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Efficacy of dietary curcumin supplementation as bactericidal for silver catfish against *Streptococcus agalactiae*

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ABSTRACT

The antibiotics were frequently used for combating bacterial infections in aquaculture, but this treatment causes antibiotic resistance, negative impact on the environment and on health, and accumulation of residual in edible tissues. Several evidences have considered the dietary supplementation with natural products an interesting alternative to antibiotics, as the use of curcumin, a polyphenol with anti-inflammatory, antioxidant and antimicrobial properties. Thus, the aim of this study was to evaluate whether fish fed with a diet containing 150 mg curcumin/kg feed is able to enhance the resistance of silver catfish to *Streptococcus agalactiae* infection. Our results demonstrated that curcumin dietary supplement exerts potent bactericidal action against *S. agalactiae*, presenting 100% of therapeutic efficacy when compared to infected and non-supplemented animals. Also, the treatment prevented the occurrence of clinical signs of disease, as erratic swimming, corneal opacity, skin lesions in the fin and tail, and loss of appetite. In summary, curcumin can be a promising dietary supplement for improving disease resistance.

1. Introduction

Aquaculture strives to produce large quantities of fish in biological and economically efficient way, since the fish or fishery products represent a source of proteins and essential micronutrients that are important for human health [1]. However, infectious diseases are considered the major impediment to development of aquaculture and the most significant cause of economic lost, such as caused by *Streptococcus agalactiae* [2].

Streptococcus agalactiae, known as group B Streptococcus (GSB), is a Gram-positive pathogenic bacterium which possess the capacity to infect a wide range of hosts, being recognized as a major etiologic agent of septicemia and meningoencephalitis outbreaks in fish farms worldwide [3]. In fish, this disease is characterized by loss of appetite, exophthalmia, eye hemorrhage, corneal opacity, abnormal behavioral and severe inflammation [4,5], causing high mortality and severe economic losses in several freshwater species such as Nile tilapia (*Oreochromis niloticus*) [6], rohu carp (*Labeo rohita*) [7] and the silver catfish (*Rhamdia quelen*) [8]. To control the infection caused by *S. agalactiae,* strategies such as anti-microbial agents and vaccination are employed in aquaculture, but the use of antibiotics have negative impacts on the environment and on human health, which includes the emergence of antibiotic-resistant bacterial strains, the accumulation of residual in edible tissues and the depression of immune system [9,10]. For these reasons, alternative methods of disease prevention or treatment have been employed in aquaculture as alternative to the use of chemical products, such as the administration of functional dietary additives [1], including curcumin [11].

Curcumin is a polyphenolic compound naturally obtained from the dried rhizome of *Curcuma longa,* being considered the foremost active ingredient responsible for a range of multifaceted biological properties, as hepatoprotector [12], antiparasitic [13], and immunomodulatory and bactericidal [14] for fish. Recently, evidence has suggested that improvement on antioxidant and immune systems contributes directly to resistance against bacterial infections [15]. In this regard, a diet containing curcumin increased the resistance of Nile tilapia experimentally infected with *Aeromonas hydrophila* due to the improvement of immune and antioxidant status [16]. Thus, our hypothesis is that antioxidant and immunomodulatory effects of curcumin can enhance the resistance of silver catfish to *S. agalactiae* infection.

Thus, the aim of this study was to evaluate whether fish fed with a diet containing 150 mg curcumin/kg feed is able to enhance the

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Table 1
Formulation of the experimental diet.

Ingredient	g/kg
Soybean meal	300
Meat and bone meal	350
Rice bran	120
Corn	150
Canola oil	30
Salt	10
Vitamin and mineral premix[a]	30
Dicalcium phosphate	10

[a] Vitamin and mineral mixture (security levels per kilogram of product): folic acid (200 mg), pantothenic acid (5000 mg), antioxidant (0.60 g), biotin (125 mg), cobalt (25 mg), copper (2000 mg), iron (820 mg), iodine (100 mg), manganese (3750 mg), niacin (5000 mg), selenium (75 mg), vitamin A (1,000,000 UI), vitamin B1 (1250 mg), vitamin B2 (2500 mg), vitamin B6 (2485 mg), vitamin B12 (3750 μg), vitamin C (28,000 mg), vitamin D3 (500,000 UI), vitamin E (20,000 UI), vitamin K (500 mg) and zinc (17500 mg).

resistance of silver catfish to *S. agalactiae* infection.

2. Materials and methods

2.1. Curcumin

Curcumin powder extracted from *C. longa* using solvent (ethanol) (molecular weight: 368.38 g/mol) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA) for posterior incorporation in fish feed.

