SALTED SARDINE RESIDUE TO FEED COBIA: ZOOTECHNIC ADVANTAGES AND HISTOLOGICAL RESPONSES

Eduardo Gomes Sanches[1]

Leandro Amaral Herrera[2]

Luciana Yuri Sato[2]

João Shimada Borges[3]

Thais Moron Machado[1]

Vanessa Villanova Kuhnen[1]

[1]Governo do Estado de São Paulo, Secretaria da Agricultura e Abastecimento, Agência Paulista de Tecnologia dos Agronegócios – APTA, Instituto de Pesca, Rua Joaquim Lauro Monte Claro Neto, 2275, Itaguá, CEP 11680-000, Ubatuba, SP, Brasil. E-mail: esanches@pesca.sp.gov.br (corresponding author).

[2]Instituto de Pesca, Programa de Pós-graduação em Aquicultura e Pesca, Av. Francisco Matarazzo, 455, Água Branca, CEP 05001-900, São Paulo, SP, Brasil.

[3]Faculdades Metropolitanas Unidas – FMU, Rua Vergueiro, 2009, Vila Mariana, CEP 04101-905, São Paulo, SP, Brasil.

ABSTRACT

Industrial fish processing generates a significant amount of wastes, which are high-value nutritional by product. Therefore, the use of fish residues could be a sustainable practice for fattening marine finfishes. In the present study, we have evaluated the benefits of feeding cobia juveniles with three different diets based on residues of salted sardine: (i) formulated with acid silage of salted sardine residue; (ii) formulated with salted residue combined with an acidity regulator; and (iii) pure salted sardine residue. Fishes that were feed with pure salted sardine residue had significant body weight gain and also expressed a lower feed conversion rate. Fishes feed with the other two diets presented a similarly lower zootechnic performance. Also, no significant changes indicating a harmful effect of salted sardine residue for cobia feed were observed in the digestive tract of any of the fishes. However, the diet based only on salted sardine residue showed higher organosomatic indexes. That can be attributed to the rich lipid and fatty acid contents of pure residue and it can be an indicative that silage processing was unable to provide the same amount of fatty acids, as pure residue diet did. In conclusion, our results indicate that pure salted sardine residue was the best choice of feed for the cobia. The use of salted sardine residue as diet complementation should be further evaluated, since its use can improve aquaculture development as an instrument of fishery resources conservation.

Key words: aquaculture; hepatosomatic; mariculture; *Rachycentron canadum*; *Sardinella brasiliensis*.

RESÍDUO SALGADO DE SARDINHA COMO ALIMENTO PARA BIJUPIRÁ: VANTAGENS ZOOTÉCNICAS E RESPOSTAS HISTOLÓGICAS

RESUMO

O processamento industrial do pescado gera uma quantidade significativa de resíduos, que são de alto valor nutricional. Portanto, o uso de resíduos de peixe pode ser uma prática sustentável para a engorda de peixes marinhos. No presente estudo, avaliamos os benefícios da alimentação de juvenis de bijupirá com três diferentes dietas à base de resíduos de sardinha salgada: (i) formulada com silagem ácida de resíduo de sardinha salgada; (ii) formulada com um regulador de acidez; e, (iii) puro. Os peixes que foram alimentados com resíduo de sardinha salgada puro tiveram ganho significativo de peso e também expressaram uma menor taxa de conversão alimentar. Os peixes alimentados com as outras duas dietas apresentaram um desempenho zootécnico similarmente menor. Além disso, não foi observada nenhuma alteração significativa no trato digestório dos peixes que pudesse indicar um efeito prejudicial do resíduo de sardinha salgada para alimentação de bijupirá. No entanto, a dieta baseada no resíduo de sardinha salgada puro apresentou maiores índices organossomáticos. Isso pode ser atribuído ao rico conteúdo lipídico e de ácidos graxos do resíduo puro e pode ser um indicativo de que o processamento da silagem foi incapaz de fornecer a mesma quantidade de ácidos graxos. Em conclusão, nossos resultados indicam que o resíduo de sardinha salgada puro foi a melhor escolha dentre os tratamentos avaliados para a alimentação do bijupirá. O uso de resíduos de sardinha salgada como complementação da dieta deve ser melhor estudado, uma vez que seu uso pode melhorar o desenvolvimento da aquicultura como um instrumento de conservação dos recursos pesqueiros.

Palavras-chave: aquicultura; hepatossomatico; maricultura; *Rachycentron canadum*; *Sardinella brasiliensis*.

INTRODUCTION

The fish farming industry, especially the marine extractive fishery, is an activity of great economic importance worldwide. However, industrial fish processing operations generate a significant amount of wastes, 35-50% of which are discarded (Pessatti, 2001). This waste is a high-value nutritional product, rich in proteins and long-chain fatty acids, among which the unsaturated omega-3 fatty acids are prominent (Feltes et al., 2010; Broggi et al., 2017). In parallel, marine finfishes are carnivorous and its farming requires high protein and lipid rations. Therefore, the use of fish processing residue for fattening could be a sustainable practice for marine finfish aquaculture (Ramos et al., 2012).

Marine finfish farming confronts several issues for its development as a productive activity. Among the numerous challenges, we can draw attention to the lack of information about nutritional requirements for marine species (Trushenski et al., 2006). Besides the lack of knowledge, marine species are mostly carnivorous and require high protein diet levels, which significantly increases production costs (Fraser and Davies, 2009; Sanches et al., 2014). For some species, such as the cobia (*Rachycentron canadum*), which have been thoroughly researched, knowledge of the specific requirements of the species is advanced and commercial feeds are already available (Fraser and Davies, 2009).

Experimental protocols captive production have been developed for species with high commercial value, such as dusky grouper *Epinephelus marginatus* (Sanches et al., 2009), lane snapper *Lutjanus synagri*s (Sanches and Cerqueira, 2010) and snapper mutton snapper (Sanches et al., 2013), however, the species of marine fish that has aroused the greatest interest for cultivation has been the cobia (Benetti et al., 2010; Nhu et al., 2011; Philipose et al., 2013). Besides its white meat and economic high value, the species has several zootechnic advantages such as easy captive breeding, good adaptation to confinement, accelerated growth among others (Cavalli et al., 2011). Currently, cobia is the most important marine fish cultured in Brazil and commercial feeds for the cobia are already available in Brazil. Nonetheless, due to the carnivorous needs of the species, cobia's ration is expensive. An alternative used by many farmers to minimize production costs is feeding with trash fish or low-cost fishes (priced below US$3.50 kg^{-1}), such as sardine (*Sardinella brasiliensis*).

