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Probiotics in Aquaculture Review: Current Status and Application in Tambaqui Cultivation (*Colossoma macropomum*)

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***Abstract*— *The development of aquaculture guarantees the supply of animal protein of great nutritional value, contributing to food security. Currently one of the main problems faced is the occurrence of diseases, responsible for a worldwide economic loss, equivalent to US $ 9 billion per year. Aiming to increase resistance to diseases, increasing growth rates and food efficiency in intensive crops, some strategies have been developed, one of them is the use of probiotic bacteria. These, when in contact with the digestive tract of the host generates a series of benefits, among them, the modulation of the immune system, developing defense mechanisms and increasing resistance to stress. However, there are few documented reports on the efficiency of probiotics in native species, such as tambaqui (Colossoma macropomum). This species shows some resistance to stress, through physiological mechanisms of adaptation, such as lip expansion when subjected to hypoxia situations, which added to the positive effects of using probiotics would represent an increase in its resistance. The purpose of this work is to review the literature on the use of probiotics in aquaculture in order to provide a comprehensive synthesis of the current knowledge about its use in aquaculture, with emphasis on the intensive cultivation of tambaqui.*
Keywords*— *probiotic; immunity; stress; tambaqui.

I. INTRODUCTION

Intensive cultivation with the improvement of modern sustainable techniques contributes to reduce the pressure on natural stocks, increasing the supply of fish with good nutritional quality, with essential protein and essential fatty acid indexes for human consumption, as well as increasing the reliability of the consumer (Ibrahem, 2015, Sartori and Amancio, 2012).

Fish is the most widely produced animal protein with a share of 36.36%, higher than that of poultry - 24.70%, pork - 24.44% and bovine - 14.52% (FAO 2017). In 2017, world aquaculture contributed 83.6 million tonnes, corresponding to 48.04%, close to half of total fish production, presenting a constant growth rate equivalent to about 4.5% and a growing associated demand the recovery of some major emerging markets, like Brazil (FAO, 2018).

In 2016, Brazilian aquaculture reached a production value corresponding to US $ 1.22 billion with fish farming contributing 70.9% of this total (IBGE, 2016). More recent data points to a growth of 8% in relation to the year 2016 and a production of 691,700 tons, with tilapia (*Oreochromis niloticus*) being the most produced species with 51.7%, followed by native species (*Colosoma macropomum*), with 47% of the total production, where production is concentrated in the states of Rondônia and Amazonas (North region), Mato Grosso and Goiás (Central-West region) and Maranhão (Northeast region) (Peixe BR, 2018).

The tambaqui is the main native species produced in the Brazilian fishery, belongs to the order of Characiformes, family Serrasalmidae. It occurs naturally in the basins of the Amazon and Orinoco River (Azevedo et al., 2016; Ferreira, 2014). It can reach up to one meter in length standard and weigh up to 30 kg. Their natural diet is composed of zooplankton, fruits and seeds, being classified as an omnivorous species with a tendency to herbivore, filtering and frugivore (Ferreira, 2014; Lopera-Barreto et al., 2011).

Its success in fish farming is related to the presence of characteristics favorable to cultivation such as acceptance of artificial rations, good(Azevedo et al., 2006). In addition, the results obtained in the present study were similar to those reported in the present study.

Under hypoxia conditions, tambaqui presents physiological adaptation represented by lip expansion, as well as morphological and molecular adjustments related to oxygen uptake by hemoglobin (Val, 1995). In the same condition Val (1986) observed an increase in the number of erythrocytes and the hemoglobin content for this species, to favor the transport of respiration gases.

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The stress occurrence is verified by intensive management practices, common in fish farming, represented mainly by excessive handling, transportation and densification that favor its installation, a condition that weakens the immune system of the fish leading to a greater susceptibility to diseases (Dawood et al. Hunsuke, 2016, Yuji-sado, 2014, Mohapatra et al., 2013, Gabbay, 2012).

The emergence of diseases is mainly due to the imbalance of the epidemiological triad composed by pathogen, host and environment, considered an emerging problem limiting the growth of the activity, as it entails large mortalities and consequently economic losses (Jesus et al., 2016; Boijink et al. Mourino et al., 2008). The disease can occur in different stages of growth of the cultured animals and represents an estimated economic loss for the world aquaculture corresponding to US $ 9 billion per year (Jesus et al., 2016; Boijink et al., 2015).

