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Morphometry in Coronary Arteries of Beijupira (*Rachycentron canadum*) Fish with Tunics Hyperplasia

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Authors' contributions

This work was carried out in collaboration among all authors. Author VFP made the analysis and wrote the study. Author RFM collaborated making the statistical analysis of the data. Author CNB collaborated in the collection of animals and material for analysis. Authors ESM, FLS and LAR were guiding the conduct of research. All authors read and approved the final manuscript.

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ABSTRACT

Tunics hyperplasia of blood vessels can be quantified by a range of techniques that relate to modifications of area or thickness on histological sections. We studied hyperplasia of tunics of coronary arteries in a fish species, *Rachycentron canadum* for establishing the relationship between tunics and luminal obstruction by morphometry. The morphometric analysis was performed on 158 arterial vessels of 40 fish samples. The histological slides of cardiac and vascular tissue were stained using hematoxylin and eosin and observed under an optical microscope. The data relating to the proportion of the lumen area compared to the area of the tunic

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media were analyzed according to Beta regression models. The lumen proportion showed a decreasing relationship with weight and length of the analyzed animals. A linear relationship was not observed between lumen proportion and areas and/or radius of arterial tunics. It is concluded that the increase in weight gain contributes to a reduction of lumen area, leading to arterial obstruction without increasing or decreasing relationship between the proportion of vascular lumen and the thickness of arterial tunics. The set of abnormalities described are likely to be attributed to nutritional factors during the fish cultivation.

Keywords: Arteriopathy; histology; nutrition; Rachycentron canadum.

1. INTRODUCTION

Morphological and morphometric anatomical studies of coronary arteries are widely made in human pathology by using X-rays and various types of molds [1]: morphometry of the coronary microvasculature [2,3], the modified technique of Wearn and patterns of branching of arterioles, capillaries and venules of domestic animals [4] and recent studies on the length of capillaries in humans, studying the microvascular structure through three-dimensional reconstruction [5].

The hyperplasia of blood vessels tunics is an accumulation of smooth muscle cells aggregated to a set of tissue elements which lodge in the inner vascular layer (intimate tunic). It can be measured by a series of techniques, by relating the measurement of blood vessel modifications related to area or thickness in histological sections [6].

The intimal hyperplasia commonly accompanies the progression of a more severe arterial disease, such as atherosclerosis [7]. The etiology and relationship between them is well elucidated in other species, not being quite researched or even notified in non-anadromous teleost fish. Researches involving vascular lesions in fish are restricted to coronary alterations associated with sexual maturation and spawning in salmon [8,9,10] and trouts [11,12].

The atherosclerotic lesion, previously characterized by accumulation of cholesterol in vessels, is currently related to the interaction of a series of risk factors such as accumulation of modified lipids, macrophages, recruitment of smooth muscle cells (SMC), circulating and tissue molecules [13]. Such facts allow us to affirm that the inflammation process is present in all the phases of atherosclerosis process [14].

Among the predisposing factors for onset of atherosclerosis, metabolic disorders, visceral obesity, hypertriglyceridemia, hyperglycemia and low HDL (High Density Lipoprotein) are identified as important elements that are associated with oxidative stress representing an important factor for developing cardiovascular complications [15].

The coronary artery hyperplasia in beijupirá (*Rachycentron canadum*, Linnaeus, 1766) was reported from our laboratory [16]. The present work aimed to establish the relationship between tunics and luminal obstruction through morphometry, evaluating the thickening of the tunic during from offshore cultivation period, with the growth of the animal.

2. MATERIALS AND METHODS

The specimens of beijupira were obtained from offshore cultivation situated 5 km away from the coast, in the state of Pernambuco/Brazil, for seven months of farming. The animals were transported alive under constant aeration and renovation of water to the port of Recife/PE [16], where sedation was carried out followed by euthanasia, by immersion in benzocaine hydrochloride (p-Aminobenzoic acid ethyl ester) [17]. Then, the fish were subjected to external examination and necropsy with left side opening and heart samples collection for histopathological and morphometric examination [18].

Heart tissue samples were fixed in 10% buffered formalin and, after dehydration in an increasing ethanol series, the tissues were embedded in paraffin and sectioned in the microtome to five microns.

