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Rigor Index of juvenile cobia (*Rachycentron canadum*): study with anesthesia (eugenol) and hypothermia

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***Abstract* - *Consumers rigorously assess the quality of fishing products, considering mainly freshness and appearance. The slaughter method is an important factor in relation to the freshness of the fish, because it maintains important organoleptic characteristics that will influence the marketing of these products, therefore, in this study an evaluation of the Rigor Index (RI%) of cobia was performed. The following methods were used: in desensitizing with eugenol and hypothermia. Immediately after the slaughter, the monitoring of rigor mortis began. In the method of slaughter with eugenol reached full rigor after 6 hours, and in the method by hypothermia lasted 4 hours. The slaughter method with lower stress showed a significant difference, with a positive influence on the duration of the stages of rigor mortis.*

I. INTRODUCTION

The evaluation of the freshness of the fish can be done through sensory, physicochemical and microbiological methods, but due to the subjectivity of sensory methods and high costs and delays in microbiological tests, the evaluation fish freshness is often made by chemical methods that count the products that enzyme and bacterial activities produce [1]. Some factors such as: postmortem degree of energy exhaustion (*rigor mortis*); physical damage, cleaning and hygiene influence the freshness of the fish [2].

The process of *rigor mortis* is marked by the muscle contraction of an animal after its death, so the animal muscle loses elasticity [1]. It is correct to affirm that *rigor mortis* is a direct consequence of ATP (adenosine triphosphate) present in the body. When ATP concentrations are low, when there is a large energy expenditure before or during slaughter, the stiffness of muscle fibers appears faster because of this ATP expenditure [3]. This ATP expenditure is linked to metabolic anaerobic due to the lack of oxygen availability of the muscle, then moving to the degradation of muscle glycogen to be the main source of metabolic energy. Thus, the muscle starts to produce lactic acid, which comes from glycolysis (glycogen breakage) and hydrolysis of ATP, causing a decrease in muscle pH and a stiffening of the muscle [4].

The period of *rigor mortis* will vary according to each type of species, physiological factors, degree of exhaustion of fish, size, water temperature and cultivation [2]. In addition, the stress associated with the management at the time of slaughter will directly influence the reduction of *rigor mortis* time, consequently, on shelf life.

Over time, the choice of slaughter methods is based almost exclusively on ease of application and those with the lowest cost, and not on those that cause less suffering

[5]. Several behavioral, anatomical, and physiological studies have proven that similarly to birds and mammals, fish could feel pain and fear [6].

The slaughter process of fish can be defined in two stages, the first is the stunned stage, where organisms are sensitized; the second stage is the sacrifice. These two stages can occur simultaneously or in separate actions [5].

Quality slaughter, considered ideal, should be easy and fast to perform, hygienic, in addition to causing the least possible damage to the integrity of the meat [7]. Among the most used, we highlight the techniques of humane slaughter, which use methods of in desensitizing such as termonarcose, bone marrow section, gill sangria, immobilization by electrical impulses, and asphyxia in CO_2.

The use of eugenol is among the most used in desensitizing methods in fish slaughter. In addition to the low acquisition cost [8], the use of eugenol does not cause any harmful effect on fish tissues, thus maintaining the quality of the product [9], and causes rapid deep anesthesia in fish [10]. Therefore, this method, in addition to possibly bringing benefits to the productive chair such as support in logistics and longer shelf life, also ensures a humane slaughter, considering the animal welfare which consumers are increasingly interested in.

As opposed to methods with anesthesia, thermal shock slaughter by immersing fish in cold water is one of the most used for slaughtering fish and is not well accepted in terms of animal welfare, since the loss of function is not immediate, leading individuals to prolonged stress [11].

The cobia, *Rachycentron canadum* (Linnaeus, 1766) is a coastal pelagic species with a large presence in the Atlantic Ocean, which in a natural environment can reach up to 60 kg and measure 2 m in length, which currently has great potential in aquaculture, this potential is given by easy obtaining of spawning in captivity, high fertility, rusticity of the species in addition to the high rate of adaptation to artificial systems, having great commercial capacity in Brazil [12].

The present study aimed to observe the process and determine the rate of *rigor mortis* of the juveniles cobia (*Rachycentron canadum*) created in captivity, comparing the results to two different methods of slaughter.

