assessment and fish biological performance

The daily feed ration, 3% of the body wet weight, was offered to fish in two equal portions fed at 11:00 and 19:00 hours. For the first 25 days of the 8-week experiment, feces were collected for the assessment of digestibility of feeds. Fifteen min. after each feeding, uneaten feed was removed from the tanks. Two hours after each feeding, feces were collected from each tank with a glass tube, placed into a 5 µm sieve, and slightly rinsed with distilled water to remove salts. Then, they were stored frozen in 15-mL Falcon tubes (Corning Inc., Corning, New York, USA). Apparent digestibility coefficients of dry matter (ADC of DM) of the experimental feeds were calculated following an *in vivo* method outlined by Montaño-Vargas *et al*. (2002), in which acid-insoluble ash (AIA) is used as an internal marker. In summary, feces and feed samples (2 g) were individually dried (one sample per tank) to constant weight and then incinerated at 450 °C. The ashes were boiled for 2 h in 2 N HCl in 50-mL beakers with closed glass lids to avoid evaporation. After 5 min, the samples were passed through an ashless paper filter and the acid was rinsed away with boiling distilled water. Subsequently, the samples were ashed again at 450 °C and the dry matter weight was determined. The AIA was determined as:

$$AIA\ (\%) = \{[(\text{sample dry weight} + \text{ash}) - (\text{crucible weight})] / \text{sample dry weight}\} \times 100$$

Tab. 1: Ingredient (a) and proximate composition (% of dry weight)(b) of experimental feeds for *C. othonopterus*.

a)

Ingredient	Treatment (crude protein/crude fat)									External
	(40/8)	(40/12)	(40/16)	(45/8)	(45/12)	(45/16)	(50/8)	(50/12)	(50/16)	Ref. diet
Fishmeal (sardine)[a]	43.75	44.50	45.30	51.10	51.90	52.70	58.50	59.25	60.00	N/A[n]
Wheat flour[b]	31.30	26.5	21.6	23.85	18.95	14.05	16.30	11.45	6.65	N/A
Soybean meal[c]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	N/A
Corn gluten[d]	6.00	600	6.00	6.00	6.00	6.00	6.00	6.00	6.00	N/A
Wheat starch[d]	4.00	400	4.00	4.00	4.00	4.00	4.00	4.00	4.00	N/A
Sardine fish oil[a]	4.85	7.65	10.45	4.90	7.75	10.55	5.00	7.85	10.65	N/A
Soybean oil[e]	1.70	2.95	4.25	1.75	3.00	4.30	1.80	3.05	4.30	N/A
Soybean lecithin[f]	1.00	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N/A
Vitamin premix without choline[g]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	N/A
Mineral premix[g]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	N/A
Choline chloride[h]	0.20	0.20	0.20	0.2	0.20	0.20	0.20	0.20	0.20	N/A
Vitamin Stay C 35%[i]	0.20	0.20	0.20	0.2	0.20	0.20	0.20	0.20	0.20	N/A
Methionine[j]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	N/A
Lysine[k]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	N/A
α-Tocopherol[l]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	N/A
Total	100	100	100	100	100	100	100	100	100	N/A

Tab. 1: continued; b)

