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EFFECTS OF STEROID SAPOGENINS ON GROWTH AND MOLTING OF THE GIANT FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN)

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ABSTRACT

Effects of steroid sapogenins on growth and molting of the giant freshwater prawn Macrobrachium rosenbergii *were studied in two experiments. In the first experiment, the giant freshwater prawn was injected intramuscularly with 5 different doses (0.0, 0.1, 1.0, 2.0 and 4.0 µg/kg prawn body weight). Sapogenins at 0.1 ug/kg prawn body weight generated the best feed conversion rate in this experiment. The administration of 4.0 ug/kg prawn body weight gave the best growth rate (0.08±0.07 g/day), and the shortest molt period (19.3±1.7 days). In the second experiment, four different concentrations of steroid sapogenins (0.0, 1.0, 5.0 and 10.0 ug/kg diet) were mixed with the prawn diet. The dose of 10.0 ug/kg diet gave the best growth rate (0.08±0.05 g/day) and the shortest molt period (19.0±1.8 days), compared to the control group, for which the growth rate was 0.05±0.02 g/day and the molt period was 24.9±3.1 days. The control groups given no sapogenins manifested the best survival rate in both experiments.*

INTRODUCTION

Steroid sapogenins were successfully extracted from *Agave sisalana* Perr byproducts from agricultural and industrial use by Ditsayaboot and Sappinant in 1990[1]. These sapogenins are 27 dihydroxy-2-ketosteroids (27 C-atom and 2 heterocyclic rings). Agave's steroid sapogenin is a mixture of Tigogenin and Hecogenin. Their molecular structures are close to molting hormone and cholesterol. It has been reported that cholesterol is an initiator in molting hormone synthesis[2] (Figure 1). Consequently, steroid sapogenins may trigger molting and accelerate growth in crustaceans. Hence, they could potentially play an important role in diet improvement in aquaculture of many species including the giant freshwater prawn (*Macrobrachium rosenbergii*, de Man).

Freshwater fishery products are important protein sources for Thai people. This protein comes from natural waters and aquaculture farms. The Department of Agricultural Economic Research, Ministry of Agriculture reported in 1992[3] that fishing products from 10,974 natural water resources, including running surface waters (0.5 million hectares), 66 rivers and 10,223 canals and dams has tended to decline. On the other hand, aquaculture farming covering areas of 46,600 hectares (78% of the total farming areas) in the central part of Thailand in 1988 has increased to compensate for the degraded natural resources. The giant freshwater prawn is one of the economic freshwater species which offers good yields and benefits to farmers. However,

growth of the giant freshwater prawn is slow and it takes at least 5 months to reach marketable size. The objective of the present study was to determine the effects of steroid sapogenins on molt, growth and survival of the giant freshwater prawn. Results from this study may be useful for improving giant fresh water prawn production.

