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Biological responses in mullet *Mugil liza* juveniles fed with guar gum supplemented diets

L.R.V. Ramos[a], L.A. Romano[a,b], J.M. Monserrat[a,c], P.C. Abreu[a,d], P.E. Verde[e], M.B. Tesser[a,f,*]

[a] *Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande – FURG, RS, Brazil*
[b] *Laboratório de Imunologia e Patologia de Organismos Aquáticos, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, RS, Brazil*
[c] *Instituto de Ciências Biológicas, Universidade Federal do Rio Grande – FURG, RS, Brazil*
[d] *Laboratório de Fitoplâncton e Microorganismos Marinhos, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, RS, Brazil*
[e] *Coordination Center for Clinical Trials, Heinrich-Heine-University, University Hospital Düsseldorf, Düsseldorf, Germany*
[f] *Laboratório de Nutrição e Alimentação de Organismos Aquáticos, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, RS, Brazil*

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ABSTRACT

This study aimed to evaluate the effects of adding guar gum, a non-starch polysaccharide, to the diet of *Mugil liza* juveniles. The juveniles (mean weight = 0.38 ± 0.01 g) were fed one of three diets with increasing supplement levels (4%, 8%, and 12%) and a control diet without additional gum for 60 days, in order to evaluate the effects on zootechnical performance, proximate composition, liver parameters, morphological alterations to the intestinal tract, and modulation of gastrointestinal microbiota. The animals fed 8% and 12% gum presented a significantly lower mean final weight, weight gain, specific growth rate, food intake rate, and protein intake rate than the control. Adding gum to the diets also reduced the dry matter, crude protein, and carcass fat levels. All treatments with added gum resulted in increased liver glycogen, and the cholesterol levels were significantly reduced in fish fed 4% and 8% supplement levels. No intestinal morphological alterations were observed in the animals. However, a modulating effect was noted on the microbial community, altering the bacterial quantity and composition throughout the tract segments. The use of guar gum is not recommended in *Mugil liza* diets, at least above 4%.

1. Introduction

Unlike formulated food for terrestrial animals, aquaculture diets require adequate processing to ensure sufficient stability in the water for the animals to consume them without loss to the environment (Paolucci et al., 2012). Therefore, "inert" additives, termed binders, of either organic or inorganic origin are typically included in diet formulas. Organic binders consist of complex carbohydrate polymer chains called non-starch polysaccharides (NSPs) and include pectin, laminarin, guar gum, agar, carrageenan, alginate, and chitosan (Paolucci et al., 2012).

* Corresponding author at: Universidade Federal do Rio Grande – FURG, RS, Brazil. Tel.: +55 53 32368132; fax: +55 5332368042.
E-mail addresses: rochavr.leo@gmail.com (L.R.V. Ramos), dcluis@yahoo.com (L.A. Romano), josemmonserrat@pq.cnpq.br (J.M. Monserrat), docpaca@furg.br (P.C. Abreu), peverde@googlemail.com (P.E. Verde), mbtesser@gmail.com (M.B. Tesser).

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However, NSPs can be considered antinutritional factors when present in a fish diet (Francis et al., 2001). Recently, Sinha et al. (2011) reviewed the antinutritional effects of these compounds, many of which are associated with changes in the diet's viscosity resulting in slowed gastric emptying and reduced gastrointestinal transit time, altered morphology and physiology of the digestive tract, changes in intestinal microbial community and additionally altered the levels of glucose and cholesterol. Moreover, the increased tendency to substitute fishmeal with plant-based protein ingredients leads to an increased of the presence of NSPs in fish diets. In that sense, studies dealing with the usage of purified NSPs are important to simulate the effects of higher levels of plant feedstuffs, which have been widely used in fish diets.

Guar gum (galactomannan) is an NSP derived from *Cyamopsis tetragonolobus* (Indian bean endosperm) and acts as an excellent thickener because it is a water-soluble polymer (Storebakken, 1985). Some studies have evaluated the addition of guar gum to fish diets, yet most have only observed the physical attributes of the feces (Amirkolaie et al., 2005; Brinker, 2007, 2009; Brinker and Reiter, 2012), nutrient digestibility (Leenhouwers et al., 2006), oxidative status, gastrointestinal tract morphology (Enes et al., 2012), and the effects on glucose and lipid metabolism (Enes et al., 2013). However, the mechanisms by which guar gum acts on body composition and modulates the microbial community in the tract remain uncertain.

The mullet *Mugil liza* reach approximately 60 cm in length and may weigh between 6 and 8 kg (Vieira and Scalabrin, 1991). They are consumers of the low trophic layers, being therefore suitable for both monoculture and polyculture with other fish and shellfish (Benetti and Fagundes Netto, 1991). Studies on the farming of this species have focused on nutritional aspects (Ito and Barbosa, 1997; Carvalho et al., 2010; Zamora-Sillero et al., 2013), rearing environment (Fonseca Neto and Spach, 1999; Okamoto et al., 2006; Poersch et al., 2007), stocking densities (Scorvo Filho et al., 1992; Sampaio et al., 2001), ontogenetic development of the digestive system (Galvão et al., 1997a,b), and reproductive biology (Vieira and Scalabrin, 1991; Esper et al., 2001). Many organisms like tilapia, carp, mullet and shrimp have been recognized as more suitable for aquaculture in tropical countries as they forage primarily on detritus (Moriarty and Pullin, 1987). By definition, detritus consists of dead organic matter primarily formed by plant material (Bowen, 1987). Ecologically, in the Lagoa dos Patos estuary (Southern Brazil), mullet primarily forage on detritus and microalgae that are undergoing microbial decomposition (Seeliger et al., 1997). However, it is unknown if the mullet is able to use any fibrous fraction of the detritus or if ingesting this material is a strategy for consuming the microbial matter that decomposes it.

