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Research Article

The Effects of Dietary Cottonseed Oil on Growth Performance of Juvenile Red Drum (*Sciaenops ocellatus* L.) and Hybrid Striped Bass (*Morone chrysops* × *M. saxatilis* S.)

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Various cottonseed products containing higher levels of protein and lipid than traditional cottonseed meal have been evaluated in recent years with several carnivorous marine fish species. In previous studies in this laboratory, distinct differences in the acceptability of diets containing those cottonseed products were noted for red drum and hybrid striped bass. Therefore, the present study consisted of two concurrent feeding trials in which the effects of increasing levels of dietary cottonseed oil were evaluated with both juvenile red drum and hybrid striped bass. Three experimental diets were formulated to contain either 0, 2, or 4% of cottonseed oil in place of menhaden fish oil. All diets were formulated to contain approximately 9% lipid, with that of the basal diet (0% cottonseed lipid) primarily contributed by menhaden fishmeal and menhaden oil. In concurrent trials, each diet was fed twice a day to triplicate groups of juvenile red drum and hybrid striped bass initially averaging 1.86 g/fish and 1.41 g/fish, respectively, for 6 weeks. Results of the feeding trial showed that there were no significant differences among red drum fed the different dietary treatments based on growth parameters (weight gain and feed efficiency) or whole-body proximate composition. On the contrary, hybrid striped bass showed significant ($P \leq 0.05$) differences in responses to the dietary treatments with weight gain decreasing as the inclusion levels of cottonseed oil increased while their hepatosomatic index significantly increased. Thus, results of this study showed distinctly different responses of red drum and hybrid striped bass to increasing levels of dietary cottonseed oil, with hybrid striped bass being much more sensitive than red drum in terms of reduced weight gain and altered body condition which appeared to be related to reduce diet palatability from cottonseed oil.

1. Introduction

As world aquaculture continues to expand to meet the increasing demand for seafood, some resources required to intensively culture fish such as balanced diets continue to increase in both demand as well as price. One of the crucial feedstuffs in compounded aquafeeds is fishmeal (FM) which has traditionally been the most nutritious feedstuff included in diet formulations, especially for carnivorous species, as it is rich in protein and lipids. Fishmeal and fish oil are finite resources from reduction fisheries which have historically provided approximately 5 million metric tons of FM and 1 million metric ton of fish oil annually [1]. As world aquaculture has expanded, the demand for FM and fish oil also has increased, resulting in considerable escalation in their prices. As such, major research efforts around the world have been pursuing the development and/or identification of suitable and cost-effective replacements for FM and FO. A wide variety of protein feedstuffs from plant products, rendered animal products and other by-products, as well as microbial biomass and insect meals have been evaluated [2] and will continue to receive attention. Of the plant protein feedstuffs, cottonseed meal is a common byproduct of cotton which can be produced in nearly every corner of the globe.

The greatest advantage to cottonseed meal is that it typically costs only a fraction of that of FM and is also typically less expensive than other protein alternatives such as soybean meal [3].

