International Journal of Fisheries and Aquatic Studies 2014; 1(3):4-8

ISSN: 2347-5129
IJFAS 2014; 1(3): 4-8

www.fisheriesjournal.com
Received: 10-10-2013
Accepted: 18-11-2013

Maria Mojena Gallo Gonzales
College of Fisheries and Aquatic Sciences - Western Philippines University - Puerto Princesa City.
College of Fisheries and Ocean Sciences - University of the Philippines Visayas
Email: mojenagonzales@yahoo.com

Crispino A. Saclauso
College of Fisheries and Ocean Sciences - University of the Philippines Visayas
Email: casaclauso@yahoo.com

Rex Ferdinand Traifalgar
College of Fisheries and Ocean Sciences - University of the Philippines Visayas
Email: skerferd@yahoo.com

Correspondence:
Maria Mojena Gallo Gonzales
College of Fisheries and Aquatic Sciences - Western Philippines University - Puerto Princesa City.
College of Fisheries and Ocean Sciences - University of the Philippines Visayas
Email: mojenagonzales@yahoo.com
Tel: +639177065336

Low dietary protein to energy ratios support rapid growth of Juvenile topshell, *Trochus niloticus*

Maria Mojena Gallo Gonzales, Crispino A. Saclauso, Rex Ferdinand Traifalgar

ABSTRACT
Nine practical diets containing nine protein-to-energy ratio of 0.058, 0.064, 0.077, 0.088, 0.097, 0.100, 0.115, 0.120, and 0.155 g/kcal were fed at 5-3% of body weight daily to juvenile topshell, *T. niloticus*, for 90 days.
Protein to energy ratio had significant effects on growth and nutrient deposition of juvenile topshell, *Trochus niloticus*. At P:E ratio of 0.058 and 0.064 g/kcal, SLG reached 0.20-0.21 mm, WG 7.18-9.69%, SGR 0.08-0.10% day^{-1}, and attained PER of 0.48–0.57. Survival (82-92%) and feed conversion ratios (5.81-6.77) were not affected by P:E ratio in the diet. Nutrient deposition was significantly affected by P:E ratio in the diet. High protein deposition was observed at P:E ratio of 0.058 g/kcal, while high fat deposition was observed at P:E ratio of 0.155 g/kcal.
This study demonstrated that a P:E ratio of 0.058 and 0.064 g/kcal was sufficient for good growth, PER and high crude protein deposition in juvenile topshell, *T. niloticus*.

Keywords: Protein to energy ratio, Growth, Topshell

1. Introduction
Earlier studies on topshell, *T. niloticus* dealt with its biology and natural environment [1, 2]. The feeding requirements of topshell, *T. niloticus* were also investigated particularly on the use of cultured microalgae and diatoms [3] but only few works have been documented on the use of formulated diets [4, 5]. In Thailand study of the growth of *Trochus maculatus* reared in tanks and observed that maculate topshell (cultured together with red snapper) that fed on fish leftover feeds and wastes exhibited rapid growth compared to maculate topshell alone that fed only on algal diets [6].
Commercial hatcheries for topshell, *T. niloticus* are already in operation in the Philippines indicating that the technology in producing juvenile Trochus in captivity has already matured. However, basic information on the feeding requirements of this species are few [3, 4, 7] since early research efforts focused on the hatchery aspects [8]. Understanding its nutritional requirements is an essential step towards the development of cost-effective feeding schemes that will make Trochus aquaculture commercially viable.
In nature, topshell and abalone thrive in similar habitats and feed on almost the same natural food. Studies on the use of artificial diet for the topshell, *T. niloticus* were scarce hence, available literature on artificial diets for abalone was used as a starting point for Trochus nutrition [4].
The advantage of using optimum crude protein and digestible energy levels is that these can improve growth rate, feed efficiency and protein utilization; minimize excessive accumulation of lipids and glycogen in the somatic tissues and liver; and reduce undesirable nitrogenous waste output thereby decreasing the impact of farm effluents [9].
The objective of this study was to establish the dietary protein level, energy level and protein-to-energy (P:E) ratio that support the best performance of juvenile topshell *T. niloticus*.