Sardine is the main fishing resource in Brazil, it represents 15% of Brazilian fishery industry, which means around of 75 tons of landing per year (Barone et al., 2017). Its availability, low cost, and high fatty acid (EPA) concentration make the sardine a profitable alternative to cobia feeding (Visentainer et al., 2000). Despite its abundance, sardine fishery is seasonal, with two closed fishing seasons along the year (Brasil, 2009). Therefore, due to its intermittent offer, using salted sardine residues can be a good alternative to use sardine as feed for cobia throughout the year. Beyond the pure salted residue, it is also possible to use preservatives to improve its conservation or make acid silages with the salted residue.

Silage is a liquid product prepared with whole fish or its parts through processes that cause such components to liquefy (Arruda et al., 2007). Among the many silage methods, the most prominent is the one in which the raw material is mixed with organic or mineral acids and liquefies due to the action of enzymes present in the fish, and microbial growth is inhibited by the pH acidification (Raa et al., 1982). The nutrient quality of fish silage can be improved by limiting the extent to which proteins are hydrolyzed to polypeptides and free amino acids (Fagbenro and Jauncey, 1993). An alternative to inhibiting protein hydrolysis in fish silage is the addition of salt (sodium chloride, NaCl) to increase the osmotic pressure on fish meat (Raa et al., 1982; Arruda et al., 2007). In light of this, residues obtained from the production of canned salted sardines can be an adequate source of raw material for silage production.

Studies have demonstrated that silage feeding does not exert influence on the nutritional composition of cobias' fillet (Mach and Nortvedt, 2013). However, it is still unknown what physiological consequences of using silage in cobia's diet are. In order to address that, histological approaches are of relevant concern, since its capacity of distinguishing morphological variations in cells, tissues, and organs (Saraiva et al., 2015). In parallel, other organosomatic indexes, such as hepatosomatic and viscerosomatic indexes, can represent an integrative indicator of fish condition (Barton et al., 2002).

Considering that diets have strong effects not only on fish growth but also on its stress tolerance and therefore on its welfare (Ashley, 2007), the goal of this study was to evaluate three different diets to feed cobia juveniles, based on residues of salted sardine. In addition, it aimed at assessing the effect of these diets on digestive organs and health condition of the fishes. Our hypothesis is that fish fed with pure salted sardine residue will present better zootechnical performance and less impact on the digestive tract. Our hypothesis is based on the fact that the pure residue has higher biochemical integrity - due to the absence of additional chemical process - and therefore, greater availability of fatty acids.

MATERIAL AND METHODS

Three diets, based on salted sardine residue, were tested to feed cobia juveniles. Diet 1 was formulated with acid silage of salted sardine residue. This silage was made with 3.5% of glacial acetic acid (composition was: 41.5% of crude protein, 12.4% of ethereal extract, 9.3% of ash and 0.9% of crude fiber). Diet 2 was formulated with salted residue combined with 0.1% of an acidity regulator (Trinken®). This acidity regulator is composed of ascorbic acid, citric acid, lactic acid, bioflavonoids, and polyphenols. It maintains pH acidity also improving feed palatability (composition was: 39.5% of crude protein, 11.6% of ethereal extract, 9.9% of ash and 0.9% of crude fiber). Diet 3 consisted of pure salted sardine residue (composition was: 40.3% of crude protein, 12.5% of ethereal extract, 9.6% of ash and 0.9% of crude

fiber). The sardine residue was obtained from *Indústria e Comércio de Conservas Ubatuba Ltda.* The microbiological quality of the three diets was investigated according to Downes and Ito (2001). The concentration of total coliforms, thermotolerant coliforms, viable aerobic mesophilic, molds and yeasts, *Clostridium* spp and *Salmonella* spp were evaluated for the three diets.

In order to evaluate diets benefits on individual performance, 30 juveniles of cobia (*Rachycentron canadum*) were microchipped and randomly divided into three circular tanks of 3.000 liters. Fishes were weighing 230.0 ± 20.1g, 33.0 ± 1.8 cm length, and were all obtained by captive breeding from Redemar Alevinos laboratory. Each tank had its individual recirculation system with mechanical filtration, skimmer and water sterilization by ultra-violet lamps. Tank recirculation rate was 200%, which amounts to the total water volume being renewed twice a day. The experiments were approved by the Committee of Ethics in Animal Experimentation of the Instituto de Pesca (001/2017).

The experiment lasted 60 days. Fishes were fed twice a day (9am and 5pm). Feed was offered at a feeding rate of 5% body weight/day. To clean the bottom of the tanks, 5% of the tank volume was removed by siphoning daily, after the last meal. This water volume as well as the amount of water lost by evaporation were replaced with deionized water.

Water quality was measured in the sump of each tank daily. Water temperature, dissolved oxygen and pH were monitored by multiparameter probe (model Hanna 9828), total ammonia, nitrite, nitrate and alkalinity by a colorimetric Kit (Tetratest®), and salinity by an optical refractometer (F3000 Bernauer Aquaculture). Throughout the experiment, the photoperiod was natural (13L:11D).

In order to obtain biometric data, fishes were anesthetized with benzocaine (0.05 g L^{-1} water), measured in ictiometer and individually weighed. Zootechnical parameters were calculated as follows: Survival (S,%) = (Px$_f$/ Px$_i$).100, where Px$_f$ = number of fish at the end of period; Px$_i$ = number of fish at the beginning trial. Specific Growth Rate SGR (% body weight day^{-1}) = 100. ((ln W$_2$ - ln W$_1$) Δt^{-1}), where W$_2$ = average final weight; W$_1$ = average initial weight; Δt = rearing period (days). Body weight gain BWG (g day^{-1}) = (W$_2$ - W$_1$) Δt^{-1}, where W$_2$ = average final weight; W$_1$ = average initial weight. Feed Conversion Ratio FCR = C$_f$/ D$_f$, where C$_f$ = Total amount of food consumed during the period; D$_f$ = weight gain during the experimental period.