Solvent-extracted cottonseed meal has been available for numerous years and evaluated extensively with several different species of fish [4]. The inclusion of this traditional cottonseed meal has typically been limited to approximately 20% for most omnivorous species due to the antinutritional factor gossypol, which is used by the plants as a defense mechanism to ward off insects. The toxic effects of gossypol on fish, as well as other monogastric animals, typically result in reduced growth and various pathologies [5]. Various efforts by means of traditional breeding and genetic engineering of the cotton plant have been made to reduce gossypol in cottonseed meal, in addition to various physical and/or chemical treatments [6]. Several of these efforts have resulted in cottonseed products with higher levels of crude protein and lower levels of gossypol [6]. Some of the products have been shown to have considerable potential with carnivorous marine species such as black seabass (*Centropristis striata*) in which 75% and 100% of the menhaden FM in the control diet could be replaced by solvent-extracted low-gossypol cottonseed protein and low-gossypol cottonseed protein from glandless seed, respectively, without impairing weight gain, feed efficiency, or survival [7]. Similar cottonseed products yielded comparable results with juvenile southern flounder (*Paralichthys lethostigma*) in that 75% replacement of FM was possible without negatively affecting weight gain or body composition [8]. Less favorable results were obtained by Wang et al. [9] with red drum in that replacement of greater than 50% of the protein from menhaden FM with a genetically modified cottonseed flour reduced weight gain. Also evaluated in that study with red drum were various palatability enhancers which did not increase the acceptance of diets containing relatively high levels of cottonseed flour in place of menhaden FM. In earlier studies conducted with hybrid striped bass in this laboratory, reduced growth of hybrid striped bass was observed when cottonseed flour replaced up to 50% of the protein typically provided by menhaden FM in the experimental diets. Wang et al. [9] noted that a potential reason for the reduced palatability observed in their study was increased lipid contributed by cottonseed flour which replaced fish oil in those diets with increased cottonseed flour. Thus, the current study evaluated the effects of extracted cottonseed oil to determine if higher inclusion levels limited diet palatability for both red drum and hybrid striped bass.

2. Materials and Methods

A feeding trial with both red drum and hybrid striped bass was conducted in which a reference diet was formulated to contain approximately 42% crude protein on a dry-matter basis primarily from menhaden FM, soybean meal, and soy protein concentrate (Table 1). Two different control diets that differed slightly in protein ingredients but did not contain supplemental cottonseed oil (CSO) were included in the trial, with the diet labeled reference fed to hybrid striped bass and the one labeled CSO 0% fed to red drum. The two experimental diets labeled CSO 2% and CSO 4% not only included cottonseed flour like CSO 0% but also contained incremental levels of cottonseed oil in place of menhaden fish oil and were fed to both red drum and hybrid striped bass. All diets were supplemented with lysine, methionine, and taurine to ensure the requirements of red drum and hybrid striped bass for these amino acids were satisfied [2].

Table 1: Formulations and proximate composition of the experimental diets fed to juvenile red drum and hybrid striped bass to evaluate the effects of incremental levels of cottonseed oil (CSO). The diet labeled reference was used as the 0% cottonseed oil diet for hybrid striped bass. Proximate composition results are expressed on a dry-matter basis as means of three replicate analyses.

Ingredient	Reference	CSO 0%	CSO 2%	CSO 4%
Proflo cottonseed meal	0.00	15.15	15.15	15.15
Menhaden fishmeal	28.05	14.00	14.00	14.00
Dehulled soybean meal	20.05	20.05	20.05	20.05
Wheat flour	11.55	11.55	11.55	11.55
Soy protein conc	16.6	16.6	16.6	16.6
L-Lysine	0.00	0.4	0.4	0.4
Taurine	1.00	1.00	1.00	1.00
DL-Methionine	0.50	0.50	0.50	0.50
Dextrinized starch	2.00	2.00	2.00	2.00
Celufil	7.00	5.05	5.05	5.05
Menhaden oil	5.95	6.40	4.40	2.40
Cottonseed oil	0.00	0.00	2.00	4.00
Vitamin premix[1]	3.00	3.00	3.00	3.00
Mineral premix[1]	4.00	4.00	4.00	4.00
Carboxymethyl cellulose	0.30	0.30	0.30	0.30
Analyzed composition				
Dry matter	91.8	91.9	91.6	91.7
Protein	46.9	45.3	45.3	45.5
Lipid	8.5	8.7	9.0	9.1
Ash	13.6	11.0	10.8	10.8

[1]As described by Moon and Gatlin [19].

The diet formulations were prepared by weighing dry ingredients and blending in a V-mixer. Once the dry ingredients were homogenized, they were placed into a food mixer for the addition of supplemental oil and then water for pelleting. The resulting dough was passed through a 2 mm die in a meat grinder attachment to produce strands which were dried overnight at 25°C using forced air. Strands were ground in a blender and sieved to produce appropriately sized pellets that could be readily consumed by the fish as they grew throughout the trial. Diets were stored at -20°C until used in the trial at which time they were kept at 4°C prior to feeding.