This study aimed to evaluate the effects of adding guar gum to the diets of *M. liza* mullet by comparing performance, proximate composition, digestive tract morphology, modulation of the tract microbial community, and changes in liver cholesterol, triglyceride, and glycogen levels.

2. Materials and methods

2.1. Fish conditioning

Mullet (*M. liza*) juveniles were caught by trawl (2.5 m × 1.5 m; mesh size 5.0 mm) at Cassino beach (Rio Grande, RS, Brazil), transferred to the Laboratório de Piscicultura Marinha e Estuarina of the Universidade Federal do Rio Grande – FURG, and stocked in one 300-L tanks (two fishes per liter) for feed training (hand-fed four times per day). After 1 week, the fish were weighed (0.38 g ± 0.01 g) and randomly distributed throughout a static system consisting of 12 rectangular tanks (50 L) at a density of 15 fish per tank. After the first daily feeding, the tanks were siphoned and filled with seawater previously filtered through bag filters (5 μm) and treated with chlorine. Sodium thiosulfate was used to neutralize the chlorine before the utilization. Submerged heaters maintained the temperature at 25 °C, the salinity was held near 30, and a photoperiod of 14L:10D was maintained.

The fish were hand-fed four times daily (8:00 a.m., 11:00 a.m., 12:00 p.m., 3:00 p.m.) until apparent satiation. At the end of each day, the diets were weighed in a precision analytical scale (±0.01 g, BL-3200H, Marte, São Paulo, Brazil) to record daily intake.

2.2. Water parameters

The water parameters were monitored daily. Dissolved oxygen and temperature were measured using an oximeter (YSI 50A, OH, USA), pH was measured using a digital pH meter (±0.01, YSI®-pH100, OH, USA), and salinity was measured with a handheld Atago® refractometer (model 103, Tokyo, Japan). Ammonia content was measured every other day, and alkalinity was measured weekly via the UNESCO (1983) method.

2.3. Diet formulas

The experiment was randomized, with four treatments performed in triplicate, consisting of reference diet (control) (350 g kg^{-1}% crude protein; 16.45 MJ g^{-1}) and three other diets with increasing guar gum (GG) supplement levels (Farmaquímica S.A., São Paulo, Brazil) (4%: GG4; 8%: GG8, 12%: GG12) (Tables 1 and 2). Care was taken to ensure that no ingredients contained significant crude fiber levels. The dry ingredients were homogenized with oil and distilled water at 60 °C until a consistent texture was obtained that could be pelleted in a meat grinder with a 2-mm-diameter opening. Next, the pellets were dried in a forced-circulation oven for 5 h at 60 °C, and after drying, they were maintained in a freezer at −20 °C until used.

Table 1
Feed ingredients and proximal composition of reference diet.

	Dry matter (g kg^{-1})
Feed ingredients	
Fishmeal	60.0
Casein[a]	250.0
Gelatin[a]	100.0
Maize	550.0
Fish oil	30.0
Premix[b]	10.0
Proximal composition	
Dry matter	890.0
Crude protein	338.4
Ether extract	27.2
Ashes	17.3
Crude fiber	0.43
Metabolizable energy (MJ g^{-1})	18.45
Viscosity (cP)	18.4

[a] Rhoster (São Paulo, Brazil).
[b] Premix M. Cassab, SP, Brazil (Vit. A (500,000 UI kg^{-1}), Vit. D3 (250,000 UI kg^{-1}), Vit. E (5000 mg kg^{-1}), Vit. K3 (500 mg kg^{-1}), Vit. B1 (1000 mg kg^{-1}), Vit. B2 (1000 mg kg^{-1}), Vit. B6 (1000 mg kg^{-1}) Vit. B12 (2000 mcg kg^{-1}), Niacin (2500 mg kg^{-1}), Calcium pantotenate (4000 mg kg^{-1}), folic acid (500 mg kg^{-1}), biotin (10 mg kg^{-1}), Vit. C (10,000 mg kg^{-1}). Colin (100,000 mg kg^{-1}), Inositol (1000 mg kg^{-1}). Trace elements: selenium (30 mg kg^{-1}), iron (5000 mg kg^{-1}), copper (5000 mg kg^{-1}), manganese (5000 mg kg^{-1}), zinc (9000 mg kg^{-1}), cobalt (50 mg kg^{-1}), iodine (200 mg kg^{-1}). [3] Calculated from the physiological standard values, where 1 kg of carbohydrate (N-free extract), protein and lipid yields 16.7, 16.7 and 37.6 MJ, respectively (Garling and Wilson, 1976).

Diet and carcasses proximate analyses at the onset and in the end of the experiment were conducted according to the methodology described by the AOAC (1999): Dry matter (#934.01) was obtained after drying in an oven for 5 h at 102 °C; for ash (#942.05), the samples were burned in a muffle for 5 h at 600 °C. The Kjeldahl method was utilized to determine the crude protein (#984.13) level after sample digestion and nitrogen distillation; the results were multiplied by 6.25. To obtain an ether extract (#920.39), a Soxhlet extraction was conducted for 6 h with petroleum ether as the solvent. The following methodology described by Silva and Queiroz (2009) was utilized for the crude fiber analysis: Acid and base digestions of the samples were conducted for 30 min each; then, the residues were burned in a muffle at 500 °C, and the crude fiber value was obtained from the weight difference. The non-nitrogenous extract was calculated from the difference between the total crude protein, the ether extract, the crude fiber, and the ash values. Viscosity was measured according to the adapted methodology by Refstie et al. (1999): a 50-g diet was added to 450 mL distilled water and incubated for 30 min at 25 °C under agitation (80 rpm). Next, the rations were centrifuged (10,000 × *g*, 10 min), and the supernatant was collected and analyzed using a rheometer (Brookfield, DV–III Ultra, MA, USA) rotating at 250 rpm.

