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PROOF RESEARCH PAPER

Effect of Dietary Taurine Supplementation on Growth Performance and Body Composition of Snapper, *Lutjanus colorado* Juvenile

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Abstract

This study evaluated the effect of soybean meal (SBM) diets supplemented with taurine (T) on growth performance and body composition of red snapper (*Lutjanus colorado*). Six isonitrogenous (50%) and isoenergetic (20 MJ/kg) diets were formulated with 20%, 40% and 60% proportions of SBM substitution of total fish meal. Three diets of fish meal protein were replaced by soybean protein which were supplemented with 0.6, 1.2 and 1.8% of taurine (SBM20+T, SBM40+T and SBM60+T), respectively, or without taurine supplementation (SBM20, SBM40 and SBM60). Each diet was fed to triplicate groups of 20 juvenile snapper (average initial weight 3.1± 0.41g) to apparent satiation three times a day for an 8-week period. The fish fed the SBM40+T diet with a total taurine concentration of 16.3 g kg^{-1} diet had a significantly greater final weight, specific growth rate, and a lower feed conversion ratio than fish fed the other diets. There were significant differences in feed intake and whole-body composition between fish fed different dietary treatments. Survival was not affected by the experimental diets. Considering growth, feed intake and body composition of red snapper, soybean meal could partially replace fish meal by up to 40% and supplemented with taurine in practical feeds.

Keywords: Red snapper, plant protein, soybean meal, fish meal replacement.

Introduction

The red snapper *Lutjanus colorado* is a potentially important species for marine aquaculture in Mexico and other American countries. Other snapper belonging to the Lutjanidae family readily accept pelleted diets, tolerate crowded conditions, perform well in floating sea cages and culture profitability depending partially on the price of feed (Hernández *et al.*, 2015). Despite current knowledge, it is well known that limited marine based protein sources exist in the world and a reduction in fish meal in fish diets may increase the profitability of aquaculture operations. Dietary costs generally constitute up to 60% of the total farm production costs. Therefore, the use of soy protein in feeds may provide an economically beneficial alternative. However, for carnivorous fish, a soybean meal based diet represents a number of challenges which are associated with its low methionine content; it is important for researchers to identify some less expensive and more sustainable ingredients to utilize in snapper diets, and these diets must have an equal or even better nutritional quality compared to diets based mainly on fish meal. Recently, Silva-Carrillo, Hernández, Hardy, González-Rodríguez and Castillo-Vargasmachuca (2012) demonstrated that for spotted rose snapper *Lutjanus guttatus*, fish meal can be replaced by soybean meal (SBM) constituting up to 20% of the total protein source, but at higher inclusion levels, the overall growth performance and feed utilization tend to reduce. Likewise, in other species such as gilthead sea bream (*Sparus aurata)*, turbot (*Psetta maxima*) and European sea bass (*Dicentrarchus labrax*), fish meal substitution by soybean meal is limited to 25-40% of dietary protein. The use of soy protein in feeds developed for carnivorous fish represents several challenges which include low methionine and cysteine content, lower protein digestibility, indigestible oligosaccharides, low phosphorus availability, antinutritional factors, poor palatability and undetectable levels of taurine (Francis, Makkar, & Becker, 2001). Increasing evidence indicates that some marine fish have a conditional requirement for taurine. In fish, the ability to synthesize taurine varies depending on species and age, due to differences in enzyme activity in taurine biosynthesis and during ontogenesis (Salze & Davis, 2015). Research has been conducted on several

species of larval and juvenile fish, including cobia (*Rachycentron canadum*) (Lunger, McLean, Gaylord, Kuhn, & Craig, 2007) Japanese flounders (*Paralichthys olivaceus*) (Kim *et al.*, 2008), yellowtail (*Seriola quinqueradiata*) (Khaoian, Nguyen, Ogita, Fukada, & Masumoto, 2014) and totoaba (*Totoaba macdonaldi*) (Bañuelos-Vargas, López, Pérez-Jiménez, & Peres, 2014). 2014), with results suggesting that taurine may be essential to promote optimum growth. In addition, due to its amino acid structure, taurine possesses the ability to stimulate feeding in fish (Carr, 1982). Thus, even though taurine is a non-essential nutrient, its inclusion in diets is often recommended (Salze & Davis, 2015). In fact, many animal feeds do not contain adequate levels of taurine and, as a result of processing methods, taurine content varies considerably (Rhodes, Rossi, Hanson, & Davis, 2011). Consequently, there is a lack of information on the effect of taurine supplementation on soybean meal based diets and its impact on the growth performance and body composition of red snapper. Therefore, the aim of the present study was to evaluate the efficacy of diets in which fish meal protein was replaced with soybean meal protein supplemented with taurine on growth performance and body composition of red snapper (*Lutjanus colorado*) juveniles.