The feeding trial was conducted at the Texas A&M University Aquacultural Research and Teaching Facility (ARTF) where red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*) were cultured in a recirculating system consisting of 30 glass aquaria filled with

38 liters of brackish (7 ppt) water produced from well water and synthetic sea salts (Instant Ocean). The recirculating system was equipped with both biological and mechanical filters to aid in maintaining adequate water quality parameters which were checked two times each week. In addition to the biological and mechanical filters mentioned above, the system also was equipped with a UV light filtration system for germicidal treatment. Water temperature was held at a constant 27°C by conditioning the ambient air of the building. Each individual aquarium had an airstone through which air from a low-pressure blower was provided to ensure dissolved oxygen was maintained at levels approaching air saturation.

The feeding trial utilized nine aquaria which were stocked with juvenile red drum at a density of 14 fish per aquarium with an average group weight of 26.03 ± 0.46 grams. Another nine aquaria were stocked with juvenile hybrid striped bass at a density of 14 fish with an average group weight of 19.8 ± 0.56 grams. The diets with 0, 2, or 4% CSO were fed to triplicate aquaria of both red drum and hybrid striped bass.

Each species of fish underwent a week of acclimation during which they were fed the reference diet to apparent satiation twice daily. After the week of acclimation, each aquarium was randomly assigned to an experimental diet. Any mortalities during the first week of the trial were weighed and replaced with a fish of similar weight. After the first week, any dead fish were weighed and recorded to calculate feed efficiency and survival. Prior to the commencement of the trial, a representative sample of 10 red drum and 10 hybrid striped bass was frozen for subsequent measurement of initial proximate composition of whole-body tissues.

The fish were fed twice every day for a total of 6 weeks at which time the trial was terminated. Every week, the fish in each aquarium were collectively weighed to monitor weight gain and adjust feed quantities equally among all diets. The initial feeding rate was 4% of total biomass and was gradually reduced to 3% of total biomass by the end of the trial to maintain a level close to apparent satiation without overfeeding or resulting in uneaten feed. Some dietary treatments caused noticeably lower feeding activity, and uneaten feed was periodically noticed in some aquaria. However, any uneaten feed was not removed from the aquaria and quantified so as not to disturb the fish. The amount of uneaten feed in any aquaria throughout the trial was relatively small and did not impair water quality in the aquaria. When the trial was terminated, a composite weight of all fish in each aquarium was obtained along with the total number of fish for computing average fish weight and survival. A sample of three fish per aquarium were randomly collected and processed for the calculation of Fulton Condition Factor [10], intraperitoneal fat ratio (IPF), hepatosomatic index (HSI), and muscle ratio. Visceral fat was sampled from the intraperitoneal cavity of each fish using scalpel blades and tweezers and weighed to compute the ratio using the individual total body weight. Production performance, feed efficiency, survival, muscle ratio, and condition indices were measured according to the following formulas:

$$\begin{aligned}
\text{Weight gain (\% of initial weight)} &= 100 \times \left[\left(\text{Average weight at the } 6^{\text{th}} \text{ week (g)} - \frac{\text{average initial weight (g)}}{\text{average initial weight (g)}}\right)\right],\\
\text{Viscerosomatic indices (HSI or IPF)(\%)} &= [\text{Liver or IPF weight (g)} \div \text{body weight (g)}] \times 100,\\
\text{Feed efficiency (FE)} &= \frac{\text{Weight at the 6th week} - \text{Initial weight}}{\text{dry feed intake}},\\
\text{Survival (\%)} &= 100 \times \left(\frac{\text{number of surviving fish}}{\text{initial number of fish}}\right),\\
\text{Fulton Condition Factor (CF)} &= \frac{W_t \times 100}{\text{total body length}^3},\\
\text{Muscle Ratio (MR)} &= \frac{(\text{fillet weight} \times 100)}{W_t},\\
\text{Intraperitoneal fat (IPF)ratio} &= \frac{(\text{IPF weight} \times 100)}{W_t},
\end{aligned} \tag{1}$$

where W_t is the final body weight.

