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EFFECT OF DIFFERENT DIETARY PROTEINS AND ENERGY LEVELS ON SOME BIOLOGICAL PROCESSES OF *BIOMPHALARIA ALEXANDRINA*

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ABSTRACT

The effect of four experimentally prepared diets with different protein contents and energy levels on various biological activities of Biomphalaria alexandrina snail was investigated as compared with lettuce, the usual laboratory diet. The results obtained illustrated the superior effect of the diets prepared over lettuce on the growth rate, egg-laying capacity and hatchability, mortality rate and the rate of cercarial shedding of infected snails.

INTRODUCTION

The energy level of an animal is a vital phenomenon that affects and controls different biological and physiological activities. The feeding capacity is an important factor affecting the energy production in snails since reproduction and growth rate are biological processes that need energy.

Many workers studied the factors that affect the different biological parameters of shistosomes intermediate hosts. Of these, Standen (1951); Chernin (1957): Coles (1973); De-Souza et al., (1977); Looker and Etges (1979); Milward and Andrade (1978); Eleman & Madoson (1982), Lewis et al., (1986); Thompson (1988); studied the effect of some different diets on the growth rate of the snails. Others; Sturrock and Sturrock (1970); Meier and Meier (1981) and Roushdy and El-emam (1984) have studied the effect of schitosome infection on the biology of snails. In any case results obtained by Thompson (1988) suggested that the lettuce is lacking in basic nutritional quality.

This work aims to investigate the influence of certain experimentally prepared diets with different energy levels on some biological activities including the growth rate, reproduction as well as the rate of S. mansoni infection on B. alexandrina snails.

MATERIALS AND METHODS

Adult laboratory-reared B. alexandrina snails of shell diameter 8-10 mm and weight of 100-120 mg were used in this study.

The snails were divided into five groups, each of 20 snails. The snails of each group were kept in two-litre aquarium at a water temperature of 25±1 °C under constant aeration. Each test group was fed on one of the four diets described in tables (1 and 2). A fifth group was fed on fresh

Table 1: Percentage composition of the experimental prepared diet used, and their energy levels

Ingredient	Diet No.			
	1	2	3	4
Wheat bran	40	34	27	18
Rice bran	25	17	10	6
Fish meal			23	28
Soya bean meal	8	13	18	23
Cotton seed meal	5	8	12	15
Meat meal	4	5	5	5
Vegetable-oil	3	3	3	3
Vit. min. Premix	1	1	1	1
Dicalcium phosphate	1	1	1	1
Total	100	100	100	100
Protein	25%	30.11%	35.11%	40%
Energy, KCal/Kg	2143.49	2279.92	2415.71	2545.54
C/P ratio	85.74	75.72	68.73	63.59

Vitamin and mineral per Kg food: Vit.A,5000 IU; D, 1000 IU; K, 2mg; riboflavin, 2.5 mg; niacin, 1.2 mg; choline chloride, 80 mg; Pantothenic acid, 5 mg; Pyridoxin, 0.5 mg; B12, 4 ug, 1 mg, Co, 80 ug; Se, 50 ug.

Table 2: Proximate analysis, mineral and energy values of experimental diets used in feeding of snails (B. alexandrina).

Item	Diet No.			
	1	2	3	4
Crude protein	25.00	30.11	35.15	40.08
Crude fat	6.88	7.25	7.43	7.71
Crude Fiber	8.15	7.11	6.13	5.20
Ash	9.67	10.19	10.60	11.19
Nitrogen free extract	40.31	35.51	30.97	26.32
Calcium	1.79	1.99	2.13	2.29
Sodium	0.34	0.47	0.59	0.70
Potassium	1.06	1.10	1.16	1.21
Phosphorous	3.83	2.42	2.43	2.42
CE(Kcal/Kg diet)	4151.00	4225.00	4305.00	4369.00
ME(Kcal/kg diet)	2134.00	2280.00	2416.00	2546.00
C/P ratio	86	78	69	64

lettuce and served as control group. The water and diets were changed every three days.

After the establishment of the experiment, the following biological parameters were studied for six successive weeks:

1. Determination of the growth rate of snails:

Criteria used in assessment of the growth rate include determination of the increase in shell diameter and in the total body weight of the snails. The shell diameter as well as the weight of the snails were determined weekly. Data obtained were analysed using student "t" test (T-test was employed to detect any significant differences at the 5% level).