At the end of the experiment, all fishes were sacrificed by cold-shock and dissected in the abdominal area to remove viscera and liver. Hepatosomatic and viscerosomatic indexes were calculated as follows: viscerossomatic index (VSI,%) = W$_f$/ (W$_t$ 100), where, W$_v$ = weight of the viscera, W$_t$ = total weight. Hepatosomatic index (HSI,%) = W$_f$/ (W$_t$ 100), where W$_f$ = liver weight.

For histological analysis, we followed Stroband et al. (1979) which sections intestine into four histophysiological segments: foregut, midgut, hindgut, and rectum. Fragments from the four parts of the digestive system were sampled and putin McDowell cold solution for 24 hours (4% paraformaldehyde in glutaraldehyde solution at 1% in 0.1M phosphate buffer pH 7.2) (McDowell and Trump, 1976). To dehydrate the tissues, samples were immersed in alcohol 3 times, in ascending series (70% ethanol, 90% ethanol, absolute ethanol), for 1 hour each. After this process, the tissues were submersed for one hour in xylene, three times, until the tissues bleached. Subsequently, samples were submitted to two baths, of1 hour each, in paraffin for embedding. Histological sections of 5µm thick were obtained using microtome (Tolosa et al., 2003). In order to analyze the intestinal tract, hematoxylin, eosin and toluidine fuchsin were used (Bancroft and Stevens, 1982). After that, tissue sections were scanned at 40x magnification using Cannon-EOS 300 camera (Zeiss Standard 25). Three pictures of each blade containing tissues fragments were taken and scanned. Then, three measurements of epithelium depth for each scanned picture were performed using Image-J, a specific measurement program. The measurements and quantification of the number of goblet cells were always performed on a site where there were at least three contiguous and intact crypts in four random fields, a total of 12 crypts being studied in each segment. To measure the thickness of crypts and goblet cell count the selected image was focused by an ordinary optical microscope and captured by a video camera previously coupled to the microscope body. The captured image was digitized and transferred to a microcomputer and analyzed using Image-J. To measure the thickness of the crypts in segments the program marke, the apical and basal limits of each crypt with a cross. After the measurement of 12 crypts, the program automatically provided the average of measured values with the standard error of each segment. Immediately after measuring the thickness, the number of goblet cells was quantified in the same crypt. Thus, the values for the two variables were always obtained in the same crypt.

The experiment was conducted in a completely randomized design with three treatments and ten replications. All data was tested for normality and homogeneity of variances. Average of evaluated parameters were subjected to analysis of variance (ANOVA) for comparison between treatments. Values expressed in percentage (survival, viscerosomatic and hepatosomatic indexes) were transformed according: to the formula = transformed variable arcsen√x. Groups' mean difference was compared using one–way ANOVA. In case of significant differences, the Tukey test (P <0.05) was applied.

RESULTS

The recirculation system used was efficient in keeping the tank water with low suspension solids. The water quality parameters remained within similar values for the three tanks, not influencing experiment results. Mean temperature was 28-30 °C, salinity was 31-34, pH 7.8-8.4, oxidation-reduction potential was 221-245 µS cm^{-3}, ammonia was lower than 1 mg L^{-1}, nitrite was lower than 1 mg L^{-1}, nitrate was 10-25 mg L^{-1} and dissolved oxygen was not less than 5.0 mg L^{-1}. Alkalinity was

Table 1. Microbiological analysis diets based on salted sardine residue *Sardinella brasiliensis* used for cobia *Rachycentron canadum*.

Parameter	Salted Sardine Residue		
	Acid silage*	plus Trinken**	Pure
	diet 1	*diet 2*	*diet 3*
Total coliforms (NMP)	< 3.0	< 3.0	< 3.0
Coliformes a 45 °C.g^{-1} (NMP)	< 3.0	< 3.0	< 3.0
Viable aerobic mesophiles (UFC.g^{-1})	< 10^2	< 10^2	< 10^2
Yeast and molds (UFC.g^{-1})	< 10^2	< 10^2	< 10^2
Clostridium spp (UFC.g^{-1})	< 10	< 10	< 10
Salmonella ssp	Absent	Absent	Absent

*3.5% glacial acetic acid 0.1% additive acidity regulator; **0.1% additive acidity regulator.

Table 2. Zootechnical performance (means ± SD[1]) of cobia *Rachycentron canadum* subjected to different diets.

Parameter	Salted Sardine Residue		
	Acid silage*	plus Trinken**	Pure
	diet 1	*diet 2*	*diet 3*
Length final (cm)	33.1 ± 3.2	34.4 ± 2.4	33.5 ± 3.7
Weight final (g)	345.9 ± 27.4[b]	310.7 ± 28.6[b]	425.3 ± 18.6[a]
Survival rate (%)	100.0	100.0	100.0
Specific Growth Rate (% BW.day^{-1})	1.35 ± 0.62[b]	1.15 ± 0.26[b]	1.66 ± 0.32[a]
Daily Weight Gain (g.dia^{-1})	2.43 ± 1.81[b]	1.46 ± 1.20[b]	3.22 ± 0.80[a]
Feed Conversion Ratio	6.9	6.4	5.2

*3.5% glacial acetic acid; **0.1% additive acidity regulator; [1]values in the same line with different letters indicate significant differences (P <0.05); SD: standard deviation.

110 a 156 mg $CaCO_3$ L^{-1}. The microbiological quality of the three diets was according to tolerance limit values, indicating that the conservation methodology used promoted a sanitary product (Table 1). Consequently, there were no mortalities during the entire trial.

Fishes grew equally in length receiving any of the three diets. However, fishes that were feed with the diet based on pure salted sardine residue had a significant weight gain (Table 2). In addition, these fishes also expressed a higher specific growth rate and lower feed conversion rate. Fishes feed with the other two diets presented a similarly lower zootechnic performance.

Fishes that were fed with pure salted sardine presented higher hepatosomatic and viscerosomatic indexes (Figure 1). There was no difference between the other two treatments.