2. Materials and Methods
2.1 Diets: Nine varying P:E ratio of 0.058, 0.064, 0.077, 0.088, 0.097, 0.100, 0.115, 0.120, and 0.155 g/kcal were formulated for juvenile topshell *Trochus niloticus* (Table 1).

Table 1: Formulation (kcal $100g^{-1}$) and composition, of the experimental diets fed to topshell, *T. niloticus* juveniles.

Dietary treatment (T)	T1	T2	T3	T4	T5	T6	T7	T8	T9
Feed composition (%):									
Casein	0.0	0.0	7.70	0.0	7.70	15.30	15.30	7.70	15.30
Cod liver oil	15.92	11.30	12.90	6.70	8.20	9.80	5.20	3.60	0.60
Diatomaceous earth (DE) [a]	43.08	47.70	38.40	52.30	43.10	33.90	38.50	47.70	43.20
Basal ingredients [b]	41.00	41.00	41.00	41.00	41.00	41.00	41.00	41.00	41.00
Proximate composition (% DM):									
Crude protein	16.30	14.20	21.00	15.84	21.80	26.65	23.10	20.45	24.20
Crude fat	17.05	12.43	14.04	7.83	9.34	10.94	6.34	4.74	1.69
Ash	47.43	51.09	45.11	54.48	48.68	39.01	44.42	52.02	46.58
Crude fiber	2.55	3.27	3.30	3.13	2.94	2.03	3.15	3.02	3.21
Nitrogen-free extract [d]	16.67	19.01	16.55	18.72	17.24	21.37	22.99	19.77	24.32
Estimated energy (kcal $100g^{-1}$)	281.87	222.52	272.72	180.21	225.55	266.95	201.29	169.79	156.01
P:E ratio (g/kcal)	0.058	0.064	0.077	0.088	0.097	0.100	0.115	0.120	0.155

([a]) Composition of DE: 90% silica, 5% sodium, 3% magnesium and 2% iron (Delta Romeo Trading, Manila, Philippines).
([b]) Common ingredients in the diet: fish meal, (2%); squid meal, (15%); corn starch, (2%); vitamin and mineral mix (5%) [c]; seaweed *Gracilaria* sp., (5%); gelatin (7%); and carboxymethyl cellulose, (3%); dicalcium phosphate (2%).
([c])Vitamins and mineral mixes: Vitamin A (10,000,000 IU), Vitamin D_3 (3,000,000 IU), Vitamin E (10,000 IU), Vitamin B_1 (400 mg), Vitamin B_2 (1,200 mg), Vitamin B_6 (1,200 mg), coated Vitamin C (25,000 mg), Folic acid (600 mg), Niacin (6,000 mg), Calcium pantothenate (10,000 mg), Biotin (20,000 mcg), Choline Chloride (10,000 mg), Iron (12,000 mg), Copper (1,200 mg), Iodine (400 mg), Manganese (5,000 mg),Zinc (6,000 mg), Cobalt (20 mg), Selenium (20 mg), Carrier q.s ad to make 1 kg (Source: Progressive Laboratories, 149 Dangay St. Project 7, Quezon City, Manila, Philippines).
([d]) NFE = 100 – [crude protein + crude fat + ash + crude fiber].

These diets contained fixed amounts of basal ingredients while the levels of casein, cod liver oil, and diatomaceous earth (DE, feed grade) varied. DE contains 90% silica, 5% sodium, 3% magnesium and 2% iron (Delta Romeo Trading, Manila, Philippines) and was used as filler. Physiological values used for calculation of energy were computed based on standard gross energy equivalents for fish food as: of 4.5 kcal g^{-1} protein, 8 kcal g^{-1} lipid and 3.3 kcal g^{-1} NFE [10].

For the preparation of artificial diets, pre-weighed, finely ground (passed through sieve number 60) ingredients including vitamins and minerals were thoroughly mixed in a food mixer for five minutes. After mixing, the resulting dough of even consistency was passed through a pelletizer with a 2-mm diameter die holes and then oven dried at 60 °C.