Outbreaks of bacterial and parasitic diseases are responsible for productive and economic losses in the intensive cultivation of tambaqui, with emphasis on the bacterioses caused by mobile Aeromonas, Flavobacterium columnare and Streptococcus agalactiae (Lacerda, et al., Kotzent, 2017).

In order to minimize such losses, the use of probiotic has been considered a preventive sanitary practice, since it helps to increase zootechnical parameters, as well as to mitigate the effects caused by stress by increasing the immunological capacity of the fish. It is considered an alternative to the use of antibiotics and an important factor for health management in aquaculture (Dawood and Koshio, 2016; Newaj-FyzuL, 2014; Qi et al., 2009).

It is known that the use of antimicrobials causes a serious impact on the aquatic environment due to the release of their residues into the water, as well as to generate economic impact due to residues present in the carcass represent a barrier to export to the United States and Europe (Kotzent, 2017).

Thus, it is well known that probiotics can be considered a sustainable and promising strategy, since it represents an alternative for the generation of a product of high quality in terms of size, health and safety, allowing an improvement in the quality and quantity of aquaculture production. (Paixão et al., 2017, Jesus et al., 2016, Ibrahem, 2015).

The aim of this work was to carry out a review in the literature about the advances in the use of probiotics in aquaculture, in order to provide a comprehensive synthesis of the current knowledge about its use in aquaculture, especially in the cultivation of tambaqui (Colossoma macropomum).

Probiotics Definition

The term probiotic means "in favor of life" originates from the Latin term PRO - Para, and from the Greek word BIOS - Life, being its concept, continually revised since 1965 (Jesus et al., 2016). For Fuller (1989) probiotics are defined as "food supplements composed of living microorganisms that benefit host health by balancing the intestinal microbiota." Ferreira (2014) reports that probiotics are non-digestible, non-hydrolyzed and inabsorbed ingredients in the gastrientestinal tract, beneficially affecting the host by selectively stimulating the growth and / or activity of desirable bacteria, improving their microbiotaIn the most recent literature, the use of the term food supplement is commonly used to report the use of probiotic in aquaculture (Yu et al., Zhai et al., 2017, Azevedo et al., 2016 and Gabbay, 2012). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) define that probiotics are living microorganisms that when administered in adequate amounts confer a health benefit to the host (Newaj-fyzul and Al-harbi, Austin, 2014).

More broadly, Mourino et al. (2008) defines probiotic as: "living microorganisms, which when added to the culture so that they enter the digestive tract of the animals and stay alive, acting beneficially on the target species, improving the alimentary efficiency, the immune system and / or balance of beneficial and pathogenic bacteria in the digestive tract ".

Among the various definitions found, they all have one point in common, in the assertion that probiotics are living organisms that are administered orally and brings benefits to the health of the animal of interest (Jesus et al., 2016; Newaj-Fyzul, et al., 2014, Ibrahem, 2015, Iribarren et al., 2012, Nayak, 2010).

II. MECHANISMS OF ACTION

Several claims to the mode of action of probiotics in aquaculture are currently found. Many, verified from in vitro tests. This questionthe fact that the efficiency of a probiotic tested in vitro can change significantly when administered to the host, that is, in vivo, generating a correlation incompatibility between the two forms of investigation (Ibrahem , Balcazar et al., 2006).

Among the different forms of action of probiotics, competitive exclusion is one of them. Consisting of the ability to prevent the growth of pathogenic bacteria in the intestinal tract of the host. For aquatic animals the evidence is that this occurs through the colonization of probiotic bacteria in the digestive tract, especially in the epithelium of the gastrointestinal mucous (Lazado and Caipang, 2014; Mahayhi et al., 2012). Balcazar et al. (2007), verifying the form of adhesion of probiotic bacteria in fish, summarized the process in the following stages: attraction, association to the surface secreting

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substances and binding the cells of the animal tissue. As adhesion to the surface is an important protection mechanism against pathogens due to competition for binding sites, nutrients and consequently for modulation of the immune system (Ibrahem, 2015).

According to Nayak (2010), the immune stimulator capacity of probiotics may be affected by some factors, such as: source, type, dose and duration of supplementation. Luis-Villaseñor et al. (2015) found that the use of two probiotic mixtures composed of an experimental mixture of Bacillus *(Bacillus tequilensis + B. endophyticus*) and a commercial probiotic, contributed positively in modulating the bacterial community of larvae of shrimp *Litopenaeus vannamei* against the challenge with *Vibrio parahaemolyticus*.