Proximate composition[m]	Treatment (crude protein/crude fat)									External
	(40/8)	(40/12)	(40/16)	(45/8)	(45/12)	(45/16)	(50/8)	(50/12)	(50/16)	Ref. diet
Moisture	7.3±0.2	5.6±0.1	4.0±0.1	6.6±0.3	6.0±0.1	6.0±0.2	8.3±0.1	5.0±0.1	3.5±0.0	3.7±0.1
Crude protein	39.4±0.0	40.6±1.0	40.6±1.0	45.2±1.0	44.0±1.0	44.6±0.0	49.7±0.0	50.9±1.0	50.3±1.0	36.6±1.0
Crude fat	8.5±0.2	11.6±0.2	16.6±0.4	7.9±0.3	12.8±0.2	16.4±0.6	8.6±0.3	13.1±0.3	17.1±0.1	7.0±0.3
Ash	6.9±0.3	8.3±1.5	7.8±1.2	8.2±0.2	8.1±0.0	8.4±0.1	9.0±0.2	9.0±0.2	9.6±0.7	11.2±0.4
Gross energy (kJ/g)	20.5±0.4	20.5±2.1	20.9±2.9	19.3±0.8	19.3±1.3	20.9±0.4	19.3±2.9	21.8±0.4	22.2±0.4	17.6±2.9
Protein (P)/energy ratio (mgP/kJ)	19.2±4.6	19.8±4.8	19.4±3.4	23.5±11.9	22.8±8.0	21.3±5.2	25.8±3.8	23.4±3.9	22.7±3.8	20.8±3.4

[a]Selecta de Guaymas, S.A. de C.V., Guaymas, Sonora, Mexico.

[b]Los Gallos, Molino La Fama S.A. de C.V., Hermosillo, Sonora, Mexico.

[c]Sumilab S.A. de C.V., Mazatlán, Sinaloa, Mexico.

[d]Gluten y Almidones Industriales, S.A. de C.V., Mexico City, Mexico

[e]Aceite Nutrioli, Ragasa Industrias S.A. de C.V., Guadalupe, Nuevo León, Mexico.

[f]Golden Harvest, Impulsora Golden, S.A. de C.V., Mexico City, Mexico.

[g]MP Biomedicals Inc., Solon, Ohio, USA, g/kg of premix: thiamine HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL pantothenic acid 5.0, niacin 5.0, biotin 0.05, folic acid 0.18, cyanocobalamin 0.002, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D_3 (400,000 IU/g) 0.002, DL- α-tocopheryl acetate (250 IU/g) 8.0, α-cellulose 865.266.

[g]MP Biomedicals Inc., Solon, Ohio, USA, g/100 g of premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate heptahydrate 4.0, magnesium sulfate pentahydrate 28.398, manganese sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, α-cellulose 53.428.

[h]Sigma Aldrich, Saint Louis, Missouri, USA;

[i] Stay C® (L-ascorbyl-2-polyphosphate 35% active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[j]Fagalab, S.A de C.V., Mocorito, Sinaloa, Mexico.

[k]Jalmek, Monterrey, Nuevo León, Mexico.

[l]General Nutrition Centers, Co., Pittsburg, Pennsylvania, USA.

[m]Values are means±standard deviation of triplicate samples.

[n]N/A = Not applicable.

Finally, ADC of DM of the feeds was calculated as: *ADC of DM (%) = 100 – [100 × (AIAfeed/AIAfeces)]*

After the 25-day feces collection period and until termination of the feeding trial, uneaten feed and feces were siphoned out of all tanks prior to each morning feeding.

The biological performance of fish was evaluated in terms of weight gain (g) = (final weight, g – initial weight, g); percent weight gain (%) = [(final weight, g – initial weight, g) /initial weight, g] × 100; thermal growth coefficient (TGC) = [(final weight$^{1/3}$ - initial weight$^{1/3}$)/(Temperature, °C × time, days)] × 100 (as adapted from Iwana and Tautz (1981) by Cho (1990); feed conversion ratio (FCR) = feed consumed, g/weight gain, g; and survival = (final No. organisms × 100)/initial No. organisms).

Statistical analysis

Employing a significance level of $p \leq 0.05$, two-way analysis of variance (ANOVA) was applied to the digestibility and fish biological performance data: weight gain, percent weight gain, SGR, FCR, and survival rate. Survival rate data were arcsine-transformed, but untransformed data are presented. When significant differences were detected, mean separation procedure was performed by the Tukey's honestly significant difference method. Statistical analyses were performed using the Statistical Analysis System software (1999–2000, Software Release 8.1; SAS Institute Inc., Cary, NC, USA).