Materials and Methods

Experimental Diets

Six isonitrogenous (50% crude protein) and isoenergetic (20 MJ/kg) diets were formulated. In three of the experimental diets, 20, 40 and 60% of fish meal (FM) protein had been replaced with soybean meal (SBM) protein and denominated SBM20, SBM40 and SBM60, respectively (Table 1). In the remaining three experimental diets the same proportions of fish meal had been substituted for soybean meal, but had been additionally supplemented with taurine (99% purity) in the following proportions: 0.6, 1.2 and 1.8% and the diets were denominated SBM20+T, SBM40+T and SBM60+T, respectively. The corresponding levels of dietary taurine by analysis were 0.96%, 1.63% and 1.89 %, respectively (Table 2). These proportions of FM substituted for SBM were selected based on data from previous growth response observations that were obtained from spotted rose snapper fed diets containing 210 or 315 g kg^{-1} SBM, which was used to replace 40% and 60% respectively, which resulted in a reduction in growth and feed intake in fish (Silva-Carrillo, Hernández, Hardy, González-Rodríguez, & Castillo-Vargasmachuca, 2012). Diets were balanced

Table 1. Ingredients and proximate composition of experimental diets for snapper *Lutjanus colorado*

	Diets					
	SBM20	SBM40	SBM60	SBM20+T	SBM40+T	SBM60+T
Ingredients (g 100g-[1] wet wt.)						
Fish meal[1]	53.63	40.05	29.46	53.63	40.05	29.46
Soybean meal[2]	19.92	39.83	57.75	19.92	39.83	57.75
DL-methionine[3]	0	0.10	0.34	0.00	0.10	0.34
Taurine[3]	0	0	0	0.60	1.20	1.80
Glutamic acid[3]	0.50	0.40	0.16	0.50	0.40	0.16
Fish oil[4]	4.13	4.86	5.60	4.13	4.86	5.60
Starch[4]	18.39	11.28	3.16	17.79	10.08	1.36
Alginate[4]	2.0	2.0	2.0	2.0	2.0	2.0
Antioxidants[5]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix[5]	0.6	0.6	0.6	0.6	0.6	0.6
Vitamin C[5]	0.2	0.2	0.2	0.2	0.2	0.2
Choline HCI[5]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[5]	0.08	0.08	0.08	0.08	0.08	0.08
CaP dibasic[5]	0	0.05	0.10	0	0.05	0.10
Dry matter	92.64	91.1	91.79	93.22	91.32	92.9
Crude protein	51.75	51.17	48.08	51.05	50.79	48.21
Crude lipid	9.45	8.63	9.52	9.75	9.35	9.40
Ash	9.67	9.65	8.77	9.93	9.56	9.03
NFE[6]	29.13	30.55	33.63	29.27	30.30	33.36
Gross energy (MJ/kg)	20.0	20.0	20.0	20.0	20.0	20.0