2.2 Experimental procedure

The study was conducted at the Western Philippines University (WPU) Trochus Hatchery in Binduyan, Puerto Princesa City, Palawan. From the wild, 2,000 topshell T. *niloticus* juveniles were collected and placed inside containers with seawater provided with aeration, and transported to the hatchery. They were acclimatized for one month in tank conditions and fed with a SEAFDEC/AQD abalone formulated diet prior to the experiment. No feeding was done 24 hours before the start of the experiment.

Twenty seven experimental concrete tanks (71 cm x 210 cm x 200 cm), each having 500 L of seawater were stocked with 20 juvenile topshells, *T. niloticus* (shell diameter of 20–25±0.01 mm weighing 1.0-4.5±0.01 g). Each compartment had its own inlet and outlet and an aeration system. Ranges of water parameters such as salinity, temperature, dissolved oxygen (DO), ammonia, and pH were maintained at 35-36 ppt, 26-27 ˚C, 5.0-5.5 mg l^{-1}, 0-0.4 ppm, and 7.5-8.5, respectively.

Each diet was tested in three replicate tanks. Experimental feeds were assigned randomly among 27 tanks and given at a rate of 5-3% body weight per day for abalone [11]. Feeding was done at 0700 h daily for 90 days.

Sampling was done every 18 days and each topshell was picked out gently prior to the cleaning of the tanks. Each topshell was weighed using a digital weighing scale sensitive to 0.01 g, and its shell length or basal diameter was measured using a Vernier caliper.

Water exchange at a rate of 30% was carried out daily and during sampling, 100% water replacement was done.

2.3 Analytical method

Proximate analyses of juvenile topshell, *T. niloticus* formulated feeds were carried out at the beginning of the experiment according to the methods of AOAC (1985) [12].

Growth, survival, and feed utilization of topshell, *T. niloticus* were computed as follows:

1. Weight gain (WG)

$$\%\ \mathrm{WG} = 100 \times \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}$$

2. Survival (%)

$$\%\ \text{survival} = 100\left(\frac{\text{Final no. of fish}}{\text{Initial no. of fish}}\right)$$

3. Protein efficiency ratio (PER) [13]

$$\mathrm{PER} = \frac{\text{Weight gain (g)}}{\text{Protein fed (g dry weight)}}$$

4. Feed conversion ratio (FCR) [13]

$$\mathrm{FCR} = \frac{\text{Feed fed (g)}}{\text{Gain in wet weight (g)}}$$

5. Specific growth rate (SGR) [13]

$$\mathrm{SGR}\ \%\ \mathrm{day}^{-1} = 100 \times \frac{\text{In final wt.} - \text{In initial wt.}}{\text{No.of days}}$$

6. Protein deposited* (Wilson 1989)

$$= \frac{\text{final body protein } - \text{ initial body protein content } \times 100}{\text{Total protein fed}}$$

7. Crude fat deposited*

$$= \frac{\text{final body fat} - \text{initial body fat content} \times 100}{\text{Total lipid fed}}$$

*Each sample was composed of flesh including conical shell apex

2.4 Experimental design and statistical analysis

Performance and body composition means for juvenile topshell, *Trochus niloticus* was computed using the SPSS Version 10 software. Tukey test was performed to test differences among means at $p<0.05$.

3. Results

Among nine Treatments, the lowest protein to energy ratio of 0.058 and 0.064 g/kcal (T3) produced significantly (<0.001) better growth performance in SLG, WG, SGR, and PER. Protein to energy ratio did not significantly affect the survival and FCR of juvenile topshell, *Trochus niloticus*. Increasing P:E ratio showed decrease in growth performance. Nutrient deposition for protein and fat was significantly affected by P:E ratio in the diet.

At P:E ratio of 0.058 g/kcal, SLG reached 0.21±0.01 mm but was not significantly different ($p>0.05$) from that of topshells fed with 0.064g/kcal P:E ratio (Table 2). Similarly, at P:E ratio of 0.058 g/kcal weight gain reached 9.69±0.01% but with no significant difference ($p>0.05$) from that of 0.064 g/kcal P:E ratio. Highest SGR of 0.010±0.00% was attained at P:E ratio of 0.058 g/kcal but with no significant difference ($p<0.05$) from that of 0.064 g/kcal P:E ratio. Topshell attained 0.57±0.07 PER at P:E ratio of 0.058 g/kcal but was not significantly different (p>0.05) with that (0.48±0.04) of 0.064 g/kcal P:E ratio.