Results

Fish biological performance and digestibility assessment

The levels of dietary crude protein and crude fat tested, or their interaction, did not have a significant influence on final weight, weight gain, percent weight gain, TGC, FCR, or survival (Tab. 2).

Tab. 2: Growth performance indices, feed utilization, and survival[a] of *C. othonopterus* fed different levels of dietary crude protein and crude fat.

Treatment means Crude protein (%)/Crude fat (%)	IW (g)	FW (g)	WG (g)	PWG (%)	TGC	FCR	S (%)
40/8	108.5 ±20.8	181.2 ±31.9	72.6 ±17.6	68.4 ±17.6	0.057 ±0.011	1.9 ±0.6	100 ±0
40/12	104.9 ±5.6	177.5 ±14.0	72.6 ±11.1	69.2 ±9.7	0.058 ±0.007	1.8 ±0.3	100 ±0
40/16	107.4 ±16.4	191.2 ±21.9	83.8 ±22.6	80.8 ±31.3	0.065 ±0.018	1.8 ±0.5	100 ±0

Tab. 2: continued

Treatment means Crude protein (%)/Crude fat (%)	IW (g)	FW (g)	WG (g)	PWG (%)	TGC	FCR	S (%)
45/8	95.7 ±15.0	164.7 ±34.4	69.0 ±20.1	71.0 ±11.8	0.058 ±0.010	1.8 ±0.3	100 ±0
45/12	101.1 ±9.6	168.4 ±18.2	67.3 ±11.2	66.5 ±9.4	0.055 ±0.007	1.8 ±0.3	100 ±0
45/16	102.9 ±16.0	176.0 ±29.7	73.0 ±14.4	70.8 ±7.3	0.059 ±0.006	1.7 ±0.2	100 ±0
50/8	99.3 ±15.7	171.5 ±24.2	72.2 ±14.5	73.7 ±16.1	0.059 ±0.010	1.7 ±0.4	100 ±0
50/12	107.3 ±14.4	170.0 ±24.3	62.7 ±24.2	60.2 ±28.3	0.050 ±0.019	2.1 ±0.8	100 ±0
50/16	93.2 ±6.9	179.0 ±19.4	85.8 ±13.3	91.7 ±9.8	0.071 ±0.007	1.4 ±0.1	100 ±0
Main effects means Crude protein (%)	**IW (g)**	**FW (g)**	**WG (g)**	**PWG (%)**	**TGC**	**FCR**	**S (%)**
40	106.9 ±14.6	183.3 ±22.8	76.3 ±17.3	72.8 ±20.7	0.060 ±0.012	1.8 ±0.5	100 ±0
45	99.9 ±13.2	169.7 ±26.7	69.8 ±14.7	69.4 ±9.2	0.057 ±0.008	1.8 ±0.3	100 ±0
50	100.0 ±13.4	173.5 ±21.4	73.5 ±19.3	75.2 ±22.6	0.060 ±0.015	1.8 ±0.5	100 ±0
Crude fat (%)	**IW (g)**	**FW (g)**	**WG (g)**	**PWG (%)**	**TGC**	**FCR**	**S (%)**
8	101.2 ±17.0	172.5 ±29.1	71.3 ±16.3	71.0 ±14.4	0.058 ±0.010	1.8 ±0.4	100 ±0
12	104.4 ±10.1	172.0 ±18.3	67.5 ±16.0	65.3 ±17.2	0.055 ±0.012	1.9 ±0.5	100 ±0
16	101.2 ±14.2	182.1 ±23.3	80.9 ±17.0	81.1 ±20.0	0.064 ±0.012	1.6 ±0.4	100 ±0
ANOVA *Pr > F*	**IW (g)**	**FW (g)**	**WG (g)**	**PWG (%)**	**TGC**	**FCR**	**S (%)**
Crude protein	0.3098	0.3210	0.5807	0.6722	0.7485	0.9566	[b]
Crude fat	0.7701	0.4667	0.1030	0.0593	0.0632	0.2490	[b]
C. Protein x C. Fat	0.5932	0.9972	0.8313	0.4769	0.5250	0.4054	[b]

[a]Values are means± S.D. of five replicate samples.

