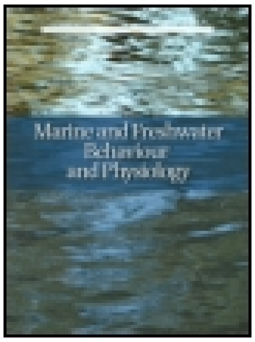

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Hematology, proximal composition and fatty acid profile comparison from wild and farm-raised juveniles of green guapote *Mayaheros beani* (Jordan, 1889)

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ABSTRACT
Sustainable aquaculture of the green guapote *Mayaheros beani* requires better understanding their physiology and composition. In that context, this research focused on the study of hematology, proximal and fatty acid (FA) composition of wild and farmed green guapote. Results showed no significant differences between wild and farmed fish in red blood cell count (1.77-2.57 $\times 10^6$ cel μL^{-1}), hemoglobin (11.6-15.1 g dL^{-1}), glucose (68-115 mg dL^{-1}) and the crude protein of whole body (51-58%). However, farmed fish showed significantly higher crude lipid (21.2 ± 0.33%) of whole body compared with wild ones (6.80 ± 0.42). Otherwise, wild fish had higher n-3 FA (14.18 ± 1.84%) than farmed fish (11.50 ± 1.97%). Because farmed fish showed a feed conversion ratio of 2.03 ± 0.8, results seem to indicate that *M. beani* require a specific balanced diet to improve its performance. Further research is required to understand the nutritional requirement of *M. beani*.

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Mayaheros beani; farmed; wild; hematology; proximal composition; fatty acids

Introduction

Aquaculture fish production has rapid growth, and aquaculture has been predicted to be the main source of fish by 2030, firstly, because of high demand from consumers (FAO 2016). However, the rise in farmed fish has led to an increasing demand for control and monitoring techniques to improve fish nutrition and health conditions.

The complete blood count profile is an important diagnostic tool with laboratory protocols and reference ranges well established in both human and veterinary medicine. Knowledge and research of hematological parameters may facilitate the development of fish health status indicators in response to changes related to nutrition, water quality and disease (Grant 2015; Powell et al. 2016; Fazio 2019). First of all, it is important to know the normal reference ranges of the hematologic parameters for assessing and monitoring fish health status.

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On the other hand, consumers expect farmed fish to be equivalent or superior to wild fish, but several studies have shown differences in fatty acid (FA) composition between the wild and farmed fish, such as the sea bass *Dicentrarchus labrax* L, sea bream *Sparus aurata* and common carp *Cyprinus carpio* (Periago et al. 2005; Mnari et al. 2007; Yeganeh et al. 2012). FA composition is strongly influenced by the environmental conditions, which determine nutrient availability and their requirement in fish farming (Yeganeh et al. 2012; Vasconi et al. 2014). Diet is an important factor that determines the composition of farmed fish, specifically the FA n–3/n–6 ratios, of which the polyunsaturated fatty acids (PUFA) linoleic acid (18:2 n–6) and a-linolenic acid (18:3 n–3, ALA) are essential dietary components (Tanamati et al. 2009). These PUFAs are modified into highly unsaturated fatty acids (HUFA) arachidonic acid (20:4 n–6, ARA), docosahexaenoic acid (22:6 n–3, DHA) and eicosapentaenoic acid (20:5 n–3, EPA) that play important biological functions (Tocher 2003; Powell et al. 2016). A comparison of lipid content and FA composition between wild and farmed fish can be a useful approach to study their requirements (Cejas et al. 2004).

The green guapote *Mayaheros beani* (AphiaID 1044168) is a native Mexican cichlid highly appreciated by aquarists, and its fishery is an important source of food for the regional population (Miller et al. 2005; García-Lizárraga et al. 2011; Aragón-Flores et al. 2014; Martinez-Cardenas et al. 2014; Rícan et al. 2016). Some efforts have been made to know its wild status (De León et al. 2008; Gómez-Balandra et al. 2012). Moreover, some studies have been performed to know their behavior, survival and growth at different temperatures (Martinez-Cardenas et al. 2014) and densities (Aragón-Flores et al. 2014). However, there is still a huge gap of knowledge about fundamental aspects of the physiology and nutritional requirements of the green guapote. No reports have been published yet about the differences between the farmed and wild green guapote and similarities and differences between these groups on hematology and fatty acid composition of this important native species. This study contributes to understanding the physiology, proximal and fatty acid composition of juveniles of green guapote in the wild and farmed conditions while our data may be useful to design future diets to improve the nutrition and health of reared green guapote.