Whole-body proximate composition of composite samples of three fish per aquarium also was determined according to established procedures of the AOAC Official Method 942.05 for moisture and ash [11]. The Dumas method ([11] Method 968.06) was used to determine total nitrogen with crude protein computed as $N \times 6.25$, and the method of Folch et al. [12] was used for determining total lipids. All the analyses were performed in triplicate. Statistical analyses were performed via JMP Pro 15 (SAS Institute, CA, USA) for Mac IOS, and significance was set at $P < 0.05$. Treatments were subjected to analysis of variance (ANOVA) and

Table 2: Production performance and condition indices of red drum fed incremental levels of cottonseed oil for 6 weeks.

Diet designation	Weight gain (%)	Feed efficiency	Survival (%)	Hepatosomatic index (%)	Intraperitoneal fat ratio (%)	Fillet yield (%)	Fulton factor (g/cm^3)
CSO 0%	651	0.59	88.1	1.93	31.6	0.21	1.09
CSO 2%	692	0.61	90.5	1.96	31.0	0.22	1.07
CSO 4%	616	0.58	88.1	2.18	31.9	0.29	1.08
PSE	0.46	0.02	5.8	0.07	0.6	0.05	0.02
Model	Quadratic	Quadratic	Linear	Quadratic	Quadratic	Linear	Linear
Pr > F[1]	0.54	0.62	0.85	0.075	0.55	0.47	0.52
Adj R^2	-0.09	-0.14	-0.14	0.44	-0.09	-0.055	-0.08

Abbreviations: PSE: pooled standard error. [1]Significance probability associated with the F-statistic.

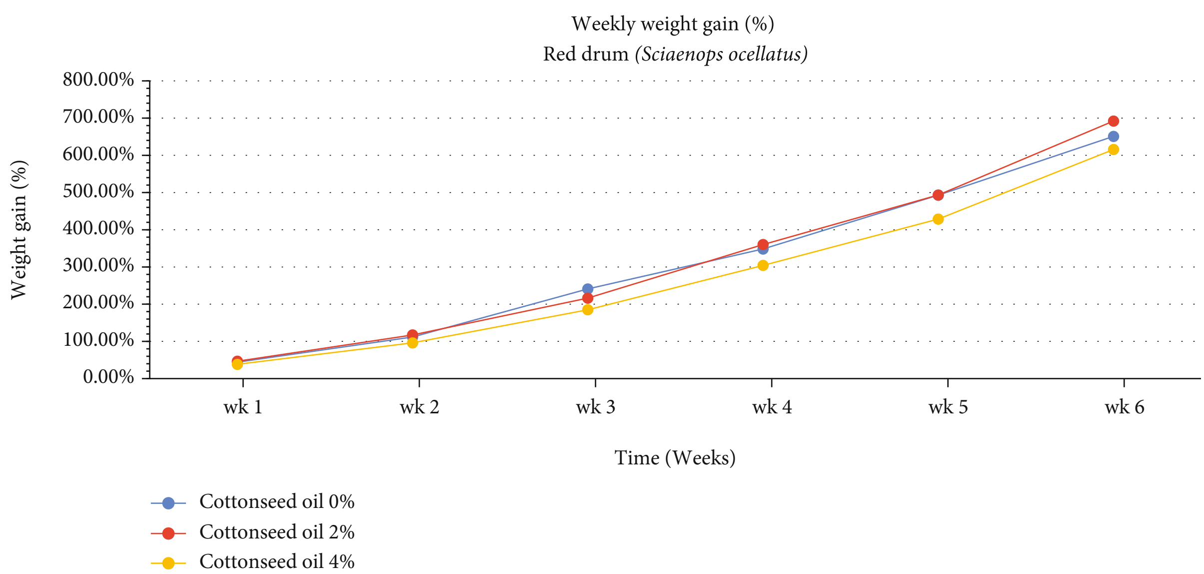

Figure 1: Weekly weight gain of the red drum fed incremental amounts of cottonseed oil over the course of the 6-week trial.

regression analysis to determine if any relationships were evident with increasing levels of cottonseed oil in the diet.