The survival ranged from 80% to 92% among dietary treatments and was not significantly affected ($p>0.05$) by P:E ratio in the diet (Tables 2). In the same way, P:E ratio of the diet did not significantly affect (p>0.05) the FCR of topshells. FCR ranged from 5.81±0.09 to 6.77±0.73.

Deposition of protein was affected by P:E ratio. The highest protein deposition (64.61±7.02) was attained at P:E ratio of 0.058 g/kcal, increasing P:E ratio in the diet decreased protein deposition.

The ration of P:E affected the crude fat deposited in the juvenile topshell, *T. niloticus* (p<0.001; Table 3). Crude fat deposit reached 16.16±1.01% for T7 and lowest fat deposition was attained under T6 with P:E ratio of 0.077 g/kcal.

Table 2: Effects of different levels of dietary protein and energy on shell length gain (SLG), weight gain (WG), specific growth rate (SGR), survival, feed conversion ratio (FCR), protein efficiency ratio (PER) of juvenile topshell, *T. niloticus*

Variable	T1	T2	T3	T4	T5	T6	T7	T8	T9
SLG (mm)	0.21±0.01^a	0.20±0.00^a	0.17±0.00^c	0.17±0.01^e	0.17±0.01^b	0.17±0.01^d	0.18±0.00^g	0.17±0.01^f	0.18±0.00^h
WG (%)	9.69±0.01^a	7.18±0.01^a	5.82±0.01^d	6.41±0.01^h	6.26±0.00^f	4.85±0.00^b	5.45±0.00^c	6.19±0.00^e	6.38±0.01^g
SGR (% day^{-1})	0.10±0.01^a	0.08±0.01^a	0.06±0.00^d	0.07±0.01^h	0.07±0.00^f	0.05±0.00^b	0.06±0.00^c	0.07±0.00^e	0.07±0.00^g
Survival (%)	90.0±5.0	91.7±1.7	90.0±0.0	81.7±6.0	80.0±8.7	88.3±3.3	83.3±4.4	86.7±6.0	88.3±1. 7
FCR	6.08±0.37	5.81±0.09	6.08±0.03	6.35±0.49	6.77±0.73	6.17±0.25	6.54±0.35	6.51±0.77	6.04±0.03
PER	0.57±0.07^a	0.48±0.04^a	0.27±0.03^c	0.39±0.05^{ac}	0.27±0.01^c	0.14±0.0b^c	0.17±0.02^c	0.29±0.02^c	0.22±0.02^c

* Initial shell length (SL), weight and number of stocked topshells were 20.1±0.01 mm, 1.0-4.5±0.01 g, and 20 juveniles per tank, respectively. Means (±SEM) with the same superscript are not significantly different ($p>0.05$), n=3.

Table 3: Body composition (% dry matter) of juvenile topshell, *Trochus niloticus* including the shell apex.

	Deposited Protein*(% DM)	Deposited Fat* (% DM)
Initial	44.53±0.02	2.4±0.01
T1	76.57 ± 2.92^a	7.88 ± 0.34^b
T2	65.00 ± 4.93^b	2.83 ± 0.27^b
T3	10.29 ± 0.05^d	0.33 ± 0.08^e
T4	52.26 ± 1.30^c	6.77 ± 0.34^c
T5	49.17 ± 0.46^c	0.93 ± 0.23^d
T6	12.31 ± 0.07^d	3.46 ± 0.19^d
T7	22.33 ± 0.23^d	5.77 ± 0.17^b
T8	53.37 ± 1.21^c	3.32 ± 1.45^b
T9	0.87 ± 0.25^e	16.16 ± 1.01^a

*Means (±SEM) with the same superscript are significantly different ($p < 0.001$), n=3.

4. Discussion

The present study showed that for better growth, topshell, *T. niloticus* required a P:E ratio of 0.058 g/kcal. This protein level is much lower than the P:E ratio (0.101-0.1179 g/kcal) required by abalone *Haliotis asinina* [11]. The species size, dietary protein quality, and environmental conditions are some factors why shellfish require different levels of protein and energy in the diet [14].

