Lipid Nutrition Research for the Development of Practical Commercial Diets for Corvina and Alternative Ingredients for Aquafeeds

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Abstract

In the state of Sonora, Mexico, several local sciaenids are promising options to diversify the local aquaculture and venture into the marine fish culture industry; however, cost-effective aquafeeds for the development of this farming business are required. Aquafeeds for carnivorous fish, like sciaenids, typically contain high levels of fish meal and fish oil, but the high prices of these dietary ingredients, well above $2000.00 US dollars per ton (aqua grades) in January of 2015, makes the use of high inclusion levels unprofitable and unacceptable. Research work with sciaenids such as the Gulf corvina at the Nutrition Laboratory of the Department of Scientific and Technological Research of the University of Sonora has focused on establishing the minimum dietary lipid requirements as well as the replacement of fish oil, as a starting point. In addition, alternative oil and protein sources, such as ray fish liver oil and tilapia by-products silage, are being explored and evaluated using a sustainable approach. Ray fish constitute an important fishery in the Mexican shoreline, but only their meat is consumed, the rest is discarded. Their liver however, is a rich source of lipids and essential fatty acids. Recent studies showed high lipid levels (30.67-46.41%) in 5 ray species distributed in Sinaloa, Mexico, with a high proportion of unsaturated fatty acids (58-69%), and considerably high levels of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (EPA+DHA= 4.79-14.47 g/100 g of liver oil), thus, ray fish liver oil can be considered an interesting alternative to fish oil as a source of EPA and DHA. Furthermore, aquaculture and fisheries are activities that generate tonnes of by-products. For tilapia, about 30% of the weight is recovered as fillet; the remaining by-products can be processed for the production of

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silage through fermentation in the presence of *Lactobacillus* spp. and molasses as a carbohydrate source, which, in turn, can be incorporated as an ingredient in diets for other cultured aquatic organisms, a viable and environmentally friendly usage of discarded remnants of tilapia to generate a value added fish by-product for commercialization. These are some of the first steps leading towards the diversification of the aquaculture industry in Sonora.

Keywords: Marine fish culture, Sciaenids, lipid dietary requirements, fish oil replacement, by-products.

I-Introduction

The culture of marine fish species on a large scale has been a very successful economic activity in Europe for several decades now (FAO, 2014) and include a number of species such as salmon (*Salmo salar*), gilthead sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*) and turbot (*Psetta máxima*), among others. Currently, the prices per kilogram in Europe for each of these species are $25.31 €/Kg (salmon fillet), $14.03 €/Kg, $15.45 €/Kg, and $45.20 €/Kg, respectively (http://thecornishfishmonger.co.uk; August 10, 2015). According to FAO (2014), finfish mariculture reached a world production volume of 5,551,905 tonnes in 2012, which represent only 12.6 percent of the total farmed finfish production by volume, but their value (US$23.5 billion dollars) represents 26.9 percent of the total value of all farmed finfish species.

In Mexico the culture of marine fish is emerging and it has great potential for the diversification of the species currently cultured on a commercial scale, coupled with the fact that it is a profitable choice for aquaculture producers, who, particularly in the state of Sonora, are mainly focused on shrimp culture. The shrimp culture industry has suffered a major drawback due to the manifestation of the white spot syndrome virus (WSSV) and the early mortality syndrome (EMS), and from 81,422.8 tonnes produced in 2009, only 32,616.0 tonnes were produced in 2014 (COSAES, 2014). Therefore, the mariculture of marine fish species presents itself as a promising and lucrative alternative to diversify this economic activity.

Among the marine finfish available within the Gulf of California with aquaculture potential are some species from the family Scianidae, commonly called drums or croakers because of the repetitive drumming sound they produce with the air bladder (Nelson, 1994). There are at least 30 known species belonging to the family in this area (Van der Heiden, 1985), and 3 of them are endemic to this region, the bigeye croaker *Micropogonias megalops*, totoaba *Totoaba macdonaldi*, and the Gulf corvina *Cynoscion othonopterus* (Thompson y McKibbin, 1978).