[b]100% survival was recorded for all experimental units. Hence, there was no variability of data.

- Initial Weight = IW (G), Final Weight (G), Weight Gain = WG, Percent Weight Gain = PWG, Survival = S

ADC of DM of experimental feeds ranged from 74.1 to 80.2%, with the 45% protein diets having slightly higher numerical value (79.1%, main effects means) than the other protein levels (75.6 and 75.4% for 40 and 50% protein, respectively) but no apparent trend was observed. No significant effects of crude protein or crude fat, or their interaction, were detected on ADC of DM (Tab. 3).

Tab. 3: Apparent digestibility coefficients of dry matter (ADC of DM) of experimental feeds with different levels of crude protein and crude fat for *C. othonopterus*.

Treatment means Crude protein (%)/Crude fat (%)	ADC of DM (%)
40/8	75.2±3.5
40/12	77.0±2.1
40/16	74.6±2.0
45/8	77.5±3.0
45/12	80.2±2.9
45/16	79.7±2.7
50/8	75.6±4.9
50/12	76.7±1.8
50/16	74.1±2.9
Main effects means Crude protein (%)	**ADC of DM (%)**
40	75.6±3.3
45	79.1±2.9
50	75.4±3.4
Crude fat (%)	**ADC of DM (%)**
8	76.1±3.7
12	78.0±3.3
16	76.1±3.6
ANOVA *Pr > F*	**ADC of DM (%)**
Crude protein	0.0550
Crude fat	0.2338
C. Protein x C. Fat	0.7656

[a]Values are means±S.D of five replicate samples.

Proximate composition of fish muscle tissue

Moisture content of muscle tissue increased significantly with dietary crude protein, from 71.2±2.5% (at 40% crude protein), to 75.9±1.4% (at 50% crude protein), but it was not affected by dietary crude fat (main effects means, Tab. 4). The content of crude protein decreased significantly with both dietary crude protein and fat. Muscle tissue from fish fed the highest crude fat level (16%) had significantly greater crude fat content than those of fish receiving lower levels (8 or 12%), but the crude fat content in this tissue decreased significantly with dietary crude protein. The ash content also decreased significantly with both dietary crude protein and fat. No significant crude protein-crude fat interactions were observed on any of components of the proximate composition of fish muscle tissue (Tab. 4).

Discussion

Results of the present study indicate that a dietary crude protein level of 40% was sufficient to promote adequate growth of the Gulf corvina, from an overall individual mean wet body weight of 102.6±14.1 g to 170-191.2 g, a size range that provides insight into the magnitude of growth rate of this fish, aiming at,

Tab. 4: Determined values of the proximate composition[a] (% of wet weight) of muscle tissue of *C. othonopterus*.