There were also no histological changes in the intestinal tract of animals submitted to the different diets. Also, no significant differences were observed for intestinal mucosa epithelium depth average (Table 3). It was only observed that, in general, epithelium presented brush border, enterocytes, goblet cells, basal lamina (connective tissue) and possible lymphocytic infiltrates in the four digestive tract sections analyzed. The difference lies in the number of goblet cells, which decreased over the intestine. Thereby, the foregut (Figure 2) had the most goblet cells, followed respectively by the medium (Figure 3), the
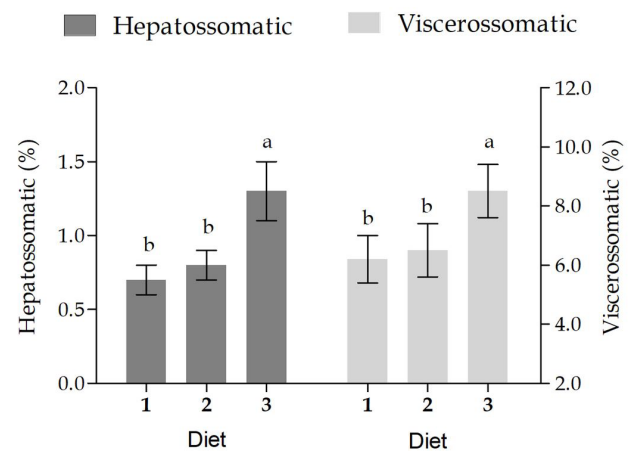

Figure 1. Hepatosomatic and Viscerossomatic index of cobia *Rachycentron canadum* subjected to different diets based on salted sardine residue: Diet 1 = acid silage; Diet 2 = plus Trinken; Diet 3 = pure. (values with different letters indicate statistical differences between treatments at the 5% level and bars represent standard deviation of the mean).

further (Figure 4), and the rectum (Figure 5). No significant changes were observed in the digestive tract in any of the fishes that could indicate a harmful effect of salted sardine residue as cobia feed.

Table 3. Epithelium (means ± SD) of the intestine tract of cobia *Rachycentron canadum* fed different diets.

| Parameter (µm) | Salted Sardine Residue | | |
| | Acid silage* | plus Trinken** | Pure |
	diet 1	*diet 2*	*diet 3*
Foregut	26.03 ± 6.31	25.80 ± 4.69	32.20 ± 4.57
Medium	34.82 ± 6.79	31.91 ± 6.54	31.77 ± 4.54
Further	36.75 ± 7.70	40.10 ± 7.81	31.16 ± 9.00
Rectum	33.45 ± 8.26	35.33 ± 8.35	37.10 ± 8.67

*3.5% glacial acetic acid 0.1% additive acidity regulator; **0.1% additive acidity regulator; SD: standard deviation.
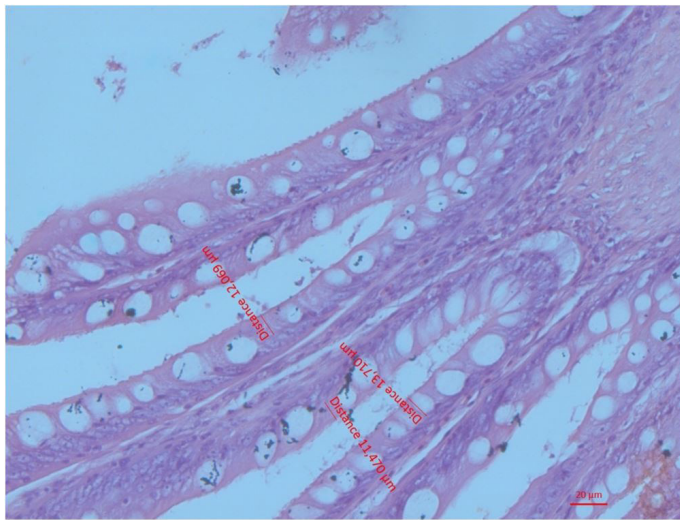

Figure 2. Photomicrograph of the intestinal tract (foregut) of cobia. Rachycentron canadum subjected to different diets based on salted sardine residue.
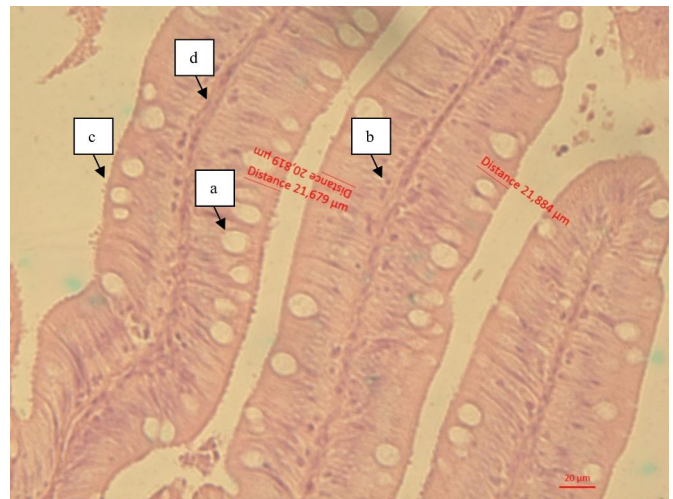

Figure 4. Photomicrograph of the intestinal tract (further) of cobia *Rachycentron canadum* subjected to different diets based on salted sardine residue: (a) goblet cells; (b) enterocytes; (c) brush border; (d) basal lamina.
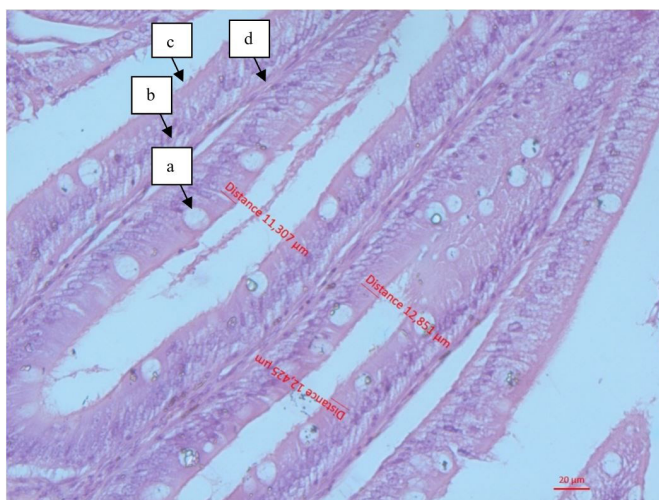

Figure 3. Photomicrograph of the intestinal tract (medium) of cobia *Rachycentron canadum* subjected to different diets based on salted sardine residue: (a) goblet cells; (b) enterocytes; (c) brush border; (d) basal lamina.

