Aquaculture 486 (2018) 161–169

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Investigation of farmed Nile tilapia health through histopathology

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ARTICLE INFO

Keywords:
Fish farming
Welfare
Histology
Epitheliocystis
Parasites

ABSTRACT

Confinement is a stressful practice for fish, which can culminate in tissue damage and production losses. Histopathological examination is a technique capable of forthcoming the normal and pathological conditions in fish. It reflects the animal's health and welfare, being able to apply measures to mitigate the stress and diseases in fish farming. This study evaluated the health status of cultured Nile tilapia using histopathological examination as a tool. Fragments of gills, liver, heart and spleen of 60 tilapias from fish farms in southern Brazil were collected and fixed. All crops were free of sanitary problems and fish were clinically healthy. This study showed a positive correlation between *Trichodina* species, protozoans and hyperplasic epithelium in the secondary lamellae and lamellar fusion, as well as the role of parasites in the inflammatory response in tilapia gills. Nonetheless, alterations such as hyperplasia of interlamellar epithelium were persistent even in non-parasitized animals, suggesting being an adaptation to the confinement environment. The presence of melanomacrophage centers in the liver and spleen were characteristic of the chronic stress that the cultured fish are exposed to. Histological findings such as epitheliocystis in the gills and bacterial colonies in the liver, spleen and heart, showed that even with absence of mortality, bacteremia were present in healthy farmed fish, as well as the parasitic diseases culminating in mixed infections. This study presented unprecedented results on tissue changes in farmed tilapia, serving as an assistance for what can be considered normal in the histopathological examination in farmed fish. Fish herein used did not present any production loss and did not have the relative condition factor (Kn) influenced by the changes, indicating a good health status. Thus, this study reports the first occurrence of epitheliocystis in tilapia gills grown in southern Brazil.

1. Introduction

Aquaculture continues to be the fastest growing animal production sector, currently accounting for half the world's supply of fish for human consumption (FAO, 2016). Nile tilapia (*Oreochromis niloticus*) is the second most widely cultured fish species in the world and the first in Brazil (Vicente et al., 2014) being widely marketed (Fitzsimmons et al., 2011). According to the topic, the search for well-being in farmed fish has been highlighted, resulting in legislation in most European countries (Galhardo and Oliveira, 2006). However, scientific information on well-being in farmed fish is still scarce (Ashley, 2007; Chandroo et al., 2004; Huntingford and Kadri, 2014; Saraiva et al., 2015; Tavares-Dias and Martins, 2017).

Various methods of assessing the health status of aquatic organisms may reflect on fish welfare and lead to significant improvement in productivity. Among them, the parasitological and histopathological diagnoses are presented as tools for analysis and prevention of diseases (Resende, 2009; Sampaio et al., 2013). Another commonly used way to determine fish welfare is the relative condition factor (Kn), a parameter measured by the relationship between the observed weight and the expected weight for a given length (Guidelli et al., 2009). It is expected that, under normal conditions, Kn will be equal to 1, but it is known that it can be influenced by numerous factors such as nutrition, contamination and parasites (Yamada et al., 2008). In turn, histopathological analyzes are highlighted, as they are characterized as confirmatory and definitive diagnosis, in the face of adverse situations (Mcgavin and Zachary, 2013; Santos et al., 2017).

Histopathological changes result from a variety of biochemical and physiological changes in the organism (Hinton and Laurén, 1990) being able to integrate the effects of abiotic and biotic factors on organ function and fish health (Handy et al., 2002; Zimmerli et al., 2007; Van Dyk et al., 2009). Tissue alterations in fish may be due to stressing

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https://doi.org/10.1016/j.aquaculture.2017.12.021
Received 15 September 2017; Received in revised form 24 November 2017; Accepted 15 December 2017
Available online 16 December 2017
0044-8486/

agents of the environment (Brum et al., 2014) or induced by pathogens (Guerra-Santos et al., 2012; Santos et al., 2017), compromising the growth and survival of animals. Thus, histopathological examination is a good indicator of fish health status, and the relevance of each lesion depends on how it affects organ function and the fish's ability to survive (Bernet et al., 1999).

This tool is widely used in fish, most of which are for experimental purposes (Figueiredo-Fernandes et al., 2007; Santos et al., 2012; Alim and Matter, 2015) and also as biomarkers (Liebel et al., 2013; Lins et al., 2010), but rarely applied in farmed fish (Coz-Rakovac et al., 2005; Raskovic et al., 2013; Saraiva et al., 2015).

In Brazil, studies of this nature were carried out by Jerônimo et al. (2014) and Santos et al. (2017), exclusively related to tissue changes by parasites, so that little is known about tissue changes and adaptations of Nile tilapia under farming conditions. This study aimed to evaluate the health status of Nile tilapia by histopathological examination, providing a ranking of the degree of lesions in fish kept under normal culture conditions.