2.4. Growth trial

The experiment lasted 60 days, after which all fish (15 per tank) were weighed and measured to obtain the following zootechnical and biometric indexes:

1. Weight gain (g): final weight – initial weight.
2. Apparent feed conversion: diet supplied/weight gain.
3. Specific growth rate (% day^{-1}): [(ln final weight – ln initial weight)/days farming] × 100.
4. Protein efficiency rate: weight gain (g)/protein intake (g).

Table 2
Formulation and proximal composition of experimental diets. GG4, GG8 and GG12: means the % of inclusion of guar gum in the reference diet.

	Diets		
	GG4	GG8	GG12
Diet formulation (g kg^{-1})			
Reference diet	960	920	880
Guar Gum	40	80	120
Dietary component (g kg^{-1} dry matter)			
Dry matter	890.4	892.4	897.0
Crude protein	324.0	323.7	301.2
Ether extract	29.2	28.1	3.02
Ashes	17.1	17.1	16.6
Crude fiber	0.55	0.58	0.84
Viscosity (cP)	41.1	101.0	135.8

5. Condition factor: 100 × body weight (g)/body length (cm)^3.
6. Hepatosomatic index: ($weight_{liver}/weight_{body}$) × 100.
7. Viscerosomatic index: ($weight_{viscera}/weight_{body}$) × 100.
8. Intestinal quotient relative to length: $length_{intestine}/length_{body}$.
9. Intestinal quotient relative to weight: $length_{intestine}/weight_{body}$.

Afterwards, all the fishes were euthanized with an overdose of Benzocaine (300 ppm) and the liver and gastrointestinal tract were collected and weighted; intestines were also measured for intestinal quotient quantifications. All livers were separated and frozen at −80 °C for subsequent analyses; the stomach and intestines (three fishes per tank) were fixed in 20% formalin for histological analysis. For microbial quantification, prior to removing the tract from the animals (three fishes per tank), the outer surfaces were sterilized with povidone iodine. Then, the tract was collected and fixed in 4% formalin solution. At the beginning nine fish were subjected to the same procedure for initial microbial quantification. The carcasses of all the animals were used for body composition analysis at the conclusion of the experiment, 50 other fish were euthanized with overdose of Benzocaine (300 ppm) for initial whole body composition.

2.5. Glycogen, cholesterol, and liver triglyceride levels

The frozen liver samples were mixed for 40 min in a sonicator with perchloric acid (6%) in a volume 7.5 times the sample weight according to Zamora-Sillero et al. (2013). After sonication, the homogenates were neutralized with the same volume of potassium bicarbonate (1 M). Then, the homogenates were centrifuged (13,000 × g × 30 min), and the supernatants were used for the analyses. The total triglyceride and cholesterol levels were estimated using commercial kits (Triglicérides Enzimático Líquido, Colesterol Enzimático Líquido, Doles, Goiânia, GO, Brazil).

The liver glycogen content was estimated in duplicate according to the method of Carr and Neff (1984), later modified by Nery and Santos (1993). The glycogen content was obtained via enzymatic breakdown (amyloglucosidase, Sigma) into glucose. The product was measured using a commercial kit (Glicose enzimática, Doles, Goiania, GO, Brazil). All measurements were obtained on a spectrophotometer with a microplate reader at a wavelength of 490 nm (ELx808, Biotek Instruments Inc., Winooski, Vermont).

2.6. Histological analysis

The fixed material was processed in a LUPE PT 05 automatic processor embedded in Paraplast® and cut into 5-μm-thick sections in a LUPETEC MRPO3 microtome. The sections were stained with hematoxylin–eosin (HE).

2.7. Microbial enumeration

The fixed samples were taken to the Laboratório de Fitoplâncton e Microorganismos Marinhos/IO–FURG for bacterial count; for this, the intestines and stomach were carefully removed from the solution and sectioned into pieces in previously autoclaved petri dishes. After being opened, the stomach and intestines were washed with 10 mL distilled water. The solution was transferred to 40-mL glass jars and sonicated (Cole-Parmer Instrument Co., Chicago, IL, USA) in three 10-s increments with 10-s intervals between them. One 0.5-mL aliquot was removed and filtered through polycarbonate membrane filters (Nucleopore, Kent, UK, 0.2-μm porosity) that were previously darkened with 12% Irgalan black. The filtrate was then stained with acridine orange (1 μg mL^{-1}) (Hobbie et al., 1977). The bacteria were counted in 30 random fields using a Zeiss Axioplan epifluorescence microscope (Oberkochen, Germany) equipped with a blue filter (487709 – BP 450–490, FT 510, LT 520) and a Watec CCD (Watec Co., Yagamata, Japan) (0.0003 lx).

The results of performance, body composition and liver parameters were subjected to analysis of variance (ANOVA). Since that bacteria count were performed along the digestive tract (stomach, proximal, mid and distal intestine) and these measurements were clustered within each animal, a Poisson Generalized Linear Mixed Effects model was used to identify the influence of the levels of inclusion of guar gum in the multivariate microbial counts. The treatments groups were used as fixed effects and the animal as random effect. The contrasts of interest analyzed were the control group vs. 4%, 8% and 12% of gum guar supplemented diets. Interactions between treatments and the different localizations in the digestive tract were also analyzed by a two-way Poisson Generalized Linear Mixed Effects method. Statistical significant differences were declared for P-values less than 0.05. The corresponding statistical test for group comparison used in the analysis was a likelihood ratio test. Statistical computations were performed with the statistical software R version 3.1.0 (R Core Team, 2014) and using the package lme4 (Bates et al., 2014).