3. Results

Red drum fed the various diets demonstrated rapid growth and efficient diet utilization over the course of the 6-week trial with fish fed all diets achieving over 600% increase in initial weight (Table 2). These responses tended to decline as the level of cottonseed oil increased to 4% of diet (Figure 1); however, the ANOVA based on the regression model indicated no significant differences among the experimental groups in terms of growth parameters. Red drum body condition indices (Table 2) and proximate composition of whole-body tissues (Table 3) after the 6-week trial also were not significantly different among treatments. Hybrid striped bass fed the diet without supplemental cottonseed oil also increased their weight by over 600% after 6 weeks of feeding which was similar to red drum fed the control diet without CSO (Table 4). However, weight gain of hybrid striped bass fed the other diets significantly decreased as the inclusion of CSO increased (Figure 2). The relative differences in weight gain of red drum and hybrid striped bass fed different levels of cottonseed oil can be seen in Figure 3. Not only was weight gain and feed efficiency of hybrid striped bass significantly reduced as the amount of CSO increased in the diet, but hepatosomatic index also increased (Table 4). The other body condition indices (intraperitoneal fat ratio, fillet yield, and Fulton factor) were not significantly different in response to increasing levels of CSO (Table 4). There were slight but significant differences in ash and protein percentages in whole-body tissues of hybrid striped bass fed the various diets, but they did not show consistent patterns with increasing CSO inclusion (Table 5). Moisture and lipid content of whole-body tissues were not significantly different.

4. Discussion

This study showed how incremental levels of CSO differentially affected growth performance of red drum and hybrid striped bass. The hybrid striped bass showed a much more dramatic reduction in the weight gain as CSO levels in the diet increased compared to red drum. However, both species

Table 3: Proximate composition of whole-body tissues of red drum fed incremental levels of cottonseed oil for 6 weeks. Results are expressed in percentage of fresh weight.

Diet designation	Moisture (%)	Protein (%)	Ash (%)	Lipid (%)
CSO 0%	72.8	18.2	4.91	3.71
CSO 2%	73.0	18.1	4.53	3.72
CSO 4%	73.0	17.8	4.16	3.80
PSE	0.32	0.31	0.69	0.12
Model	Linear	Linear	Linear	Linear
$Pr > F^1$	0.78	0.33	0.12	0.70
Adj R^2	-0.06	-0.0004	0.09	-0.05

Abbreviations: PSE: pooled standard error. [1]Significance probability associated with the F-statistic.

Table 4: Production performance and condition indices of hybrid striped bass fed incremental levels of cottonseed oil for 6 weeks.

Diet designation	Weight gain (%)	Feed efficiency	Survival (%)	Hepatosomatic index (%)	Intraperitoneal fat ratio (%)	Fillet yield (%)	Fulton factor (g/cm^3)
Reference	617^A	1.06	90.5	1.37^A	3.13	29.1	1.17
CSO 2%	436^B	0.95	64.3	1.59^A	3.46	30.0	1.18
CSO 4%	320^B	0.87	76.1	2.02^B	3.5	31.8	1.15
PSE	28.7	0.06	6.5	0.054	0.247	1.2	0.02
Model	Linear	Linear	Linear	Linear	Linear	Linear	Linear
$Pr > F^1$	0.0002	0.04	0.27	0.001	0.29^A	0.14	0.69
Adj R^2	0.87	0.4	0.05	0.88	0.03	0.17	-0.12

Abbreviations: PSE: pooled standard error. [1]Significance probability associated with the F-statistic. Different superscript letters designate significant ($P < 0.05$) differences.