2. Determination of the mortality rate:

Any dead snails could be distinguished by lack of response to gentle prodding with a needle. The mortality percentage was calculated every week and statistically analysed by X^2 test).

3. Determination of the fecundity and hatchability of the snails:

The fecundity was considered as the number of viable eggs laid by the snails, and the fertility as the number of these eggs that hatched successfully (Frank, 1963). Polyethelene sheets were immersed in the snail aquaria to serve as a good subtratum for egg deposition. Eggs deposited on the sheets were counted under the dissecting microscope, while those

deposited on the walls of the container were counted with the help of a magnifying lens. The mean number of egg. masses / snail / week as well as the mean number of egg cell / snail / week were counted.

For determinig the hatchability rate of the deposited eggs, polyethelene sheets containg 300 egg cells were collected from every group after four weeks from feeding. These were placed in aereated 250 ml - beaker with 150 ml of dechlorinated water and egg hatching was examined every day under the dissecting microscope and the hatchability percentage was calculated.

4. Effect of diet on the infection rate of B. alexandrina with S. mansoni miracidia and on emergence of cercariae:

For snail infection, five freshly hatched miracidia were pipetted into a test tube containing one snail in three ml of dechlorinated water. Snails were left under artificial light for at least five hours. Then five groups, each composed of 25 snails, were put in an aquarium containing two liters of dechlorinated water. Enough amount of one of the four prepared diets was added to each group. Fresh lettuce was used as a control diet for the control group. The snails were examined for cercarial shedding 6 weeks post-infection. Results of each expriment were compared statistically.

RESULTS

Effect of diet on the growth rate of B. alexandrina snails:

a) Effect on total body weight:

The results presented in table (3) illustrate the effect of the different types of diets on the total body weight of the snails. It was found that snails fed on diet No. 1 exihibited significant increase during the 2nd, 3rd, 4th, 5th and 6th weeks compared with the control group. Concerning diet No. 2 and No. 3 significant increase was observed during the 3rd., 4th. 5th. and 6th. weeks. However feeding the snails on diet No. 4 induced a significant increase on body weight only during the 4th, 5th, and 6th, weeks of the experimental period. The significant increase in the body weight was recorded from 2nd. week for diet No. 1, from 3rd. week for diet No. 2 and 3 and from the 4th. week for diet No. 4.

b) Effect on growth rate of shell diameter:

Table (4) showed the effect of the different diets on growth rate of shell diameter of B. alexandrina. A significant increase in shell diameter of all animal groups was observed during the 5th. and 6th. weeks compared with the control group.

c) Effect of different diets on snails mortality rate:

It was found that feeding the snails on diets No. 1, 2 and 3 resulted in a lower mortality rate, while diet No. 4 that

contained higher energy level showed a higher mortality rate compared with other diets but still relatively lower than the control snails (Table 5).

d) Effect of different diets on egg laying capacity of B. alexandrina:

As presented in Table (6), the data obtained showed that all studied diets induced marked increases in the egg laying capacity during the experimental period compared with the control snails. Whereas diet No. 3 induced the highest effect but diet No. 4 showed the lowest effect during the 6th. week.

e) Effect of different diets on egg fertility and hatchability of snails:

The data obtained in Table (7) indicated that feeding of snails on diet No. 1, 2 and 3 produced no significant changes in the deposited fertilized eggs compared with the snails fed on lettuce as a control diet. While diet No. 4 showed no significant effect. However all the four prepared diets increased the percentage of hatchable eggs.

f) Effect of diet on the infection rate of B. alexandrina with S. mansoni and on emergence of cercariae:

Results in Table (8) showed that B. alexandrina infected with S. mansoni and fed on diet No. 1 (25% protein and energy level 2143.49 Kcal/Kg) showed the hitghest infection rate (88.8%) compared with the control snails (36.3%). Snails fed on

Table 3: Effect of experimental diets on wet weight of B. alexandrina(gram/Snail)

Diet No.	Curde Protein %	Calorie Protein ratio	Experimental period (weeks) 1	2	3	4	5	6
Control			167.75±29.87	182.00±51.27	185.30±41.81	182.70±33.20	212.87±58.47	200.00±49.48
1	25	2143.49	181.00±29.74	213.90±43.86*	200.00±46.81*	215.62±51.94*	261.25±54.76*	285.60±50.74**
2	30.11	2279.92	175.40±41.60	197.26±49.67	198.66±52.63*	215.52±31.57*	274.62±64.50*	301.06±71.13**
3	35.15	2415.71	168.00±39.50	190.75±35.62	204.10±44.30*	210.66±30.05*	247.50±26.28*	260.00±55.15**
4	40	2545.54	177.00±40.30	192.00±24.75	194.10±25.39	212.80±35.68*	251.00±34.73*	294.33±46.52**

Average weight of one group of snails, 20 snails, 20 snails/2 Liters water ± S.D.
* Significant at the 0.05% level (T. test). Initial weight (9) 110 ± 10.

