Fish & Shellfish Immunology 43 (2015) 295–300

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Energy deficit does not affect immune responses of experimentally infected pacu (*Piaractus mesopotamicus*)

Rodrigo Y. Gimbo [a], Gisele C. Fávero [a], Luz N. Franco Montoya [a], Elisabeth C. Urbinati [a, b, *]

[a] Faculdade de Ciências Agrárias e Veterinárias, UNESP Univ Estadual Paulista, Via de Acesso Prof. Paulo Donato Castelane, 14884-900 Jaboticabal, SP, Brazil
[b] Centro de Aquicultura, UNESP Univ Estadual Paulista, Via de Acesso Prof. Paulo Donato Castelane, 14884-900 Jaboticabal, SP, Brazil

ARTICLE INFO

Article history:
Received 7 September 2014
Received in revised form
2 January 2015
Accepted 5 January 2015
Available online 10 January 2015

Keywords:
Innate immune responses
Energy deficit
Starvation
Pacu

ABSTRACT

We investigated if the energy deficit following a 30-day starvation period could affect the ability of fish to mount immune responses after experimental exposure to *Aeromonas hydrophila*. Fish were submitted to two feeding strategies during 30 days: starvation and continuously feeding. Fish were then sampled to allow for the assessment of baseline metabolic and immune system indicators, were next intraperitonially inoculated with *A. hydrophila*, and finally were sampled at 3 and 24 h after the challenge. The respiratory activity of leukocytes was lower in starved fish at baseline, increasing after bacterial inoculation to levels similar to those seen among fed fish. Levels of serum lysozyme were higher in starved fish at baseline. The same response profile was observed 3 h after inoculation, but among fed fish, these levels increased to values similar to those of starved fish 24 h after infection. Among starved fish, lysozyme concentration did not change over the course of the experiment. The serum ACH activity was lower in starved fish at baseline and increased after bacterial inoculation in both fish groups. Baseline levels of blood glucose of starved fish were lower than those of fed fish and increased 3 h after bacterial inoculation in both fish groups, decreasing in both groups at 24 h after inoculation. Baseline liver glycogen levels were similar in both fish groups and higher than at 3 and 24 h after inoculation. Three hours after bacterial inoculation, liver glycogen was less reduced in fed fish. Baseline levels of blood triglycerides were lower in starved fish and the profile remained unchanged 3 h after inoculation. There was a gradual decrease in fed fish, and the levels of starved fish remained unchanged throughout the observation period. Blood glycerol levels at baseline were higher in starved fish than in fed fish and remained unaltered at 3 h after inoculation. However those levels increased at 24 h. In fed fish there was a gradual increase of glycerol levels up to 24 h after bacterial inoculation. Baseline liver lipid levels of starved fish were lower and this difference in the response profile remained unchanged 3 and 24 h after inoculation. The liver lipid levels of starved fish decreased after inoculation, and remained unchanged in fed fish. As observed in liver lipid, muscle lipid levels of starved fish were lower than in fed fish, throughout the experiment. Starved fish levels remained unchanged; however fed fish levels decreased 24 h after bacterial inoculation. Levels of cortisol were higher in starved fish at baseline and increased in both fish groups 3 h after bacterial inoculation, reaching intermediary levels 24 h after inoculation. Our results show that in pacu, although mounting an immune response triggered after bacterial exposure is an energy-expensive process, fish under energetic deficit status were able to display protection against infection.

1. Introduction

Immune defense is an energetically costly physiological process. The production and maturation of immune cells in response to a pathogen, as well as the increased synthesis and activity of humoral components, such as proteins of the innate and acquired immune systems, are energetically costly [1,2]. Both starvation and infection promote important alterations in animal physiology, which are sustained by mobilization of reserves to meet this energy demand [3–6]. The adaptive physiological response of fish to starvation is aimed at conserving the health and function of key organs and redistributing essential resources for biological systems [7].

* Corresponding author. UNESP Univ Estadual Paulista, 14884-900 Jaboticabal, SP, Brazil. Tel.: +55 16 3209 2654.
E-mail addresses: bethurb@fcav.unesp.br, bethurb@uol.com.br (E.C. Urbinati).

http://dx.doi.org/10.1016/j.fsi.2015.01.005
1050-4648/

Previous research on the effects of starvation in fish has focused mainly on metabolic responses and endocrine pathways [5,7–9]. Immune responses have also been evaluated in starved fish [5,8,10–12], however, little is known about immune responses after infection in starved fish [5]. Thus, it is still unclear how energy deficits affect the different metabolic pathways involved in the immune responses triggered after bacterial exposure.

Understanding the modulation of immune responses under conditions of energetic deficit is particularly important considering that food restriction is a feeding strategy widely used in the fish farming industry to promote compensatory growth [13] while improving growth efficiency [14] and preventing water quality deterioration [15]. However, it is possible that while growth is more efficient at restricted levels of energy, this reduction of the energy budget might affect biological processes such as mounting of immune responses. This possibility would be in line with the notion that host defense against parasites and pathogens is a costly life-history trait that can generate trade-offs with other fitness components [16]. In this study, we investigated the effects of energetic deficit following starvation on innate immune and metabolic responses of pacu, an important farmed fish in South America, after experimental pathogen exposure. Published studies have concerned pacu response to starvation [17,18], stress [19], metabolism [20] and immune responses [21] but none associated metabolic mobilization to an experimental bacterial infection.

