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# Cinnamon (*Cinnamomum* sp.) inclusion in diets for Nile tilapia submitted to acute hypoxic stress

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ABSTRACT

The aim of this study was to evaluate the possible effects of diets supplemented with probiotics and different cinnamon levels (powder and essential oil) on immunological parameters of Nile tilapia after being subjected to acute stress by hypoxia. Three hundred and thirty juvenile male tilapia fish (66.08 ± 2.79 g) were distributed in 30 tanks of 100 L capacity (11/cage) with a water recirculation system. The animals were fed for 71 days with diets containing extruded cinnamon powder at different levels (0.5, 1, 1.5, 2%), cinnamon essential oil (0.05, 0.1, 0.15; 0.2%) and probiotics (0.4%), all in triplicate. At the end of the experiment, the fish (200.36 ± 19.88 g) of the different groups were subjected to stress by hypoxia. Hypoxia was achieved by capturing the animals with a net, keeping them out of the water for three minutes, and then sampling the blood 30 min after the procedure to determine the levels of cortisol, glucose, haematocrit, lysozyme, bactericidal index, total protein, and its fractions. The animals kept blood homeostasis after hypoxic stress. Diet supplementation with 0.5% cinnamon powder improved the fish immune response, since it resulted in an increase of 0.5% in γ-globulin level. Administration of 0.15% cinnamon essential oil resulted in an increase of $\alpha_1$ and $\alpha_2$-globulins, which may be reflected in increased lipid content of the carcass and the hepatosomatic index. More studies are necessary to better understand the effects of these additives for fish immunity.



## 1. Introduction

In recent years, fish farming has been consolidating and occupying greater space in both national and international markets. The improvement of production systems, nutrition, and choice of species that are best adapted to these systems are largely responsible for this growth. Among freshwater fish species, the Nile tilapia (*Oreochromis niloticus*) stands out for its hardiness, rapid growth during intensive farming, and meat quality.

In intensive production systems, stress is inevitable and can manifest itself in acute or chronic form, according to the duration of animal exposure to the stress source [1]. Changes to the blood levels of cortisol, glucose, and total protein are some indicators of a stress response in fish. The total protein and its fractions may indicate the general state of fish health and indirectly, reflect the immune status of the animal [2,3].

Hypoxia is an important stressor that can occur during fish farming [4], mainly in high-density storage when the aeration system is suspended, there is a lack of appropriate food, or during transportation. As the essential metabolic processes require oxygen, exposure to hypoxic conditions can adversely affect a wide variety of biochemical and physiological processes [5] and hence, reduce the resistance of fish to pathogens, thereby increasing their susceptibility to disease [6].

A promising method in disease control in fish farming is based on the enhancement of fish defence mechanisms through administration of immunostimulants. Some plants [7–9] and probiotic bacteria [10,11] have immunomodulatory properties that can be efficient replacements for antibiotics in fish farming. An example is cinnamon (*Cinnamomum* sp.), used worldwide as a flavouring spice. Among its proven biological activities, we highlight its antimicrobial activity [12,13], primarily attributable to cinnamaldehyde present in the bark of the plant [14]. Concentrations of this active ingredient may vary greatly depending on various environmental factors [15] as well as to the form used (powder or essential oil).

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The use of cinnamon could improve conditions during fish farming by enhancing the innate immune system of fish, allowing a better response to stressful environmental conditions [16,17], such as hypoxia.

The aim of this study was to evaluate the possible effects of diets supplemented with probiotics and different levels of cinnamon (powder and essential oil) on the immunological parameters of Nile tilapia after being subjected to acute hypoxic stress.

## 2. Materials and methods

For this work, the methodology was approved by the Ethics Committee on Animal Experimentation under protocol 75/2011 CEUA/UFMG.

### 2.1. Animals and experimental conditions

The experiment was conducted at the Laboratório de Aquacultura of the Universidade Federal de Minas Gerais, Brazil. In the experiment, 330 juvenile male Nilotic tilapia were studied, having an average weight of 66.08 ± 2.79 g, and were distributed in ten treatments with three replicates each (11 fish/tank).

The fish were kept in 100 L tanks with a water recirculation system and the temperature was maintained at 28 ± 1 °C with a photoperiod of 9 h light and 15 h dark. Food was provided thrice daily for 71 days *ad libitum*.

### 2.2. Experimental diets

The design was completely randomized with 10 treatments and three replications. Ten extruded diets were prepared (Table 1), four with cinnamon powder (0.5%, 1%, 1.5%, and 2%), four with essential cinnamon oil (0.05%, 0.1%, 0.15%, and 0.2%), one with probiotics (0.4%), and a control (no supplementation). The probiotic used was constituted of live bacteria, in the lyophilized form, from the genus Bacillus (*Bacillus cereus* and *Bacillus subtilis*).