Materials and methods

The specimens of wild green guapote used in this study were captured in the Iguanero Lagoon, Sinaloa, México (22° 59′ 22.70” LN, 105° 51′ 35.98” LW) in June 2016. Immediately after fish were caught with a net, blood samples were collected by heart puncture using insulin syringes containing 0.1 mL of ethylenediaminetetraacetic acid (EDTA) anticoagulant solution (5 mg mL^{-1} blood) following the modified method described by Valenzuela et al. (2002) and subsequently transported to the laboratory to be processed. The body weight (25.3 ± 8.4 g) and the length (11.0 ± 1.3 cm) of wild fish captured were measured.

Farmed juveniles of green guapote were bred (F1) at the Faculty of Marine Sciences, Universidad Autonóma de Sinaloa, Mexico. Ninety fish (average initial body weight of 11.6 ± 0.89 g and length of 8.2 ± 0.3 cm) were reared in three 80-L tanks supplied with filtered and recirculated freshwater. To maintain dissolved oxygen contents above 6 mg L^{-1}, water was constantly aerated with air stones. Rearing was subjected to natural photoperiod of approximately 12:12 h (light: dark) cycle. Fish were fed twice daily by hand until apparent satiation with a commercial feed for tilapia (Nutripec™, Purina, Mexico). Every day, all tanks were siphoned to remove uneaten feed and fecal matter. After 8 weeks of rearing, fish were deprived of diets for 24 h; then, a total of eight fish were randomly sampled per tank. After blood collection, body weight (± 0.01 g) and length (± 0.1 cm) of farmed fish were measured, and the survival rate (SR), specific growth rate (SGR) and feed conversion rate (FCR) were calculated using the following equations (Guillaume et al. 2004): SR = (final fish/initial fish) × 100; SGR (% day^{-1}) = [(ln final weight − ln initial weight)/day of the experiment] × 100; FCR = (feed intake/weight gained). The condition index was estimated by Fulton's condition factor (K) (Ricker, 1975), calculated as $K = (W/L^3) \times 100$, where W: wet weight (g) and L: standard length (cm).

Finally, wild and farmed fish were killed with an overdose of anesthesia 2-phenoxyethanol and ice bath (AAZV 2006) and stored at −20°C to analyze proximate and fatty acid composition. All blood and fish samples were analyzed as described further below.

Each blood sample was analyzed individually. Total red blood cell (RBC) counts were determined following the modified methods described by Blaxhall and Daisley (1973). Concentration of hemoglobin (HB) was performed using the methemoglobin method (Pointe Scientific kits, USA). Packed cell volume (PCV) values were determined by micro-capillary glass tube and centrifugation (10 000 rpm, 5 min). Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were calculated with PCV, HB and RBC. The remaining blood samples were centrifuged at 12,000 rpm at 4°C for 5 min to obtain plasma (Atencio-García et al. 2007). Total protein (TP), albumin (AL) and glucose (GLU) were measured by a standardized procedure using Pointe Scientific kits (USA). Globulin content (GB) and the albumin:globulin ratio (AL:GB) were determined from PT and AL values.

Proximate composition of moisture, crude protein (CP) and ash of commercial diet, muscle and whole body were performed according to the Association of Official Agricultural Chemists (AOAC) methods (2000). Crude lipid (CL) was determined gravimetrically by chloroform:methanol (2:1) extraction according to the modified method of Folch et al. (1957). The fatty acid methyl esters (FAMEs) were extracted from the whole body. Lipid extraction was carried out by using dichloromethane: methanol as a solvent in a 2:1 ratio (Cequier-Sánchez et al. 2008). After esterification, FAMEs were dried under a nitrogen gas atmosphere and stored at −20°C until analysis (Kates 1986). Posteriorly, FAMEs were analyzed using a gas chromatograph equipped with a mass spectrum detector and an autosampler (Agilent Technologies, Santa Clara, CA, USA). The FA was confirmed by comparing retention times against two standards: 37 component FAME Mix and PUFA-1 Marine source (Supelco Inc., Bellefonte, PA, USA) and expressed as percentages of the total fatty acids identified.