The Gulf corvina is a heavily overfished sciaenid, the second most important fishery in the Northern Gulf of California near the Colorado River delta, where it is captured during spawning aggregations, thus, it is listed as a vulnerable species but its capture is overseen by the norm NOM-063-PESC-2005, which establishes a yearly closed fishing season from May 1st to August 31st, and 65 cm as the legal minimum capture length during the open season (Castro-González, 2011). A catch quota of 3,620 tonnes was established for the 2013-2014 season by SAGARPA-INAPESCA (2013), less than the 3,727 tonnes registered capture for 2010 (Carta Nacional Pesquera, 2012).

Corvinas are appreciated in the regional and international markets for their firm mild-flavor white meat. Although the price in the local market is low, close to MXN$16.00 pesos per Kg during the first trimester of 2014 (http://datamares.ucsd.edu/eng/projects/fisheries/kilos-and-pesos-interactions-between-fisheries-catch-and-market-value/; August 11th, 2015), the international market for other corvinas is much better, average price per kilo for fillet is US$10-12 (http://eatwineblog.com/2006/03/24/debunking-the-chilean-seabass-myth/; August 11th, 2015).

Unlike other marine finfish, the Gulf corvina reaches sexual maturity at a relatively early size and age, at approximately 2 years old or less. This is a fortunate advantage because individuals can become the broodstock in a short period of time at a lesser cost, and they may be kept within smaller tanks or less expensive infrastructure, also implying a lesser cost for the mass production of fry. There is regional awareness and interest in pursuing the culture of corvina in Sonora, hence, research work with the Gulf corvina at the Nutrition Laboratory of the Department of Scientific and Technological Research of the University of Sonora (DICTUS) has focused on establishing the minimum dietary lipid requirements as well as the replacement of fish oil, as a starting point (Minjarez-Osorio *et al.* 2014; González-Félix *et al.* 2015; Maldonado-Othón, 2015). This research is essential to limit the utilization of fish oil in aquafeeds developed for these species.

Up until now, fish oil is the most important source of the essential fatty acids for aquafeeds, particularly eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acid. However, the sustained increment in fish oil demand over the last years and the projected increment in the near future, together with the limited availability and high cost, have driven the search for alternative sources of these essential fatty acids (González-Félix *et al.* 2010). An alternative oil source explored is ray fish liver oil. Ray fish constitute an important fishery in the Mexican shoreline, but only their meat is consumed, the rest is discarded. Their liver however, is a rich source of lipids and essential fatty acids, such as EPA and DHA, thus, ray fish liver oil can be considered an important and interesting alternative to fish oil (Ould el Kebir *et al.* 2017; Navarro-García *et al.* 2004a,b, 2014).

On the other hand, alternative protein sources for aquafeeds also have one of the most important research topics for some time now. An interesting alternative for fish meal replacement is the utilization of by-products from the fish-processing industry (e.g. heads, tails, guts, fins, bones and/or skin). It is estimated that about 25% of the total fisheries and aquaculture production is discarded. Only a small share of by-products is used for human consumption, the rest is used for production of fish meal, silage, and other goods (Venugopal, 1995). Taking into consideration that the amino acid profile and digestibility of fish proteins are exceptional, it would be valuable to intensify the use of the protein fractions present in fish by-products, for example, for the production of silages, peptide and amino acid concentrates, surimi, collagen, gelatin, etc. (Rustad, 2007). The preparation of silages is one of the most popular uses for animal by-products (Gildberg, 2002); their manufacture from aquacultural by-products, as well as their use in aquafeeds for several cultured species is under study. This work presents current research in aquaculture nutrition developed at the University of Sonora for the Gulf curvina, as well as research on alternative ingredients produced from by-products for the replacement of fish oil and fish meal in aquafeeds.