2.2. Diet preparation

A basal diet was formulated based on a recent protocol established in detail by Zeppenfeld et al. [17], that provides all nutritional requirements to silver catfish (Table 1). All ingredients were finely grounded, weighed and kneaded until homogenous. In order to prepare a supplemented diet, inclusion of curcumin was performed by premixing it with the corn fraction before mixing with the other ingredients. The mixtures were dried in a forced air circulation oven at 35 °C for 24 h. Finally, the pellets were broken, sieved and stored at 2 °C until use. The concentration of curcumin in the supplemented diet was 150 mg/kg feed, in accordance with that preconized by Cui et al. [18] against *Streptococcus iniae.*

2.3. Collection, maintenance of fish and water quality variables

Healthy fish were collected for experimental purpose from a fish farm located in Cruz Alta, Rio Grande do Sul, Brazil. The animals were analyzed for the absence of ectoparasites or endoparasites in the gills, skin and fins, as well as observed for the absence of skins lesions compatible to possible bacterial infections, that prove the animal's health. The fish were transported and maintained in 250 L fiberglass tanks with continuous aeration with controlled water parameters (21 − 23 °C, pH 7.3–7.7, dissolved oxygen levels: 5.7–7.0 mg/L), in fresh water for seven days. Dissolved oxygen and temperature were measured with a YSI oxygen meter (Model Y5512, Ohio, USA). The pH was measured using a DMPH-2 pH meter (Digimed, São Paulo, Brazil). Total ammonia levels were determined according to Verdouw et al. [19], and non-ionized ammonia (NH_3) levels were calculated using a conversion table for fresh water. The animals were fed once a day with commercial fish feed. The water quality variables were maintained stable during all experimental period as follows: water temperature 22 ± 1 °C, pH 7.4 ± 0.6, dissolved oxygen 6.78 ± 0.61 mg/L, total ammonia 0.98 ± 0.002 mg/L and non-ionized ammonia 0.006 ± 0.0005 mg/L.

2.4. Bacterial culture and inoculum preparation

Streptococcus agalactiae was isolated from a patient in southern Brazil and identified according to colony characteristics and biochemical tests, as recently published in detail by Baldissera et al. [8]. The bacterial culture was maintained frozen in glycerol, and was seeded in nutrient agar for 24 h. Following this, the bacterial culture was grown on sheep blood agar for use in this experimental model. The suspension of *S. agalactiae* was washed twice in sterile saline (NaCl 0.9%), turbidity (OD_{600}) adjusted to 0.8–1.0 (equivalent to 7×10^7 CFU/mL) and used for infection (100 μL via oral), according to the protocol established by Baldissera et al. [8].

2.5. Animals and experimental design

A total of 40 juvenile silver catfish (205.55 ± 18.93 g; 29 ± 2.2 cm) were used as the experimental model to evaluate efficacy of curcumin against *S. agalactiae* infection. The animals were assigned into four groups with ten animals in each: group A was composed by uninfected and non-supplemented animals (negative control), the group B was composed by uninfected and supplemented animals, group C was composed by infected and non-supplemented animals (positive control), and group D was composed by infected and supplemented animals. The groups A and C were fed with a basal diet, while the groups B and D were fed with a diet supplemented with curcumin at 150 mg/kg feed. Fish received the experimental diets once a day (2 p.m.) at a proportion of 3% of total biomass for 14 days, according to the protocol established by Cui et al. [18] with some modifications.

The methodology used in the experiment was approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Maria under protocol number 74/2014.

2.6. Treatment efficacy

The animals were evaluated for 30 days, and the longevity, mortality and therapeutic efficacy was evaluated followed the protocol stablished by Souza et al. [15]. Moreover, the presence of lesions was observed in intervals of two days during 30 days. After 30 days, the surviving animals were euthanized with eugenol followed by spinal cord section, according the Ethics recommendation.

2.7. Statistical analysis

Normality and homoscedasticity were analyzed through the Shapiro–Wilk and Levene tests, respectively. Statistical analysis was performed using a bilateral two-way analysis of variance (ANOVA) for independent samples, followed by Tukey post hoc analysis. Significance was set at $p < 0.05$.

3. Results

3.1. Clinical signs

The infected and non-supplemented animals (group C) presented erratic swimming, corneal opacity, skin lesions in the fin and tail, and loss of appetite. The infected and curcumin-supplemented animals (group D) did not present any clinical sign, similarly to observed in the uninfected and non-supplemented animals (group A).

3.2. Mortality, longevity and therapeutic efficacy

No uninfected animal (groups A and B) died during the experimental period. The infected and non-supplemented animals (positive control) showed 100% mortality, while the no fish fed curcumin-supplemented diet died during the experimental period. A significant increase on animal longevity was observed in the infected and curcumin-