Data normality (Shapiro–Wilks) and variance homogeneity (Cochran test) were previously checked. The transformation $\log_{10}$ (microbial counts + 1) was applied in order to satisfy analysis assumptions. A Tukey test was applied to identify any significant difference from experimental groups. All statistical tests were performed using a 5% of significance level (Zar, 1984).

Table 3
Growth performance and biometric index of juvenile mullets *Mugil liza* fed with increasing levels of guar gum.* GG4, GG8 and GG12: means the % of inclusion of guar gum in the reference diet.

Parameters	Control	GG4	GG8	GG12	PSE	P
$AW_{initial}$	0.38	0.38	0.38	0.38	0.01	–
AW_{final}	3.67[a]	2.63[ab]	2.52[ab]	2.42[b]	0.20	0.031
WG	3.29[a]	2.26[ab]	1.94[ab]	1.86[b]	0.24	0.032
SGR	3.78	3.47	3.16	3.09	0.11	0.090
FI	6.90[a]	4.63[b]	4.32[b]	4.00[b]	0.44	0.007
FCR	1.94	2.01	2.29	2.12	0.08	0.466
PI	2.41[a]	1.62[b]	1.51[b]	1.40[b]	0.15	0.007
PER	1.47	1.42	1.28	1.35	0.04	0.501
Biometric indexes						
K	1.29	1.31	1.26	1.29	0.02	0.827
HSI	1.62	1.68	1.49	1.62	0.04	0.545
VSI	10.57	10.96	10.16	11.32	0.22	0.316
QIR_{CL}	2.49	2.6	2.42	2.52	0.05	0.678
QIR_{BW}	4.51	5.35	5.63	5.97	0.25	0.209

AW, average weight; WG, weight gain; SGR, specific growth rate; FI, feed intake; FCR, feed conversion rate; PI, protein intake; PER, protein efficiency rate; *K*, condition factor; HSI, hepatic somatic index; IVS, viscera somatic index; QIR, Quotient intestinal relative (CL, corporal length; BW, body weight).
* A mean value of triplicates groups. Mean with different subscript letters in the same column differ e significantly (P<0.05). PSE, pooled standard error.

3. Results

3.1. Water quality

Throughout the experiment, the mean temperature was 24.9 °C ± 0.03 °C, the dissolved oxygen content was 6.82 ± 0.04 mg L^{-1}, the pH was 8.12 ± 0.05, and the mean alkalinity was 147 ± 12.93 mg L^{-1} $CaCO_3$. The mean values for ammonia in the control, GG4, GG8, and GG12 treatments were 0.69 ± 0.38, 0.62 ± 0.34, 0.42 ± 0.23, and 0.39 ± 0.22 mg L^{-1}, respectively.

3.2. Zootechnical performance

The zootechnical performance results are provided in Table 3. No mortality occurred during the experimental period. Final weight, weight gain, feed intake rate, and protein intake rate were significantly higher (P<0.05) in the control group than in the 8% and 12% added guar gum treatment groups. No significant differences (P>0.05) were found in apparent feed conversion, protein efficiency rate, and biometric indexes.

3.3. Body composition

Increased guar gum supplementation in the fish diets resulted in reduced dry matter, crude protein, and carcass fat levels (Table 4). The control treatment exhibited significantly higher (P<0.05) dry matter and ether extract levels than the treatments with added guar gum; the crude protein was significantly higher (P<0.05) in the control and GG4 treatments, with the lowest value found for the 8% guar gum treatment. The ashes were significantly reduced (P<0.05) by the addition of 8% guar gum (Table 4).

Table 4
Proximal body composition of juvenile mullet *Mugil liza* fed with increasing levels of guar gum in diets.* GG4, GG8 and GG12: means the % of inclusion of guar gum in the reference diet.

Body composition	Initial	Final				PSE	P
		Control	GG4	GG8	GG12		
Dry matter	22.25	28.59[a]	27.47[b]	24.66[c]	16.36[d]	1.28	0.000
Crude protein	15.70	17.60[a]	17.51[ab]	16.26[c]	16.59[bc]	0.18	0.030
Ether extract	0.89	7.53[a]	7.02[b]	6.06[c]	6.24[c]	0.16	0.000
Ashes	5.34	3.25[a]	3.26[a]	3.05[b]	3.19[ab]	0.03	0.006

* Values are means of triplicate groups. Means with different superscript letters in the same column differ significantly (P<0.05). PSE, pooled standard error.

Table 5
Levels of hepatic glycogen, triglycerides and cholesterol (mg g^{-1}) of juvenile mullets *Mugil liza* fed with increasing levels of guar gum in diets.* GG4, GG8 and GG12: means the % of inclusion of guar gum in the reference diet.

Treatment	Glycogen	Triglycerides	Cholesterol
Control	2.95^{b}	5.50	0.058^{ab}
GG4	6.25^{a}	4.43	0.039^{b}
GG8	5.87^{a}	4.88	0.034^{b}
GG12	5.09^{a}	4.69	0.144^{a}
PSE	0.34	0.43	0.01
P	0.000	0.924	0.022

* Values are means of triplicate groups. Means with different superscript letters in the same column differ significantly (P<0.05). PSE, pooled standard error.

3.4. Liver parameters

All guar gum treatments caused significantly higher (P<0.05) glycogen values compared with the control group. Liver cholesterol content for the GG8 and GG4 were below those for the GG12 and control treatments (P<0.05). No significant difference was noted in the triglyceride levels between the treatment groups (Table 5).

3.5. Digestive tract histological analysis

No morphological or pathological alterations associated with guar gum supplementation to the diets were observed.