In addition to the increased immune stimulator capacity, the adhesion and colonization of probiotic bacteria are important in the competition for nutrients and energy sources, an extremely necessary condition in the composition of the intestinal tract microbiota (Dawood and Koshio, 2016; Newaj-Fyzul et al., 2014). In the present study, the use of probiotic agents in the digestion of nutrients by stimulating and / or producing digestive enzymes, such as amylase, lipase and protease, was observed with the addition of probiotic in the fish diet (Lazado, et al., 2014, Qi et al., 2009). In a study conducted by Wang et al. (2008) showed an increase in protease activity in common carp - Cyprinus carpio, fed a diet containing Bacillus spp.

Another important mechanism of action is the production of several antimicrobial compounds, such as bacteriocin, commonly produced by bacteria of the genus Bacillus that are capable of inhibiting the growth of undesirable bacteria (Mohapatra et al., 2012; Ali et al., 2000; Gildberg et al. ., 1997). The compounds produced in an antagonistic way, have also been shown to be efficient against viruses, as verified by Balcazar (2007).

Vitamin production is another important action of probiotics, observing the ability of some strains to produce water-soluble vitamins such as complex B and folic acid (Leblanc, 2011).

Required Characteristics and Selection Form

In order to use microorganisms as a probiotic in aquaculture, it is necessary that they present some essential characteristics among which they are safe for the cultured animal, for the environment in which they live and for humans, being innocuous and not presenting resistance genes antibiotics (Moubareck et al., 2005).

It should also have anticancer properties, be able to colonize the digestive tract of the host and be resistant to the enzymes present in it and bile, besides being stable to the process of inoculation in the ration, the time of storage and transport (Gabbay, 2012).

According to Balcazar (2006), the colonization of the host gastrointestinal tract is only verified when the probiotic is administered for a long period of time. In the literature, there is a variation in the time of action of probiotics for different species in aquaculture (Paixão et al., 2017).

However, the use of autochthonous probiotic strains is more likely to colonize the intestinal tract of the host and to remain viable, as well as being part of the culture environment (Kotzent, 2017; Jesus et al., 2016). According to Cahill (1990), bacteria present in the aquatic environment influence the composition of the intestinal microbiota, in the same way that the intestinal microbiota influences the aquatic environment.

In the absence of a microorganism with all the characteristics mentioned above, several studies have aimed at the simultaneous use of several probiotics (Paixão et al., 2017; Torres, 2014) or of probiotics with prebiotics (Azevedo et al., 2016 Ganguly et al., 2010),satisfying the necessary characteristics and generating greater benefits to the host.

Microorganisms Used as Probiotics in Aquaculture

It is now possible to find in the literature a variety of probiotic groups used in aquaculture, from Gram-positive and Gram-negative bacteria, unicellular algae, bacteriophages and yeasts (ibrahem, 2015; Das et al., 2008). In this work the focus was given to gram-positive bacteria (Table 1).

Table.1: Gram positive bacteria used as probiotics in Aquaculture

Probiotic microorganism	Target species	Source	Study developed	Referencies
Lactobacillus plantarum	*Oreochomis nilotícus*	Target species	Acute inflammatory response in Nile tilapia fed probiotic *Lactobacillus plantarum* in diet	Dotta et al. 2011
		Colection	Dietary *Lactobacillusplantarum* supplementation enhances growth performance and alleviates aluminum toxicity in tilapia	Yu et al. 2017
		Colection	Dietary *Lactobacillusplantarum*	Zhai et al. 2017

			supplementation decreases tissue lead accumulation and alleviates lead toxicity in Nile tilapia (*Oreochromisniloticus*)	
	Yoshitomi tilapia	Soy	Deoxynojirimycin from *Bacillussubtilis* improves antioxidant and antibacterial activities of juvenile *Yoshitomi tilapia* Lining	Tang et al. 2017
	Colossoma macropomum	Commercial	Prebiotic, probiotic and symbiotic supplementation for tambaqui juveniles at two storage densities	Azevedo et al. 2016
Bacilllus subtilis	*Colossoma macropomum*	Commercial	Effect of commercial probiotics (*Bacillussubtilis*and *Saccharomycescerevisiae*) on growth performance, body composition, hematology parameters, and disease resistance against *Streptococcusagalactiae* in tambaqui(*Colossomamacropomum*)	Paixão et al. 2017
Bacillus spp.	*Colossoma macropomum*	Commercial	Use of probiotic during transport of tambaqui (*Colossoma macropomum*) juveniles in a closed system	Ferreira. 2014
Weissellacibaria	Surubins hibrídos	Target species	Immunological parameters of surubins vaccinated against and supplemented withprobiotic	Pereira, 2013
lactic acid bactéria	*Oncorhynchus mykiss*	Target species	Identification and characterization of lactic acid bacteria isolated from rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792), with inhibitory activity against *Vagococcus salmoninarum* and *Lactococcus garvieae*.	Didinen et al., 2017
Lactobacillus spp	*Oncorhynchus mykiss*		Evaluation of the effect of probiotic microorganisms on the zootechnical, hematological and stress tolerance performance of rainbow trout *Oncorhynchus mykiss*	Torres, 2014