The SLG of topshell, *T. niloticus* in the present study showed a narrow range (0.17-0.21 mm) compared to those reported by other investigators. Hatchery-bred topshells, *T. niloticus* exhibited inconsistent shell length which might be due to shortage of food supply in the rearing tanks [3, 6, 8]. Decreasing trend in weight gain in maculate topshell due to insufficient availability of natural food in the hatchery was also observed [6].

At P:E ratio higher than 0.058 and 0.064 g/kcal, topshell, *T. niloticus* exhibited lower growth, indicating that further increase in P:E ratio are no longer necessary and thus excessive. High dietary protein in relation to energy reduced the growth of juvenile topshell, *T. niloticus* that might be due to a high demand for energy in nitrogenous waste excretion [15]. Excessively high energy in the diet can restrict nutrient intake [16] and remains to be investigated for topshells.

The FCR in this experiment was not affected by levels P:E ratio. On diet development for abalone attained lower FCR of 6.89 [11] compared to the FCR (5.81) of topshell in the present study.

The increase in PER in topshells fed with P:E ratio of 0.058 and 0.064 g/kcal diet could be attributed to the protein sparing effect of higher energy diets (222.52 to 281.87 kcal $100g^{-1}$). This pattern was also observed in finfishes where low protein diet resulted in better utilization of protein [17, 18].

In this study, the highest amount of crude protein level (76.57±2.92%) in the flesh included the conical shell apex of topshell, *T. niloticus* in the chemical analysis; thus, was almost the same compared to the foot muscle crude protein level (77.7%) [7].

The results of the present study corroborate with the findings on the increasing protein contents in fish such as red tilapia fry [19] and Asian seabass [20] with respect to the increasing levels of energy in the diet but only at 15.84, 14.20 and 16.30% dietary crude protein. The highest protein deposition was at 281.87 kcal $100g^{-1}$ dietary energy indicating that at this dietary energy level, juvenile topshell, *T. niloticus* was able to deposit more protein. High deposition of crude protein at 281.87 kcal $100g^{-1}$ dietary energy is due to protein sparing effect supported by the result obtained in PER.

Crude fat of topshell, *T. niloticus* foot muscle was 6.1% [7], higher than that of the highest level (3.57%) obtained in the present study due to the inclusion of shell apex to the flesh samples. High crude fat deposited in juvenile topshell, *T. niloticus* fed high dietary protein level (24.7%) suggested that dietary protein might have been converted to crude fat which was supported by the low PER and low protein deposited.

Diatomaceous earth was used in the present study as the inert filler and at high inclusions (33.9-52.3%) it is difficult to assume that it had no effect on growth. For snapper (*Pagrus auratus*), at 40% in the diet, DE did not significantly alter the apparent digestibility of protein, lipid and phosphorus, but feed intake and growth were reduced [21]. Future studies on nutritional requirements of topshells will need to look closely on the types of fillers that would have minimal effects on nutrient digestibility as well as feed intake.

5. Conclusions

Protein to energy ratio had significant effects on growth (based on gain in shell length, weight gain and specific growth rate) and protein efficiency ratio (PER). Survival (82-92%) and feed conversion ratio (5.81-6.77) were not affected by P:E ratio in the diet. Nutrient deposition was significantly affected by P:E ratio in the diet. High protein deposition was observed at P:E ratio of 0.058 g/kcal, while high fat deposition was observed at P:E ratio of 0.155 g/kcal.

This study demonstrated that a P:E ratio of 0.058 and 0.064 g/kcal was sufficient for good growth, PER and high crude protein deposition in juvenile topshell, *T. niloticus*.

This is the first report using artificial diets to determine the nutritional requirements for topshell, *T. niloticus,* further investigations particularly on sources and types of protein and lipids are needed to be able to develop cost-effective feeds for topshells that will promote good growth, survival, nutrient utilization, and attractive shell coloration.

6. Acknowledgment

I thank the DOST-PCAMRD for providing the necessary funding to undertake this study.