II-Dietary lipid requirements of the Gulf corvine

The Gulf corvina, *Cynoscion othonopterus* (Figure 1) supports one of the most important seasonal commercial fisheries in northwest Mexico, and it is currently a potential aquaculture candidate in the state of Sonora. Early studies (Perez-Velazquez *et al.*, 2015) demonstrated that organisms near 100 g of individual weight and up to market size, could grow well on diets with 40% crude protein (CP) and high lipid levels, close to 16%, although the optimal dietary lipid level had not been accurately determined. Gonzalez-Félix *et al.*

(2015) carried out a 56-day study at the Wet Nutrition Laboratory of Kino Bay Experiment Station (KBES, DICTUS) located in Kino Bay, Sonora, Mexico, to evaluate the dietary lipid requirement of juvenile Gulf corvina raised in a recirculation aquaculture system.

Diets with an iso-proteic dietary content of 40% evaluated the effects of incremental levels of dietary crude fat at levels of 2, 5, 8, 11, 14, 17, 20, 23 and 26%, on the performance of *C. othonopterus* juveniles, with an initial mean body weight of 32.86 ± 0.48 g. Fish were reared in a clear-water recirculating culture system (Figure 2), composed of 48 circular tanks of 250 L (0.4 m^2 bottom area) filled with 200 L filtered seawater, at a density of 3 fish $tank^{-1}$. Each treatment was assigned to five replicate tanks. Fish were fed approximately 3% of their wet body weight daily and the daily ration divided into three equal portions. Overfeeding was minimized while maintaining the feeding rate close to apparent satiation.

Figure 1. Gulf curvina, *Cynoscion othonopterus*.

A clear dose-response effect of dietary crude fat was observed on growth of the Gulf corvina, with the best results corresponding to fish fed 11% crude fat, while growth performance was reduced as dietary crude fat departed from this level. These results were significant for specific growth rate and thermal growth coefficient data (P = 0.0283 and 0.0450, respectively), and although not statistically significant, the same numerical pattern was observed for additional growth response variables measured (Table 1).

Figure 2. Indoor clear-water recirculating culture system at the Wet Nutrition Laboratory of Kino Bay Experiment Station, DICTUS.

A quadratic broken line analysis of the thermal growth coefficient data estimated a requirement for dietary crude fat of 11.4% for this species when fed a diet containing 40% crude protein, with a 95% confidence interval of 9.8 to 13.0%. Significantly increased lipid deposition and reduced moisture content for muscle and whole body were observed in response to incremental levels of dietary crude fat. This study provided the first documented data on lipid nutrition of the Gulf corvina.

Table 1. Growth performance of *C. othonopterus* fed graded levels of dietary lipid. After González-Félix *et al.* 2015.

Dietary Lipid (%)	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (%/d)	TGC
2	31.3 ± 1.1	42.7 ± 3.9	11.7 ± 2.6	0.6[ab] ± 0.1	0.025[ab] ± 0.004
5	32.3 ± 1.1	49.0 ± 2.8	16.7 ± 2.5	0.7[a] ± 0.1	0.034[a] ± 0.004
8	33.9 ± 2.0	51.1 ± 2.7	17.2 ± 1.5	0.7[a] ± 0.1	0.034[a] ± 0.003
11	33.7 ± 1.6	55.4 ± 6.8	21.8 ± 6.2	0.9[a] ± 0.2	0.041[a] ± 0.009
14	33.3 ± 1.6	49.2 ± 0.5	15.9 ± 0.8	0.7[a] ± 0.1	0.029[ab] ± 0.003
17	30.7 ± 1.0	48.7 ± 0.7	18.0 ± 1.5	0.8[a] ± 0.1	0.038[a] ± 0.003
20	32.7 ± 1.1	45.8 ± 3.0	13.1 ± 2.5	0.6[ab] ± 0.1	0.027[ab] ± 0.005
23	32.8 ± 2.0	45.1 ± 4.8	12.2 ± 3.1	0.5[ab] ± 0.1	0.025[ab] ± 0.005
26	34.4 ± 2.0	42.4 ± 3.9	8.0 ± 2.4	0.4[b] ± 0.1	0.016[b] ± 0.004
External[1]	31.9 ± 1.7	48.4 ± 2.5	16.5 ± 2.2	0.7 ± 0.1	0.034 ± 0.004
ANOVA $Pr > F$	0.7563	0.3270	0.1040	0.0283	0.0450

Values are means ± standard error of five replicate samples.