Figure 5. Photomicrograph of the intestinal tract (rectum) of cobia *Rachycentron canadum* subjected to different diets based on salted sardine residue: (a) goblet cells; (b) enterocytes; (c) brush border; (d) basal lamina.
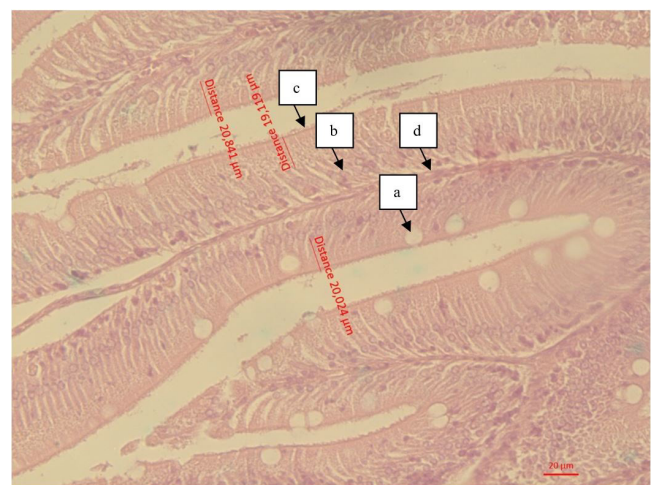
DISCUSSION

Three commercial diets for cobias juveniles (weighing of 26.7 ± 0.9 g in initial density of 1.2 m^{-3} kg) were tested in recirculating systems (Wills et al., 2013). The commercial diets presented 50% and 22%, 49% and 17%, 48% and 17% of crude protein and ethereal extract, respectively. After 57 days, animals fed with higher lipid diet reached superior growth performance (203.3 g body weight and SGR of 3.6). Therefore, although sardine salted residue is not a balanced diet for cobia, the performance parameters results obtained are similar to those achieved using commercial diets. Thus, increases the potential for using fish industry waste for marine fish fattening.

Specific Grow Rate can be an effective parameter to productive performance evaluations. For dusky grouper initial growth stage (from 5.5 g), Ramos et al. (2012) obtained SGR of 1.68 using a 40% protein and 8% ethereal extract. Sanches et al. (2014), incorporating 4% fish oil on dusky grouper diet, obtained SGR of 1.95. Resley et al. (2006) studding cobias weighting 5.7g obtained SGR between 4.7 and 5.4. Those values are higher than obtained by Craig et al. (2006), who reached SGR between 0.74 and 0.75, and by Lunger et al. (2007) who reached SGR between 2.0 and 5.1 for cobias. Evaluating the growth rate in cobias cages located on Caribbean, Benetti et al. (2010) obtained an average of 2.0 SGR, using a commercial diet containing 53% protein and 10% lipids, for fish fattening since 3g through the final weight of 6.0kg. More recently, Silva Junior et al. (2011), evaluated the inclusion of plant ingredients in cobias diet weighing 12 g, obtaining SGR 0.05 to 0.64, which was explained due to the low nutrient absorption from proposed diets. In this study, SGR (1.15 to 1.66) for cobia, remained about the range compared with the reviewed researches. However, the small experimental period (60 days) and the size of the fishes, could justify the presented results.

By combining zootechnic parameters and histological analysis, we were able to demonstrate that salted sardine residues can be used to feed the cobia and its use results in high weight gain and lower food conversion ratio. Both of the other two diets tested (the acid silage and the sardine with acidity regulator), did not present quality satisfying growth results as the pure salted sardine diet did. Although the acidity regulator was supposed to improve palatability, its addition on the residue was noticed by the fishes, which often rejected it. In addition, our findings have demonstrated that diet based solely on salted sardine silage negatively influenced cobia's growth performance.

A similar study of Mach et al. (2010) also found out that diets based on raw fish were better utilized and thus supported growth better than diets based on fish silage. This tendency may be due to the high enzymatic hydrolysis of fish proteins during the silage process. The low pH induces liquefaction and creates an ideal environment for protein autolysis, transforming silage in a source of free amino acids and low molecular weight peptide fractions (Arruda et al., 2007; Olsen and Toppe, 2017). These free aminoacids are absorbed earlier and asynchronously and may thus be more prone to catabolism (Hevrøy et al., 2005). Besides, there is a limited number and specificity of aminoacids transporters in the intestine. Therefore, increasing concentration of free aminoacids in the intestinal tract of fishes will not necessarily represent increasing in metabolic profiteering.

Lipids are other nutrients that can be an alternative to reducing proteic catabolism, thus boosting fish growth. They are an important source of energy and play a vital role in maintaining cell membranes (Carvalho et al., 2018; Xu et al., 2018). High lipid occurrence in diets can be identified by an increase in viscerosomatic and hepatosomatic indexes (Nunes et al., 2011). The liver is an essential organ not only to the metabolic processes but also in stocking proteins, lipids, and carbohydrates. Hence, the accumulation of fat in the liver normally suggests an increase in glycogenesis processes in response to high lipid levels in the diet (Wang et al., 2005). A significant difference in the viscerosomatic and hepatosomatic indexes was observed between the pure salted sardine diet and the other diets, the first showing higher viscerosomatic and hepatosomatic indexes. That may be attributed to the rich lipid contents and fatty acid composition of *S. brasiliensis* (Visentainer et al., 2000). Therefore, the differences in the organosomatic indexes between the diets may be an indicative that the silage process was unable to provide the same amount of fatty acids as pure residue diet did.

Food intake and eating habits affect the digestive system morphology. Therefore, diet can change specific cell types (Kuperman and Kuz'mina, 1994). Bowel size can be associated to dietary habits. Carnivorous species have shorter gut, compared to herbivores, which have long intestine to better use low digestibility diets (Gonçalves et al., 2016). Cobia presents most carnivorous fish digestive tract characteristic, such as developed stomach and short intestine (Kapoor et al., 1975). Individual or grouped, pyloric cecum can be found in previous intestine, along with duodenum opening. Cobias presents numerous small pyloric cecum which can expand digestive capacity (Resley et al., 2006) and should receive more attention in futures researches about this species. Hernandez-Blazquez et al. (2006) related that the anatomical distinction between the hindgut and rectum is difficult, such as observed in this research.