7. Reference

1. Nash WJ. Trochus: nearshore marine resources of the South Pacific. (Eds.) Wright A, Hill and Suva. Forum Fisheries Agency, Institute of Pacific Studies, 1993, 451-497.
2. SEAFDEC AQD. Stock Enhancement. Vol. 23, 5-6, A publication of the Aquaculture Department of the Southeast Asian Fisheries Development Center, Philippines, 2001, 27.
3. Dwiono SAP, Makatipu PC, Pradina. Hatchery of the topshell (*Trochus niloticus*) in Indonesia. SPC Trochus Information Bulletin 1997; 5:33-40.
4. Renaud SM, Djafar M, Parry DL. 1996a. Preliminary investigation of an artificial diet for the marine topshell, *Trochus niloticus* (Mollusca: Gastropoda). Trochus: Status, Hatchery Practice and Nutrition Workshop, 1996.
5. Do HH, Tuyen HT, Lu HD. Growth rate of *Trochus niloticus* (Linnaeus, 1767) fed different food types. SPC Trochus Information Bulletin 2008; 14:7-11.
6. Chunhabundit S, Chunhabundit P, Aranyakananda P, Moree N. Dietary effects on shell microstructures of cultured maculate topshell (Trochidae: *Trochus maculatus*, Linnaeus, 1758). SPC Trochus Information Bulletin 2001; 8:15-21.
7. Rebhung F, Renaud SM, Parry DL, Lee CL. Fatty acid composition and proximate biochemical composition of wild and hatchery held broodstock of the marine topshell, *Trochus niloticus* (Mollusca: Gastropoda). SPC Trochus Information Bulletin 1997; 5:34-40.
8. Heslinga GA, Hillman A. Hatchery culture of the commercial topshell *Trochus niloticus* in Palau, Caroline Island. Aquaculture 1981; 22:35-43
9. Bicudo AJA, Sado RY, Cyrino JEP. Growth and haematology of pacu, *Piaractus mesopotamicus*, fed diets with varying protein to energy ratios. Aquaculture 2009; 40:486-495.
10. Brett JR, Groves TDD. Physiological energetics. In: Fish Physiology. Bioenergetics and Growth. Vol. 8, Academic Press, New York, 1979, 279-352.
11. Bautista-Teruel MN, Millamena OM. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: Protein/energy levels. Aquaculture 1999; 178:117-126.
12. Association of Official Analytical Chemist (AOAC). Official methods of analysis, Edn 14, Association of Official Analytical Chemists, Washington, DC, 1985, 547-548.
13. Halver JE, Hardy RW. Fish Nutrition. Edn 3, Academic Press Elsevier Science, USA, 2002, 589-839.
14. Daniels WH, Robinson EH. Protein and energy requirements of juvenile red drum *Sciaenops ocellatus*. Aquaculture 1986; 53:243–252.
15. National Research Council. Nutrient requirements of fish. National Academy Press, Washington DC. 1993, 49-54.
16. Lovell T. Nutrient and feeding of fish. Van Nostrand Reinhold, New York, NY. 260pp Nash, W.J. (1993) Trochus: nearshore marine resources of the South Pacific. (Eds.) Wright A and Hill L.

Honiara and Suva. Forum Fisheries Agency, Institute of Pacific Studies, 1989, 451–497.
17. El-Dahhar AA, Lovell RT. Effect of protein to energy ratio in purified diets on growth performance, feed utilization and body composition of Mozambique tilapia, *Oreochromis mossambicus* (Peters). Aquaculture Research 1995; 26:451-457.
18. Webster CD, Tiu LG, Tidwell JH, Van WV, Howerton RD. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Moronechrysops* x *M. saxatilis*) reared in cages. Aquaculture 1995; 131:291-301.
19. Santiago C, Laron M. Growth response and carcass composition of red Tilapia fry fed diets with varying protein and protein to energy ratio. Special Publication, Asian Fisheries Society. Manila, Philippines, 1991, 55-62.
20. Catacutan MR, Coloso RM. Effect of dietary protein to energy ratios on growth, survival and body composition of juvenile Asian seabass, *Lates calcarifer*. Aquaculture 1995; 131:125-133.
21. Saxby SA, Williams IH, Glencross BD. Dietary fillers reduce growth and affect apparent digestibilities of pink snapper. Recent Advances in Animal Nutrition in Australia, 2001, 13, 10A.