Among the gram-positive bacteria, there is a group composed of lactic acid, anaerobic or aerotolerant bacteria, in general they present catalase negative and do not present spores and movement. They produce lactic acid as the largest or only product of their metabolism (Gabbay, 2012; Poffo e Silva, 2011).Within this group, several bacterial species of the genus Bacillus, Lactobacillus and Lactococcus were successfully isolated from the intestinal tract of fish and are widely known as the main probiotic species in aquaculture (Kotzent, 2017; Balcázar et al., 2006).

The genus Lactobacillus has been frequently used as a probiotic in aquaculture (Yu et al., 2017, Gabbay, 2012, Dotta et al., 2011, Jatobá et al., 2008). Some results point to an improvement in host response to the presence of pathogens. Aly et al. (2008) found that Lactobacillus acidophilus contributed to improvement in the immune modulation of Nile tilapia increasing its resistance to the pathogen *P. fluorescens*. Ferreira (2014) found that the probiotic Bacillus spp. was not efficient in suppressing stress responses and in improving the innate immune system of tambaqui submitted to the transport procedure. Similar, Passion et al. (2017) did not observe a difference in performance and body composition. already Azevedo et al. (2016) identified improvement in growth and better use of food with *Bacillus subtilis* in conjunction with a symbiont. However, there may be differences in the immunological effect of fish among different probiotics (Mohapatra et al., 2013).

According to Balcázar et al. (2007), species of the genus Lactococcus have favorable characteristics to be used as a probiotic in aquaculture. In a recent study by Kotzent, (2017) six strains of bacteria isolated from the intestinal tract of tambaqui were tested. Only

Staphylococcus hominis, *Enterococcus hirae*, *Pediococcus pentosaceus* and*Lactococcus lactis* were shown to be potential probiotics in diets for the species. *Lactococcus lactis* presents coccus formations; measuring between 0.5 and 1.5 µm, presents hemolysis and catalase negative, develop at temperatures between 30 ° C and 40 ° C, with 37 ° C at optimum temperature (Kotzent, 2017).

Didinen et al. (2017) observed that *L. lactis* administration in the rainbow trout diet resulted in a significant reduction in mortality caused by *Lactococcus garvieae*, being a viable alternative for the management of lactococcosis in cultured fish.

Linh, et al. (2018) evaluated the probiotic properties of *L. lactis* for application in aquaculture from fermented vegetables and found its efficacy against Gram-positive and Gram-negative pathogenic bacteria.

There are few studies in the literature regarding the use of probiotics for tambaqui. Ferreira et al. (2014), Azevedo et al. (2016) and Paixão et al. (2017), tested the use of allochthonous strains, that is, from another species, making evident the need for evaluation of autochthonous strains, especially with in vivo studies (Kotzent, 2017).

Stress in pisciculture and probiotics

In fish culture, the most effective way of administering probiotics is through feed, with the microorganisms incorporated into the feed, using soybean oil as a vehicle, in order to guarantee the adhesion of the cells to the grain of the food (Ferreira; Torres, 2014; Gabbay, 2012).

From its ingestion, one of the most important roles is the development of innate immunity in cultured animals, which are subject not only to the action of pathogenic microorganisms but also to changes in the environment, which can seriously affect their physiological state (Mohapatra et al., 2013).

Brandão et al. (2006) states that the response to stress occurs in three ways: primary - related to hormonal responses; secondary - changes in physiological and biochemical parameters; behavioral changes, changes in behavior and increased susceptibility to diseases.

To be effective for the organism grown, probiotics must exert physiological importance on the consumer, when they reach populations above 10^6 to 10^7 CFU / g or mL of bioproduct. However, there is a need to establish reference values according to the microorganism used the target species and their health status (Torres, 2014).