3.6. Digestive tract bacterial count

Significant differences were detected (P<0.05) between the guar gum inclusion and the control in the total microbial counts. In the stomach, control treatment exhibited higher counts than GG4 and GG8 treatments; in the mid intestine, control was higher than GG4, but lower than GG8 treatment; in the distal intestine, stomach had more total bacteria than GG4 treatment (Table 6).

Regarding the bacterial morphotypes, three bacterial groups were identified: cocci, bacilli and filamentous. Significant differences (P<0.05) were observed between the counts in the different tract sections. Cocci bacteria showed higher counts in the control when compared with GG4 treatment in the distal intestine section. Bacilli count in the proximal intestine had

Table 6
Summary of results from bacterial morphotypes count (total bacteria: 10^7 org mL^{-1}; cocci: 10^7 org mL^{-1}; bacilli: 10^6 org mL^{-1}; filamentous: 10^5 org mL^{-1}) in the different tract sections of juvenile mullets *Mugil liza* fed with increasing levels of guar gum.* GG4, GG8 and GG12: means the % of inclusion of guar gum in the reference diet. I.: intestine.

	Treatments				*P*-values		
	Control	GG4	GG8	GG12	*(avs b)*	*(avs c)*	*(avs d)*
Total bacteria							
Stomach	4.17	**3.52**	**3.71**	4.27	**0.020**	**0.006**	
Proximal I.	1.99	1.91	1.91	1.99			
Mid I.	1.10	**0.77**	**1.27**	1.10	**0.016**	**>0.001**	
Distal I.	1.06	**0.60**	**1.27**	1.32	**0.0563**	**>0.001**	
PSE	**0.462**	**0.500**	**0.406**	**0.505**			
Cocci							
Stomach	24.24	27.09	28.01	31.24			
Proximal I.	18.08	17.08	17.01	16.77			
Mid I.	7.61	6.38	9.46	9.08			
Distal I.	12.31	**5.23**	9.62	11.31	**>0.001**		
PSE	**2.802**	**14.261**	**3.092**	**3.652**			
Bacilli							
Stomach	6.69	5.15	6.31	7.46			
Proximal I.	0.84	**1.85**	1.07	2.00	**0.022**		
Mid I.	1.92	**0.54**	1.84	1.23	**0.024**		
Distal I.	1.30	**0.38**	0.76	1.15	**0.027**		
PSE	**0.774**	**0.718**	**0.873**	**1.090**			
Filamentous							
Stomach	8.77	**3.00**	**2.77**	**4.00**	**>0.001**	**>0.001**	**>0.001**
Proximal I.	1.61	**0.23**	1.07	1.15	**>0.001**		
Mid I.	0.61	0.84	**1.46**	0.69		**>0.001**	
Distal I.	0.23	0.38	**0.76**	0.69		**0.033**	
PSE	**1.301**	**0.354**	**0.403**	**0.584**			

Bold values are significantly different ($P < 0.05$) from Control treatment.
* Values are mean of triplicate groups. PSE, pooled standard error.

lower results in the control than GG4, while in the mid and distal intestine sections the control showed elevated counts when compared with this same treatment. Finally, filamentous count in the stomach was higher in the control treatment than the treatments with guar gum inclusion; the proximal intestine had more filamentous bacteria in the control than GG4 treatment; mid intestine had less filamentous bacteria in the control than the GG8 treatment; and in the distal intestine GG4 and GG8 had more filamentous counts than control (Table 6).

Interactions between guar gum inclusion and tract section in the bacterial count tract were identified. The inclusion of 4% guar gum showed higher ($P<0.05$) total bacteria, cocci, bacilli and filamentous counts in the stomach and proximal intestine than distal intestine. The inclusion of 8% guar gum exhibited higher ($P<0.05$) for filamentous counts in the stomach and proximal in comparison to distal intestine. Also, filamentous counts were significantly higher ($P<0.05$) in the stomach section than distal intestine in the treatment with 12% of guar gum (data not shown).

4. Discussion

Although the mullet *M. liza* in its natural environment forages for foods such as algae and detritus that contain soluble and insoluble polysaccharide (Vieira, 1991; Oliveira and Soares, 1996), the addition of soluble guar gum polysaccharide to its diet caused a negative growth response. Despite their presence in a wide variety of plant-based ingredients, soluble NSPs are related to depressed growth in some monogastric species (Sinha et al., 2011). The antinutritional effects of guar gum are due to its physical properties. According to Paolucci et al. (2012), the main property of guar gum is its ability to rapidly hydrate, creating a highly viscous material even at relatively low levels, a response that was observed in this study (Table 2). Leenhouwers et al. (2006) demonstrated that among the adverse physiological effects associated with viscosity, soluble NSPs (guar gum) induce increased digesta viscosity in fish. Delayed gastric emptying is among the factors responsible for reduced food intake (Sinha et al., 2011), which affects animal performance, and could explain the reduced food intake and consequent reduced zootechnical performance in the fish fed with guar gum.

Furthermore, adding guar gum to the diets markedly affected the carcass proximate composition. As the gum supplement increased, water quantity increased, with a concurrent reduction in carcass lipid, protein, and ash levels. The same result was observed in studies using soluble polysaccharide-rich plant sources, including those by Hossain et al. (2001, 2003), Siddhuraju and Becker (2001), Krogdahl et al. (2003) and Kumar et al. (2011). Some physiological effects of high viscosity induced by guar gum may be related to reduce carcass lipid and protein levels. Sinha et al. (2011) explain that the addition of NSP supplements to diets reduces protein digestibility and consequently interferes with amino acid absorption, which in turn, influences body protein formation. Pasquier et al. (1996) used *in vitro* assays to demonstrate that soluble polysaccharides (guar gum, pectin, and arabic gum) reduce fat emulsification and triglyceride lipolysis, hindering digestion and absorption, while Vahouny et al. (1980) demonstrated how soluble fibers can bind to bile salts, hindering their intestinal absorption. This latter mechanism is described by Potter (1995), and according to the authors, increased bile salt excretion creates an environment in which cholesterol is removed from the body, making the liver to provide them for bile acid re-synthesis.