[1] Premium" grade fish meal was obtained from Selecta de Guaymas, S.A. de C.V. Guaymas, Sonora, México.
[2] Proteínas marinas y Agropecuarias, S.A. of C.V., Guadalajara, Jalisco, México.
[3] Sigma-Aldrich Chemical, S.A. de C.V. Toluca, México State, México.
[4] Droguería Cosmopolita, S.A. de C.V. México, D.F., México.
[5] Trouw Nutrition México S.A. de C.V. (by cortesy). *Vitamin premix composition: Vitamin A, 10,000,000 IU o mg/g; Vitamin 8 D3, 2,000,000 IU; Vitamin E, 100,000 g; Vitamin K3, 4.00 g; Thiamine B1, 8.00 g; Riboflavin B2, 8.70 g; Pyridoxine B6, 7.30; 9 Vitamin B12, 20.00 mg; Niacin, 50.00 g; Pantothenic acid, 22.20 g; Inositol, 153.80 g; Nicotinic Acid, 160.00 g; Folic Acid, 4.00 g; 10 80 mg; Biotin, 500 mg; Vitamin C, 100.00 g; Choline 300.00 g, Excipient c.b.p. 2000.00 g. Mineral premix composition: 11 Manganese, 100 g; Magnesium, 45.00 g; Zinc, 160 g; Iron, 200 g; Copper, 20 g; Iodine, 5 g; Selenium, 400.00mg; Cobalt 600.00 12 mg. Excipient c.b.p. 1500.00 g.
[6] Nitrogen-free extract (including fibre) = 100 – ((% protein + %lipid + % ash).

Table 2. Total amino acid composition and taurine of experimental diets (g AA/ 100 g of sample dry weigh) for snapper *Lutjanus colorado* [1]

Amino acids[2]	Body[3]	Diets					
		SBM20	SBM40	SBM60	SBM20+T	SBM40+T	SBM60+T
Essential							
Arg	3.51	5.44	5.42	5.42	7.73	6.01	5.89
Lys	2.75	3.10	2.74	2.74	2.70	3.11	3.66
His	1.10	1.36	1.21	1.21	1.13	1.09	1.18
Leu	4.20	1.95	2.12	2.12	1.85	1.89	1.80
Ile	2.58	2.60	2.59	2.57	2.60	2.59	2.57
Val	2.93	3.44	3.08	2.86	3.44	3.08	2.85
Thr	0.90	2.63	2.51	2.48	3.49	2.58	2.38
Met	1.42	2.58	2.93	2.92	2.48	2.75	2.72
Phe	2.61	2.67	2.65	2.52	2.66	2.67	2.52
Nonessential							
Glu	7.88	10.48	9.84	9.83	10.79	9.80	9.51
Gly	5.86	6.45	5.96	5.75	6.44	5.92	5.70
Asp	5.58	5.75	5.63	5.56	5.75	5.62	5.56
Ala	4.28	4.50	4.45	4.35	4.55	4.50	4.26
Ser	0.86	3.03	2.76	3.25	2.71	3.02	3.05
Tyr	0.90	1.52	1.54	1.60	1.54	1.56	1.55
Tau[4]	*1.02*	*0.66*	*0.49*	*0.28*	*0.96*	*1.63*	*1.89*

[1] Values are means (n=3).
[2] Arg-arginine; His-histidine; Ile-isoleucine; Leu-leucine; Lys-lysine; Met-methionin; Phe-phenylalanine; Thr-threonine; Val-valine; Ala-alanine; Asp- 18 aspartate Glu-glutamate; Gly-glycine; Ser-serine; Tyr-tyrosine; Tau-taurine.
[3] Mean amounts of amino acids in whole body of red snapper juvenile
[4] Taurine is shown separately since it only exists in the free form and expressed in (g AA/100 g of sample dry weigh).

with free crystalline DL-methionine (99% purity) using the carcass amino acid profile of *L. colorado* as a target value (Table 2), to avoid unbalanced diets. All the diets were manufactured as described by Silva-Carrillo *et al.* (2012).

Dry matter, crude protein, crude lipid and ash levels in the experimental diets and fish samples were determined using standard methods (AOAC, 1990). Dry matter was analyzed by drying the samples to a constant weight at 105°C. Crude protein was determined by the Dumas combustion method (AOAC, 2000) using a Leco FP-528 nitrogen analyzer. Crude lipid was analyzed using a micro Foss Soxtec after extraction with petroleum ether. Ash was examined by combustion of the samples in a muffle furnace at 550 °C for 16 h. Gross energy was determined according to the following energy coefficients (Miglavs & Jobling, 1989) 23.6 kJ/g for protein, 38.9 kJ/g lipids, and 16.7 kJ/g for carbohydrates. The amino acid profile (except for tryptophan) in the experimental diets was quantified (Table 2) following Vázquez-Ortiz, Caire, Higuera-Ciapara, and Hernández (1995) method by high performance liquid chromatography (HPLC, Varian 9012, Walnut Creek, CA, USA).