2. Material and methods

2.1. Places of study

Sixty adult tilapia (average weight 480.9 ± 210.2 g and average length 28.1 ± 4.2 cm) were collected in four mesoregions of the state of Santa Catarina, southern Brazil. In each region 15 specimens were evaluated, totaling 60 animals. All fish were from non-sanitary crops and were clinically healthy. The collections were carried out in 2015 in 12 fish farms, three of which were located in the city of Braço do Norte (28° 16′ 30″ S 49° 09′ 57″ W) in the south of the state, three in Joinville (26° 18′ 14″ S 48° 50′ 45″ W) in the northern region, three in Gaspar (26° 55′ 51″ S 48° 57′ 32″ W) in the Itajaí Valley, and three in the west of Santa Catarina, in the cities of Caxambú do Sul (27° 09′ 39″ S 52° 52′ 44″ W), Pinhalzinho (26° 50′ 52″ S 52° 59′ 31″ W) and Barra Bonita (26° 39′ 14″ S 53° 26′ 24″ W).

2.2. Physico-chemical parameters of water

During the sampling, the following water quality parameters were evaluated: transparency (18.7 ± 8.4 cm) with Secchi disk, temperature (24.4 ± 3.4 °C), pH (6.5 ± 0 °C), and dissolved oxygen (4.7 ± 2.8 mg·L^{-1}) using multiparameter (model HI 9828 - Hanna instruments®, São Paulo, Brazil) and ammonia (0.6 ± 0.5 mg·L^{-1}) with Hanna® commercial kit.

2.3. Histopathological analysis

After capture, the fish were anesthetized with eugenol (75 mg·L^{-1}) and euthanized by rapid cerebral concussion (CEUA n° PP00928), followed by detailed macroscopic examination. Then, the first right branchial arch (Debortoli et al., 2016), and fragments of liver, spleen and heart were removed and fixed in 10% formalin buffered with monobasic and dibasic sodium phosphate at pH 6.9. The samples were dehydrated in progressive graduation of alcohol, diaphanized in xylol and embedded in paraffin. Using a microtome PAT-MR10 (The Pathologist®, Brazil), samples were sectioned in 3 μm and stained with Harris haematoxylin and eosin (HH & E) (Howard et al., 2004), and by prussian blue by the Perl method (Howard et al., 2004), mounted on permanent blades with Entellan® and analyzed by DIC (Differential Interference Contrast) microscope model Axio Imager A2 (Zeiss®, Germany). The rest of the gills were fixed in 5% formalin, for further analysis and quantification of parasites under light microscope and stereomicroscope.

2.4. Ranking of lesions

In addition to a qualitative description, the histological changes in the gills, liver, spleen and heart were evaluated using the semi-quantitative method proposed by Schwaiger et al. (1997), adapted to an increasing scale of mean values of change (MVA), according to the degree of severity of the lesions, in the following scale: 0 (alterations absent), 1 (mild alterations or focal process), 2 (moderate alterations or multifocal) and 3 (severe alterations or diffuse process). Based on this scale, a mean histological alteration value (MVA) was given for each animal, being classified as mild (0.1–1.0) moderate (1.1–2.0) and intense (2.1–3.0). The prevalence of each lesion was also calculated.

2.4.1. Gills

For classification of the gill lesions, longitudinal sections of the filaments were made, and the median portion of the organ (excluding the apex and filaments base) was used in a field covered by a 5 × objective. The following tissue alterations were considered: interlamellar epithelial hyperplasia, secondary lamella epithelial hyperplasia, fusion of secondary lamellae, epithelial detachment, edema justalamelar, venous sinus dilation, mononuclear inflammatory infiltrate, eosinophilic granular infiltrate, telangiectasia, primary lamella congestion, hemorrhage at the base of the secondary lamina, vascular dilation in the central filament vessel, vascular congestion in the filament central vessel, epitheliocystis and presence of parasite.

2.4.2. Liver

A cubic section of the liver was performed, which was analyzed thoroughly on the histological slide. The following hepatic alterations were considered: congestion, mononuclear inflammatory infiltrate, eosinophilic granular inflammatory infiltrate, melanomacrophage centers, vacuolar degeneration and bacterial colonies.

2.4.3. Spleen

A longitudinal section of the organ was performed, which was analyzed in its entirety on the histological slide. The splenic lesions considered were: congestion, mononuclear inflammatory infiltrate, eosinophilic granular inflammatory infiltrate, melanomacrophage centers, bacterial colonies and granuloma. The quantification of melanomacrophage centers in the spleen was performed using the Weibel graticule (Weibel, 1963) coupled to a light microscope. The methodology was adapted from Garcia and Magalhães (2008).

2.4.4. Heart

As in the spleen, a longitudinal section of the heart was performed, which was analyzed in the histological slide. The cardiac lesions considered were: congestion, mononuclear inflammatory infiltrate, eosinophilic granular inflammatory infiltrate, melanomacrophage and bacterial colonies.

2.5. Relative condition factor (Kn)

Kn values were calculated according to the method described by Le Cren (1951), in which Kn corresponds to the ratio between the observed weight and the theoretically expected weight for a given length (Kn = Wt / We). The animals in this study had Kn = 1.00 ± 0.01.

2.6. Statistical analysis

The data were submitted to the Shapiro-Wilk and Bartlett tests to verify normality and homoscedasticity of variance, respectively. When the assumptions were guaranteed, the data were submitted to Anova unifatorial test. When necessary, the means of the variances were separated by the Tukey test. The possible correlations were verified using Spearman's correlation coefficient. All tests were performed at a significance level of 5% using the software Statistica 10.0 (StatSoft, United