Surprisingly, adding 12% guar gum increased the liver cholesterol levels. Several studies of fish have reported that soluble NSP supplements reduce dietary lipid use (Sinha et al., 2011), and they have subsequently been indicated for the human diet (Tungland and Meyer, 2002). However, Enes et al. (2013) observed increased plasma cholesterol levels when 8% and 12% guar gum was added to the diets of *Diplodus sargus* (sea bream). The increased liver cholesterol levels and reduced carcass fat observed in this study indicate that muscular fat reserves are more mobile than liver fat reserves, as noted previously by Potter (1995).

Enes et al. (2013) evaluated the effect of guar gum on liver glycogen in *D. sargus*; however, the authors found no alterations, concluding that guar gum aids in reducing endogenous glucose production in this fish species. In our study, fish fed diets containing guar gum supplement exhibited significantly higher liver glycogen values than the control group, regardless of supplement level. Non-ruminant animals derive an additional source of energy from the fermentation products that are not digestible by endogenous enzymes, some of which are absorbed and used as a source of glucose in the liver (Montagne et al., 2003); this process may have occurred in this study.

Typically, direct microbial counts in the fish digestive tract only evaluate the intestine; the stomach is often neglected. In this study, the highest bacterial counts were observed in this organ and decreased further along the tract toward the distal intestinal segment regardless of diet. Conversely, studies conducted on marine herbivorous fish caught in their natural environment found increased bacterial density toward the distal intestinal segment (Rimmer, 1986; Clements, 1991; Fidopiastis et al., 2006). According to Clements (1991), the absence of microorganisms in the anterior portion of the gastrointestinal tract and their abundance in the terminal portions indicate that the organisms present are not only consumed together with the food particles but also form an endosymbiotic bacterial community profile that assists with polysaccharide digestion.

However, histological analysis of the mullet stomach (Galvão et al., 1997a,b) demonstrated that the pyloric region – with deep folds, highly developed muscles, and no digestive glands – has the primary function of grinding food, comparable to the gizzard in birds. The absence of secretory glands for both enzymes and hydrochloric acid in the pyloric region creates a proper environment for microbial colonization, which could explain the high bacterial density in the stomachs of these animals. Thus, it is very likely that *M. liza* utilizes the bacterial biomass produced after the decomposition of consumed food. If this is the case, mullet fish should have a ruminant-like feeding behavior, with the incorporation of bacteria into its biomass.

In ruminant animals, microorganisms are the main source of high-quality protein and are subsequently digested in the abomasum (Allison, 1993). The hypothesis that the stomach microbial community can be used as a protein supplement may also apply to mullet because the microbial density follows a trend wherein gradually decreases throughout the intestinal tract, which could denote absorption of digested bacteria throughout the intestines. Chun-Fang et al. (2012) also evaluated the effect of purified NSPs (raffinose and stachyose) on the microbial profile of the silver crucian carp digestive tract and verified that the bacterial community remained unchanged.

To our knowledge, this is the first study that has evaluated the effect of guar gum in microbial population in the fish tract. In this work, guar gum modulated the bacterial community of the mullet tract, and markedly the inclusion 4% guar gum had impacted not only in the total counts, but in all bacteria morphotype composition, mainly in the bacilli group, which are the major group of bacteria that present some role as a probiotic (Balcázar et al., 2006). However, this manipulation does not seem to affect the animal's performance, since the worst results were observed in the inclusion of 12% of guar gum. In grower pigs, guar gum causes the increasing of intestinal bacteria populations, mainly bacteria of bacilli morphotypes (lactobacilli, clostridia, enterobacteria and bifidobacteria), and this effect is related with changes in the physiology and ecosystem caused by the increased viscosity in the gut (Owusu-Asiedu et al., 2006).

5. Conclusion

Supplementing the diets of *M. liza* mullet with guar gum caused an antinutritional effect, reducing growth and feed intake. Furthermore, the supplementation altered the body composition and increased cholesterol and glycogen levels in the liver. Guar gum modulated the bacterial profile and the bacteria densities in the different tract sections. This research indicates the use of guar gum is not recommended in *M. liza* diets at levels exceeding 4%.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Allison, M.J., 1993. Microbiology of fermentative digestion in the rumen and large intestines. In: Swenson, M.J., Reece, W.O. (Eds.), Duke's Physiology of domestic Animals. , 12th ed. Conell University Press, Ithaca, NY, p. 1024.

Amirkolaie, A.K., Leenhowers, J.I., Verreth, A.J., Schrama, J., 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia. Aquacult. Res. 36, 1157–1166.

Association of Official Analytical Chemists, 1999. Official Methods of Analysis of the AOAC, 16th ed. AOAC International, Washington, pp. 1141, http://CRAN.R-project.org/package=lme4

Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1. 1–6, http://CRAN.R-project.org/package=lme4

Balcázar, J.L., de Blas, I., Ruiz-Zazuela, I., Cunningham, D., Vendrell, D., Múzquiz, J.L., 2006. The role of probiotics in aquaculture. Vet. Microbiol. 114, 173–186.

Benetti, D.D., Fagundes Netto, E.B., 1991. Preliminary results on growth of mullets (*Mugil liza* and *Mugil curema*) fed artificial diets. World Aquacult. 22, 115–122.