II. MATERIAL AND METHODS

The cobias (*Rachycentron canadum*) (Fig. 1) used in the experiment were acquired from the Laboratory of Nutrition and Propagation of Aquatic Organisms (LANPOA) of the Federal Institute of Espírito Santo Campus Piúma.

Fig.1. Specimen of juvenile cobia (Rachycentron canadum) used in the study.

A random sample of 6 specimens was removed from the recirculating aquaculture systems, and were immediately slaughtered. In order to verify the influence of the form of slaughter on the Rigor Index (RI%), two different slaughter methodologies were tested as described in Table 1.

Table 1. Description of the slaughter methods

Method slaughter	**Description**
Eugenol and bone marrow section	Immersion for 10 minutes in eugenol solution at the ratio of 1.5ml/L and subsequently sacrifice by means of a cross section of the spinal cord immediately after the occipital region.
Hypothermia	Immersion for 10 minutes in water/ice slurry (ratio of 1:1).

After slaughter, the total length of the fish and the weight of each specimen were measured. According to the methodology proposed the Rigor Index (RI%) was measured and calculated according to the equation (1) [13], where D0 is the distance value that separates the base from the caudal fin to the reference point, immediately after death and Dt is the distance value that separates the base from the caudal fin to the reference point at the time intervals selected as illustrated in Fig. 2.

Equation (1):

$$\text{Rigor Index (RI\%)} = [(D0 - Dt) / D0] \times 100$$

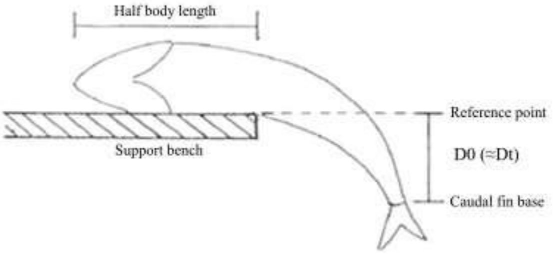

Fig.2. Illustrated adapted from the methodology proposed for the calculation Rigor Index (RI%) [13].

The process of determining the stage of *rigor mortis* began shortly after the slaughter, being measured every 20 minutes. The experiment lasted 15 hours and was carried out at the Ifes Piúma Fish Processing Laboratory. Initial temperature and temperature variation throughout the day were measured with the aid of a portable digital thermometer with an ION© skewer probe with temperature range from -50ºC to 200ºC.

The values of the Rigor Index (RI%) were submitted to statistical analysis of variance (ANOVA) for the significance of 5.0% with Post-hoc Tukey's test, using the program BioStat (AnalystSoft).

III. RESULTS AND DISCUSSION

For the method of slaughter with eugenol and bone marrow section the mean total length of these, it was 23.2 cm and the average weight 61.74g; hypothermia slaughter was 22.9 cm and the average weight of the specimen's equivalent to 68.49g.

In the method of slaughter of fish by hypothermia there was no muscle contraction of the fish for approximately 20 min., the mean D0 was 4.57 cm, *pre-rigor* stage. The process of *rigor mortis* occurred in a growing way and lasted 4 hours to reach *full rigor*, after slaughter. Staying for 4:40 hours in *full rigor*, and then the *post-rigor* stage begins.

The method of desensitizing with eugenol the mean D0 was 4.77 cm, *pre-rigor* stage. The process of *rigor mortis* occurred in a growing way, but more time-consuming reaching *full rigor* after 6 hours remaining for 2:40 hours in *full rigor* and later starting the *post-rigor* stage.

The initial ambient temperature at the site of the measurements was 24.7ºC and ranged from 23.8ºC to 27.2ºC throughout the day. Fig. 3 shows the development of the Rigor Index (RI%) in juveniles cobia in the two slaughter methods.

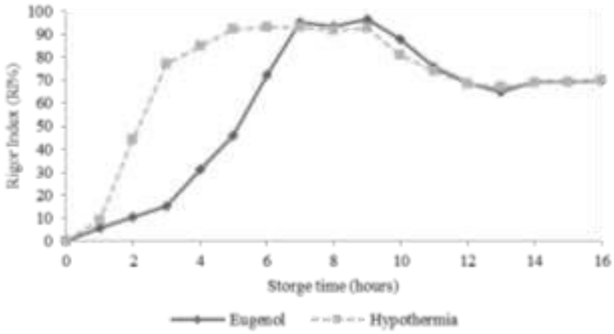

Fig.3. Development curve Rigor Index (RI%) in juveniles cobia (Rachycentron canadum) slaughtered by hypothermia methods and unsensitized with eugenol.