Few studies have histomorphological references to digestive tract sections. The lack of references complicates the correlation between the observed phenomenon in cellular structures among the anatomical occurrence (Barbieri et al., 1989). This study adopted as reference Stroband et al. (1979) which teleost intestines can be histophysiological sectioned into: proximal segment, containing cells specialized in lipids absorption; middle segment, responsible for protein absorption; distal segment, for water and electrolytes absortion. In the present work it was observed differences in the goblet cells distribution along the intestine. Santos and Duarte (2007) reported that in proximal middle and distal intestine of *Pimelodus maculatus* (Pimelodidae, Siluriformes), mucosa showed villi, with cylindrical simple epithelium and numerous goblet cells in duodenum, as well as in this research.

Diet composition can affect not only zootechnic performance and organosomatic indexes but also the digestive system morphology (Kuperman and Kuz'mina, 1994). The digestive system is segmented according to specialized absorption functions such as lipids absorption (foregut), protein absorption (midgut) and water and electrolytes absorption (hindgut) (Stroband et al.,

1979). The characteristic of cobia digestive system reflects its carnivorous diet, with developed stomach and short intestine (Kapoor et al., 1975). Besides, cobia also presents numerous small pyloric cecum, which can expand digestive capacity. From our histological analysis, it is possible to state that salted sardine residue caused no damage to the intestinal epithelium. It is of extreme importance that more efforts are applied in the construction of the knowledge on the histology of marine finfish as it will lead to a better understanding of their nutrition and advances in diet formulation. Few studies are available with histomorphological graphic references, which could improve the interpretation of the observed phenomenon in cellular structures (Faulk et al., 2007; Romarheim et al., 2008). Due to the rate of renewal of the intestinal epithelium, future studies in this subject should address the evaluation of biochemical or physiological alterations aiming at amplifying the effect of the use of salted sardine residue to feed cobia.

In conclusion, our findings provide evidence that acidity regulators do not improve salted sardine palatability and should not be used in cobia diet. In addition, our results have also demonstrated that diet based only on silage is not appropriate for cobia feed. However, we strongly support further studies that evaluate the use of such residue as diet complementation (Olsen and Toppe, 2017; Soltan et al., 2017). Finally, pure salted sardine residue was the best choice for cobia feeding. This represents a promising alternative to reducing residue waste in fish industry, lowering feed costs for producers, and producing an environmentally friendly aquaculture feed. Since fish stocks are a limited resource, a more efficient and intelligent use is needed (Barbieri and Doi, 2012; Blanco et al., 2007). Consequently, a diet based on fish industry residues can improve aquaculture status as an instrument of fishery resources conservation. Further studies are needed to better elucidate the mechanism of salted sardine residue in promoting growth performance of cobia. We are aware that salted sardine residue availability is limited to specific regions. Nonetheless, it is our belief that the dissemination of such findings is of utmost importance to the advancement of aquaculture, as it can incite the conscientious use of the diverse fishery waste available worldwide.

CONCLUSION

Pure salted sardine residue present better zootechnical performance and less impact on the digestive tract of cobia.

ACKNOWLEDGEMENTS

REFERENCES

Arruda, L.F.D.; Borghesi, R.; Oetterer, M. 2007. Use of fish waste as silage: a review. Brazilian Archives of Biology and Technology, 50(5): 879-886. **http://dx.doi.org/10.1590/S1516-89132007000500016**.

Ashley, P.J. 2007. Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science, 104(3-4): 199-235. **http://dx.doi.org/10.1016/j.applanim.2006.09.001**.

Bancroft, J.D.; Stevens, A. 1982. Theory and practice in of histological technicians. London: Churchill. 320p.

Barbieri, E.; Doi, S.A. 2012. Acute toxicity of ammonia on juvenile cobia (*Rachycentron canadum*, Linnaeus, 1766) according to the salinity. Aquaculture International, 20(2): 373-382. **http://dx.doi.org/10.1007/s10499-011-9467-3**.

Barbieri, R.L.; Leite, R.G.; Hernandez-Blazquez, F.J. 1989. Morphologie du tract digestif du curimbatá *Prochilodus scrofa.* Ciência e Cultura, 41(1): 67-70.

Barone, R.S.C.; Lorenz, E.K.; Sonoda, D.Y.; Cyrino, J.E.P. 2017. Fish and fishery products trade in Brazil, 2005 to 2015: a review of available data and trends. Scientia Agrícola, 74(5): 417-424. **http://dx.doi.org/10.1590/1678-992x-2016-0300**.

Barton, B.A.; Morgan, J.D.; Vijayan, M.M. 2002. Physiological and condition-related indicators of environmental stress in fish. In: Adams, S.M. (Ed.). Biological indicators of ecosystem stress. Bethesda: American Fisheries Society. p. 111-148.

Benetti, D.D.; O'Hanlon, B.; Rivera, J.A.; Welch, A.W.; Maxey, C.; Orhun, M.R. 2010. Growth rates of cobia (*Rachycentron canadum*) cultured in open ocean submerged cages in the Caribbean. Aquaculture, 302(3-4): 195-201. **http://dx.doi.org/10.1016/j.aquaculture.2010.02.021**.

Blanco, M.; Sotelo, C.G.; Chapela, M.J.; Pérez-Martín, R.I. 2007. Towards sustainable and efficient use of fishery resources: present and future trends. Trends in Food Science & Technology, 18(1): 29-36. **http://dx.doi.org/10.1016/j.tifs.2006.07.015**.

Brasil. 2009. Portaria nº 15, de 22 de maio de 2009. Regulamenta a captura da sardinha *Sardinella brasiliensis*. Diário Oficial da União, Brasília, 22 de maio de 2009, nº 96, Seção 1: p. 81.

Broggi, J.A.; Wosniak, B.; Uczay, J.; Pessatti, M.L.; Fabregat, T.E.H.P. 2017. Hidrolisado proteico de resíduo de sardinha como atrativo alimentar para juvenis de jundiá. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 69(2): 505-512. **http://dx.doi.org/10.1590/1678-4162-8348**.