III. PROBIOTICS AND STRESS VARIABLES

Hematologic Parameters

Studies on the hematological picture of Brazilian fish in fish farming have increased greatly, since blood parameters can be used as biological indicators in identifying the stress that the environment and parasites can impose on cultured animals (Dias, et al. 2009).

According to Dias et al. (2009) the inhibitory effects of acute or chronic stress can affect the immune response of the fish implying a significant reduction in resistance to diseases. Variables related to leukogram help in the diagnosis of infectious processes and states of homeostatic imbalance, or erythrogram, in the identification of anemiemic processes. The reference values for the tambaqui erythrogram are shown below (Table 2).

Table.2: Reference values of the erythrogram for tambaqui

Parameters	Minimum/Maximum	Reference range
Erythrocytes (x 106 µL)	1,250 - 2,960	1,625 - 3,383
Hematocrit (%)	26,0 - 38,0	36,0 - 40,0
Hemoglobin(g/dL)	6,3 - 13,7	8,9 - 10,9
VCM (fl)	70,8 - 123,7	112,7 - 192,6
CHCM (g/dL)	20,2 - 30,5	26,2 - 49,6

Source: Dias et al., 2009

Generally, in stressed fish there are changes in hematocrit, hemoglobin concentration and number of lymphocytes followed by hyperglycemia (Torres, 2014). For Nile tilapia fed with *Lactobacillus plantarum* in the diet, submitted to an inflammatory response by the injection of carrageenan, there was an increase in the number of neutrophils (Dottaet al., 2011).

Paixão et al. (2017) testing *B. subtilis* in the diet of tambaqui juveniles subjected to *Streptococcus agalactiae* infection found an increase in the number of erythrocytes. However, there was no difference in this parameter using *Saccharomyces cerevisiae*.

Histopathological Parameters

Histological methods have been considered an important tool to evaluate pathological changes in tissues and have been used in toxic studies for different aquatic organisms (Díaz-de-alba et al., Yu et al., 2017), providing information on biochemical changes at the cellular level and the presence of pathologies in the tissues (Mohapatra et al., 2013). On the other hand, tissue analysis can serve as a biomarker of environmental pollution (Zelikoff, 1998). Lesions observed, for example, by histopathological analysis of the liver, may present hepatocytes with wide vacuolization, reduction of glycogen stores, inflammation, alteration in the shape of sinusoidal vessels, and are even considered markers of the quality of the environment in which these animals are inserted (Teh et al., 1997).

Enzymatic Parameters - Antioxidant Enzymes

In addition to the presence of contaminants in the aquatic environment, other factors such as the physical-chemical parameters of the environment may be related to changes in the physiological state of the cultured animals being responsible for the oxidative stress in the animal (Mohapatra et al., 2013).

hai, et al. (2017) found that the use of *L. plantarum* in the diet of *Nile tilapia*, decreased the oxidative stress caused by lead. Similar was observed by Yu et al. (2017) that verified improvement in the effects caused by the oxidative stress generated by the aluminum concentration, in addition to an improvement in the growth performance for the species. For Castex (2009), the use of probiotic in the diet plays an important role in the antioxidant activity.

The effects caused by oxidative stress can be verified by means of enzymatic analyzes. The enzyme superoxide dismutase (SOD) and catalase (Cat), are among the major antioxidant defense enzymes. SOD is a metalloenzyme acting on the O_2 radical - disrupting it to H_2O_2 and protecting the targets of the superoxide anion attack (Trevisan, 2008).

The oxidative stress and the activity of antioxidant enzymes in the liver and white muscle of *Nile tilapia* submitted to chronic exposure to ammoniacal nitrogen were investigated by Hegazi et al. (2010), showing an increase in levels of stress biomarkers and enzymatic activity according to the increase in nitrogen concentration in the environment.

IV. FINAL CONSIDERATIONS

Aquaculture is an extremely important activity in ensuring the planet's food security. The emergence or improvement of techniques used to promote the supply of a high quality product is desirable by all involved with the aquaculture production area.

In promoting a product offering safety, good growth, health and production time, the use of probiotics has proven to be a promising alternative. Its use is already a reality worldwide and its application is already considered part of the aquaculture of the future.

Based on the results shown in this work, it is evident the need for studies related to the effects of probiotics, for native species, especially for tambaqui, this important species for Brazilian fish farming.

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