The arterial morphometric analysis was carried out in 40 fish samples of *Rachycentron canadum*, eight fingerlings and 32 juveniles, where 158 arterial vessels were evaluated (Fig. 1). The histological slides of heart and vascular tissue were stained using hematoxylin and eosin (HE) [19] and then observed under optical microscopy.

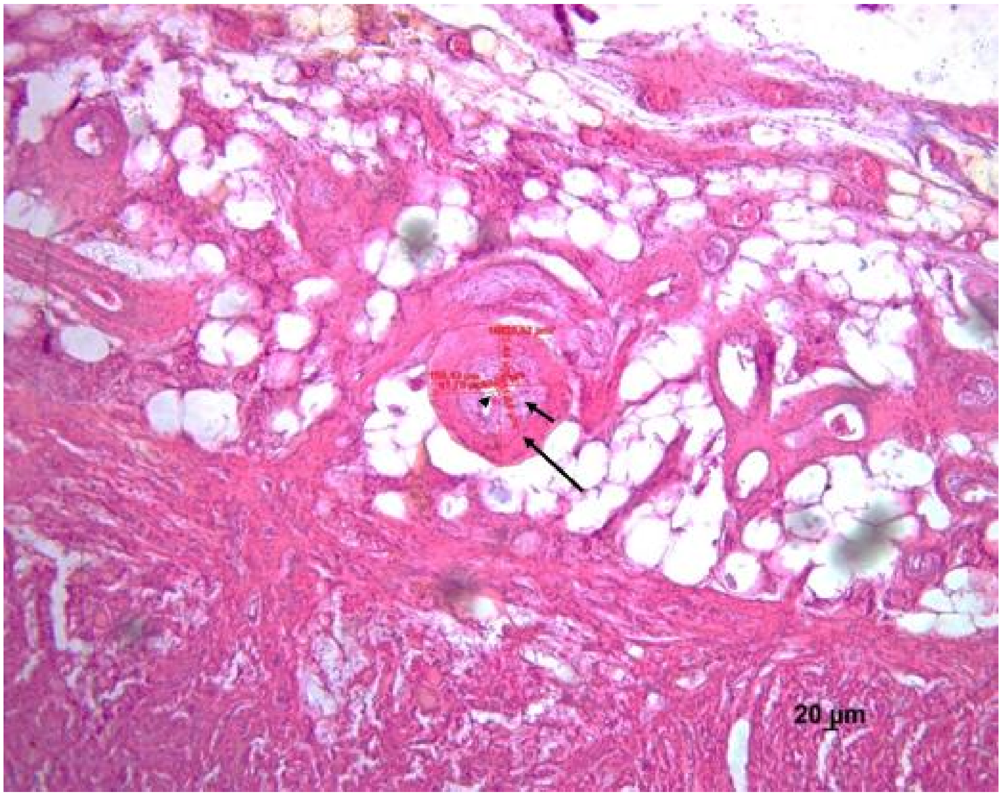

Fig. 1. Morphometric analysis of the coronary arteries in *Rachycentron canadum*. Vascular Lumen (arrow head); Intimate Tunic (short arrow); Media Tunic (long arrow). H&E-staining, Bar = 20 51x37 mm

The images of cardiac tissue sections were digitalized for morphometric analysis with light microscope Primo Star Zess and the digital camera AxioCam ERc5s. The image analysis was carried out with software AxioVision 4.8.2.0 and the planimetry of the lumen areas of coronary arteries and intima, media and adventitia tunics was carried out randomly.

Initially, a descriptive analysis of the variables was performed, being of interest the proportion of vascular lumen area (*proplum*). The purpose of the study was to evaluate the relationship of this ratio with other variables.

For the modeling proposal, data regarding the ratio of lumen area and tunica media area was analyzed according to Beta regression models for rates and proportions [20,21]. We investigated the influence of the following variables *areaext*: area of the outer layer (tunica adventitia), *arealum*: lumen area, *areamed*: area of the tunica media, areaint: Area of the tunica intima, *radiusext*: radius of the tunica external, *radiusmed*: radius of the tunica media, *radiusint*: radius of the tunica intima, *radiuslum*: lumen radius, *diamvessel*: vessel diameter, *diamlum*: lumen diameter, weight and length of the specimens of beijupira collected and cultivation time (in months).

