Use of Kaolin For Protection Against Flavobacteriosis In *Oreochromis niloticus (b)*

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Abstract

Because of the difficulties facing the treatment of flavobacteriosis, this study aimed to evaluate kaolin (a type of clay) for the prevention of such disease in *Oreochromis niloticus*. Addition of kaolin at rate of 1 g/L, to the water aquaria, 5 min prior the challenge of *O. niloticus*, protected the fish against columnaris disease (100%) and improved survival (93.7%) as compared to untreated fish (50 and 66.7%, respectively). The re-isolated strain from untreated challenged fish confirmed through a pair of 16S RNA gene-based PCR primers specific for *F.columnare* and produced unique and clear PCR bands of the expected size (675 base pair).

Key words: Kaolin, protection, *F. columnare*, flavobacteriois, *O. niloticus*

Introduction

Columnaris disease can infect fish at any age, under a variety of water conditions, and during any season of the year ***(Griffin, 1992).*** In USA, columnaris disease considered the second most prevalent bacterial disease causing economic losses secondary to Enteric septicemia of catfish ***(USDA, 2003)***. In addition, ***Eissa (1993 and 1994)*** recorded that Falvobacterium *colamnare* is the primary cause of mass mortalities in cultured *Oreochromis niloticus* and *Mugil cephalus*, respectively in Egypt.

Despite its importance, columnaris disease still faces some of the difficulties regarding the diagnosis and chemotherapy. Recently, the use of antibiotic as growth promotors in animal diets has been eliminated or limited in many countries because of concern related to cross-resistance to antimicrobial agents that are used in human medicine ***(Barton, 2000; Anonymous, 2002; Castro, 2005 and Chen et al., 2005)***. This limitation prompted the search and development of alternatives such as organic acids, enzymes, probiotics, plant extract and essential oils. Of these alternatives, Kaolin, a feed additive in animal nutrition affects growth performance, has been investigated formerly and recently ***(Savory, 1984; Sakata, 1986 and Owen et al., 2012).*** The use of Dietary Kaolin improves the

nutrient digestibility and enzymatic activity of gastrointestinal secretion ***(Ouhida et al, 2000 and Alzueta et al, 2002).***

Kaolin, a natural aluminum silicate hydrate (Al2(OH)4SiO5), is authorized for use as a food additive. In addition, according to last revision of Code of Federal Regulations (2014), issued by US-FDA, kaolin is permitted as generally recognized as safe (GRAS) used as an indirect human food ingredient with no limitation. Additional benefits related to the use of kaolin are that there is no risk of antibiotic resistance and no harmful chemical residues are introduced into the environment or the eventual consumer product.

Due to its adsorption character and it has no toxicity ***(Anonymous,1998),*** using of kaolin is recommended to overcome the adverse effects produced by toxic agents such as aflatoxins ***(Abdel-Wahhab et al, 1999; Phillips, 1999),*** metabolites of plant (alkaloids, tannins), heavy metals pathogenic microorganisms, ***(Hassen et al, 2003; Katsumata et al, 2003),*** enterotoxins caused by diarrhea ***(Dominy et al, 2004).*** Besides, Using of kaolin in aquaculture as bulking agents in pelleted feeds ***(Grove et al, 1978 and Jobling, 1981)*** and to reduce egg adhesion in hatchery operations ***(Mizuno et al, 2004)*** have been demonstrated.

Recently, ***Beck et al (2015)*** demonstrated survival improvement, reduction of gill infection by using kaolin and reduction of bacterial attachment to target tissues of columnaris disease in channel catfish by binding ability to *F. columnare* .

This study was carried out to evaluate the role of kaolin in prevention of columnaris disease in *Oreochromis niloticus* experimentally infected with *Flavobacterium columnare.*

Materials and methods

1) Fish:

A total of 120 alive and apparently healthy *O.niloticus* with an average body weight 50±5 g were collected from a fish farm from Ismailia governorate. They were transported to Fish Diseases and Mangement Depatment of Suez Canal University. Fish were fed on standard commercial ration containing 25% crude protein. The diet was daily provided as 3% of body weight.