2. Material and methods

The experimental procedures were approved by the Comissão de Ética no Uso de Animais (CEUA – Protocol 002112/12) and performed in accordance with the guidelines of the ethical principles in animal experimentation, adopted by the Colégio Brasileiro de Experimentação (COBEA).

2.1. Fish and experimental conditions

We used a total of 96 (345.6 ± 16.4 g) fish, obtained from the Centro de Aquicultura da UNESP and randomly distributed in 8 fiber tanks of 460 L (12 fish tank^{-1}) for one week acclimatization, being fed a commercial diet. During this period, water temperature (28.5 ± 1.0 °C) and oxygen levels (>5.0 mg L^{-1}) were monitored. Photoperiod was 13 h light: 11 h dark.

2.2. Experimental design

Fish were submitted to two different strategies during 30 days: starvation (S) and continuous feeding (F) groups (4 tanks per treatment). Fed fish were fed until apparent satiation with experimental diet twice a day (9:00 AM and 5:00 PM). At the end of the 30-day trial, 12 fish per treatment were quickly sampled to allow for the assessment of baseline metabolic and immune system indicators. The remaining fish of each tank were intraperitoneally injected with *Aeromonas hydrophila* and then sampled at 3 and 24 h after the challenge (n = 12). The suspension of 1×10^2 CFU cells mL^{-1} of bacteria was previously determined to provoke only infection and not severe mortality (data not shown), to allow the stimulation of the fish immune system.

2.3. Sampling and experimental infection

Fish (12 from each treatment) were anaesthetized with benzocaine (0.1 g L^{-1}) and blood samples, drawn from the caudal vessel, were dispensed in heparinized microtubes (plasma), and microtubes without anticoagulant (serum). Whole blood was immediately used to measure the leukocyte respiratory burst. For plasma separation, blood samples were quickly centrifuged (10 min at 3.000 × g), and glucose and triglyceride concentrations were determined immediately. Blood was allowed to clot at room temperature for 3 h, thereafter centrifuged and serum was stored at −80 °C until analysis of cortisol, glycerol, complement system activity, and serum lysozyme concentration. After blood sampling, fish were euthanized (benzocaine, 0.4 g L^{-1}) for mesenteric fat removal, whose weight was used to calculate the mesenteric fat index (MFI) [(mesenteric fat weight/body weight) × 100] and liver and dorsal portions of white muscle were removed and stored at −20 °C for further determination of lipids and glycogen.

2.4. Specific procedures

2.4.1. Experimental diets

The practical diets were formulated and prepared aiming to supply pacu requirements (22% crude protein and 4200 kcal kg^{-1}) [22] and stored at −20 °C until needed.

2.4.2. Metabolic assays

The blood glucose and triglyceride concentrations were measured by enzymatic method (Labtest kit, Sao Paulo, Brazil, codes 84 and 87, respectively). Serum was used to determine glycerol by enzymatic method (Bioclon, Belo Horizonte-MG, Brazil, code 76) and cortisol concentration by ELISA (Enzyme-Linked ImmunoSorbent Assay) with commercial kit (DRG International, Inc., USA; Cortisol ELISA – EIA – 1887) following manufacturer's instructions. Liver glycogen level was measured by lysis in amyloglycosidase after extraction with perchloric acid (7%) [23] and liver and muscle lipid levels were determined after extraction in chloroform and methanol solution [24].

2.5. Immunological assays

2.5.1. Leukocyte respiratory burst

The production of reactive oxygen species (ROS) was measured using NBT (Nitrotetrazolium Blue chloride – Sigma–Aldrich – N6876), following protocol [25] with modifications as suggested by Ref. [21]. Immediately after fish bleeding, 100 μL of heparinized blood was incubated with an equal volume of NBT buffer (0.2%) at room temperature for 30 min. Thereafter, 1 mL of dimethylformamide (DMF, Sigma, St Louis, MO, USA – 227056) was added to the samples, and they were read using a spectrophotometer (Thermo Scientific; Genesys 10S), at room temperature, and set to 540 nm.

2.5.2. Serum lysozyme concentration

Serum lysozyme concentration was determined following protocol [26] modified for use with pacu blood. The assay is based on the lysis *Micrococcus lysodeikticus* (Sigma–Aldrich – M3770) suspension using hen egg white lysozyme (Sigma–Aldrich – L6876) as standard. The assay was performed into a 96-well plate in triplicate. The rate of decrease in absorbance for each sample (ΔOD) was then compared to that obtained with the standard curve so that lysozyme concentration could be expressed in ng μL^{-1}.

2.5.3. Complement system activity: alternative pathway (ACH activity)

Serum complement hemolytic activity was measured according to [27,28] modified for use with pacu blood. Rabbit blood was collected and processed to isolate red blood cells (RaRBC) whose suspension was added to serum, then complement hemolytic activity was measured as time (in seconds) necessary to lyse 50% of RaRBC in kinetic assay at 700 nm, using a Genesys 10S spectrophotometer (Thermo Scientific®).