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Culture techniques for *Limnodrilus hoffmeisteri* (Annelida: Oligochaeta)

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summary

The Oligochaete worm *Limnodrilus hoffmeisteri* is one of the best and cheapest live food for fish. The worm grows fastest (8.0 mg in 50 days) on the substrate containing 75% cow dung and 25% fine sand; the culture system (100*100*75 cm) requires continuous running water at the rate of 250 ml min-1 to maintain 3 mg O_2 L^{-1}. Addition of fresh cow dung (250 mg cm-2) one in 4 days is the optimum frequency. As the worm is photophobic, so the best time for collection the worm from the substrate is before dawn.

تقنيات في اعداد اوساط زرعية تجريبية للديدان قليلة الاهلاب *Limnodrilus hoffmeisteri* (Annelida: Oligochaeta)

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الخلاصة

تعد ديدان L. hoffmeisteri قليلة الاهلاب من افضل وارخص غذاء الاسماك. اعدت مزرعة لتربية هذه الديدان ساعدت على نموها بسرعة وبكفائة بلغت 8ملغم في 50 يوما لمزرعة احتوت على 75% سماد حيواني و25% رمل ناعم في حوض تربية ابعاده 100× 100×75سم ويتطلب مياه جارية بصورة مستمرة بمعدل 250مل /الدقيقة ليحافظ على نسبة الاوكسجين المذاب بمقدار 3ملغم/لتر . ان افضل اضافة للسماد الحيواني كان بنسبة 250غم/سم (مرة كل اربعة ايام). بينت النتائج ان افضل وقت للجمع هو وقت الفجر .

Introduction

In practical aquaculture, the largest problem of the farm is the cost of food, so attention has been given to the development of artificial feeds as well as to the problems of manufacture and storage facilities. Among the natural feed organisms *L.hoffmeisteri* is one of the best candidates for many reasons: 1.it has short generation time on a single medium containing organic waste like cow dung. 2. It occurs in different habitats and tolerates many environmental variables (Brinkhurst and Kennedy, 1965; Birtwell and Arthur, 1980) and 3. It has a high fecundity of 85 to 325 eggs and reproduces within a temperature range of 0.4-32C 0 (poddubnaya, 1980)

Material and Methods

L. hoffmeisteri was collected from irrigation canal in Babylon University in 2000 and cultured in aquarium (100* 100*75 cm) in laboratory. Continuous water flow through the aquarium was maintained.

1/ Substrate Combination:

The worms (10 individual per container, mean live weight of an individual, 1.5 ± 0.1 mg) were maintained on different combination substrate with three replicates for each.

The component were fresh cow dung, fine sand, medium sand, coarse sand. Each combination was prepared so that the cow dung comprised 0,25,50,75 or 100% of the total weight. The Oxygen concentration of water flow was around 3.0 ml . L^{-1}.

After 3 month the weight and number of worms were estimated.

2/ The rate of water flow:

L. hoffmeisteri can survive in low levels of dissolved Oxygen (1.7 mg /L at the minimum). Different quantities (50,125,250 or 500 g L^{-1}) of fresh cow dung were separately immersed in water, and Oxygen depletion was followed. To determine the optimum flow rate, which would maintain the dissolved Oxygen at 1.7 mg /L in the media containing a mixture of 75% cow dung and 25% fine sand.

The following flow rates were tested: 1500, 1000,500,250 and 100 ml min^{-1}.

The Oxygen content of the water at the outlet was determined.

3/ Optimum frequency of cow dung application:

A series of experiments indicated that the application of 2g of cow dung per cm2 ensures the highest individual growth rate. The schedules of application shown in table 1 were tested to select the optimum quantity and frequency of application.

Table (1): Cow dung application schedules.

Application schedules	Quantity added (g /cm2)	Frequency application
1	1.000	once in 16 days
2	0.500	once in 8 days
3	0.250	once in 4 days
4	0.125	once in 2 days
5	0.063	once in per days

4/ Growth estimation:

To study the growth rate of *L. hoffmeisteri* the worms were cultured at a density of 15 ± 3 worms (age of 28 days) in culture substrate selected. Weight of the individuals was noted at different time intervals.

Result

1/ Substrate Combination:

During the experiment period of 3.0 month survival of *L.hoffmeisteri* in different substrate combination ranged from 70% to 80%. Growth was significantly affected by both quantity of cow dung added and the kind of other substrate component. Table (2) reveals that growth was almost doubled and then redoubled when the addition of cow dung was increased from 25% to 75% and among the other components the fine sand have fastest growth. Hence a combination of fine sand (25%) with cow dung

(75%) is ensure rapid growth of *L. hoffmeisteri.*

Table(2):Growth of *L. hoffmeisteri* as a function of substrate composition.

Composition of substrate %	**Growth rate (mg live weight per worm) in substrate /day**		
Cow dung	Fine sand	Medium sand	Coarse sand
0	0.6	0.4	0.1
25	1.4	0.9	1.0
50	3.7	2.3	2.3
75	6.0	2.9	3.2
100	-	-	-

Analysis of variance

Source	ss	d.f.	MS	F
Between cow dung quantities	101.3	3	33.8	344.9
Between other combination	125.5	3	4.5	45.9
Error	3.3	33	1.3	13.2
Total	117.1	39	0.098	-

SS- sum of squares; MS -mean square

2/the rate of water flow:

In sample containing 50,125,250 or 500g cow dung L^{-1}, the dissolved oxygen was completely d was completely depleted within 24h. Oxygen content of the water at inlet was 5.8 mg L^{-1} indefinitely (Table 3). So rearing in a culture media containing 75% cow dung and 25% fine sand flushed by water flowing at the rate of 250 ml min-1 is recommended.