Carvalho, M.; Peres, H.; Saleh, R.; Fontanillas, R.; Rosenlund, R.; Oliva-Teles, A.; Izquierdo, M. 2018. Dietary requirement for n-3 long-chain polyunsaturated fatty acids for fast growth of meagre (*Argyrosomus regius* Asso, 1801) fingerlings. Aquaculture, 488(1): 105-113. **http://dx.doi.org/10.1016/j.aquaculture.2018.01.028**.

Cavalli, R.O.; Domingues, E.C.; Hamilton, S. 2011. Desenvolvimento da produção de peixes em mar aberto no Brasil: possibilidades e desafios. Revista Brasileira de Zootecnia, 40(1): 155-164.

Craig, S.R.; Schwarz, M.H.; Mclean, E. 2006. Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics. Aquaculture, 261(1): 384-391. **http://dx.doi.org/10.1016/j.aquaculture.2006.08.004**.

Downes, F.P.; Ito, K. 2001. Compendium of methods for the microbiological examination of foods. 4th ed. Washington: Edwards Brothers. 230p. **http://dx.doi.org/10.2105/9780875531755**.

Fagbenro, O.; Jauncey, K. 1993. Chemical and nutritional quality of raw, cooked and salted fish silages. Food Chemistry, 48(4): 331-335. **http://dx.doi.org/10.1016/0308-8146(93)90313-5**.

Faulk, C.K.; Benninghoff, A.D.; Holt, G.J. 2007. Ontogeny of the gastrointestinal tract and selected digestive enzymes in cobia *Rachycentron canadum* (L.). Journal of Fish Biology, 70(2): 567-583. **http://dx.doi.org/10.1111/j.1095-8649.2007.01330.x**.

Feltes, M.M.C.; Correia, J.F.G.; Beirão, L.H.; Block, J.M.; Ninow, J.L.; Spiller, V.R. 2010. Alternativas para a agregação de valor aos resíduos da industrialização de peixe. Revista Brasileira de Engenharia Agrícola e Ambiental, 14(6): 669-677. **http://dx.doi.org/10.1590/S1415-43662010000600014**.

Fraser, T.W.; Davies, S.J. 2009. Nutritional requirements of cobia, *Rachycentron canadum* (Linnaeus): a review. Aquaculture Research, 40(11): 1219-1234. **http://dx.doi.org/10.1111/j.1365-2109.2009.02215.x**.

Gonçalves, E.L.T.; Sanches, E.G.; Martins, M.L.; Tsuzuki, M.Y. 2016. Fotobacteriose em cultivo de cobia no Brasil. Pesquisa Agropecuária Brasileira, 51(5): 465-472.

Hernandez-Blazquez, F.J.; Guerra, R.R.; Kfoury Junior, J.R.; Bombonato, P.P.; Cogliati, B.; Silva, J.R.M.C. 2006. Fat absorptive processes in the intestine of the Antarctic fish (Richardson, 1844). Polar Biology, 29(10): 831-836. **http://dx.doi.org/10.1007/s00300-006-0121-x**.

Hevrøy, E.M.; Espe, M.; Waagbø, R.; Sandnes, K.; Ruud, M.; Hemre, G.I. 2005. Nutrient utilization in Atlantic salmon (*Salmo salar* L.) fed increased levels of fish protein hydrolysate during a period of fast growth. Aquaculture Nutrition, 11(4): 301-313. **http://dx.doi.org/10.1111/j.1365-2095.2005.00357.x**.

Kapoor, B.G.; Smith, H.; Verighina, I.A. 1975. The alimentary canal and digestion in teleosts. Advances in Marine Biology, 13(1): 109-139.

Kuperman, B.I.; Kuz'mina, V.V. 1994. The ultrastructure of the intestinal epithelium in fishes with different types of feeding. Journal of Fish Biology, 44(2): 181-193. **http://dx.doi.org/10.1111/j.1095-8649.1994.tb01197.x**.

Lunger, A.N.; McLean, E.; Craig, S.R. 2007. The effects of organic protein supplementation upon growth feed conversion and texture quality parameters of juvenile cobia (*Rachycentron canadum*). Aquaculture, 264(1-4): 342-352. **http://dx.doi.org/10.1016/j.aquaculture.2006.12.012**.

Mach, D.T.N.; Nguyen, M.D.; Nortvedt, R. 2010. Effects on digestibility and growth of juvenile cobia (*Rachycentron canadum*) fed fish or crab silage protein. Aquaculture Nutrition, 16(2): 305-312.

Mach, D.T.N.; Nortvedt, R. 2013. Comparison of fillet composition and initial estimation of shelf life of cobia (*Rachycentron canadum*) fed raw fish or fish silage moist diets. Aquaculture Nutrition, 19(3): 333-342. **http://dx.doi.org/10.1111/j.1365-2095.2012.00969.x**.

McDowell, E.M.; Trump, B.F. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Archives of Pathology & Laboratory Medicine, 100(8): 405-414. PMid:60092.

Nhu, V.C.; Nguyen, H.Q.; Le, T.L.; Tran, M.T.; Sorgeloos, P.; Dierckens, K.; Reinertsen, H.; Kjorsvik, E.; Svennevig, N. 2011. Cobia *Rachycentron canadum* aquaculture in Vietnam: recent developments and prospects. Aquaculture, 315(1-2): 20-25. **http://dx.doi.org/10.1016/j.aquaculture.2010.07.024**.

Nunes, A.J.P.; Pinto, R.C.C.; Sá, M.V.C. 2011. Labomar study defines optimal dietary lipid, energy content for fat snook. Global Aquaculture Advocate, 78(5): 74-75.

Olsen, R.L.; Toppe, J. 2017. Fish silage hydrolysates not only a feed nutrient, but also a useful feed additive. Trends in Food Science & Technology, 66(1): 93-97. **http://dx.doi.org/10.1016/j.tifs.2017.06.003**.

Pessatti, M.L. 2001. Aproveitamento dos sub-produtos do pescado. Itajaí: MAPA/UNIVALI. 130p.