Table : Effect of experimental diets on shell diameter of B. alexandrina(mm/snail)

Diet No.	Crude Protein	Calorie Protein ratio	Experimental periods (weeks) 1	2	3	4	5	6
Control			1.000±0.130	1.010±0.130	1.040±0.110	1.060±0.120	1.060±0.120*+	1.060±0.100*
1	25.00	2143.49	1.020±0.100	1.040±0.051	1.060±0.080	1.120±0.080	1.150±0.100	1.090±0.100*
2	30.11	2279.92	1.010±0.110	1.020±0.080	1.060±0.090	1.100±0.120	1.130±0.090*	1.200±0.120*
3	35.15	2415.71	1.010±0.091	1.020±0.089	1.050±0.060	1.070±0.110	1.140±0.070*	1.170±0.100*
4	40.00	2545.54	1.015±0.067	1.031±0.074	1.053±0.061	1.090±0.070	1.136±0.070*	1.140±0.050

Average shell diameter of one group of snails, (20 snails/liter water ± S.D.).
* Significant at the 0.05% level (T-test). Initial shell diameter 0.9±0.1.

Table 5: Effect of different diets on mortality rate of B. alexandrina snails

Type Diet	Crude Protein %	Calorie Protein ratio	Number of snails	Number of surviving snails after 6 weeks from feeding	Survival rate	Number of death	Mortality rate
Control			20	11	55%	9	45%
1	25.00	2143.49	20	15	75%	5	25%
2	30.11	2279.92	20	15	75%	5	25%
3	35.15	2415.71	20	15	75%	5	25%
4	40.00	2540.54	20	12	60%	8	40%

Table 6: Effect of experimental diets on egg-laying of B. alexandrina

Diet No.	Crude Protein %	Calorie Protein ratio	Experimental Periods (Weeks)					
			1	2	3	4	5	6
Control			5.500±0.707*	7.166±2.136*	7.220±3.500*	7.500±0.350*	6.280±5.219*	6.400±1.732*
1	25.00	2143.49	10.530±8.935*	11.580±4.043*	13.760±5.190*	14.140±7.890*	12.000±3.380*	11.880±2.820*
2	30.11	2279.92	9.000±1.000*	11.033±5.641*	16.230±6.070*	13.210±7.260*	11.450±5.880*	12.040±3.365*
3	35.15	2415.71	8.330±0.577*	14.000±2.846	13.890±5.440*	12.430±3.650*	12.480±3.650*	12.450±2.500*
4	40.00	2546.54	12.142±2.734	12.530±4.560	13.800±6.127	12.407±3.815	11.460±3.942	11.840±2.739

Average egg laying of one group of snails (20 snails/2 liter of water ± S.D.).
* Significant at the 0.05% level (T-test).

Table 7: Effect of different diets on fertility and hatachbility of eggs of *B. alexandrina* snails.

Diet No.	Mean No.of egg/masse	Mean No.of fertilized eggs/masse	% of fertilized eggs	Mean No. of hatched eggs masses	% of hatched eggs
Control	21.428±6.185	21.071±6.157	98.33%	20.071±7.730	93.66%
1	23.076±7.852	22.615±7.963	-98.00%	22.307±8.209	+96.66%
2	20.000±9.388	19.600±9.325	-98.00%	19.000±9.805	+96.66%
3	16.666±6.633	16.444±6.696	+98.66%	16.500±6.732	+99.00%
4	23.076±6.473	22.307±6.316	-96.66%	22.307±6.316	+96.66%

* Significant at the 5% level (X^2-test). Total number of eggs examined was 300 eggs

Table 8: Effect of diet on the infection rate of *B. alexandrina* with *S. mansoni* miracidia and emergence of cercariae.