Means with different superscripts are significantly different ($P \leq 0.05$).

[1]Not included in the statistical analysis.

Abbreviations: SGR = specific growth rate; TGC = thermal growth coefficient.

An additional 56-day study was conducted to evaluate the effects of three levels of dietary CP (40, 45, and 50%) and three levels of crude fat (CF: 8, 12, and 16%) on the growth performance and body compositions of *C.* juveniles (initial mean body weight of 102.6 ± 14.1 g) using a 3 x 3 factorial experimental design (Perez-Velázquez *et al.*, 2015). The levels of dietary CP and CF tested, or their interaction, did not influence significantly the various growth responses evaluated (*e.g.*, final weight, weight gain, thermal growth coefficient-TGC, or survival; Table 2). However, in muscle tissue, increased crude fat deposition was observed in response to increasing levels of this nutrient, as described in the previous study. However, the content of CP and ash decreased significantly with both dietary CP and CF. In turn, moisture content increased significantly with dietary crude protein, from 71.2% ± 2.5 (at 40%

CP), to 75.9% ± 1.4 (at 50% CP), but it was not affected by dietary CF. However, no significant of the CP × CF interactions were observed on any of components of the proximate composition of fish muscle tissue.

It was suggested that, apparently, 40% dietary CP was sufficient to promote adequate growth of the Gulf corvina. This species tolerated the manipulation of dietary levels of CP (40-50%) and CF (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 (Perez-Velázquez *et al.*, 2015). Additional research is necessary to evaluate if the dietary protein requirements of the Gulf corvina might be below 40%, and to further elucidate the extent of the protein-sparing effect of dietary CF.

III-Alternative ingredients for aquafeeds

The Gulf of California is an important ray fish fishing area in Mexico; particularly the state of Sinaloa has a high diversity of elasmobranchs (CONAPESCA, 2010). The ray fish species incidentally captured in shrimp trawlers include the families Urotrygonidae, Myliobatidae, Narcinidae, Rhinobatidae, and Rhinopteridae. Most of the ray fish species are underutilized; only the meat is used and its price depends on its color (Navarro *et al.,* 2004a, b). No information is available on the liver quality and oil yield of the captured species that would encourage the utilization and commercialization of this resource in this area. However, reports (Ould El Kebir *et al.,* 2007) confirm that ray fish liver oil of three species from the Republic of Mauritania have concentrations of EPA and DHA ranging from 1.88-5.01% and 10.00-13.04%, respectively. In Mexico Navarro *et al.* (2004a,b) reported that liver of some rays from the Gulf of California represents between 5 and 11% of the animals' wet weight, and the lipid content of the liver corresponds to 50% of that weight. The liver oil had 16-18% of EPA+DHA.

Table 2. Growth performance and survival of *C. othonopterus* fed different levels of dietary crude protein and crude fat. After Perez-Velazquez *et al.* 2015.

Main effects means	Final weight (g)	Weight gain (g)	TGC	Survival (%)
Crude protein (CP, %)				
40	183.3 ± 22.8	76.3 ± 17.3	0.060 ± 0.012	100 ± 0
45	169.7 ± 26.7	69.8 ± 14.7	0.057 ± 0.008	100 ± 0
50	173.5 ± 21.4	73.5 ± 19.3	0.060 ± 0.015	100 ± 0
Crude fat (CF, %)				
8	172.5 ± 29.1	71.3 ± 16.3	0.058 ± 0.010	100 ± 0
12	172.0 ± 18.3	67.5 ± 16.0	0.055 ± 0.012	100 ± 0
16	182.1 ± 23.3	80.9 ± 17.0	0.064 ± 0.012	100 ± 0
ANOVA *Pr > F*				
Crude protein	0.3210	0.5807	0.7485	[b]
Crude fat	0.4667	0.1030	0.0632	[b]
CP × CF	0.9972	0.8313	0.5250	[b]

