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TRIALS FOR CONTROL OF EDWARDSIELLOSIS BY IMMERSION VACCINATION (B) THE ROLE OF SOME ENVIRONMENTAL FACTORS ON THE IMMUNE RESPONSE OF NILE TILAPIA (OREOCHROMIS NILOTICUS) AGAINST EDWARDSIELLA TARDA CRUDE LIPOPOLYSACCHARIDE

(With 2 Tables)

By

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محاولات لمقاومة الأدواردسيلوزس بالتحصين بالتغطيس
(ب) دور بعض العوامل البيئية على الأستجابة المناعية فى البلطى النيلى
ضد الأدواردسيلاتاردا ليبوبولى سكاريد الخام

أحمد بدران ، محمد الدناصورى

فى عدة تجارب معملية تم دراسة تأثير درجة الحرارة ومعدلات التغذية وكثافة الأسماك على الأستجابة المناعية فى البلطى النيلى المحصن بالأدواردسيلاتاردا ليبوبولى سكاريد الخام. وقد وجد أن البلطى النيلى المربى تحت تأثير العوامل البيئية المثلى وكذلك المعرض لدرجات الحراره العالية أو المغذى على معدلات مختلفة من العليقة أو المربى بكثافات عالية تستجيب للتحصين بإنتاج معدلات عالية من الأجسام المناعية المفرزة فى السائل المخاطى المحيط لجسم الأسماك تصل الى (٥) فى الأسبوع الأول بعد التحصين والى (١٠: ١١) بعد الأسبوع العاشر من التحيصن. على النقيض فإن البلطى النيلى المعرض لدرجات الحرارة المنخفضة (١٦+١ م°) ينتج كميات قليلة من الأجسام المناعية تصل الى (٦) فى الأسبوع العاشر بعد التحصين.

SUMMARY

Effect of water temperature, feeding levels and stocking densities on the immune response of Nile tilapia, *Oreochromis niloticus*, vaccinated with *Edwardsiella tarda* crude lipopolysaccharide (LPS) was determined in laboratory controlled experiments. *O. niloticus* reared under optimal environmental factors and those exposed to other factors, with exception of low water temperature, responded to immersion (DP) vaccination in crude LPS by production of high levels of secreted antibody in the body surface mucus pronounced from first week post-vaccination (PV), 5 by Log_2, and reached 10:11 at 10 week PV. By contrast, *O. niloticus* exposed to low water temperature (16 ± 1C°) had low levels of secreated antibody in the body surface mucus (2 at 1 week and 6 at 10 week PV).

Keywords: *Trials, Control, Edwardsiellosis, immersion Vaccination, Nile Tilapia.*

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INTRODUCTION

The occurrence of edwardsiella septicemia as a fish disease of cultured fishes have been reported among different fish species,eels *(WAKABAYASHI and EGUSA, 1973)* , channel catfish *(MEYER and BULLOCK, 1973)*, mullets *(KUSUDA et al., 1976)*, carps *(SAE-ONI et al., 1984)* and Nile tilapia *(BADRAN, 1993)*. As it is considered a dangerous septicemic disease affecting cultured fish and leading to high economic losses *(WAKABAYASHI and EGUSA, 1973)*, research on preventive measures became a natural concern. Many experimental and practical approaches to stimulate the immune response of fish by *Edwardsiella tarda* bacterin were established *(SONG and KOU, 1981; SALATI et al., 1983; LIO-PO and WAKABAYASHI, 1986; SALATI, 1988 and BADRAN, 1995)*. On the other hand, there are different number of environmental factors- more or less inevitable in fish culture- may affect the immune response of fish and the development of an effective vaccine *(ELLIS, 1988)*. Such factors are environmental water temperature, quality and quantity of feed intake and stocking density of fish.

The present work was planned to study the effect of water temperature, feeding level and stocking density on the immune response of Nile tilapia, O. niloticus, vaccinated with *Edwardsiella tarda* crude LPS.

MATERIAL and METHODS

(1) Fish:-

A total of 330 healthy Nile tilapia *O. niloticus*, with an average body weight of 70±5 g. were used. Fifty fish divided into equal 5 groups (from A to E) each contained 10 fish were used for determination of the maintenance level of feeding. Two hundred and eighty fish were divided into 8 groups for studying the effect of different environmental factors on the immune response. The groups from 1 to 6 contained 20 fish/ each group, while groups 7 and 8 contained 60 and 100 fish respetivelly.

(2) determination of maintenance level of feeding:-

The groups from A to E (Table 1) were supplied with diet in a ratio of 1.5,2 , 3, 4 and 5% of their body weight/ day respectivelly. The fish were placed under examination during one week. The maintenance level was determined as the level of diet sufficient to fish maintenance where the fish not need more diet or the diet not more than their requirment and considered to point out of over feeding.

(3) Environmental conditions:-

Each environmental condition was adjusted and fixed for 2 weeks before fish vaccination and throughout the experimental period.