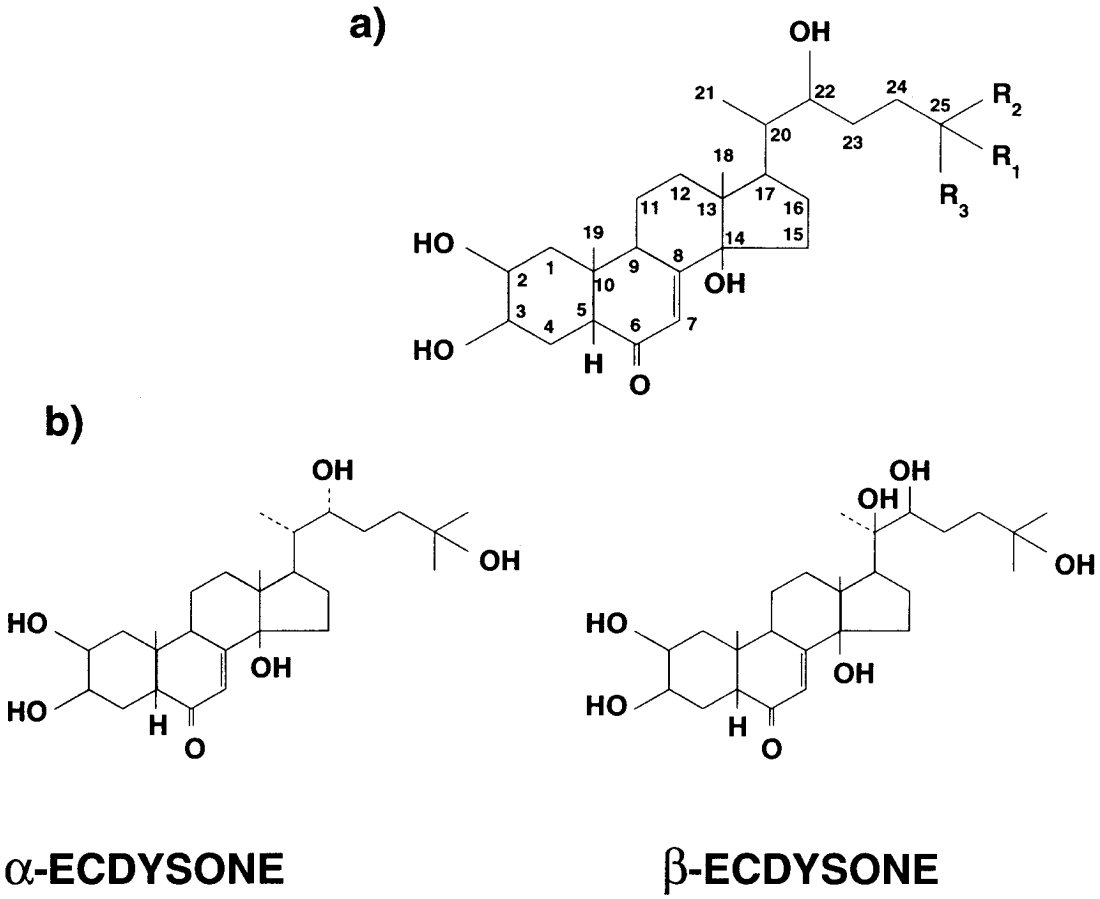

Fig. 1. a. General molecular structure of molting hormone.
b. Molecular structure of α and β ecdysone.

MATERIALS AND METHODS

Three hundred *M. rosenbergii* females (age 5 months) with individual weight of 10-15 grams were transported from a farm in Ayudhya province by packing in 100 liter-styrofoam boxes filled 1/3 with aerated water. On arrival, prawns were acclimatized for one week in recirculating water ponds at ambient temperature (28±1°C).

Experimental Design

Tested animals were divided into 2 groups. In the first group, prawns were injected with different doses of steroid sapogenins, and the second group, they were fed with different levels of steroid sapogenins mixed in their diet.

1. Steroid sapogenin injection treatment

Prawns were injected once on the third day after molting with 5 doses of sapogenins (0.0, 0.1, 1.0, 2.0 and 4.0 ug/kg body weight). A microsyringe was used for muscular injection at the third abdominal segment.

Preparation of sapogenins

Steroid sapogenin powder was dissolved in chloroform to make a 10,000 mg/ml stock solution. Then, the solution was diluted with absolute ethanol to a concentration of 1,000 ug/ml. This solution was finally diluted in isosaline (NaCl 0.65 g, KCl 0.42 g and $CaCl_2$ 0.025 g in 100 ml distilled water) to make a standard solution of 10 ug sapogenins for later use.

2. Steroid sapogenin mixed diet

Pelleted prawn diet (containing 45% protein and 6% fat) was sprayed and mixed with sapogenin solution to make 4 sapogenin concentration-diets (0.0, 1.0 5.0 and 10.0 ug sapogenins/kg diet).

Feeding

Prawns were divided into 10 groups (shown in Table 1). For the injection and the control groups, they were fed control pelleted diet (no steriod added) at an amount of 3% body weight twice a day (9.00 a.m. and 17.00 p.m.). In the sapogenins mixed diet treatments, prawns were fed diet containing different concentrations of sapogenins following the same feeding regime.