2) Aquaria

Fish were kept in glass aquaria measuring 40x50x100 cm supplied with decolorinated tap water and air pumps for continuous aeration and filter for continuous filtration.

3) Kaolin:

Kaolin (Al2 Si205(OH) 4) was obtained from (Duggan veterinary company, Ireland) in form of 1 Kg white powder package.

4) *Flavobacterium columnare* strain:

A well identified *Flavobacterium columnare* strain was kindly obtained from Dept. of Fish Diseases and Management, Faculty of Vet. Medicine, Suez Canal University.

Challenge experiment:

One hundred and twenty apparently healthy *O.niloticus* were equally divided into 4 groups (30 fish for each group). The flow-through system provided with water at a flow rate of approximately 1 L/min and the temperature was maintained at 26 ± 0.5 C. According to Mitchell and Farmer (2010), fish were acclimiated to the experimental conditions for 10 days. The four aquaria were randomly assigned to one of four treatment groups: group one remained not treated with kaolin and challenged by cutaneous scrubbing and waterborne exposure to *F. columnare* (positive control); group two was treated with kaolin then challenged; group three was kaolin-treated non-challenged group and group four remained non-treated non-challenged (negative control).

Kaolin was added slowly to water at a rate of 1 g/L, near air pumping, to facilitate mixing and 5 minutes prior to challenge, to give enough mixing time and to initiate the ultra-low flow **(Beck *et al*, 2015).**

Fish were challenged by adding 5 ml of the bacterial suspension (4 x 10^7) from the virulent identified strain to each 10-L water.

Molecular characterization:

Bacterial isolates:

For re-isolation of bacteria from challenged-diseased fish, a sterile swab was rasped in the characteristic lesions of the fish. The material was then immediately transferred to Ordals agar and incubated at 22° C for 72 hrs. The bacterial isolate was identified using PCR .

Results

Kaolin protection against flavobacteriosis:

Addition of kaolin at rate of 1 g/L, to the water aquaria containing *O. niloticus*, 5 min prior the challenge protected fish against columnaris disease as it prevented the appearance of lesions (100%) as compared to untreated fish (0%) (table 1).

Molecular Identification of *Flavobacterium columnare* by polymerase chain reaction (PCR).

A pair of 16S RNA gene-based PCR primers, were used for detection of *F.columnare* re-isolates from challenged fish. The re-isolated strain from diseased fish challenged and untreated (group 1) produced unique and clear PCR bands of the expected size (675 base pair) (Fig. 1) PCR confirmed that the re-isolated strain was *F.columnare*.

Table (1) *Effect of kaolin on diseased O. niloticus with flavobacteriosis*

Group	No. of fish	Kaolin treatment	Challenge with *F.columnare*	Dead fish		Diseased fish	
				No.	%	No.	%
1	30	Non	Challenged	10	33.3	20	66.67
2	30	Treated	Challenged	2	6.7	0	0
3	30	Treated	Non	0	0	0	0
4	30	Non	Non	0	0	0	0

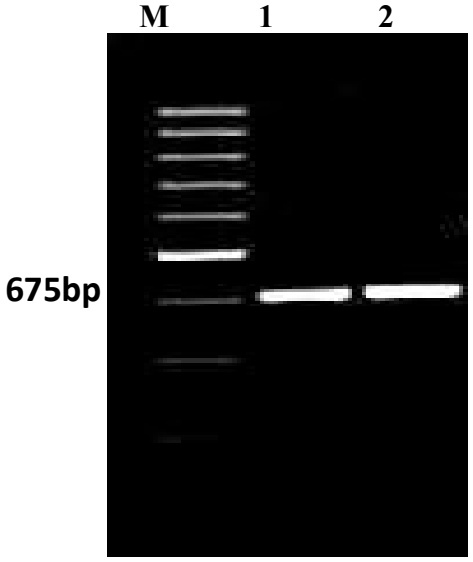

Fig. (1): Agarose gel electrophoresis of PCR for re-isolated strain.
M: marker- Lane 1= control positive (strain of challenge).
Lane 2= reisolated strain from diseased fish.