Treatment means Crude protein (%)/Crude fat (%)	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)
40/8	70.2±2.7	21.1±1.7	3.2±1.2	1.6±0.2
40/12	71.3±3.1	20.7±2.6	2.6±0.8	1.5±0.2
40/16	72.1±2.1	18.3±0.8	4.6±1.8	1.4±0.1
45/8	73.4±0.6	18.7±1.2	3.4±0.9	1.3±0.1
45/12	74.4±0.1	18.1±0.7	2.5±0.4	1.3±0.1
45/16	74.7±0.3	17.5±0.9	3.8±1.0	1.3±0.1
50/8	76.5±2.2	17.8±0.4	2.2±0.3	1.3±0.0
50/12	76.1±2.2	17.6±1.0	2.6±1.1	1.3±0.1
50/16	75.0±0.9	18.0±1.1	3.5±1.3	1.3±0.1
Initial fish	76.9±0.6	17.8±1.0	2.3±0.4	1.5±0.1
External reference diet[b]	75.3±0.8	18.6±0.7	3.3±0.8	1.3±0.1
Main effects means Crude protein (%)	**Moisture (%)**	**Crude protein (%)**	**Crude fat (%)**	**Ash (%)**
40	71.2^{c}±2.5	20.0^{a}±2.2	3.5^{a}±1.5	1.5^{a}±0.2
45	74.1^{b}±1.0	18.1^{b}±1.1	3.2^{ab}±1.0	1.3^{b}±0.1
50	75.9^{a}±1.4	17.8^{b}±0.8	2.8^{b}±1.1	1.3^{b}±0.1
Crude fat (%)	**Moisture (%)**	**Crude protein (%)**	**Crude fat (%)**	**Ash (%)**
8	73.4±3.0	19.2^{a}±1.8	2.9^{b}±1.0	1.4^{a} ±0.2
12	73.9 ±2.8	18.8^{a} ±2.1	2.5^{b}±0.8	1.4^{ab} ±0.2
16	73.9 ±1.9	17.9^{b} ±0.9	4.0^{a} ±1.4	1.3^{b} ±0.1
ANOVA *Pr > F*	**Moisture (%)**	**Crude protein (%)**	**Crude fat (%)**	**Ash (%)**
Crude protein	< 0.0001	< 0.0001	0.0478	< 0.0001
Crude fat	0.3753	< 0.0019	< 0.0001	0.0119
C. Protein x C. Fat	0.1385	0.0564	0.2632	0.1808

[a]Values are means±standard deviation of triplicate samples.

Main effects means with different superscript are significantly different ($P \leq 0.05$).

[b]Treatment added as a reference, not included in the statistical analysis

potentially, grow-out operations. Taking into account that the Gulf corvina is a carnivorous species that feeds mainly on sardine, *Cetengraulis mysticetus* (Román-Rodríguez, 2000), this result agrees with the relatively high dietary crude protein levels needed for maximum growth of other sciaenids with similar feeding habits, such as the red drum *Sciaenops*

ocellatus requiring 44% dietary crude protein (Jirsa *et al*, 1997; Thoman *et al*., 1999), the meagre *Argyrosomus regius* with 50% crude protein (Chatzifotis *et al*., 2012) or 47% crude protein in combination with 20% crude fat (Martínez-Llorens *et al*., 2011), and the brown meagre *Sciaena umbra* with 42% at a dietary energy content of 17 kJ/g (Chatzifotis *et al*., 2006). However, it has been established that the dietary crude protein requirement is often influenced by other dietary factors, such as the crude fat and/or the overall energy content of the diet. McGoogan and Gatlin (1999) observed that growth of red drum juveniles (7.7 g) fed incremental levels of dietary crude protein (35, 40, and 45%), in combination with crude fat levels from 9.9 to 28.9%, increased with dietary protein level, but within a protein level, growth of fish was negatively affected by high dietary energy, with the best results obtained with 45% crude protein in combination with 15% crude fat. Following similar approaches, more studies have confirmed this phenomenon with other sciaenids, such as the large yellow croaker, *Pseudosciaena crocea* (Duan *et al*., 2001), the meagre *A. regius* (Chatzifotis *et al*., 2012), totoaba, *Totoaba macdonaldi* (Rueda-López *et al*, 2011), the mulloway *A. japonicus* (Pirozzi *et al*. 2010; Woolley *et al*., 2010), and other seawater and freshwater fish species such as cobia *Rachycentrum canadum* and tilapia, *Oreochromis niloticus* (Sweilum *et al*., 2005; Wang *et al*., 2005). For some species, these results have been explained in terms of reduced ingestion of diets that are too dense in protein and/or energy which, in turn, limits fish growth (McGoogan and Gatlin, 1999; Wang *et al*., 2005). However, some fish appear to tolerate a wide range of dietary crude protein and lipid levels without impairing their growth or survival, i.e., showing the so-called protein-sparing effect of dietary lipid. In some instances, excess energy is stored under the form of body fat. One example is the cod, which had comparable growth when fed dietary crude protein and fat levels ranging from 49 to 63% and 11-28%, respectively, but progressively stored more fat in liver and viscera (Grisdale-Helland *et al*., 2008). Similarly, in two experiments conducted with subadult red drum of 145 and 186 g of initial weight and after being fed dietary crude protein and fat levels ranging from 36 to 44%, and from 7.6 to 16.3%, respectively, fish did not show differences in somatic growth, but accumulated more intraperitoneal fat at increasing levels of dietary lipid (Turano *et al*., 2002). These results appear to contradict the negative effects of high dietary energy observed on growth of juvenile red drum (7.7 g) reported by McGoogan and Gatlin (1999). Nevertheless, they actually indicate that for organisms of the same species, life stage may influence their capacity to manage these nutrients. As a new prospective species for aquaculture, no information is available on the dietary requirements of Gulf