Rearing unit

A static system with partial water exchange was used for prawn rearing. Aquaria (30 x 30 x 60 cm.) was filled with 40 litres of dechlorinated tap water. The density of the rearing unit was five prawns per aquarium. Each prawn was number-tagged on the carapace for individual identification. The rearing units were cleaned every morning by siphoning feces and then 20% of the total volume was replaced. The sediment was collected with a nylon net, dried and weighed to determine feed conversion ratio and consumption rate. The experiment was carried out for 12 weeks.

Water quality of the rearing units (e.g., water temperature, pH and dissolved oxygen) was monitored daily and maitained in normal condition.

Weight, length, survival and feed consumption of the prawns in each experimental group were determined weekly and molting of individuals was observed every morning.

TABLE 1. Ten experimental groups with different levels of sapogenin treatment.

Experiment groups	Treatments	Levels of sapogenins
A	sapogenins injection	0.1 μg/kg body weight
B	sapogenins injection	1.0 μg/kg body weight
C	sapogenins injection	2.0 μg/kg body weight
D	sapogenins injection	4.0 μg/kg body weight
S	solvent injection	0.0 μg/kg body weight
Fa	Sapogenins mixed diet	0.0 μg/kg diet weight
Fb	Sapogenins mixed diet	1.0 μg/kg diet weight
Fc	Sapogenins mixed diet	5.0 μg/kg diet weight
Fd	Sapogenins mixed diet	10.0 μg/kg diet weight
CT	Control group	non sapogenin diet

RESULTS

Effect of sapogenins on daily weight gain

1. Sapogenin injection treatment

The effect of sapogenin injection on growth of the giant freshwater prawn is shown in Figure 2. The highest growth rate was seen in sapogenin injection group D (0.08±0.06 g/day). This can be compared to the CT (0.05±0.02 g/day) and S (0.03±0.02 g/day) groups. In groups A, B and C, the growth rate was similar to that in the control group (0.05±0.05, 0.06±0.04,

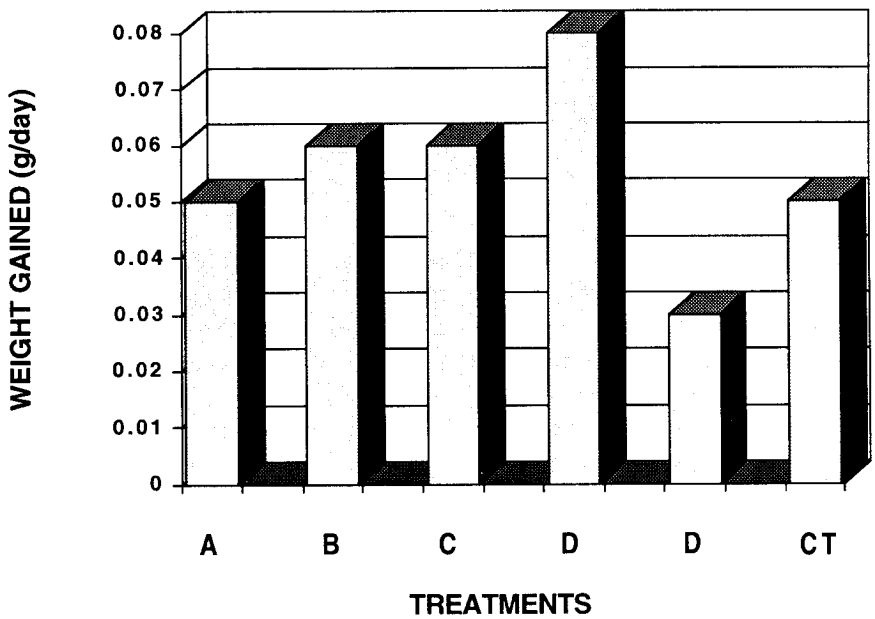

Fig. 2. Average growth rate (grams/day) of *M. rosenbergii* injected with different concentrations of sapogenins.

0.06±0.06 g/day, respectively). Statistical analysis revealed that only group D showed a significantly higher growth rate than did groups S and CT ($p<0.05$).

2. Sapogenin diet treatment

Effect of dietary sapogenins is shown in Figure 3. The highest growth rate was found in the Fd (0.08±0.05 g/day) and Fc groups (0.07±0.05 g/day) which were significantly different from that of CT and Fa groups. The growth rates of the other groups (Fb, Fc and CT) showed no significant differences.