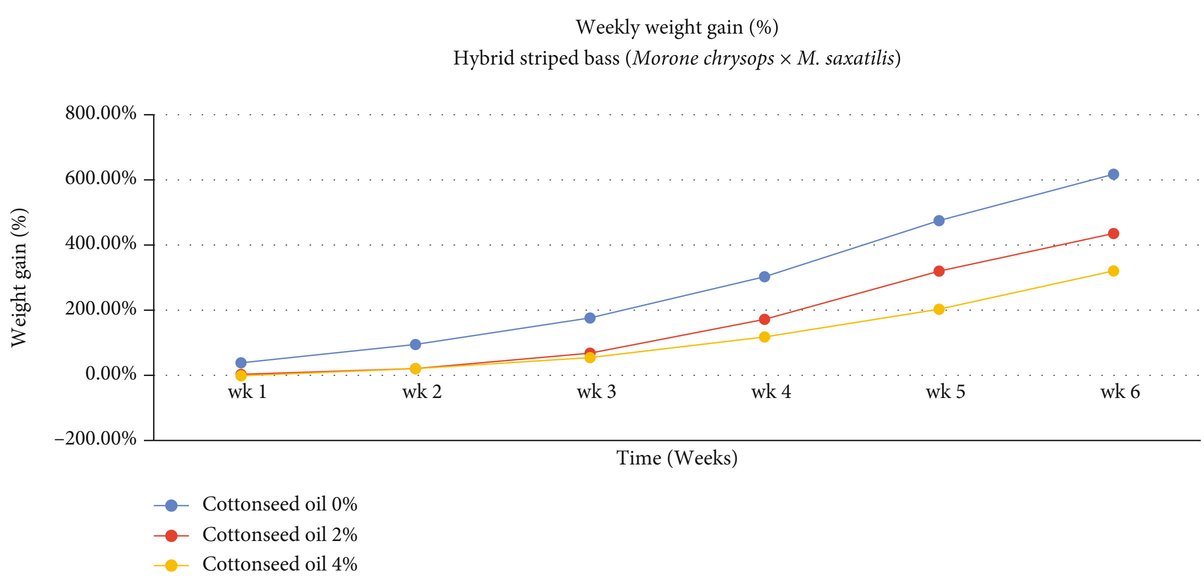

Figure 2: Weekly weight gain of hybrid striped bass fed incremental amounts of cottonseed oil over the course of the 6-week trial.

showed a tendency for reduced weight gain as the level of CSO in the diet increased to 4% of the diet. The reduced weight gain and feed efficiency of hybrid striped bass fed higher inclusion levels of CSO appeared to have been due to reduced palatability as theorized by Wang et al. [9]. The study of Wang et al. [9] reported lower feeding activity of red drum fed increasing levels of genetically modified cottonseed flour in the diet which also contributed to increased CSO in place of fish oil. It was that observation as well as reduced weight gain of red drum fed higher levels of the cottonseed flour that prompted Wang et al. [9] to conduct a second trial to evaluate potential palatability enhancers in a