The results are shown as means ± SD. Normality of distributions and homogeneity of variances were tested using the Kolmogorov–Smirnov test and Levene's test, respectively. Data from wild *versus* farmed green guapote were statistically analyzed by Student's *t*-test. Where the assumption of normality was not satisfied even after data transformation, a Kruskal–Wallis nonparametric test was performed. The level of significant difference was set at $p < 0.05$. All statistical analyses were carried out using SPSS version 20.0 for Windows (SPSS, Inc.).

Results

At the end of the 8 weeks of rearing, the juveniles of green guapote fed with a commercial feed for tilapia (Table 1) obtained an SR of 99 ± 1.7%. The average final fish weight was 15.0 ± 2.8 g with a final length of 9.2 ± 0.6 cm. The fish showed an SGR of 0.48 ± 0.1% day^{-1} while the FCR was 2.03 ± 0.8; however, the K index of the farmed fish (1.93 ± 0.07) did not show significant differences with respect to those from the wild (1.86 ± 0.14).

On the other hand, the hematologic results showed no significant differences between farmed and wild green guapote in the RBC (1.77 to 2.57 × 10^6 cell μL^{-1}) and HB (11.6 to 15.1 g dL^{-1}) (Table 2). Nonetheless, farmed fish showed significantly higher values of PCV and MCV than those in the wild. In contrast, the values of MCHC and MCH were significantly higher in wild fish compared with those farmed.

The GLU concentration in the blood of wild and farmed fish showed no significant differences with the interval of values of 68 to 115 mg dL^{-1} (Table 3). Nevertheless, the contents of TP, AL, GB and the AL:GB ratio in the blood of wild fish were significantly lower than those in farmed fish (Table 3).

The CP from the whole body of fish ranged from 51.6% in farmed green guapote to 57.9% in wild fish without statistical differences (Table 4). However, moisture and ash contents in the whole body of wild fish were higher compared to farmed fish, contrary to CL that was higher in farmed fish. Muscle composition showed no significant differences in moisture and CP between wild and farmed fish, but CL was significantly higher in farmed fish while ash was higher in wild green guapote.

The analysis of fatty acid composition in green guapote indicated that saturated fatty acids (ΣSFA) were the more abundant in wild (41.76 ± 8.74%) compared to farmed fish (34.95 ± 8.39%) whereas monounsaturated (ΣMUFA) were significantly higher in farmed (39.52 ± 10.05%) compared to wild fish (30.34 ± 6.21%); however, ΣPUFA (27.90 ± 2.47 and 25.54 ± 3.67%, for wild and farmed fish, respectively) showed no significant differences between the origin of the samples (Table 5). With respect to the content of n–3 from wild green guapote, it was significant (14.18 ± 1.84%) compared to farmed fish (11.50 ± 1.97%) while n–6 showed no differences between wild and farmed fish.

The most abundant FA in the whole fish body was 16:0 both in the wild (23.09 ± 2.6%) and farmed fish (21.9 ± 1.0%) without significant differences between them, followed by

Table 1. Proximate composition of commercial diet (Nutripec™, Purina, Mexico) used to feed juveniles of green guapote *Mayaheros beani* under farming conditions.

Proximate composition (in % of dry matter)	
Moisture	4
Crude protein	46
Crude lipids	9
Ash	6
Nitrogen-free extract*	35

*Nitrogen-free extract was calculated like follows: 100% – (moisture + crude protein + crude lipids + ash).

Table 2. Red blood cell indices of juveniles of green guapote (*Mayaheros beani*) from wild (n = 24) and farmed (n = 24). The minimum and maximum values from each blood index are shown.