Control system:-

Each of control groups (1 and 2) contained 20 *O. niloticus* with total biomass 1400 g. resulting in stocking density of 12.5 g/l. The fish diet was offered daily at the rate of 3% of the total biomass (Maintenance level). The water temperature was adjusted at 22±1 C°. The flow rate of aquarium water through the filter was approximately 1.9 l/ minute and the water volume of each aquarium was 112 l. resulting in com-

plete water turnover/ hour *(COLLINS et al., 1976).*

(A) Effect of environmental water temperature:-

All environmental conditions were identical to the control system with exception of the water temperatures were adjusted at 16±1 and 28±1 C° to groups 3 and 4 respectivelly.

(B) Effect of feeding level:-

All environumental conditions were identical to control system with exception that, the feeding level was offered at 4% and 2% (above and below maintenance level) to the fish of groups 5 and 6 respectivelly.

(C) Effect of stocking density of fish:-

All environmental conditions were identical to the control system with exception that the stocking densities of groups 7 and 8 were 37.5 g and 62.5 g/l respectivelly. The flow rate of water in both aquaria through the filter was increased to 5 l/ minute.

(4) Vaccine preparation and vaccination:-

E. tarda crude LPS prepared in the previous study *(BADRAN, 1995)* was used. *O. niloticus* of groups from 2 to 8 were vaccinated by DP in crude LPS at concentration of 20 ug wet weight/ ml for 2 hs *(Baba et al., 1988). O. niloticus* of group1 were remained as unvaccinated control.

(5) Antibody titeration:-

The body surface mucus was collected from 5 *O. niloticus* at 1, 4, 7 and 10 week PV (KAWAI *et al., 1981);* salted out in 50% saturated solution with ammonium sulfate and then dialysed and concentrated in polyethylene glycol powder *(AUSTIN and RODGERS 1981).* The mucus was concentrated to a ratio of 0.2 ml/ fish. The secreted antibody titer in collected mucus to *E. tarda* was determined using bacterial agglutination test. For agglutination, formalin-killed bacterial cells were washed twice with phosphate buffer saline (PBS), and prepared to a concentration of 3 mg wet-weight/ ml in PBS *(BABA et al., 1988).*

RESULTS

The maintenance level of feeding for *O. niloticus* in the present experiment was 3% of their body weight day (Table 1).

The results of immune response in *O. niloticus* to E. tarda crude LPS under different environmental conditions were reported in Table (2). The results revealed that *O. nilotucus* remained without vaccination had low level of natural secreted antibody (1 by Log_2) in the body surface mucus. While *O. niloticus* vaccinated with *E. tarda* crude LPS and reared under all optimal environmental conditions had high levels of secreted antibody (5, 9, 10 and 11 at 1, 4, 7 and 10 week PV respectivelly). Likewise, *O. niloticus* exposed to high water temperature, above and below maintenance levels and overcrowding had high levels of secreted antibody similar to those of fish reared under optimal environmental conditions. On the other hand, the immune response of *O. niloticus* exposed to low water temperature was greatly suppressed as the secreted antibody titers were 2, 4, 5 and 6 at 1, 4, 7 and 10 week PV respectivelly.

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immune response did not different from those of the control. Yet, the immunosuppression recorded in over-crowded blue gouramis *(PERL-MUTTER et al., 1973)* to the 2nd injection of Infectious Pancreatic Ni-crosis Virus vaccine was attributed to a water-born methylchloroform soluble factor produced by this species of fish.

From the present study, it could be concluded that, vaccine application in pisciculture, particularly in enzootic ar-eas, should be performed at the begin-ning of summer season to get rid of the immunosuppressive effect of low water temperature.

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Table (1): Experimental desige for determination of the manitenance level of O. niloticus feeding.

Fish group	Total No.	Stocking density (g/L)	Water temp. (C°)	Water flow rate (L/min)	Feeding level (%)
A	10	6.3	22±1	1.9	1.5
B	10	6.3	22±1	1.9	2
C	10	6.3	22±1	1.9	3 *
D	10	6.3	22±1	1.9	4
E	10	6.3	22±1	1.9	5

* = Maintenance level of feeding.

Table (2): Antibody response of O. niloticus reared under different environmental factors.

Fish group	Total No.	Stocking density (g/L)	Water temp. (C°)	Feeding level (%)	Flow rate (L/min)	Secreated antibody titers (Log / week PV) 1	4	7	10
1	20	12.5	22±1	3	1.9	1	1	1	1
2	20	12.5	22±1	3	1.9	5	9	10	11
3	20	12.5	16±1	3	1.9	2	4	5	6
4	20	12.5	28±1	3	1.9	5	9	10	11
5	20	12.5	22±1	4	1.9	5	9	10	11
6	20	12.5	22±1	2	1.9	5	9	10	10
7	60	37.5	22±1	3	5	5	9	10	11
8	100	68.5	22±1	3	5	5	9	10	10