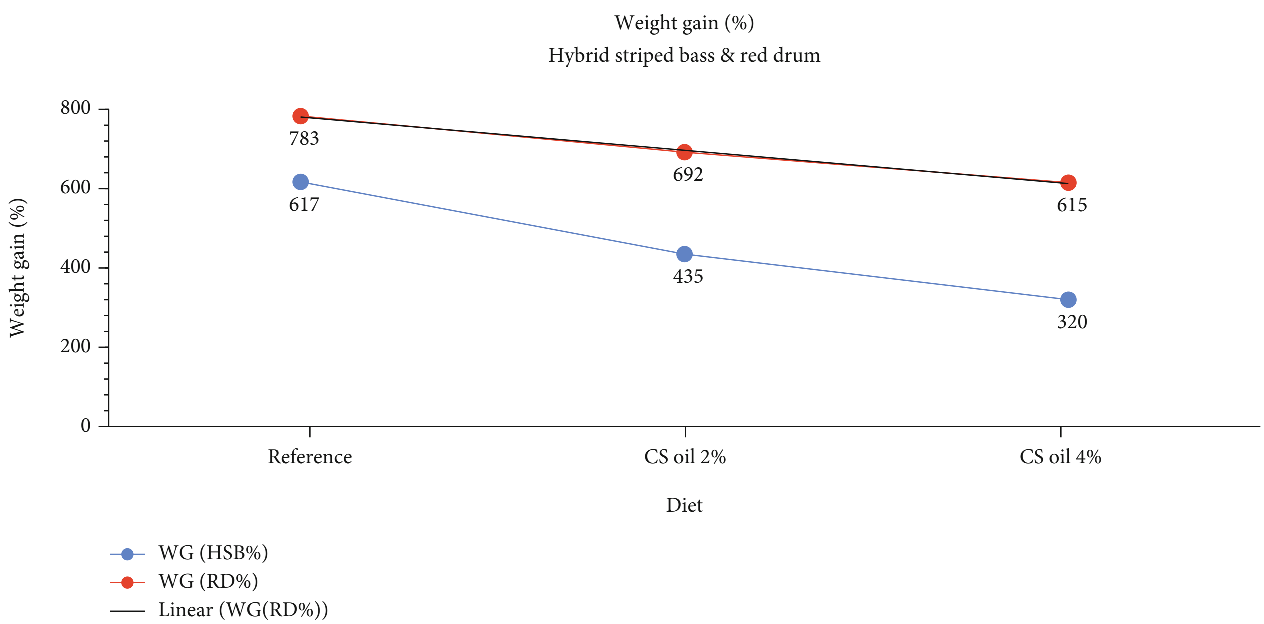

Figure 3: Weight gain percentage of hybrid striped bass (blue) and red drum (red) after 6 weeks of being fed incremental amounts of cottonseed oil in the diet.

Table 5: Proximate composition of whole-body tissues of hybrid striped bass fed incremental levels of cottonseed oil for 6 weeks. Results are expressed in percentage of fresh weight.

Diet designation	Moisture (%)	Protein (%)	Ash (%)	Lipid (%)
Reference	70.0	17.7^{A}	3.96^{A}	7.38
CSO 2%	72.8	16.3^{B}	3.61^{B}	6.74
CSO 4%	70.1	18.0^{A}	4.08^{A}	7.06
PSE	1.73	0.12	0.05	0.18
Model	Quadratic	Quadratic	Quadratic	Quadratic
$Pr > F$[1]	0.48^{A}	<0.0001	<0.0001	0.09^{A}
Adj R^2	-0.05	0.85	0.71	0.18

Abbreviations: PSE: pooled standard error. [1]Significance probability associated with the F-statistic. Different superscript letters designate significant ($P < 0.05$) differences.