Type Diet	Crude Protein %	Calorie Protein ratio	No. of No. of snails	No. of surviving snail	Survival rate	No. of snails shedding cercariae	Infection rate based on total snails used	Infection rate based on surviving snails	No. of cercariae shed/snail 2 days	% difference
Control			25	22	88%	8	32%*	36.3%*	537.3±462.9	
1	25.00	2143.49	25	20	80%	16	64%	88.8%	995.0±550.0	85.10%
2	30.01	2279.92	25	18	72%	9	36%	45.0%	589.2±457.5	9.65%
3	35.15	245.71	25	22	88%	9	36%*	40.9%*	650.0±530.6	20.97%
4	40.00	254.54	25	21	84%	15	60%	71.4%	703.4±593.0	23.61%

* (X^2) for infection rate based on surviving snail at the 5% level and infection rate based on total snails used.

(T-test) for No. of cercarial shed snail/2days.

diet No. 2, 3 and 4 showed infection rates of 43%, 40.9 and 71.4% respectively (Table 3).

Concerning the effect on production of cercariae, results revealed that snails fed on diet No. 1 produced a significant increase in the number of cercariae (Table 8). On the other hand, the other three diets "2, 3 and 4" did not differ significantly from the control treatment as shown in Table (8). In respect to the effect on the survival rate of infected snails results showed no effect of the experimental diets on the survival rate of the host during the experimental period (Table 8).

DISCUSSION

Although lettuce is widely used as a diet in most laboratory cultures of the snails, it does not support the maximum growth rate of the snail intermediate host (Eveland, 1972). In the present study all the four experimentally prepared diets containing proteins, carbohydrates, oils, vitamins and dicalcium phosphate (in the proportions described in tables 1 and 2) and having different energy level, increased significantly the growth rate of B. alexandrina snails. These results are comparable with those obtained by Vierira (1967) who found that the addition of wheat germ rich in vitamin E to the diet of B. glabrata promotes its growth rate. He explained this increase in the view that vitamin E protects some essential nutrients against oxidation, hence increases the growth rate. Also the addition of dicalcium phosphate to all the four diets has an important effect in increasing the size of

snail. This result is supported by Milward and Andrade (1978) who found that addition of calcium salts to the food produced an increase in the shell diameter of snail. Another important factor which could play a role on the growth rate is the addition of vegetable oil which was also reported by Frank (1963) and Thamas, et al., (1983), who found that the increase in the density of plant food as lettuce and lucerence increased the growth rate of snails. The egg laying capacity of B. alexandrina fed on the prepared diets (of energy levels of 2143.49, 2279.92, 2415.71 and 2545.54 K Cal/Kg) exihibited significant increases compared with those snails fed on lettuce only. It is to be noted that snails fed on diet No. 1 that has the highest energy / protein ratio (85.74) displayed the highest egg laying group. It is possible that the increase in the ferility rate of the snails is partly correlated to the existence of dicalcium phosphate. This view agrees with that reported by Frank (1963) who also found that the addition of calcium salts to snail diet gave optimum fecundity. On the other hand it is suggested that this increase in the fecundity of B. alexandrina is also due to the high protein ratio presented in the cotton seed meal and vegetable oil. Similar results were reached by Eveland (1972), Looker and Etges (1965), Stanislawski and Becker (1979) and Eleman and Madson (1982) who found a decline in the reproduction of Biomphalaria snails fed carbohydrate diet. This might be due to deficiency of lipids or proteins or might be due to a lack of protein enzymes. It is known that the infected snails need carbohydrates, proteins and energy to compensate the loss of nutrients consumed by the developing daughter sporocysts. Similarly snails fed on

diet No. 1 gave the highest infectivity rate and cercaria production in B. alexandrina snails. This finding agreed with that of Kendall (1949) Wright (1967), Etges and Gresso (1979), Eveland (1972) and Coles (1973) who found that a high protein food increased the number of cercariae and suggested that this is due to the large size of snails which increase the space available for parasite growth or increased availability of nutrients for the parasite.

REFERENCES

Chernin, E. (1957): Studies on the control of Schistosome - bearing snails. VII. Streptomycin-induced inhibition of growth and reproduction in the Schistosome-bearing snail, Austrolobis glabratus. Amer. J. Hyg., 66: 321-330.

Coles, G.C. (1973): The effect of diet and crowding on the Shedding of Schistosoma mansoni cercariae by Biomphalaria glabrata. Ann. Trop. Med. Parasitol., 67: 419-423.