Philipose, K.K.; Loka, J.; Sharma, S.R.; Divu, D.; Srinivasa Rao, K.; Sadhu, N.; Dube, P.; Gopakumar, G.; Syda Rao, G. 2013. Farming of cobia, *Rachycentron canadum* (Linnaeus 1766) in open sea floating cages in India. Indian Journal of Fisheries, 60(4): 35-40.

Raa, J.; Gildberg, A.; Olley, J.N. 1982. Fish silage: a review. Critical Reviews in Food Science and Nutrition, 16(4): 383-419. **http://dx.doi.org/10.1080/10408398209527341**. PMid:7047081.

Ramos, F.M.; Sanches, E.G.; Fujimoto, R.Y.; Cottens, K.F.; Cerqueira, V.R. 2012. Growth of juvenile dusky grouper *Epinephelus marginatus* at three different diets. Boletim do Instituto de Pesca, 38(1): 81-88.

Resley, M.J.; Webb Junior, K.W.; Holt, J.G. 2006. Growth and survival of juvenile cobia, *Rachycentron canadum*, at different salinities in a recirculating aquaculture system. Aquaculture, 253(1-4): 398-407. **http://dx.doi.org/10.1016/j.aquaculture.2005.08.023**.

Romarheim, O.H.; Zhang, C.; Penn, M.; Liu, Y.-J.; Tian, L.-X.; Skrede, A.; Krogdahl, Å.; Storebakken, T. 2008. Growth and intestinal morphology in cobia (*Rachycentron canadum*) fed extruded diets with two types of soybean meal partly replacing fish meal. Aquaculture Nutrition, 14(2): 174-180. **http://dx.doi.org/10.1111/j.1365-2095.2007.00517.x**.

Sanches, E.G.; Cerqueira, V.R. 2010. Refrigerated storage of lane snapper *Lutjanus synagris* sperm. Boletim do Instituto de Pesca, 36(1): 293-305.

Sanches, E.G.; Oliveira, I.R.; Serralheiro, P.C.S. 2009. Semen cryopreservation of dusky grouper *Epinephelus marginatus* (Lowe 1834) (Teleostei, Seranidae). Boletim do Instituto de Pesca, 35(3): 389-399.

Sanches, E.G.; Oliveira, I.R.; Serralheiro, P.C.S.; Cerqueira, V.R. 2013. Cryopreservation of mutton snapper (*Lutjanus analis*) sperm. Anais da Academia Brasileira de Ciências, 85(3): 1083-1092. **http://dx.doi.org/10.1590/S0001-37652013005000047**. PMid:23969847.

Sanches, E.G.; Silva, F.C.; Leite, J.R.; Silva, P.K.A.; Kerber, C.E.; Santos, P.A. 2014. Can fish oil incorporation in diet improve the growth performance of dusky grouper *Epinephelus marginatus?* Boletim do Instituto de Pesca, 40(2): 147-155.

Santos, C.M.; Duarte, S. 2007. Histologia e caracterização histoquímica do tubo gastrintestinal de *Pimelodus maculatus* (Pimelodidae, Siluriformes) no reservatório de Funil, Rio de Janeiro, Brasil. Iheringia, Série Zoologia, 97(1): 113-120.

Saraiva, A.; Costa, J.; Serrão, J.; Cruz, C.; Eiras, J.C. 2015. A histology-based fish health assessment of farmed seabass (*Dicentrarchus labrax* L.). Aquaculture, 448(2): 375-381. **http://dx.doi.org/10.1016/j.aquaculture.2015.06.028**.

Silva Junior, R.F.; Nova, W.V.; Farias, J.L.; Costa-Bomfim, C.N.; Tesser, M.B.; Druzian, J.I.; Correia, E.S.; Cavalli, R.O. 2011. Substituição do óleo de peixe por óleo de soja em dietas para beijupirá (*Rachycentron canadum*). Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 63(4): 980-987. **http://dx.doi.org/10.1590/S0102-09352011000400025**.

Soltan, M.A.; Fouad, I.M.; El-Zyat, A.M.; Zead, M.A. 2017. Possibility of using fermented fish silage as feed ingredient in the diets of Nile Tilapia, *Oreochromis niloticus*. Global Veterinaria, 18(1): 59-67.

Stroband, H.W.; Van deer Meer, H.; Timmermans, L.P. 1979. Regional functional differentiation in the gut of the grasscarp *Ctenopharyngodon idella*. Histochemistry, 64(3): 235-249. **http://dx.doi.org/10.1007/BF00495024**. PMid:521316.

Tolosa, E.M.C.; Rodrigues, C.J.; Behmer, O.A.; Freitas-Neto, A.G. 2003. Manual de técnicas para histologia normal e patológica. 2ª ed. São Paulo: Manole. p. 331.

Trushenski, J.T.; Kasper, C.S.; Kohler, C.C. 2006. Challenges and opportunities in finfish nutrition. North American Journal of Aquaculture, 68(2): 122-140. **http://dx.doi.org/10.1577/A05-006.1**.

Visentainer, J.V.; Carvalho, P.D.O.; Ikegaki, M.; Park, Y.K. 2000. Concentração de ácido eicosapentaenóico (EPA) e ácido docosahexaenóico (DHA) em peixes marinhos da costa brasileira. Ciência e Tecnologia dos Alimentos, 20(1): 90-93. **http://dx.doi.org/10.1590/S0101-20612000000100017**.

Wang, J.T.; Liu, Y.J.; Tian, L.X.; Mai, K.S.; Du, Z.Y.; Wang, Y.; Yang, H.J. 2005. Effects of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (*Rachycentron canadum*). Aquaculture, 249(1-4): 439-447. **http://dx.doi.org/10.1016/j.aquaculture.2005.04.038**.

Wills, P.S.; Weirich, C.R.; Baptiste, R.M.; Riche, M.A. 2013. Evaluation of commercial marine fish feeds for production of juvenile cobia in recirculating aquaculture systems. North American Journal of Aquaculture, 75(2): 178-185. **http://dx.doi.org/10.1080/15222055.2012.750635**.

Xu, H.; Cao, L.; Wei, Y.; Zhang, Y.; Liang, M. 2018. Lipid contents in farmed fish are influenced by dietary DHA/EPA ratio: a study with the marine flatfish, tongue sole (*Cynoglossus semilaevis*). Aquaculture, 485(2): 183-190.