corvina. In the present study, this species also appeared to tolerate dietary manipulations, within the levels tested of crude protein (40-50%) and fat (8-16%), without compromising its growth, while storing significantly more crude fat in muscle tissue with increasing dietary lipid. However, it is possible that the levels of nutrients tested were too narrow to detect significant interactions between dietary crude protein and fat. Therefore, more studies should be conducted with this species, including wider ranges of crude protein and fat, in order to evaluate if the Gulf corvina could meet its dietary protein requirements at levels below 40%, and to further elucidate the extent of the observed protein-sparing effect of dietary lipid.

Taking into consideration that crude protein not only is widely recognized as an expensive and the major component of aquafeeds (Tacon and Metian, 2008; Tacon *et al*., 2011), but it also interacts with crude fat and/or the overall energy content of the diet to determine growth, much attention has been paid to the optimization of the dietary crude protein to fat ratio for seawater and freshwater fish inhabiting cold, temperate, or warm waters (Bowyer *et al*., 2013). Using a variety of approaches and strategies, researchers have conducted complete and incomplete factorial experiments, with either wide or narrow ranges of dietary crude protein and fat, expressing the protein-to-energy, or alternatively, the energy-to-protein ratio, in various units. In addition, dietary gross energy (GE) or digestible energy (DE) has been used to estimate such ratios. In this respect, the proportions of dietary crude protein and fat that were tolerated well by the Gulf corvina in the present study correspond to protein to energy (GE) ratios between 19.2 and 25.8 mg of protein (mg P)/kJ. These values are comparable to those reported as adequate, ranging from 20.7 to 28.6 mg P/kJ (values taken or calculated from dietary protein and energy content data reported by authors), for other members of the family Sciaenidae, such as *S. ocellatus*, *T. macdonaldi*, *A. japonicus*, *A. regius*, *Nibea japonica*, and *N. miichthioides* (McGoogan and Gatlin, 1999; Turano *et al*., 2002; Wang *et al*., 2006; Pirozzi *et al*. 2010; Wooley *et al*., 2010; Martínez-Llorens *et al*., 2011; Rueda-López *et al*, 2011; Chatzifotis *et al*., 2012; Chai *et al* 2013). Similarly, they are comparable to optimal protein to energy ratios of other seawater carnivorous fish, such as the Japanese seabass (*Lateolabrax japonicus*) (25.9 mg P/kJ), common dentex (*Dentex dentex*) (19.5 mg P/kJ for fingerlings of 10 g, and 23.7 mg P/kJ for juveniles of 92.4 g), winter flounder (*Pleuronectes americanus*) (26.6 mg P/kJ), cobia (*R. canadum*) (22.4-28.8 mg P/kJ), and the Mediterranean yellow tail (*Seriola dumerili*) (Hebb *et al*., 2003; Ai *et al*., 2004; Skalli *et al*., 2004; Wang *et al*., 2005; Tomás-Vidal, *et al*., 2008).