De-Souza, C.P.; Dos gracas, V.M.; De carvalho, M.A.R. and Gardillo, V.E. (1977): Rearing of the snail Biomphalaria glabrata. Under axenic conditions in isolalors. 26: 1013-1017.

Eleman, M.A. and Madson, H. (1982): The effect of temperature darkness starvation and various food types on growth survial and reproduction of Helisoma duryi, Biomphalaria alexandrina and Bulinus truncatus

gastropoda planorbidae. Hydrobiologia, 88: 266-276.

Etges, F.J. and Gresso, W. (1965): Effect of Schistosoma mansoni infection upon fecundity in Austalorbis glabratus. J. Parasitol., 51: 757-760.

Eveland, L.K. (1972): Infectivity of cercariae of Schistosoma mansoni from snails on inadquate diets. Parasitol., 64: 441-444.

Frank, G.H. (1963): Some factors affecting the fecundity of Biomphalaria preiferi (Krauss) in glass aquaria. Bull. Wld. Hlth. Org., 29: 531-537.

Kendall, S.B. (1949): Nutritional factors affecting the rate of development of Fasciola hepatrca in Limnaea trunculata J. Helminthol., 23: 179-190.

Lewis, F.A.; Stirewalt, M.; Souza, C and Gazzinelli, G. (1986): Largescale Laboratory maintenance of Schistosoma mansoni, with observations on three Schistosome / snail host combinations. J. Parasitol., 72 (6): 813-829.

Looker, D.L. and Etges, F.J. (1979): Effect of Schistosoma mansoni infection on fecundity and perivitelline fluid composition in Biomphalaria glabrata. J. Parasitol., 65: 880-885.

Meier, M and Meier, B. (1981): Schistosoma mansoni effect on growth fertility and development of distal male organs in Biomphalaria glabrata exposed to

miracidia at different ages. Z. Parasitenkd., 66: 121-132.

Milward, D. E and Andrade, R. (1978): Competition between Helisoma duryi and Biomphalaria glabrats under laboratory conditions Pulmonata planorbidae. Rev. Bras. Biol., 38: 787-880.

Roushdy, M.Z. and El-emam, M. (1984): The effect of lemperature on interrelationship between S. Haematobium and B. truncatus in Egypt, J. Egypt. Soci. Parasitol., 14: 115-123.

Standen, O. D. (1951): Some observations upon the maintenance of Australorbis glabratus in the laboratory Amm. Trop. Med. and Parasitol., 45: 80-83.

Stanislawski, E. and Becker, W. (1979): Influences of Semisynthetic diets, starvation and infection with Schistosoma Mansoni (Trematoda) on the metabolism of Biomphalaria glabrata (Gastropoda) Comp. Biochem. Physio. 63 A: 527-533.

Sturrock, B. M. and Sturrock, R.F. (1970): Laboratory studies of the hostparasite relationship of S. mansoni and B. glabrata from St. Lucia, West Indies. Ann. Trop. Med. Parasitol. 64: 357-363.

Thomas, J.D.; Grealy, B. and Fennell, C.F. (1983): The effects of varying the quantity and quality of various plants on feeding and growth of Biomphalaria glabrata gastropoda, Olkos, 14: 77-90.

Thompson, S.N. (1988): Experimental maintenance of the Schistosoma glabrata on an improved alginate gel. Ann. Trop. Med. Parasitol., 82(1): 59-63.

Vierira, E.C. (1967): Influence of vitamin E on reproduction of Biomphalaria glabrata under axenic conditions. Am. Soc. Trop. Med. Hyg., 16: 792-796.

Wright, C.A. (1967): The crowding phenomenon in laboratory colonies of fresh water snails. Ann. Trop. Med. Parasitol, 54: 224-232.

تأثير اختلاف البروتينات الغذائية ومستويات الطاقة على بعض الأنشطة الحيوية لقوقع البومفلاريا الكسندرينا

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تمت دراسة تأثير أربعة أنواع من الأغذية المعدة معمليا وذات محتيوات بروتينية ومستويات طاقة مختلفة على بعض الأنشطة البيولوجية لقوقع البيومفلاريا الكسندرينا مقارنة بالغذاء المعلمى المعتاد وهو الخس .

وقد أوضحت النتائج التى تم الحصول عليها أفضلية الاغذية المجهزة معمليا على الخس من حيث التأثير على معدل النمو ومقدرة وضع البيض ومعدل فقسه ، كذلك معدل انتاج السركاريا بالنسبة للقواقع المعدية .