Fish and Feeding Trial

Red snappers were produced in a pilot scale hatchery at the Food Research and Development Center A.C. (CIAD) Mazatlán, Mexico, following established protocols for spawning and larval rearing. The fish were randomly distributed at a stocking density of 20 fish (weight 3.1 ± 0.41g) per tank among eighteen tanks (volume 350 L). The trial was conducted under natural photoperiod and the following average water quality parameters (±SD): temperature, 25 ± 1.5 °C; dissolved oxygen, 6.4±0.5 mg/L; salinity, 32.8 ± 0.4 g L^{-1}; pH, 8.1 ± 0.3. Triplicate groups were fed by hand to apparent satiation 3 times a day (0900, 1200 and 1700) during an 8-week period. After feeding, uneaten feed was removed by siphoning, allowed to dry to calculate feed intake, feed conversion ratio and PER. The fish were caught with scoop nets and anesthetized with 2-phenoxyethanol (Sigma®, St. Louis, MO, USA) at a concentration of 0.3 ml L^{-1}. To evaluate growth performance, fish were weighed every 2 weeks to calculate mean body weight and the biomass in each tank. The growth performance and feed efficiency of the fish were assessed by calculating the specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), feed intake (FI) and survival rate (SR). At the end of the feeding trial, the fish were starved for 24 h before three snappers from each replicate tank were randomly sampled and frozen for whole-body proximate chemical analysis. Another three snappers from each replicate were dissected, and visceral organs and liver were weighed for calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI).

Calculation of Growth Performance Indices

SGR = [(Ln final weight- Ln initial weight) / number of days] × 100

FCR = feed intake (g) / weight gain (g)

Feed intake (FI) = [(total feed consumption (g)) /

(number of fish)]/number of days

SR = (Final number / initial number) × 100

PER = weight gain / protein intake

HSI= Liver weight (g) × 100/BW (g)

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

Where, BW indicates the total body weight (g) and BL the total body length (cm) of the fish.

Statistical Analysis

A two-way ANOVA with Tukey´s test for multiple comparisons was used to compare growth performance and proximate whole-body composition between the six experimental diets. The results are presented as means with the pooled standard error of the mean. Normality of distributions and homogeneity of variances were tested using the Kolmogorov–Smirnov test and Levene test, respectively. A significance level of $P<0.05$ was used in all the analyses. All the statistical procedures were performed using Sigma Plot 12.0 software (Systat Software Inc., UK).

Results

Growth Performances

Survival rate (SR) was greater than 90% and there were no significant differences among dietary treatments ($P>0.05$) detected. Feed intake of fish was significantly affected by the replacement of FM protein with SBM protein, however FI increased with taurine supplementation (Table 3). The group fed with the SBM40+T diet, comprising a total taurine concentration of 1.63%, exhibited a significantly higher final weight, specific growth rate (SGR) and a lower FCR compared to the other treatment groups (Table 3). The protein efficiency ratio values varied among treatments. The highest value was for the red snapper fed SBM60+T, while the lowest value was observed in fish fed the SBM60 diet without taurine ($P<0.05$) (Table 3). Fish fed the SBM40+T or SBM60+T diets had similar hepatosomatic index and viscerosomatic indices to those of fish fed SBM20 (Table 3).

Proximate Whole-Body Composition

The proximate whole-body composition of fish was shown in Table 4. The fish fed SBM40 and SBM40+T diets had higher protein contents than fish fed other diets ($P<0.05$). Fish fed the SBM60 diet supplemented and not supplemented with taurine had a higher whole body lipid content than fish fed diets with lower SBM. Whole body ash was significantly higher in the control group than of treatment groups supplemented with taurine. Higher moisture content was found in the control group without taurine

Table 3. Growth parameters, feed efficiency and somatic index of snapper *Lutjanus colorado* fed the experimental diets for 8 weeks. (n=3 tanks per diet)[1]