Other approaches also have been used to estimate requirements for dietary protein and

lipid/energy, using estimates expressed as g of dietary protein needed per unit weight of fish per unit time. For example, 1.5-2.5 g digestible protein (gDP)/kg of fish body weight (kgBW)/day for body maintenance, and 20-25 gDP/kgBW/d for maximum growth, have been estimated for the red drum (*S. ocellatus*) (McGoogan and Gatlin, 1998). It has been suggested that this type of approach provides estimates that are precise and more adequate than those of studies in which dietary needs for protein/lipid are expressed in terms of the dietary concentration of these nutrients (Watanabe *et al*., 2000; Tomás-Vidal, *et al*., 2008). Unfortunately, they involve measurements that have not been made in all studies, especially those using traditional methodologies, making research findings difficult to compare.

In the present study, dietary crude protein and lipid were capable of significantly influencing the proximate composition of fish muscle tissue. Increased crude fat deposition was observed in response to increasing levels of this nutrient, a phenomenon reported numerous times for a variety of fishes, such as drums, groupers, flounders, and salmonids, among others (Lee *et al*., 2000; Shiau and Lan, 1996; Hemre and Sandnes, 1999; Skalli *et al*., 2004; Woolley *et al*., 2010). Under these circumstances, fat usually increases at the expense of moisture, which for some fish, such as cobia (*R. canadum*), mullet (*Liza macrolepis*), and cuneate drum (*N. miichthioides*), decreases in whole body or tissues (Rangaswamy *et al*., 1998; Wang *et al*., 2005, 2006), but this pattern does not apply to other species, e.g., dusky cob (*A. japonicus*) and rabbitfish (*Siganus rivulatus*) (Ghanawi *et al*., 2011; Woolley *et al*., 2010). Decreased moisture content with dietary crude fat was neither observed in the present study for the Gulf corvina, perhaps because the dietary crude fat range (8-16%) was not wide enough to detect differences. On the other hand, dietary lipid elicited a significant decrease of crude protein content of muscle tissue of the Gulf corvina, which also has been observed in whole body or tissues of *D. dentex* (Skalli *et al*., 2004), *R. canadum* (Wang *et al*, 2005), and *P. olivaceus* (Lee *et al*., 2000), among others. However, for some species (*Epinephelus malabaricus, A. regius*), tissue crude protein content has not been altered by dietary lipid (Lin and Shiau, 2003; Chatzifotis *et al*., 2010). Ash content of muscle tissue also decreased significantly with dietary crude fat in the present study, agreeing with results observed for *E. malabaricus* (Lin and Shiau, 2003), but no clear trend of the body ash content in response to dietary lipid has been observed in *P. olivaceus* (Lee *et al*., 2000), *R. canadum* (Wang *et al*., 2005), and *A. regius* (Chatzifotis *et al*., 2010).

The contents of crude protein, fat, and ash of muscle tissue in the Gulf corvina decreased progressively as dietary protein increased,

which seemed to be governed by the concomitant increase of the moisture content. Similarly, Pirozzi *et al*. (2010) showed a tendency of increased moisture and decreased body lipid content of *A. japonicus* fed diets with low energy content (16 MJ/kg) and increasing protein levels, ranging from 25.5 to 55.5%. However, other reports on the body proximate composition of fish in response to dietary protein are highly variable among different fish species, body sizes, and range of dietary protein used. For example, higher body protein content has been observed, coupled with varying contents of crude fat, moisture, and ash, at increasing dietary protein levels for *E. malabaricus, P. olivaceous*, and *L. japonicus*, (Shiau and Lan, 1996; Lee *et al*., 2000; Ai *et al*., 2004). Nonetheless, for a number of species, including *N. miichthioides*, *T. macdonaldi, S. dumerili, D. dentex, A. japonicus*, and *S. ocellatus*, the contents of muscle crude protein, lipid, moisture, or ash have not been influenced significantly by dietary protein (McGoogan and Gatlin, 1999; Wang *et al*., 2006; Skalli *et al*., 2004; Tomás-Vidal *et al*., 2008; Woolley *et al*., 2010; Rueda-López *et al*., 2011).