Effect of sapogenins on length gain

1. Sapogenin injection treatment

Results in Figure 4 showed that the CT group exhibited the highest percentage length gain (1.42±0.79%). The D group, injected with the highest concentration of sapogenins, gave the lowest percentage length gain (0.89±0.41%). In groups A, B, C, and S, the percentage length gains were 1.31±0.86, 1.29±0.38, 1.09±0.63 and 1.27±0.45%, respectively. Statistical analysis indicated that length gain in the CT group differed from the D group significantly ($p<0.05$), but not from others.

2. Sapogenin diet treatment

Through 12 weeks of experiments, the average percentage length gain from every group was almost the same. There was no significant difference among the treatment groups ($p>0.05$).

Effect of sapogenins on molt duration

1. Sapogenin injection treatment

The results of sapogenin injection treatment on molting interval are shown in Figure 5. The molting periods for groups A, B, C and S were similar (22.6±3.8, 21.9±3.1, 21.8±2.0 and 22.5±0.9 days, respectively). The shortest molting period was in the D group (19.3±1.7 days), and the longest in the control group (CT) (24.9±3.1 days). Statistical analysis indicated that molting duration of the CT group differed from groups C and D significantly ($p<0.05$), but not from others. Clearly, prawns treated with sapogenins by injection exhibited shorter molting periods.

2. Sapogenin diet treatment

Results in Figure 6 show that molting duration was highest in the CT group (24.9±3.1 days) and shortest in the Fd group (19.0±1.08 days). The Fa and Fc groups presented molting periods of 23.1±3.4 days and 20.41.5 days, respectively. The data showed that the more sapogenins added to the diet, the shorter the molting interval.

Effect of sapogenins on feed consumption rate

1. Sapogenin injection treatment

The average weekly feed consumption rates of the prawn in the first few weeks of the test were high for all groups. After 12 weeks of experiment, the B group exhibited the highest consumption rate (17.50±6.84%) and the control group (CT) exhibited the lowest consumption rate (13.2±3.75%). The feed consumption rates of the others were 16.11±3.63, 15.21±5.17, 13.88±6.11, and 15.30±3.78% for A, C, D and S group, respectively. No statistical significant of the feed consumption rate was found among the treatments.

2. Sapogenin diet treatment

The results were almost the same as those from sapogenin injection treatment. During the first few weeks, the consumption rate was high and it later decreased in every group. The Fc group displayed the highest consumption rate and the Fa group displayed the lowest consumption rate (14.77±5.84% and 12.45±6.55%, respectively). No statistical difference of the feed consumption was found among the treatments.

Food conversion ratio

1. Sapogenin injection treatment

The food conversion ratio was high in the beginning weeks. The S group gave the highest food conversion ratio (21.93±21.76) and the CT group gave the lowest food conversion ratio (13.61±12.93). No statistical difference of food conversion ratio among the treatments.

2. Sapogenin diet treatment

The Fc group gave the highest food conversion ratio (18.14±20.93). Weekly variation of food conversion ratio was quite high in each treatment.

Survival rate

1. Sapogenin injection treatment

Results are shown in Figure 7. During the first week, the survival rate of every group was almost 100%. After 6 weeks of experiment, groups A and S gave the highest survival rate (60%), while group D and the control group gave the lowest survival rate (40%). By the last week, groups S and B gave the highest survival rates (about 50%), while D and control groups showed the lowest survival rate (about 20%).

2. Sapogenin diet treatment

Results are shown in Figure 8. In the first week, survival rates were almost 100%, as with the above experiment. Later they declined to reach relatively stable levels until the end of the test. When the experiment was completed, the Fa group had the highest survival rate (70%), while the control group had the lowest (20%).