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

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

Three more species from the state of Campeche, in the Gulf of Mexico, *Rhinoptera bonasus*, *Aetobatus narinari* and *Dasyatis americana*, showed an oil yield of 43.0, 41.2 and 38.2% of the liver wet weight, respectively. The highest sum of eicosapentaenoic, docosahexaenoic and docosapentaenoic n-3 LC- PUFA were found in *R. bonasus* (22.4%) and *D.* (21.6%) (Navarro-García *et al.*, 2004a, b). Thus, a study to analyze the lipid content and fatty acid composition of the liver oil from *Urotrygon chilensis*, *Urobatis halleri*, *Rhinobatos glaucostigma*, *R. steindachneri* and *D. dipterura* captured in Sinaloa, México, was carried out.

Samples of *U. chilensis* (20), *U. halleri* (20), *R. glaucostigma* (5), *R. steindachneri* (3) and *D. dipterura* (7) were obtained from experimental shrimp trawl surveys conducted in Teacapan, Sinaloa. The livers were dissected, placed in polyethylene bags and frozen at -20°C for their transportation in coolers to the DICTUS, where they were stored at -80°C until lipid extraction (Navarro-García *et al.*, 2014). Total lipid content in their liver was relatively high, ranging between 30.67-46.41%, the highest amount observed in *D. dipterura* (Table 3). All of these species showed important concentrations of poly and highly unsaturated fatty acids, particularly EPA and DHA, around 4.79-14.47 g/100 g of liver oil of EPA+DHA (Table 4). EPA and DHA have a significant nutritional value in aquafeeds, being essential for many marine finfish and crustaceans cultured around the world. Therefore, ray fish liver oil can be considered an important and interesting alternative to fish oil as a source of EPA and DHA.

Table 3. Total lipid in liver of various ray fish. After Navarro-García *et al.* 2014.

Species	Lipid (%)
U. chilensis	$36.18^{ab} \pm 10.18$
U. halleri	$36.27^{ab} \pm 7.51$
R. glaucostigma	$31.83^{b} \pm 10.27$
R. steindachneri	$30.67^{b} \pm 4.84$
D. dipterura	$46.41^{a} \pm 4.98$

Tabla 4. Fatty acid composition of liver oil from *U. chilensis, U. halleri, R. glaucostigma, R. steindachneri* and *D. dipterura* (g/100 g of liver oil). After Navarro-García *et al.* 2014.

	U. chilensis		***U. halleri***		***R. glaucostigma***		***R. steindachneri***		***D. dipterura***	
Fatty acid	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
16:0	15.28	4.17	16.70	4.94	15.20	1.76	4.74	1.11	23.44	10.85
18:0	10.71	2.93	7.21	1.95	6.48	0.66	3.10	0.87	8.41	3.24
18:1	6.24	1.78	12.37	3.38	8.15	1.30	2.86	1.13	17.61	6.96
18:2n-6	1.73	0.40	1.79	0.63	1.44	0.31	0.61	0.27	2.12	1.04
18:3n-3	0.39	0.11	0.39	0.32	0.32	0.09	0.09	0.06	0.31	0.18
20:1	2.27	0.53	2.05	0.58	0.96	0.17	1.02	0.24	2.50	1.02
20:4n-6	3.24	0.85	3.10	0.87	2.86	1.21	1.30	0.24	2.88	0.95
20:5n-3	5.41	1.54	5.09	1.75	3.28	1.20	0.99	0.18	4.33	1.42
22:5n-3	2.27	0.41	2.21	0.62	1.21	0.23	1.10	0.41	1.85	0.55
22:6n-3	8.55	1.66	8.37	3.22	11.19	1.82	5.70	2.84	4.73	1.44
24:1	3.02	0.58	2.29	0.59	0.73	0.09	1.52	0.40	2.44	0.73
24:2	2.02	0.38	1.69	0.66	1.43	0.32	2.29	0.41	1.07	0.35