Table 2. Values of the Rigor Index (RI%) between the treatments used for juveniles cobia (Rachycentron canadum) slaughter (n= 46 measurements).

	Rigor Index (RI%)
Methods	**means ± SD**
Hypothermia	73.66±3.68ª
Eugenol	60.31±6.34ᵇ

Note: Different lowercase letters indicate statistical difference, ANOVA test, with Post-hoc Tukey's test, $p < 0.05$.

According to the ANOVA variance analysis, the $p < 0.05$ value was observed (Table 2), that is, there was a significant difference between the two slaughter methods. It can be observed that in the hypothermia slaughter method, the *rigor mortis* begins immediately after the slaughter of the animal, already in the method of desensitizing with eugenol and bone marrow section was delayed for longer compared to the other method.

There are several methods of sacrificing fish, such as: asphyxiation (in air or ice), evisceration, thermal shock (hypothermia), electric shock, immersion in water saturated with CO_2, anesthetics (eugenol), bone marrow section, among others. Since the technique employed at the time of slaughter can slow or even intensify the onset of *rigor mortis* [5][14]. In addition, slaughter is one of the most stressful steps in animal production, its effects can generate undesirable sensory aspects and accelerate the processes of fish deterioration, reducing the shelf life of the product [4].

The results show that the behavioral reactions caused by each treatment end up influencing the initial time of *rigor mortis* thus, we can say that until 6 hours after slaughter, the cobia sacrificed with eugenol remained fresher in relation to those slaughtered by hypothermia.

According to study the *rigor mortis* predetermines the shelf life of the fish, so if we extend or prolong this process, consequently there will be a decrease in the development of deteriorating microorganisms, ensuring fresh and quality meat [15].

Rigor mortis occurs simultaneously with decreased amount of glucose and ATP present in the fish muscle and together with the degradation of glycogen in lactic acid [4][16], These processes occur quickly when we have an imbalance of homeostasis, the stress generated during the hypothermia slaughter process can generate panic, discomfort and leakage situations, causing fish to use their energy reserves and consequently accelerate the stages of *rigor mortis* [5].

Evaluating tambaqui quality (*Colossoma macropomum*), sacrificed by asphyxiation and kept on ice, the stage of *full rigor* occurred 30 minutes after slaughter [14]. For matrinxãs (*Brycon cephalus*) slaughtered and kept on ice, the stage of *full rigor* occurred at 75 minutes after death [15]. The use of eugenol also showed greater efficacy in reducing post-slaughter strep in the study by [16] with juvenile common snook.

These results show that both methods used in the present study were efficient for cobia slaughter, managing to considerably delay the arrival time in the stage of *full rigor*. However, the use of eugenol presented the best result because it achieved the highest results of the Rigor Index (RI%). Eugenol, in addition to having antiseptic effects it also acts in reducing stress in fish [17][18][19].

The initial period of *rigor mortis*, duration and completion time can be influenced by several factors, such as fish fat degree, species, morphometric data and slaughter methods [4][20]. Knowing the time that the cobia created in captivity enters the stage of *rigor mortis* is fundamental for creating a specific management protocol, aiming at a better use in the stage of fish processing, especially in the production of fillets, because it is essential that during this stage the fish is still in the *pre-rigor* stage, resulting significantly in sensory qualities, such as texture and color of fresh fillets [4][21][22].

This stage can be delayed with the cooling of the fish shortly after its slaughter, increasing the service life and maintaining the quality of the product. In addition, conservation is important because it acts directly in the decrease in microbial actions after the process of *rigor mortis* [20].

A higher RI% value reveals a longer period of the *rigor mortis* process, this increase gives the fish longer freshness time. At 15 hours of the experiment, it was observed that the slaughter by eugenol presented the highest value of the Rigor Index (RI%) (69.76±6.04) then by hypothermia (70.02±2.12).

IV. CONCLUSION

There was a significant difference between the different methods of slaughtering juveniles cobia to determine the Rigor Index (RI%). The eugenol slaughter method showed a delay in the beginning of the *rigor mortis* process, indicating a less stressful methodology positively influences the duration of the *rigor mortis* stages, and consequently the freshness of the fish, enabling better quality products and longer shelf life.

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