3. RESULTS AND DISCUSSION

Throughout the collection period in cultivation, the parameters of temperature, salinity, oxygen and transparency along the cultivation ranged between 26,1-29,9ºC; 31,0-37,3 gL-1; 5,2-8,0 mgL-1 and 6,0-17,5 m, respectively.

Signals of luminal stenosis can be visualized in coronary arteries subjected to morphometric analysis (Fig. 2). It was used for statistical analysis free software R (available in https://www.r-project.org/). The influence of the variables was verified by carrying out tests of marginal hypotheses according to the normal distribution, considering a significance level of 5%.

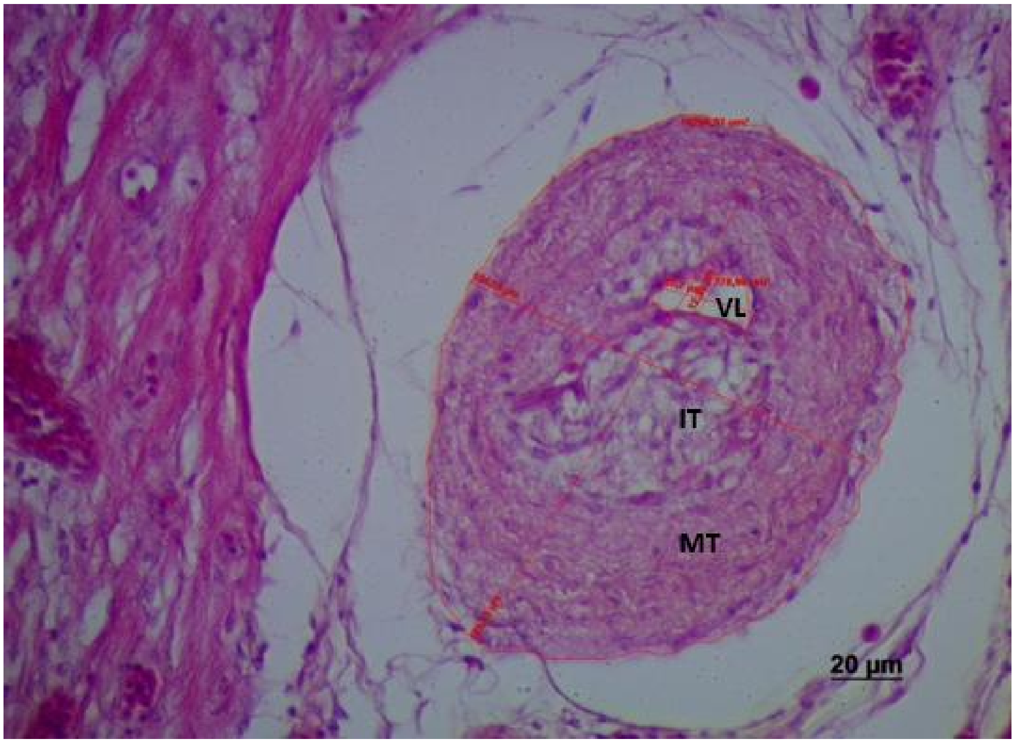

Fig. 2. Morphometric analysis of the coronary arteries with luminal obstruction in *Rachycentron canadum*. VL: Vascular Lumen; IT: Intimate Tunic; MT: Media Tunic. H&E-staining, Bar = 20 51x37 mm

The descriptive measures of the variables are observed, showing that the fish weight in a range of 0.078 - 1,025 kg. With regard to the length of the fish, the average value was 36.468 cm, ranging between 24 and 49 cm. The intervals for variables average were constructed with 95% confidence.

In Fig. 3, it can be verified that the median values of lumen proportion decrease along the months. The greater median ratio was observed in second and fourth month, while the smaller median proportions of lumen were observed in sixth and eighth month. During the third month, there was little variability between the lumen proportions registered. The higher variability in proportions was observed in the second, fifth and seventh month.

There was no visible linear relationship of the lumen proportion with areas and rays (thickness) of arterial tunics.

Considering weight and length, the lumen proportion showed a decreasing relationship with both variables mentioned, that is, the lumen ratio observed increases when the weight of the fish decreases, the same happened in relation to fish length.