Samples of the essential oil and cinnamon powder were subjected to chemical characterisation to determine the percentage of major constituents present in the samples. The analysis primarily identified cinnamaldehyde as the predominant active component, with concentrations of 59.6% and 86.2% for cinnamon powder and essential oil, respectively.

### 2.3. Stress testing and analysis

At the end of the trial period, fish from the different groups, with an average weight of 200.36 ± 19.88 g, were subjected to hypoxic stress. The fish were stressed by removing them from the tanks with a net and keeping them out of water for three minutes. Thirty minutes after returning the animals to the water, blood samples were taken from nine fish per treatment for glucose, haematocrit, protein, cortisol, lysozyme, bactericidal index, and electrophoretic profile analysis.

Glucose was measured using a portable digital glucometer (Accu Chek ACTIVE®), while haematocrit was measured using the microhaematocrit technique, and total plasma protein was determined using a refractometer, using the plasma obtained from microhaematocrit technique.

Cortisol levels were determined using a commercial kit (Direct ELISA Kit The ELAsy Way -. Cortisol, Diag Biochem Canada Inc.).

For determination of lysozyme activity, 25 μL of serum was added to a suspension of *Micrococcus lysodeikticus* (Sigma-Aldrich Co., Ltd., Athens, Greece). The activity of lysozyme was measured after 20 min at a 450 nm wavelength.

To determine the serum bactericidal index, a colony of *Escherichia coli* was inoculated into TSB medium (Trypticase Soy Broth) with stirring for 20 h. Then, the concentration was adjusted to an OD (optical density) of 0.1–0.2 (570 nm). The serum from the animals was diluted in saline (0.9%), and subsequently, the diluted serum was placed in the bacterial inoculum and incubated at 25 °C

**Table 1**
Experimental diets.

| Ingredients | Diets (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
| Soybean meal | 39.22 | 39.2 | 39.22 | 39.22 | 39.22 | 39.22 | 39.22 | 39.22 | 39.22 | 39.22 |
| Broken rice | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Salmon meal | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Wheat bran | 8.55 | 8.55 | 8.55 | 8.55 | 8.55 | 8.55 | 8.55 | 8.55 | 8.55 | 8.55 |
| Wheat gluten | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Soybean oil | 5.64 | 5.64 | 5.64 | 5.64 | 5.64 | 5.64 | 5.64 | 5.64 | 5.64 | 5.64 |
| Bicalcic phosphate | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| [a]Supplement | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL-Methionine | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 |
| [b]Antioxidant | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Chromium oxide | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Inert | 2.00 | 1.50 | 1.00 | 0.50 | – | 1.95 | 1.90 | 1.85 | 1.80 | 1.60 |
| Cinnamon powder | – | 0.50 | 1.00 | 1.50 | 2.00 | – | – | – | – | – |
| Cinnamon essential oil | – | – | – | – | – | 0.05 | 0.10 | 0.15 | 0.20 | – |
| Probiotic | – | – | – | – | – | – | – | – | – | 0.40 |
| [c]**Analysed composition** | | | | | | | | | | |
| Crude protein (%) | 38.84 | 38.89 | 38.86 | 38.72 | 38.32 | 38.73 | 39.01 | 38.89 | 39.30 | 38.61 |
| Crude energy (MJ $kg^{-1}$) | 18.01 | 17.99 | 18.26 | 18.23 | 18.32 | 18.05 | 18.07 | 17.93 | 17.96 | 17.95 |
| Ether extract (%) | 7.47 | 7.14 | 7.01 | 7.46 | 7.11 | 7.36 | 7.40 | 7.41 | 7.29 | 7.35 |
| Ash (%) | 9.99 | 10.43 | 9.94 | 9.45 | 9.09 | 10.93 | 10.80 | 10.65 | 10.65 | 10.87 |

[a] Composition of vitamin and mineral supplement: Folic Acid (Min) 2500 mg/kg, Pantothenic acid (Min) 3750 mg/kg, Biotin (Min) 125 mg/kg, Zinc (Min) 20 g/kg, Copper (Min) 2000 mg/kg, Choline (Min) 125 g/kg, Iron (Min) 15 g/kg, Iodine (Min) 125 mg/kg, Vit. K (Min) 1000 mg/kg, Manganese (Min) 3700 mg/kg, Niacin (Min) 7800 mg/kg, Selenium (Min) 75 mg/kg, Vit. A (Min) 2,000,000 UI/kg, Vit. E (Min) 15,000 UI/kg, Vit. $B_1$ (Min) 2500 mg/kg, Vit. $B_{12}$ (Min) 5000 mg/kg, Vit. $B_2$ (Min) 2500 mg/kg, Vit. $B_6$ (Min) 2000 mg/kg, Vit. $D_3$ (Min) 500,000 UI/kg.

[b] BHT - Butylated hydroxytoluene.

[c] Values analysed in laboratory, expressed on a dry matter basis.