Parameters[a]	Wild	Farmed	Wild Min–Max	Farmed Min–Max
RBC (10^6 cel μL^{-1})	2.01 ± 0.49	2.26 ± 0.50	1.77–2.32	1.93–2.57
HB (g dL^{-1})	12.7 ± 1.9	13.2 ± 2.1	11.7–13.9	11.6–15.1
PCV (%)	19.0 ± 4.10^b	26.3 ± 5.2^a	16.1–20.9	23.1–27.8
MVC (fL)	98.2 ± 21.2^b	117 ± 23.8^a	78.0–149	50.4–167
MCHC (pg)	68.4 ± 12.0^a	48.6 ± 10.4^b	60.9–76.8	41.6–53.5
MCH (g dL^{-1})	66.3 ± 13.2^a	56.7 ± 12.3^b	56.3–71.6	50.5–60.7

The values are means ± SD. Different superscript letters in the same row indicate significant differences ($P < 0.05$).

[a]RBC, red blood cell count; PCV, packed cell volume; HB, hemoglobin; MCV, mean corpuscular volume (erythrocytes); MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 3. Plasma biochemical parameters of juveniles of green guapote (*Mayaheros beani*) from wild (n = 24) and farmed (n = 24). The minimum and maximum values from each blood index are shown.

Parameter[a]	Wild	Farmed	Wild Min–Max	Farmed Min–Max
GLU (mg dL^{-1})	103 ± 53	90.3 ± 30	73–115	68–105
TP (g dL^{-1})	2.28 ± 0.34^b	3.65 ± 0.72^a	2.03–2.36	3.14–4.09
AL (g dL^{-1})	0.64 ± 0.12^b	1.40 ± 0.43^a	0.54–0.71	1.22–1.44
GB (g dL^{-1})	1.64 ± 0.30^b	2.43 ± 0.65^a	1.44–1.65	2.05–2.88
AL:GB	0.40 ± 0.09^b	0.56 ± 0.16^a	0.32–0.47	0.45–0.66

The values are means ± SD. Different superscript letters in the same row indicate significant differences ($p < 0.05$).

[a]GLU, glucose; TP, total protein; AL, albumin; GB, globulin; AL:GB, albumin: globulin ratio.

Table 4. Proximal composition (%, dry matter) of whole fish and muscle of juveniles of green guapote (*Mayaheros beani*) from wild and farmed.

	Wild	Farmed
Whole fish		
Moisture	76.2 ± 3.11^a	67.4 ± 0.53^b
Dry matter	23.8 ± 3.11^b	32.6 ± 0.53^a
Crude protein	57.9 ± 7.5	51.6 ± 3.0
Crude lipids	6.80 ± 0.42^b	21.2 ± 0.33^a
Ash	22.6 ± 0.92^a	12.5 ± 0.56^b
Muscle		
Moisture	83.9 ± 1.19	83.5 ± 1.82
Dry matter	16.5 ± 1.82	16.1 ± 1.19
Crude protein	85.0 ± 0.74	86.0 ± 3.9
Crude lipids	3.24 ± 0.03^b	3.82 ± 0.30^a
Ash	6.28 ± 0.13^a	5.98 ± 0.04^b

The values are means ± SD. Different superscript letters in the same row indicate significant differences ($p < 0.05$).

18:1 n–9, whose content was significantly higher in farmed fish (25.89 ± 0.8%) compared to wild fish (16.62 ± 3.1%) (Table 5). Regarding the PUFA content, 18:2 n–6 was the most abundant, being significantly higher in farmed fish (11.98 ± 0.55%) compared to wild fish (8.25 ± 0.09%). Concerning HUFA, the EPA content did not show significant differences between farmed (1.35 ± 0.09%) and wild fish (1.12 ± 0.23%). The DHA content was

Table 5. Fatty acid (% of total fatty acids identified, in dry matter) of whole fish of juveniles of green guapote (*Mayaheros beani*) from wild and farmed.