For the selection of the significant variables to explain the proportion of lumen area, hypothesis tests of the type Z [22] were used. To evaluate the suitability of the model, the graph of quantile was used with simulated envelope (confidence bands). The final adjusted model is given by:

$$\mu=\frac{\exp\{0.06*areaint-12.25*radiusext-24.26*radiusmed-52.16*radiusint+3,14*diamvessel-0.86*weight\}}{1+\exp\{0.06*areaint-12.25*radiusext-24.26*radiusmed-52.16*radiusint+3.14*diamvessel-0.86*weight\}'}$$

Where, µ is the average proportion of lumen area in relation to the area of tunica media.

Considering tests of the type Z, all parameters are significant at a significance level of 5%. Through graphs of the quantiles in Fig. 4, the vast majority of points are randomly located within the confidence bands, indicating that the Beta distribution for the proportion of the lumen is correctly specified.

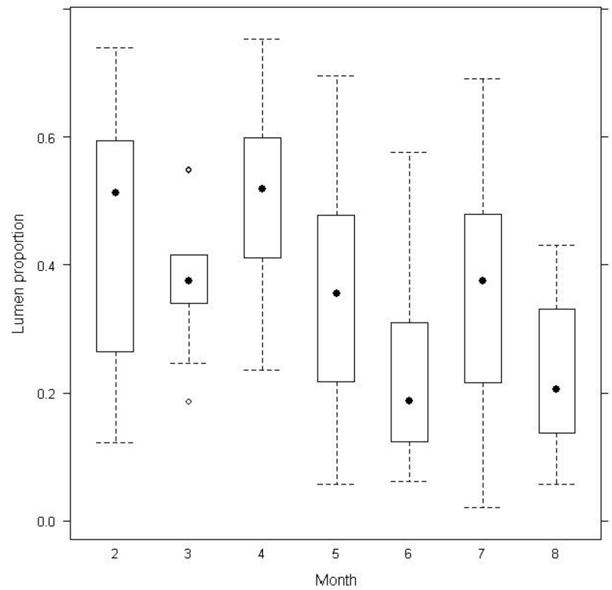

Fig. 3. Proportion of the lumen of coronary arteries in relation to the months of cultivation

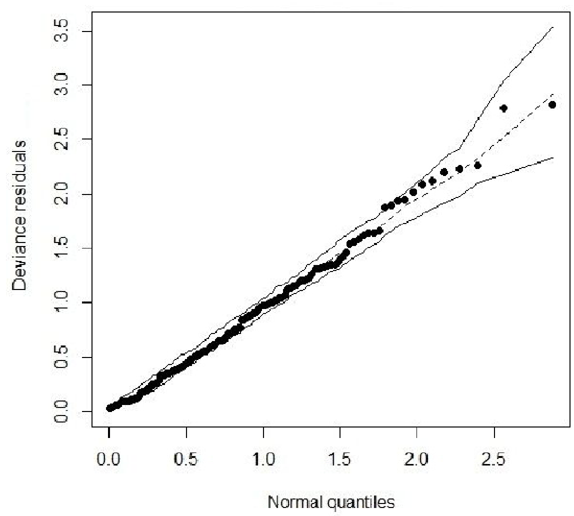

Fig. 4. Quantiles with simulated envelope for residues of the Beta model adjusted for proportions of the lumen

The occlusive lesions in coronary arteries described here have already been identified in humans because these arteries are capable of developing clinical manifestations of atherosclerotic disease, presenting a greater degree of stenosis when compared to other arteries [23].

The image analyzer enables a more precise characterization of histological areas studied [24].

Arterial lesions similar to those exposed here have been described in some species of anadromous fish. All the salmonids, when they reach the size of about 10 cm, initiate the development of coronary changes that can be identified microscopically as atherosclerosis [25]. The progression of lesions is reported to be associated with the migratory habit during sexual maturation, involving also rapid growth rate by inducing stress, contributing to the start of a coronary lesion. In contrast, the animals examined in this study exhibited a weight gain associated with a higher rate of luminal occlusion, with immature animals also being used in present work, without involving periods of maturation in the appearance of the lesion.