DISCUSSION

From the experimental results, it is obvious that steroid sapogenins promoted the giant freshwater prawn's growth rate and that the best growth rate was obtained with 4.0 ug/kg by injection treatment and 10.0 ug/kg by diet treatment. These concentrations of sapogenins also yielded the shortest molt period and this agreed with the report by Peebles[4] where crustaceans which molted more frequently had better growth rates. Steroid sapogenins have molecular structures and properties close to molting hormone and therefore at appropriate concentration apprear to induce prawns to molt more frequently. At present, 1,056 molting hormones are extracted from plants[5]. Hence, it is understandable that steroid sapogenins extracted from *Agave sisalana* could also have certain hormone properties. Our results are similar to those of Piyataratitivorakul *et al.*[6] who used 2-deoxyecdysone and b ecdysone extracted from *Vitex glabatra* in tests which showed shorter molting periods on the tiger prawn (*Penaeus monodon*). It was also reported that b ecdysone could shorten molt period of *Procambarus simuans* which lacked eyestalks in early premolt[7] and also in lobsters in the intermolt period[8].

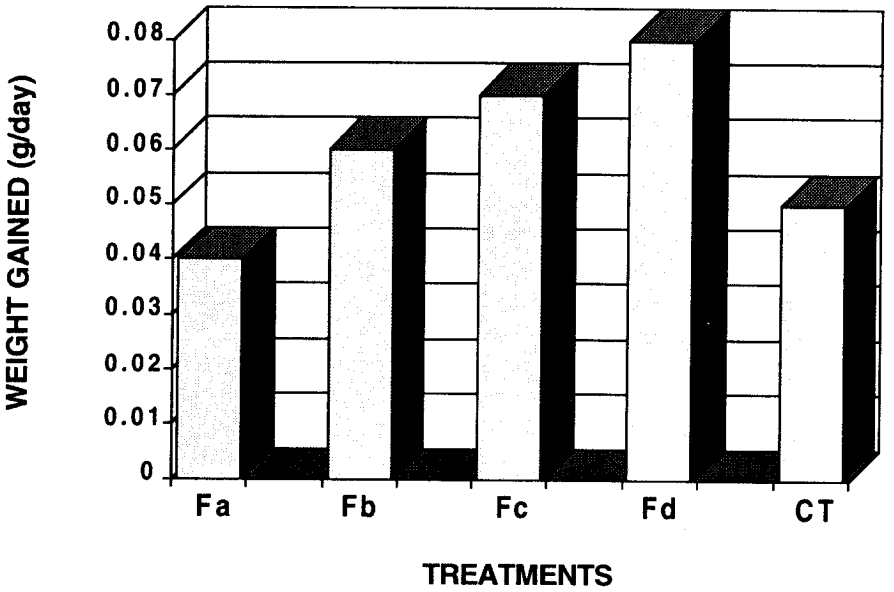

Fig. 3. Average growth rate (grams/day) of *M. rosenbergii* fed with different concentrations of sapogenins mixed in the diet.

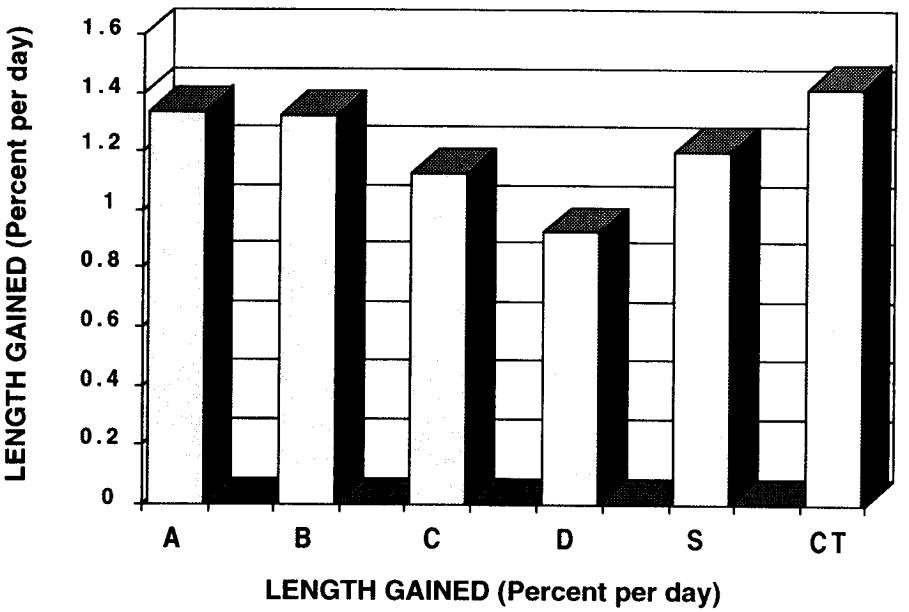

Fig. 4. Average percentage lenth gain of *M. rosenbergii* treated with various concentrations of sapogenins by injection.