	Diets							*Two-way ANOVA*		
	SBM20	SBM40	SBM60	SBM20+T	SBM40+T	SBM60+T	SEM[2]	Diet	Taurine	DxT[3]
IBW (g)	3.11	3.11	3.11	3.11	3.11	3.11				
FBW (g)	8.41^{bc}	8.40^{bc}	6.52^{d}	9.42^{b}	11.30^{a}	7.87^{c}	0.27	0.00	0.00	0.02
SGR(%/day^{-1})	1.92^{b}	1.81^{b}	1.35^{c}	2.01^{b}	2.34^{a}	1.54^{c}	0.01	0.00	0.00	0.00
FCR	1.54^{b}	1.50^{b}	1.75^{a}	1.81^{a}	1.50^{b}	1.67^{ab}	0.00	0.00	0.05	0.00
PER	1.26^{a}	1.31^{a}	1.05^{b}	1.26^{a}	1.31^{a}	1.25^{a}	0.00	0.00	0.06	0.04
FI (g)	9.21^{bc}	8.19^{cd}	6.14^{d}	10.68^{ab}	12.30^{a}	7.94^{cd}	0.69	0.01	0.00	0.02
SR (%)	93.33^{a}	100.00^{a}	95.00^{a}	90.00^{a}	90.00^{a}	100.00^{a}	0.03	0.31	0.48	0.21
HSI	3.55^{a}	2.11^{bc}	1.76^{c}	3.07^{ab}	2.84^{ab}	2.33^{bc}	0.12	0.01	0.12	0.02
VSI	10.08^{a}	7.68^{c}	8.72^{bc}	8.79^{bc}	9.80^{ab}	9.29^{ab}	0.21	0.06	0.05	0.00

IBW, initial body weight; FBW, final body weight; SGR, specific growth rate; FCR, factor conversion ratio; PER, protein efficiency ratio; FI, feed intake; SR, survival rate; HSI, Hepatosomatic index; VSI, viscerosomatic index

[1]Values in the same row with no common superscripts are significantly different ($P<0.05$).

[2] Standard error of the mean.

[3] Diet per taurine

Table 4. Body composition of snapper *Lutjanus colorado* fed experimental diets supplemented with taurine for 8 weeks (% diet on wet basis; n=3)[1]

	Diets %						*Two-way ANOVA*			
Parameters	SBM20	SBM40	SBM60	SBM20+T	SBM40+T	SBM60+T	SEM[1]	Diet	Taurine	DxT[3]
Moisture (%)	68.38^{bc}	68.51^{bc}	69.58^{ab}	70.67^{a}	67.57^{c}	68.54^{bc}	0.29	0.01	0.60	0.00
Protein % (N × 6.25)	18.32^{bc}	19.69^{a}	17.73^{c}	17.69^{c}	19.30^{a}	18.57^{b}	0.06	0.00	0.65	0.00
Lipid (%)	5.59^{c}	5.62^{c}	6.57^{a}	4.96^{d}	6.20^{b}	6.34^{ab}	0.01	0.00	0.07	0.00
Ash (%)	6.67^{a}	6.11^{b}	5.75^{cd}	6.00^{bc}	5.59^{de}	5.26^{e}	0.02	0.00	0.00	0.44

[1] Values in the same column with no common superscripts are significantly different ($P<0.05$).

[2] Diet per taurine.

supplementation than groups fed diets supplemented with taurine ($P<0.05$).