Bowen, S.H., 1987. Composition and nutritional value of detritus. In: Moriarty, D.J.W., Pullin, R.S.V. (Eds.), Detritus and Microbial Ecology in Aquaculture. Como, Italia, pp. 192–216.

Brinker, A., 2007. Guar gum in rainbow trout (*Oncorhyinchus mykiss*) feed: The influences of quality and dose on stabilization of faecal solids. Aquaculture 267, 315–327.

Brinker, A., 2009. Improving the mechanical characteristics of faecal waste in rainbow trout: the influences of fish size and treatment with a non-starch polysaccharide (guar gum). Aquacult. Nutr. 15, 229–240.

Brinker, A., Reiter, R., 2012. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part II: Effects on faeces stability and rheology. Biorheology 49, 27–48.

Carr, R.M., Neff, J.M., 1984. Quantitative semiautomated enzymatic assay for tissue glycogen. Comp. Biochem. Physiol. 77B, 447–449.

Carvalho, C.V.A., Bianchini, A., Tesser, M.B., Sampaio, L.A., 2010. The effect of protein levels on growth, postprandial excretion and tryptic activity of juvenile mullet *Mugil platanus* (Günther). Aquacult. Res. 41, 511–518.

Chun-Fang, C., Wen-Juan, W., Yuan-Te, Y., Krogdahl, A., Young-Ling, W., Yan-Mei, X., Cai-Geng, Y., 2012. Effects of soybean meal, raffinose and stachyose on the growth, body composition, intestinal morphology and intestinal microflora of juvenile allogynogenetic silver crucian carp (*Carassius auratus gibelio*♀ x *Cyprinus carpios*♂). Aquacult. Res. 43, 128–138.

Clements, K.D., 1991. Endosymbiotic communities of two herbivorous labroid fishes, *Odax cyanomelas* and *O. pullus*. Mar. Biol. 109, 223–229.

Enes, P., Pérez-Jiménez, A., Peres, H., Couto, A., Pousão-Ferreira, P., Oliva-Teles, A., 2012. Oxidative status and gut morphology of White sea bream, *Diplodus sargus* fed soluble non-starch polysaccharide supplemented diets. Aquaculture 358–359, 79–84.

Enes, P., Pousão-Ferreira, P., Salmerón, C., Capilla, E., Navarro, I., Gutiérrez, J., Oliva-Teles, A., 2013. Effect of guar gum on glucose and lipid metabolism in White sea bream *Diplodus sargus*. Fish Physiol. Biochem. 39, 159–169.

Esper, M.L.P., Menezes, M.S., Esper, W., 2001. Época reprodutiva de *Mugil platanus* (Günther, 1880), Pisces Mugilidae da Baia de Paranaguá (Paraná, Brasil). Acta Biol. Paranaense. 30, 5–17.

Fidopiastis, P.M., Bezlek, D.J., Horn, M.H., Kandel, J.S., 2006. Characterizing the resident, fermentative microbial consortium in the hindgut of the temperate-zone herbivorous fish, *Hermosilla azurea* (Teleostei: Kyphosidae). Mar. Biol. 148, 631–642.