Table (3) Effect of different rates of water flow on oxygen depletion in the culture

DO in inlet watermg/l	DO in outlet water mg/l	Flow rate of water ml/min
5.8	4.8	1500
5.8	4.3	1000
5.8	3.5	500
5.8	3.0	250
5.8	1.8	100

3/ Optimum frequency of cow dung application:

The growth of *L.hoffmeisteri* in density and biomass under the chosen cow dung application schedules showed that In term of number the population density reached a peak of 31 cm -2 on the 58th day in schedules 1 in which the cow dung applied once in 16 days. In schedules of 3,4 and 5 a peak density of about 176 cm -2 was obtained on the 40th days of culture . In term of biomass there was different picture. In schedules 3 the biomass of population was 233 mg cm -2 around 50th days . Hence schedule 3 was considered to ensure the maximum yield of *L.hoffmeisteri* (Table 4)

Table(4): Effect of frequency of cow dung application on the population density and biomass (x= SD) of L.hoffmeisteri.

Cow dung application schedules	Maximum density (ind/ cm -2)	Biomass (mg /cm)	Frequency of application
1	31±8	35±5	1/16
2	135±15	181±6	1/8
3	166±23	233±15	1/4
4	176±15	220±13	1/2
5	170±119	223±15	-

4/ Growth estimation:

L.hoffmeisteri grew slowly and a body weight of about 1.5 mg during the initial period of 30 days, this was followed by a growth phase for a period of 15 days. After the 50 days the maximum body weight become at around 8.0 mg. From this period all converted energy was channeled for reproductive rather than somatic growth.When the body weight of animal about 5mg, formation of cocoons formed only after the worm become 8.0 mg around 50th days (Table 5). The release of cocoons and the hatching of young in culture medium led to crowding and harvest of adult.

Table (5) Growth rate of *L. hoffmeisteri* (star marks the beginning of cocoon laying)

Time/day	Growth rate mg/day
10	0.9
20	1.3
30	2.0
40	5.0
50	8.0 *
60	7.5

Discussion

Most previous works have reared tubificids in mud with organic matter (e.g Brink hurst and kennedy, 1965; AL-Dargazally, 1999). Feeding Oligochaetes with lettuce (Kosiorek, 1974; Bonacina et a, 1987) observed the highest rate of growth and fecundity. Hence lettuce is recommended, as digested and absorbed by the worm, the worm feed also supports the development of a bacterial flora in the gut of the worm. In the present study *L.hoffmeisteri* was cultured on substratum containing a mixture of fine sand (25%) and fresh cow dung (75%) comparison of the growth rate and fecundity of these worm revealed that the cow dung is a better feed than lettuce for the following reason:

The weight of the worm in this study which provided with cow dung become 8.0 mg within 50 days while those provided with lettuce could grow to 4.0 mg during the same period (Table 4) and-2- . The number of cocoon per egg was higher in present study (10 ± 3 egg per cocoon) compared with results (3 egg per cocoon) reported by (Bonacina et al, 1987).

The addition of sand is likely to improve the use of cow dung for culture in many ways:

Firstly its probably reduce the Oxygen demand per unit volume of substrate. This is likely to reduce the concentration of products of anaerobiosis which might be toxic or to growth and reproduction. The reduction of oxygen demand allow higher dissolved oxygen level in the layer of mud of the substrate so that the level that the worm extracts its supply of dissolved oxygen by obserbing the gas through the epithelium of the tail.

Secondly its provides a thicker substrate which facilitates to utilization of more energy for growth and reproduction by minimizing activity (Aston &Milner, 1982).

Our culture system supported the highest density (176x10m -2) of *L.hoffmeisteri* (table 6) this value compared with that of (148x10 m-2) reported for Brinkhurst & Kennedy (1965) system.

The density in Brinkhurst,s population may have increased for need of frequent water changes (styczymska –jurwicz , 1967). Possibly the water change (once in 3 days) adopted by Brinkhurst was not sufficient and the continous water flow system provided in the present study may have supported the high population density by ensuring sufficient aeration.

Other study have shown that the reproductive potential *L.hoffmeisteri* is higher than that of other tubificids (Brinkhurst & kennedy 1965; kennedy, 1966 and poddabnaya, 1980) and the egg production is dependent on temperature, oxygen content of the water and food availability. Normal development of the embryo requires a minimum oxygen content of 2.5 – 7.0 mg L-1 (poddubnaya, 1980), an oxygen level lower than 2.0 mg L-1 inhibits feeding (Mc Call & Fisher, 1980) and reproduction(poddubnaya ,1980). Thus the high oxygen content (~ 3.0 mg L-1) maintained in our culture system supported not only the highest worm density but also ensured the highest fecundity.

Table (6): Comparison of tubificid production in field and laboratory condition.

Species	Site	Density No. m-2	References
L.hoffmeisteri	Lake Eric	10^3-104^4	Mc Call & Fisher 1980
L.hoffmeisteri	London Bridge	$5*10^5$	Birtwell & Arthur 1980
L.hoffmeisteri	Cold spring harbor	10^4-10^5	Teal 1957
L.hoffmeisteri	Laboratory culture	$176 * 10^4$	Present study
Mixture of species *T.tubifex* *L.hoffmeisteri* *L.undekmianus*	Ditton Brook	$148 * 10^4$	Brinkhurst & kennedy 1965

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