Discussions

The present study was designed to generate data on practical diets in which fish meal protein was replaced with soybean meal protein supplemented with taurine. We observed that it was possible to substitute the diets with SBM since increasing values of growth rate were attained by red snapper which were greater than those reported in Lutjanidae genus such as spotted rose snapper (Castillo-Vargasmachuca, Ponce-Palafox, Chávez-Ortiz, & Arredondo-Figueroa, 2007), and mutton snapper *L. analis* (Benetti *et al.*, 2002). In this study, we report that the supplementation of taurine in diets substituted with SBM in proportions of 40% or 60% (the content of SBM in diet was 398.3 or 587.5 g kg^{-1}) significantly improved growth performance and feed efficiency in red snapper compared to diets that lacked taurine but contained the same proportions of SBM. Therefore, it could be concluded that taurine could be a conditional amino acid for red snapper growth at this stage. *L. colorado* may not be capable of processing the quantities required for maximum growth when fed a SBM diet without taurine. Similar results have been previously reported, whereby the inclusion of taurine to all plant protein diets improve the growth performance in other carnivorous marine organisms, because they are either unable or poorly able to synthesize enough taurine to fulfill their nutritional needs (Gaylord *et al.*, 2007). Therefore, taurine supplementation is necessary in diets that are either partially or totally substituted with plant protein (Lunger, McLean, Gaylord, Kuhn, & Craig, 2007; Goto, Tiba, Sakurada, & Takagi, 2001; Kim *et al.*, 2005; Takagi *et al.*, 2006). Studies in mangrove red snapper (*L. argentimaculatus*) and spotted rose snapper (*L. guttatus)* have shown that FM protein can be replaced by up to 24% and 20% with soybean meal protein without taurine supplementation, respectively (Benetti *et al.*, 2002; Silva-Carrillo, Hernández, Hardy, González-Rodríguez, & Castillo-Vargasmachuca, 2012). The results of this study suggest that commercial soybean meal could constitute up to 40% of dietary protein in diets supplemented with taurine (13.4 g kg^{-1} diet) in *L. colorado* juveniles. These results are in agreement with other reports of feeds containing between 25% and 45% SBM and supplemented with taurine that are tolerated by different fish species such as sea bass fry (Brotons-Martinez, Chatzifotis, Divanach, & Takeuchi, 2004), Japanese flounder (Kim *et al.*, 2008), rainbow trout *Oncorhynchus mykiss* (Gaylord *et al.*, 2007) juvenile cobia (Kim *et al.*, 2008), common dentex *Dentex dentex* (Chatzifotis, Polemitou, Divanach, & Antonopoulou, 2008), and white bass *Atractoscion nobilis* (Jirsa, Davis, Salze, Rhodes, & Drawbridge, 2014).

Higher SGR was observed in fish fed SBM40+T while fish fed diets with the highest inclusion of SBM (60%) without taurine supplementation had the lowest SGR. Fish fed SBM60+T exhibited the highest PER value, while fish fed SBM60 without taurine showed low PER. These results are related to a low feed intake, where low FI results in the use of protein for maintenance rather than for protein synthesis and growth (Deng *et al.*, 2006). Diets without taurine supplementation showed a decrease in FI as SBM inclusion increased, while taurine supplementation in diets positively affected FI. A stimulant effect of taurine on olfactory organs of fish has been observed in several species (Chatzifotis, Polemitou, Divanach, & Antonopoulou, 2008), and the present results on FI confirm that palatability increases significantly with taurine supplementation in diets containing SBM as has been previously reported for other fish species (Lunger, McLean, Gaylord, Kuhn, & Craig, 2007; Kim *et al.*, 2005; Qi *et al.*, 2012; Lim *et al.*, 2013). Therefore, higher weight gain in fish fed SBM20+T and SBM40+T can be partly attributed to the increased feed intake.

In intensive aquafarming, carcass composition analysis is a useful quality assessment of whole-body content in response to dietary restriction of an essential nutrient. Protein and fat whole body contents are generally known as criterion constituents for determining the quality of fish flesh and the hepatosomatic indices which are related to glycogen and lipid energy reserves in the liver (Caulton & Bursell, 1977). In the present study, the whole-body composition was altered by diet. Snapper consuming the SBM60 diets, whether supplemented or not with taurine, had more fat in whole body composition and low protein content. This indicates that higher lipid storage is associated with dietary nutrients being allocated to energy in the form of lipid deposition in these tissues and lower protein retention.

The hepatosomatic index (HSI) was influenced by dietary treatments whereby significantly greater values of HSI were observed in fish fed 20% and 40% with or without taurine supplementation. Hepatosomatic index is related to glycogen and lipid energy reserves in the liver. HSI values of above 2 as observed in this study, are similar in fish such as European seabass, where fat deposition is very high (Dias *et al.*, 2005). There is evidence that replacement of fish meal by plant protein sources such as SBM affects hepatic lipogenic enzyme activities in red snapper. Present results do not exclude the possibility the storage of lipids is the result of direct effects of dietary plant ingredients on *L. colorado* physiology.

In conclusion, the results of this study indicated that supplementation with taurine (16.3 g kg^{-1}) increased the success of fish meal replacement with up to 40% of SBM for red snapper *Lutjanus colorado* juveniles. Further research is necessary to determine the optimal dietary requirement of taurine in snapper as well as to identify physiological processes blocked

when taurine is not supplied in the diet.

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