Fonseca Neto, J.C., Spach, H.L., 1999. Sobrevivência de juvenis de Mugil platanus Günther, 1880 (PISCES, MUGILIDAE) em diferentes salinidades. B. Inst. Pesca 25 (único), 13–17.
Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.
Galvão, M.S.N., Fenerich-Verani, N., Yamanaka, N., Oliveira, I.R., 1997a. Histologia do sistema digestivo da tainha Mugil platanus Günther, 1880 (OSTEICHTHYES, MUGILIDAE) durante as fases larval e juvenil. B. Inst. Pesca 24 (único), 91–100.
Galvão, M.S.N., Fenerich-Verani, N., Yamanaka, N., Oliveira, I.R., 1997b. Estudos preliminares sobre enzimas digestivas proteolíticas da tainha Mugil platanus GÜNTHER, 1880 (Oscteichthyes, Mugilidae) durante as fases larval e juvenil. B. Inst. Pesca 24 (único), 101–110.
Garling Jr., D.L., Wilson, R.P., 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalarus punctatus*. J. Nutr. 106, 1268–1375.
Hobbie, J.E., Daley, R.R., Jasper, S., 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33, 1225–1228.
Hossain, M.A., Focken, U., Becker, K., 2001. Galactomannan-rich endosperm of Sesbania (*Sesbania aculeata*) seeds responsible for retardation of growth and feed utilization in common carp *Cyprinus carpio* L. Aquaculture, 121–132.
Hossain, M.A., Focken, U., Becker, K., 2003. Antinutritive effects of galactomannan-rich endosperm of Sesbania (*Sesbania aculeate*) seeds on growth and feed utilization in tilapia, *Oreochromis niloticus*. Aquacult. Res. 34, 1171–1179.
Ito, K., Barbosa, J.C., 1997. Nível protéico e proporção de proteína de origem animal em dietas artificiais para a tainha, *Mugil platanus*. B. Inst. Pesca. 24, 111–117.
Krogdahl, A., Bakke-Mckellep, A.M., Barverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar*). Aquacult. Nutr. 9, 361–371.
Kumar, V., Makkar, H.P.S., Becker, K., 2011. Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus mykiss*) juveniles fed detoxified *Jatropha curcas* kernel meal. Aquacult. Nutr. 17, 451–467.
Leenhouwers, J.I., Adjei-Boateng, D., Verreth, J.A.J., Schrama, J.W., 2006. Digesta viscosity, nutrient digestibility and organ weights in African catfish (*Clarias garipinus*) fed diets supplemented with different levels of a soluble non-starch polysaccharide. Aquacult. Nutr. 12, 111–116.
Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108, 95–117.
Moriarty, D.J.W., Pullin, R.S.V., 1987. Detritus and microbial ecology in aquaculture. In: International Center for Living Aquatic Resources Management Conference Proceedings 14, p. 420.
Nery, L.E.M., Santos, E.A., 1993. Carbohydrate metabolism during osmoregulation in *Chasmagnathus granulata* Dana, 1851 (Crustacea, Decapoda). Comp. Biochem. Physiol. 106B, 747–753.
Okamoto, M.H., Sampaio, L.A., Maçada, A.P., 2006. Efeito da temperatura sobre o crescimento e a sobrevivência de juvenis da tainha *Mugil platanus* GÜNTHER, 1880. Atlântica 25 (1), 61–66.
Oliveira, I.R., Soares, L.S.H., 1996. Alimentação da tainha *Mugil platanus* GÜNTHER, 1800 (PISCES: MUGILIDAE) da região estuarino-lagunar de Cananéia. Boletim do Insituto de Pesca, São Paulo, Brasil, pp. 95–104.
Owusu-Asiedu, A., Patience, J.F., Laarveld, B., Van Kessel, A.G., Simmins, P.H., Zijlstra, R.T., 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility and performance of grower pigs. J. Anim. Sci. 84, 843–852.
Paolucci, M., Fabbrocini, A., Volpe, M.G., Varricchio, E., Coccia, E., 2012. Development of biopolymers as binders for feed for farmed aquatic organisms. In: Muchlisin, Z. (Ed.), Aquaculture. InTech, Avaliable from: <http://www.intechopen.com/books/aquaculture/development-of-biopolymers-as-inders-for-feed-for-farmed-aquatic-organisms>.
Pasquier, B., Armand, M., Castelain, C., Guillon, F., Borel, P., Lafont, H., Lairon, D., 1996. Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fibers in acidic gastric medium *in vitro*. Biochem. J. 314, 269–275.
Poersch, L.H., Santos, M.H.S., Mirando Filho, K., Wasielesky Jr., W., 2007. Efeito agudo do nitrato sobre alevinos da tainha *Mugil platanus* (PISCES:MUGILIDADE). B. Inst. Pesca 33 (2), 247–252.
Potter, S.M., 1995. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J. Nutr., 606S–611S.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, Available from: <http://www.R-project.org/>.
Refstie, S., Svihus, B., Shearer, K.D., Storebakken, T., 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharides content in different soybean products. Anim. Feed Sci. Technol. 79, 331–345.
Rimmer, D.W., 1986. Changes in diet and the development of microbial digestion in juvenile buffalo bream, *Kyphosus cornelii*. Mar. Biol. 92, 442–448.
Sampaio, L.A., Ferreira, A.H., Tesser, M.B.T., 2001. Effect of stocking density on laboratory of mullet fingerlings, *Mugil platanus* (Günther, 1880). Acta Sci. 22 (2), 471–475.
Scorvo Filho, J.D., Almeida Dias, E.R., Ayroza, L.M., Colherinas Novato, P.F., 1992. Efeito da densidade sobre o desenvolvimento de alevinos da tainha (*Mugil platanus*) em água doce. B. Inst. Pesca 19 (único), 105–109.
Seeliger, U., Odebrecht, C., Castello, J.P., 1997. Subtropical Convergence Environments: The Coast and Sea in Southwestern Atlantic. Springer Verlag, Berlin, pp. 308.
Siddhuraju, P., Becker, K., 2001. Preliminary nutritional evaluation of Mucuna seed meal (*Mucuna pruriens* var. *utilis*) in common carp (*Cyprinus carpio* L.): an assessment by growth performance and feed utilization. Aquaculture, 105–123.
Silva, D.J., Queiroz, A.C., 2009. Análise de Alimentos: Métodos Químicos e Bilógicos, 3rd ed. UFV, Viçosa, pp. 235.
Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition – a review. Food Chem. 127, 1409–1426.
Storebakken, T., 1985. Binders in fish feeds: Part I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. Aquaculture 47, 11–26.
Tungland, B.C., Meyer, D., 2002. Nondigestible oligo- and polysaccharides (dietary fiber): their physiology and role in human health and food. Compr. Rev. Food Sci. Food Saf. 3, 90–109.
UNESCO, 1983. Chemical methods for use in marine environmental Monitoring. In: Manual and Guides. Intergovernamental Oceanographic Commission, Paris, France, pp. 53.
Vahouny, G.V., Tombes, R., Cassidy, M.M., Kritchevsky, D., Gallo, L.L., 1980. Dietary fibers: V. Binding of bile salts, phospholipids and cholesterol from mixed micelles by bile acid sequestrants and dietary fibers. Lipids 15, 1012–1018.
Vieira, J.P., 1991. Juvenille mullets (Pisces: Mugilidae) in the Estuary of Lagoa dos Patos, RS, Brazil. Copeia 2, 409–418.
Vieira, J.P., Scalabrin, C., 1991. Migração reprodutiva da tainha (*Mugil platanus* GÜNTHER, 1980) no sul do Brasil. Atlântica, Rio Grande 13, 131–141.
Zamora-Sillero, J., Ramos, L.R.V., Romano, L.A., Monserrat, J.M., Tesser, M.B., 2013. Effect of dietary dextrin levels on the growth performance, blood chemistry, body composition, hepatic triglycerides and glycogen of Lebranche mullet juveniles (*Mugil liza* Valenciennes 1836, Mugilidae). J. Appl. Ichthyol. 29, 1342–1347.
Zar, J.H., 1984. Biostatical Analysis, 2nd ed. Prentice Hall, New Jersey, pp. 960.