Fatty acid	Wild	Farmed
14:0	3.44 ± 0.43^{b}	5.26 ± 0.26^{a}
14:1	1.42 ± 0.13	Nd
15:0	1.03 ± 0.23^{a}	0.41 ± 0.05^{b}
16:0	23.09 ± 2.6	21.9 ± 1.0
16:1 n–7	6.86 ± 0.33	9.14 ± 0.05
17:0	1.71 ± 0.18^{a}	0.44 ± 0.02^{b}
17:1 n–7	0.62 ± 0.07^{a}	0.41 ± 0.06^{b}
18:0	11.06 ± 3.3	6.93 ± 0.34
18:1 n–9	16.62 ± 3.1^{b}	25.89 ± 0.8^{a}
18:1 n–7	4.62 ± 0.43	3.34 ± 0.10
18:2 n–6	8.25 ± 0.09^{b}	11.98 ± 0.55^{a}
18:3 n–3	4.47 ± 1.14^{a}	0.51 ± 0.03^{b}
18:4 n–3	0.88 ± 0.01^{a}	0.67 ± 0.02^{b}
20:1 n–9	0.42 ± 0.03^{a}	0.20 ± 0.02^{b}
20:2 n–6	0.76 ± 0.08	0.58 ± 0.02
20:2 n–3	0.54 ± 0.05	0.65 ± 0.02
20:4 n–6 (ARA)	3.53 ± 0.49^{a}	1.30 ± 0.15^{b}
20:5 n–3 (EPA)	1.12 ± 0.23	1.35 ± 0.09
22:1 n–9	1.20 ± 0.13^{a}	0.54 ± 0.07^{b}
22:5 n–3	2.48 ± 0.46	2.78 ± 0.12
22:6 n–3 (DHA)	4.69 ± 0.13^{b}	5.54 ± 0.13^{a}
C24:1 n–9	1.18 ± 0.07^{a}	0.18 ± 0.03^{b}
∑SFA	41.76 ± 8.75^{a}	34.95 ± 8.39^{b}
∑MUFA	30.34 ± 6.2^{b}	39.52 ± 10.05^{a}
∑PUFA	27.90 ± 2.47	25.54 ± 3.68
∑n-3	14.18 ± 1.84^{a}	11.50 ± 1.97^{b}
∑n-6	12.54 ± 3.79	13.86 ± 6.38

The values are means ± SD. Different superscript letters in the same row indicate significant differences ($p < 0.05$). Nd: not detected.

significantly higher in farmed fish (5.55 ± 0.13%) compared to wild fish (4.69 ± 0.13%); however, farmed fish showed lower ARA content (1.30 ± 0.15%) compared to wild fish (3.53 ± 0.49%).

Discussion

This study was performed with the first generation of green guapote juveniles obtained in laboratory conditions from wild breeders. Due to the lack of important basic information on the physiological and nutritional characteristics of this species, this study showed the first data on some of the production variables, hematological and nutritional composition of farmed organisms compared to those in the wild.

Due to the lack of specific feed for green guapote, a commercial feed for tilapia was used with a content of 46% of CP and 9% lipids. After 8 weeks of feeding, results of K index showed non-significant differences between wild and farmed fish; however, the SGR of farmed green guapote in the present study was lower compared to report by Aragón-Flores et al. (2014), Aragón-Flores et al. (2017) using a commercial feed with 45% CP and 5% lipids. Due to our fish also showed a high FCR, such differences could be related to the fact that the commercial feed here used may not provide some specific nutrients for *Mayaheros beani* in juvenile stage. Therefore, these results indicate the need for improving rearing conditions and to develop a specific feed for green guapote.