An additional activity generating tonnes of by-products from aquaculture and fisheries results from gutting, heading, and filleting fish. The term by-products is used to indicate something that can be utilized, and is usually referred to all the raw material, edible or inedible, left during the production of the main product. When producing fish fillets, the left products or fractions are fillet cuts, backbone, head, liver, gonads and guts, they are all by-products (Gildberg, 2002). For tilapia, *Oreochromis niloticus*, about 30% of the weight is recovered as fillet; the composition of the remaining by-product fractions after filleting a sample of thirty organisms of an average size of 643.3 ± 4.8 g and 29.6 ± 3.5 cm is shown in Table 5. Proximate composition of these by-product fractions was analyzed. Skin (43.53 ± 6.28%) and backbone (42.56 ± 2.85%) showed the largest protein content, probably because of the leftover muscle adhered to these tissues. In contrast, the gut showed the lowest protein content (16.1 ± 2.01%) (Table 6).

Table 5. Composition (%) of the by-product fractions obtained after filleting thirty tilapias (*O. niloticus*).

By-product	Percentage (%)
Head-tail	31.0 ± 2.1
Backbone	21.3 ± 1.7
Skin	9.5 ± 1.6
Gut	8.5 ± 1.9

Tabla 6. Proximate composition of by-product fractions of tilapia (*O. niloticus*).

By-product fraction	*Moisture (%)*	*Ash* (%)*	*Crude* Protein (%)*	*Crude Fat* (%)*
Fillet	75 ± 1.0	0.35 ± 0.07	71.73 ± 8.40	12.73 ± 2.20
Gut	57.3 ± 2.3	0.21 ± 0.03	16.1 ± 2.01	68.26 ± 4.22
Backbone	61.6 ± 1.2	1.36 ± 0.25	42.56 ± 2.85	39.46 ± 9.05
Skin	63.3 ± 1.5	0.15 ± 0.05	43.53 ± 6.28	51.76 ± 6.81
Head-tail	60.0 ± 1.7	1.43 ± 0.31	33.73 ± 1.79	42.4 ± 2.36

Values are means ± standard deviation of three replicate samples.*Dry matter basis.

Additionally, the fatty acid profile of these by-product fractions was analyzed. Palmitic acid (16:0) was the most abundant saturated fatty acid in all fractions, oleic acid (18:1n-9) was the most abundant monounsaturated fatty acid, and linoleic acid (18:2 n-6) the most abundant among the polyunsaturated fatty acids. The gut fraction demonstrated to be a good source of fat, with an n-6/n-3 ratio equal to 4.67. A light brown silage with a doughy consistency of pH below 4.5 was obtained from a homogenate of all by-product fractions through fermentation in the presence of *Lactobacillus* spp. and molasses as a carbohydrate source. It contained approximately 41% crude protein and 10% crude fat. This silage can be incorporated as an ingredient in diets for other cultured aquatic organisms, such as catfish (*Ictalurus punctatus*) for instance, where an inclusion level of 5% proved to be best for promoting optimal growth, coinciding with other reports for the inclusion of silages or hydrolysates in balanced feeds (Forster *et al.*, 2011; González-Félix *et al.*, 2014). Incorporating these processed by-products in balanced feeds as a source of very digestible small peptides and amino acids may have beneficial effect on growth of cultured organisms, but their inclusion level requires evaluation. All the same, the manufacture of silage from by-products has demonstrated to be a viable and environmentally friendly usage of discarded remnants of tilapia to generate a value added fish by-product for further commercialization.

Conclusions

The opportunities to diversify the aquaculture industry in the state of Sonora with the development of marine fish mariculture are promising. Sciaenid species such as the Gulf corvina are interesting options, since corvinas are already well accepted in the national and international market. Developing cost-effective aquafeeds, while searching for alternative sources of protein and oil for fish meal and fish oil replacement is a necessary task. By-products from fisheries and aquaculture, for example, ray fish liver oil, which can be considered an important and interesting alternative to fish oil as a source of the essential fatty acids EPA and DHA, or the use of the fractions obtained from the tilapia filleting, which can be processed into silages and incorporated into balanced feeds as a source of very digestible small peptides and amino acids, are sustainable approaches to do so, and may also generate a value added fish by-product for further commercialization.

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