Apr 07, 2021

Prebiotic research protocol

Mohammad Abdus Salam[1]

[1]Bangabandhu Sheikh Mujibur Rahman Agricultural University

1 Works for me dx.doi.org/10.17504/protocols.io.bt2knqcw

Prebiotic research protocol

Mohammad Abdus Salam
Bangabandhu Sheikh Mujibur Rahman Agricultural University

ABSTRACT

The dietary chitosan promotes growth, biochemical composition, hematological parameter and morphology of internal organs by improving gut microbiota status of juvenile *Barbonymus gonionotus.*

DOI

dx.doi.org/10.17504/protocols.io.bt2knqcw

PROTOCOL CITATION

Mohammad Abdus Salam 2021. Prebiotic research protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bt2knqcw

CREATED

Apr 07, 2021

LAST MODIFIED

Apr 07, 2021

PROTOCOL INTEGER ID

48940

Euthanasia methods

1 Pure clove oil was first dissolved in ethyl alcohol in 1:9 ratio (clove oil: ethyl alcohol)

2 This solution then diluted in water in order to obtain concentrations of 0.05 mL (50 mg), and 0.20 mL (200 mg) of clove oil per 500 mL of water

3 For hematological study, experimental fish were anesthetized by using 0.05 mL clove oil per 500 mL of water

4 For histological, reproductive and intestinal microflora study, fish were euthanized by using 0.20 mL of clove oil per 500 mL of water, and death was confirmed by the destruction of the brain

Chemical analysis of carcass composition of B. gonionotus

5 The experimental fish were humanely killed by using clove oil (0.20 mL per 500 mL of water), and death was confirmed by the destruction of the brain

6 The fresh fish were weighed by an electric balance

7 Then the fishes were dried overnight using an oven at 105 ^{o}C (MC2846SL, LG Company, India) for 12 h and the dried fish weight was measured again

8 Then moisture content was determined by subtracting weight of dried fish from the weight of fresh fish

9 Then those dried fish samples were ground using an electrical blender

10 To determine crude protein, nitrogen content of fish carcass was measured according to modified Kjeldhal method following H_2SO_4 - salicylic acid digestion, distillation and titration

11 Fat content in carcass was determined by Soxhlet extraction with diethyl ether.
Ash content was determined by incineration of the samples at 550 ^{o}C for 12 h in a muffle furnace (Hayashi Denko Co. Ltd, Japan).

Analysis of mineral contents in muscles of B. gonionotus

12 Nine fishes from each treatment were anesthetized with the clove oil and gradually sacrificed.

13 The fish muscles were collected by removing head, fins, scales and intestine.

Then the muscles were dried using an oven at 105 ^{o}C (MC2846SL, LG Company, India) for 12 h and the dried muscles were ground using a blender.

Citation: Mohammad Abdus Salam (04/07/2021). Prebiotic research protocol. https://dx.doi.org/10.17504/protocols.io.bt2knqcw

14 The powdered samples were digested in boiling nitric acid and perchloric acid mixture (5:1) by following standard methods.

15 After appropriate dilution, the mineral contents such as K, Na, Ca, Mg, Zn, Fe and Mn of fish muscles were estimated using Atomic Absorption Spectrometer (170-30, serial 6268-001, Hitachi, Japan).

16 Calibrated standards for mineral estimation were prepared from commercially available standards (Buck Scientific 1-800-562-5566, BS-AQ-PB, Single element AA standard, USA).

17 The estimations of K, Na, Ca, Mg, Zn, Fe and Mn were done using a hollow cathode lamp (Hitachi, Japan).

Assessment of chitosan on the intestinal digestive enzyme activities of B. gonionotus

18 Nine fish of each treatment were randomly sampled to assay the intestinal digestive enzyme activity of *B. gonionotus*

19 Fish were sacrificed by the overdose of clove oiland the surface of each fish was sterilized using 70% ethanol

20 The peritoneal cavity was opened aseptically with a sterile scalpel

21 The intestine between the pyloric caeca and approximately 1cm anterior to the anus of the fish was excised and the feces with mucus were stripped off with sterile forceps

22 The intestine of 9 fish from each treatment were removed and frozen in liquid nitrogen then stored at -80 ^{o}C until subsequent analysis

23 The intestinal samples were homogenized in 10 vol (v/w) of ice cold PBS and centrifuged at 3000 g for 20 min at 4 ^{o}C. The supernatant was conserved and used to determine the amylase activity, protease activity and lipase activity using ELISA kits (Thermo Fisher Scientific, USA)

Histological analyses of intestine, liver and kidney of the dietary chitosan treated silver barb

24 The experimental fish were humanely killed by using clove oil (0.20 mL per 500 mL of water)

25 Death was confirmed by the destruction of the brain

26 The whole liver, kidney and part of the intestine from each fish were dissected carefully, cut to separate each other, and stored in bouins solution for 24 h

Citation: Mohammad Abdus Salam (04/07/2021). Prebiotic research protocol. https://dx.doi.org/10.17504/protocols.io.bt2knqcw

27 These samples were dehydrated in ascending grades of alcohol and cleared in xylene

28 The fixed tissues were embedded in histoparaffin (Paraplast plus; Sigma-Aldrich) and sections (7 µm) were cut using a microtome (CUT-5602, Germany)

29 Then the sections of intestinal villi, kidney and liver were selected and stained with Delafield's hematoxylin-eosin for observation under a light microscope (DM 100; Leica, Wetzlar, Germany).

30 Ten slides were prepared from the intestine of each fish through histological method.

31 Then the slides were observed under a trinocular microscope.

32 Images were captured using a digital camera (DFC 290, Leica) and the villi length of the intestine was measured using AmScope software (Version 3.7; Carl Zeiss Primo Star, Germany)

Measurement of Hematological Parameters

33 A total of 90 fish from each treatment were anesthetized with the clove oil (0.05 mL per 500 mL of water) for hematological analysis

34 Blood was collected from fish using a 3 cc syringe containing 10% blood anti-coagulant (EDTA) inserted into the caudal peduncle region to drag out blood.

35 The blood was transferred to a test tube coated with EDTA, and stored at -30 ^{O}C until use.

36 Red blood cells (RBCs) and white blood cells (WBCs) were counted using an improved Neubauer hemocytometer (MarienFeld Company Germany) under the light microscope (DM 100; Leica, Wetzlar, Germany)

37 To measure hemoglobin, fresh blood was collected from fish from each treatment and was poured in the edge of a strip of hemoglobin meter before the coagulation of blood.

38 The glucose level of blood was measured through a glucose meter from the sample.

39 To measure packed cell volume (PCV) (%), blood was taken in a capillary tube at the marked level and sealed with gum.

Citation: Mohammad Abdus Salam (04/07/2021). Prebiotic research protocol. https://dx.doi.org/10.17504/protocols.io.bt2knqcw

Citation: Mohammad Abdus Salam (04/07/2021). Prebiotic research protocol. https://dx.doi.org/10.17504/protocols.io.bt2knqcw