The proximate composition of the experimental feeds corroborated that feed preparation was adequate. Determined values of dietary crude protein and fat were always close to expected values, progressively increasing with greater dietary inclusion levels and reflecting such changes on the protein-to-energy ratio. With regard to the studies for the assessment of *in vivo* digestibility, it is worthwhile pointing out that the process of feces collection over the first 25 days of the experiment did not appear to alter feed consumption by fish. Because fish were kept in the experimental tanks and were allowed to acclimate to these conditions for 15 days prior to initiation of the experiment, they always displayed normal feeding behaviour, actively eating the experimental feeds and even attempting to bite the glass tubes used during feces collection. The same feeding behavior was observed after the feces collection period and until the end of the feeding trial. A large variability of ADC of DM, generally obtained with the indirect method using chromic oxide as a marker, has been reported in studies of digestibility of feeds and feed ingredients for a variety of fish species, including salmon, Siberian sturgeon, and gilthead seabream (Shearer *et al*., 1992; Lupatsch and Kissil, 1997; Cook *et al* 2000; Zhou *et al*., 2007; Liu *et al*., 2009). In the present study, in which acid-insoluble ash was used as an internal marker for the assessment of digestibility, high values of apparent digestibility coefficients of dry matter were observed, ranging from 74.1 to 80.2%, which compare well with typically high values observed in nutritional studies of salmon, with reported values ranging from 70.62 to 87.0% (Shearer *et al*., 1992; Cook *et al* 2000).

However, the values of ADC of DM reported herein are higher than values found for *T. macdonaldi*, ranging from to 38.1 to 43.7% (Rueda-López *et al*., 2011), which were obtained using the same methodology as the employed in the present study.

With respect to the water physico-chemical parameters, they were maintained within levels considered adequate for related sciaenids, such as the red drum and totoaba (Davis, 1990; Wurts and Stickney, 1993; Tomasso and Kempton, 2000; Minjarez-Osorio *et al*., 2012). Further studies are warranted to determine optimal physico-chemical conditions for rearing Gulf corvina. In this respect, even though the safe levels of the concentrations of nitrogenous wastes in culture water of the Gulf corvina have not yet been determined, the concentration of total ammonia nitrogen (TAN) observed in the present study (0.17±0.11 mg/L) was below the 24 and 48-h median lethal concentrations reported for red drum fingerlings (221.1±41.3 mg/L and 199.9±43.0 mg/L, respectively) (Wise *et al*., 1989). Similarly, the concentration of nitrite (0.02±0.01 mg/L) was negligible, below 48-h median lethal concentrations also reported for red drum fingerlings, 2.8 mg/L at salinity of 0.6‰, and 85.7 mg/L at salinity of 36.0‰ (Wise and Tomasso, 1989). The salinity of culture water in this study (37.3±0.5‰) was slightly above the standard value for seawater (35‰) (Gros *et al*., 2008). The value herein reported represents the normal seasonal variation of this parameter in the area, since the Laboratory's seawater intake is located directly on the shore of Kino Bay, which is part of the natural habitat of this species. Furthermore, the Gulf corvina has recently been shown to be a euryhaline species (Perez-Velazquez *et al*., 2014). Therefore, it is unlikely for salinity to have adversely affected growth and/or feed utilization in the present study.

In summary, a dietary crude protein level of 40% was sufficient to promote adequate growth of the Gulf corvina. This species tolerated the manipulation of dietary levels of crude protein (40-50%) and fat (8-16%), without compromising its growth, or survival, while storing significantly more crude fat in muscle tissue with increasing dietary lipid. In addition, increased protein deposition in muscle tissue was observed in response to increasing dietary crude protein. Further research is warranted to evaluate if the dietary protein requirements of the Gulf corvina are below 40%, and to further elucidate the extent of the protein-sparing effect of dietary crude fat.

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