diet with higher inclusion of cottonseed flour. Those observations also served as the basis for the present study which compared responses of red drum and hybrid striped bass which in separate earlier trials appeared to demonstrate reduced growth performance when fed diets with elevated levels of cottonseed products. The hybrid striped bass in the present study clearly exhibited reduced feeding activity compared to red drum when fed the diets with supplemental CSO. Hybrid striped bass fed the diets with CSO exhibited noticeably less aggressive feeding behavior compared to fish fed the diets without CSO. This was manifested in more obvious reductions in weight gain and feed efficiency and elevation of hepatosomatic index when hybrid striped bass were fed diets with increasing levels of CSO compared to the responses of red drum. However, gradually diminishing weight gain of red drum with increasing dietary CSO in the present study is somewhat similar to the finding of Wang et al. [9] although in that study weight gain was most significantly reduced in red drum fed the diet with all of the FM replaced with cottonseed flour which contributed the highest amount of CSO. Anderson et al. [7] also reported reduced feed intake as well as impaired weight gain and feed efficiency of juvenile black seabass (*Centropristis striata*) fed a diet in which all of the protein from fishmeal was replaced with regular cottonseed meal which contained 16.2% residual oil. Other forms of cottonseed meal fed to black seabass including a glandless product and solvent-extracted cottonseed meal generally did not reduce weight gain of that species. Alam et al. [8] fed southern flounder (*Paralichthys lethostigma)* diets in which the cottonseed products contained lower amounts of residual oil compared to the cottonseed products utilized by Anderson et al. [7]. After 8 weeks, there were no reported differences in feed intake among southern flounder fed the three types of cottonseed meal (genetically improved, genetically modified, and regular) nor were there any significant differences among fish fed diets with different levels of replacement [8]. Cook et al. [13] did not report any effects of cottonseed flour or oil on the ingestion rate of pompano *Trachinotus carolinus*. Similarly, Wassef et al. [14] reported no effect on the absolute ingestion rate of *Sparus aurata* fed diets containing cottonseed oil; however, the relative ingestion rate as a percent of body weight increased due to a reduction in feed efficiency and weight gain. It is apparent that the effects of cottonseed products can vary from species to species as can be seen with the southern flounder appearing to have a higher tolerance to gossypol compared to black seabass as well as the two species cultured in the current study. Researchers from the previously mentioned studies theorized that the presence of gossypol in the cottonseed products could be responsible for the overall decline in the growth performance of the fish. Anderson et al. [7] specifically attributed lower palatability of the diet containing regular CSM to higher gossypol in the diet.

Studies that explored the use of CSO as a replacement for fish oil in other species of fish such as rainbow trout (*Oncorhynchus mykiss*) [15, 16] and European seabass (*Dicentrarchus labrax*) [17] have reported elevated HSI in fish fed diets with higher inclusion of CSO similar to the responses of hybrid striped bass in the current study. It was reported by Güler and Yildiz [15] that up to 50% of the fish oil was able to be replaced by CSO without impairing the growth of the fish. In the study conducted by Eroldgan et al. [17], it was reported that up to 100% of the fish oil could be replaced by CSO without significantly reducing growth of the fish. Eroldgan et al. [17] noted that with the use of CSO in sea bass diets there was an overall decrease in n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFA) in fillet samples as the inclusion of CSO increased. The reduction of n-3 LC-PUFA with increased levels of CSO was believed to cause steatosis [17], which occurs when the deposition and synthesis of triacylglycerols in the hepatocyte vacuoles increase [18]. A reduction in n-3 LC-PUFA also was found by Wassef et al. [14] in the muscle and liver of juvenile gilthead seabream. Although in the present study dietary levels of linoleic acid (18:2n-6) increased from 6.6 to 30.9% of total fatty acids and docosahexaenoic acid (22:6n-3) decreased from 11.6 to 5.6% of total fatty acids as CSO supplementation increased from 0 to 4% of diet, there was no concern that any of the experimental diets were deficient in essential fatty acids for either red drum or hybrid striped bass [2]. The more dramatic increase in liver lipid in hybrid striped bass fed the diet with the highest level of CSO in contrast to that observed for red drum may have been related to their less efficient utilization of dietary energy which also was reflected in reduced weight gain.

In summary, both red drum and hybrid striped bass tended to show reduced weight gain when fed diets with increasing levels of CSO in place of menhaden fish oil. However, these effects were much more pronounced in hybrid striped bass compared to red drum.

Data Availability

The data used to support the findings of present study can be provided upon request to the corresponding author.

Disclosure

A preliminary report of this study was submitted and published on the conference proceedings of the conference Aquaculture America 2021 (San Antonio, Texas, USA) and presented as an oral presentation.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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