Discussion

Since 2005, the European Commission implemented an absolute ban on the use of antibiotic growth promoters in animal diets ***(Castro, 2005; Chen et al, 2005)***, mainly due to the potential occurrence of cross-resistance to antimicrobial agents that are used in human medicine.

Kaolin represents one of the alternatives that is permitted as food additives in the European Union and approved as GRAS indirect food materials in United State (CFR, 2014) without risk of antibiotic resistance or harmful chemical residues addition to the environment or the eventual consumer product.

Our results revealed that addition of kaolin prior to the challenge of *O. niloticus* protected the fish against columnaris disease as the mortality reduced to 6.7% in kaolin-trreated challenged group while

reached to 33.3% in untreated challenged group. This finding is in complete accordance with that reported recently by ***Beck et al (2015)*** who reported the reduction of gill infection by using kaolin and reduction of bacterial attachment to target tissues of columnaris disease in channel catfish lead to survival improvement of infected fish. They demonstrated that Kaolin adsorbs the bacterium and binds to it, thereby preventing it from attaching to the fish and causing the disease.

Flavobacterium species have a highly susceptiblity to be adsorped by kaolin ***(Esterman and McLaren, 1959 and Soda et al, 1999).*** Adsorption phenomenon means an accumulation of a number of molecules, ions, or atoms that occur at the boundaries of the two phases. The amount of adsorbate accumulates on the adsorbent is affected by particle size and surface area of the adsorbent and the adsorbate. Condensed molecule is called adsorbate, while the substrate surface (solid or liquid) is called adsorbent ***(Prawira, 2008).***

Because of its adsorbent capability, kaolin was added to reduce egg adhesion in hatchery operations in aquaculture ***(Mizuno et al, 2004)*** and added to animal diets to prevent enteric diseases through its ability to absorb enterotoxins and significant mycotoxins ***(Abdel-Wahhab et al, 1999; Phillips, 1999; Boranic, 2000; Dominy et al, 2004 and Trckova et al, 2004).***

Regarding the molecular identification of the re-isolated strain from lesions appeared on the challenged groups, by using PCR , the results confirmed without doubt that, it was *F. columnare*. This is in agreement with ***Mohamed and Refat (2011)*** who confirmed molecularly all of the positive isolated of *F. columnare* which isolated from Nile tilpia by means of PCR

The results concluded that Kaolin not only offers producers an alternative to antibiotics in combating columnaris disease, but it also provides an inexpensive treatment for a costly fish disease because plentiful clay is available in Egypt.

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استخدام الكاولين للحماية من الفلافوبكتريوسز (ب)

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بسبب الصعوبات التي تواجه علاج الفلافوبكتريوسز فإن هذه الدراسة تهدف إلى تقييم الكاولين (نوع من الطين) للوقاية من هذا المرض في أسماك البلطي النيلي . وجد أن إضافة الكاولين بمعدل ١ جم / لتر ، إلى أحواض المياه ، ٥ دقائق قبل التحدي ببكتريا الفلافوبكتريم كولامنر فى البلطي النيلي ، يحمى الأسماك من مرض الكولامنارس بنسبة ١٠٠% ويحسن البقاء على قيد الحياة (٩٣,٧ ٪) بالمقارنة مع الأسماك غير المعالجة (٥٠ و ٦٦,٧ ٪ على التوالي) .نوع البكتريا المعاد عزلها من الأسماك الغير معالجة بالكاولين مع اجراء التحدى لها تم تاكيده من خلال 16SRNA gene-based PCR و الخاص ببكتريا الفلافوبكتريم كولامنر وأنتجت عصابات PCR فريدة من نوعها و اضحة من الحجم المتوقع (٦٧٥ base-pair) .