Lipids have an essential role in the reproductive life of salmonids, supplying energy to the reproductive activity and being consumed during sexual migration [26]. The animals analyzed here and identified with arterial injury were young, aged less than the period of sexual maturity of the species. The coronary lesions reported in salmon [8,9,10] and trout [11,12] are related to periods of sexual maturation and spawning, leading us to believe that fat mobilization required for this phase could arise as a possible trigger for the onset of lesions.

The high level of body fat in humans is clearly associated with changes in lipid metabolism and endothelial dysfunction [27], increasing prevalence of cardiovascular disease [28].

Hyperlipidemia is also studied with young mice in relation to the emergence of atherosclerotic lesion [29] and, recently, it is reported that the reversion of hyperlipidemia after implantation of atherosclerotic lesions, even in advanced stages, generates a reduction in the levels of CD68 cells that are derived from macrophages and foam cells in atherosclerotic plaques. Phenotypic changes with migratory behavior *in vivo* and decreased expression of genes encoding inflammatory and pre-thrombotic factors can also be observed [30].

The results found here suggest that the increase in growth, weight and length contributes to the reduction of lumen area, predisposing to arterial obstruction. Previous researches underscore the importance of studies on the real involvement of dietary factors, mainly polyunsaturated fatty acids on the proliferation of smooth muscle cells in coronary arteries with consequent development of atherosclerotic lesions in salmon [25], reinforcing the importance of this factor in the cultivation of the fish species described here, and probably with similar injuries caused by dietary factors.

The fish analyzed here were fed a commercial diet with lipid content of 7.93%, with polyunsaturated fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic (EPA), in values above the requirement of the species [31]. Although polyunsaturated fatty acids present have beneficial effects on vascular function [32], their use must be controlled in diet, because of the possibility of excessive weight gain due to high energy content in the diet [33], because beijupira has a reduced ability to utilize lipids as an energy source, choosing the protein as its main energy source [34].

There are reports stating that lipid peroxidation begins in polyunsaturated fatty acids of phospholipid from the surface of LDL (low density lipoprotein), then spreading to the lipid core, contributing to oxidative modification of polyunsaturated fatty acids, cholesterol and phospholipids and finalizing with the modification and degradation of apoB, that contributes to the progression of atherosclerosis [35,36].

Obesity in rats is associated with elevated plasma concentrations of acute phase proteins, such as amyloid A protein (serum amyloid-associated protein – SAA) and C-reactive protein [37,38], which are considered predictive of the occurrence of vascular disease [39].

Experimental models using mice with apolipoprotein E (ApoE) deficiency demonstrate increased body weight, stimulating atherosclerosis when fed a diet with a high fat percentage, presenting increased plasma concentration of SAA [39]. Surveys still report that high concentrations of SSA represent an important tissue marker of acute and chronic inflammatory diseases [40], demonstrating the association of CRP with the risk of cardiovascular changes [41], that contributes to inflammation and increased tissue injury [42].

Hyperlipidemic condition is described in rabbits after a high-cholesterol diet that contributes to the formation of a thrombus in the tunica intima, inducing injury and making the neointima rich in macrophages and fibrin plates in thrombus [43].

As mentioned, some animals are used as experimental model to evaluate vascular hyperplasia. Swine are often selected as a model for monitoring the lesion, due to the similarity with human in the response to vascular injury, so morphometric studies are carried out for controlled evaluation of chronic arterial injury [44]. Similarly, rodent models are also found to be useful because they are animals with short life cycle, available for genetic manipulation, presenting relative low cost laboratory maintenance, and mainly because of the similarity with humans in the spontaneous development of atherosclerosis during their lifetime [39].

The discussion with regard to the aspect of body fat content in this study is limited, since we did not perform any body composition analysis on the species and the ration supplied. This makes it difficult to establish the possible exact relationship between dietary factors and the described lesions. The observations reported here lead to the need for detailed research about what was discussed in relation to other species where the coronary lesions are well defined.

4. CONCLUSION

The increase in weight gain contributes to the reduction of lumen area, leading to arterial obstruction in the fish *Rachycentron canadum* and there was no increasing or decreasing relationship between the proportion of vascular lumen and the thickness of arterial tunics.

ETHICAL APPROVAL

All experimental procedures were conducted in accordance with the protocol of number 23082.008952/2010 approved by the "Ethics Committee on the use of animals (CEUA)" of Universidade Federal Rural de Pernambuco.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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