On the other hand, the hematological analysis is especially important because it may provide a reliable evaluation of physiological conditions via non-lethal means (Fazio 2019). Changes in erythrocyte parameters are used for the diagnosis of the presence and type of anemia (Fazio et al. 2015). In the absence of known PCV intervals, values from 20% to 45% are considered acceptable for fish (Clauss et al. 2008), similar to the PCV values found in farmed *Mayaheros beani* in our research, which are similar to the cichlid *Cichlasoma dimerus* (Rey-Vázquez and Guerrero 2007). Otherwise, PCV and MCV were significantly lower in wild green guapote. Food is known to play an important role in the renewal of blood cells where RBC and PCV significantly decrease after prolonged periods of fasting (Lim and Klesius 2003) while fish with lower activity have a higher size of erythrocytes (consequently higher MCV values) and hemoglobin content (Hrubec and Smith 2010; Maciak et al. 2011; Grant 2015). In this research study, farmed green guapote was fed *ad libitum* with a high energy diet (42% CP and 12% CL) and confined in a reduced space, which may explain the higher PCV and MCV values that may have improved the oxygen requirements due to the high metabolic rate. These results agree with the higher concentrations of TP and AL from farmed green guapote compared to the wild organisms. Variations in AL concentrations are usually related to variations in nutritional and health conditions, and an adequate protein intake is essential for normal AL synthesis (Mazzaferro et al. 2002; Tan et al. 2018). Our results suggested that AL concentration on farmed green guapote may be related to the high protein/energy ratio of commercial fish feed, since one of the functions of AL is to transport free FA for energy use. These results also may be related to the fact that a high lipid content was observed in the whole body of farmed fish. Conversely, non-significant differences were observed in the protein content of muscle and whole body between wild and farmed green guapote, which agree with Vasconi et al. (2014), who considered that protein is a stable component of the fish body only dependent on its genetics and ontogenetic stage.

In relation to the above, farmed green guapote showed higher values of CL than wild fish in the whole body and muscle. CL and FA compositions in fish vary from species to species, and they are influenced by many intrinsic and extrinsic factors related to the biology of fish species, such as food habits, age, sex, season, temperature, among others (Rasoarahona et al. 2005; Kwetegyeka et al. 2008; Yeganeh et al. 2012; Vasconi et al. 2014; Baki et al. 2015). In this study, farmed fish were fed to satiation with commercial feed rich in proteins and lipids, which could have promoted the CL increase in the whole body of these fish. These results coincide with other studies were wild and cultured fish were compared (Mustafa and Dikel 2015; López-Huerta et al. 2018). A high accumulation of lipid content in farmed green guapote seems to indicate these fish favor proteins from diet to get energy and thus fat taken from the feed was stored in the body (Baki et al. 2015). Likewise, lipid content in muscle tissues was almost similar for wild and farmed green guapote, but a massive difference is observed for lipid content in the whole body between these, which in part could be related to farmed fish showed large amounts of visceral fat.

As in other freshwater fish, palmitic acid (16:0) and oleic acid (18:1 n−9) were the main FA in the whole body of green guapote specimens, both wild and farmed (Vasconi et al. 2014). Also, the ARA proportions in green guapote were like previous reports for freshwater fish (Prato and Biandolino 2012; Jaya-Ram et al. 2018; Sushchik et al. 2018). However, farmed green guapote showed greater content of linoleic acid (18:2 n−6) and less n−3 fatty acids compared to wild fish. Similar results have been reported in wild and cultured sea bass (Baki et al. 2015) and *Dormitator latifrons* (López-Huerta et al. 2018). Although the proportion of the different nutrients is unknown in the natural diet of wild green guapote, which is an omnivorous species with some tendency to carnivore (Martínez-Cárdenas et al. 2017), linoleic acid has been reported to be found in plant oils used for feeding farmed fish, which is accumulated and maintained unchanged in some fish (Smichi et al. 2017; Stoneham et al. 2018) except those species with the ability to bioconvert 18:2 n−6 to 18:3 n−6 via the delta-6 desaturase pathway (Vagner and Santigosa 2011), thus, the FA composition in green guapote seems to indicate that although they can be a rich source of n−3, their content may vary significantly with the composition of the available feed (Vasconi et al. 2017). Moreover, we suggest conducting further studies to analyze the fatty acid content of diets for both captive and wild green guapote, and to evaluate the effect of this on their whole-body and muscle composition.

Conclusion

This research study is the first report of hematology, proximal and fatty acid compositions of the green guapote *Mayaheros beani*, a native cichlid from Northwest Mexico. The values observed in the blood parameters between wild and farmed green guapote provide valuable information to understand their physiology and nutritional conditions. Moreover, the differences in fatty acid composition, as well as the high FCR observed in farmed fish, indicated that the green guapote required a specific diet for aquaculture production to improve their development and nutritional value for human consumption. Further research must be performed to have a better understanding of the nutritional requirements of green guapote as well as its physiological adaptations to different cultural conditions.

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Disclosure statement

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