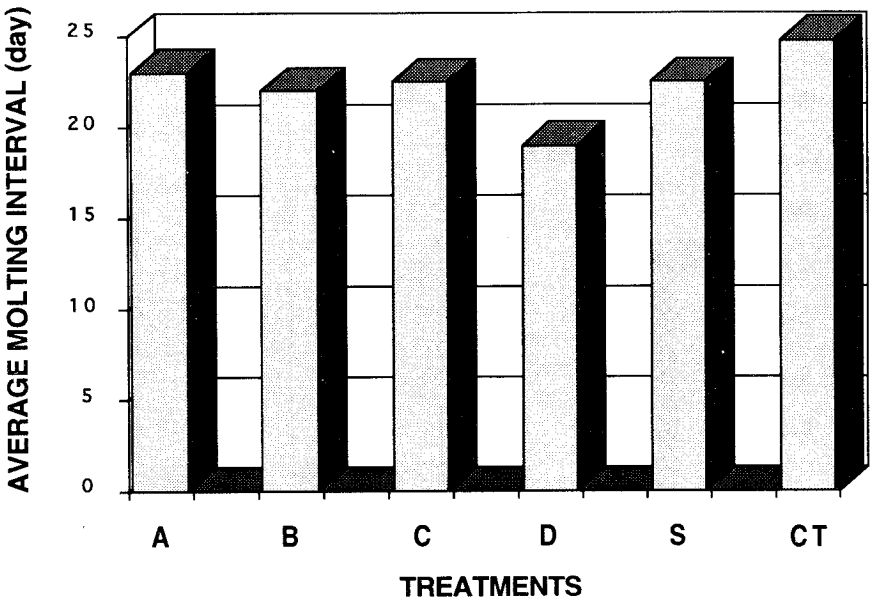

Fig. 5. Average molt cycle (days) of *M. rosenbergii* treated with various concentrations of sapogenins by injection

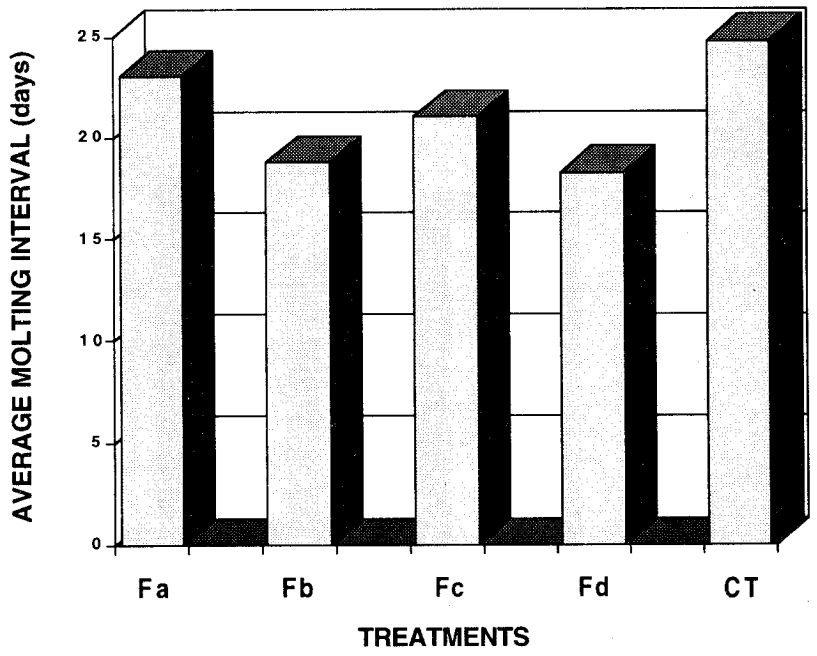

Fig. 6. Average molt cycle (days) of *M. rosenbergii* treated with various concentrations of sapogenins mixed in the diet.

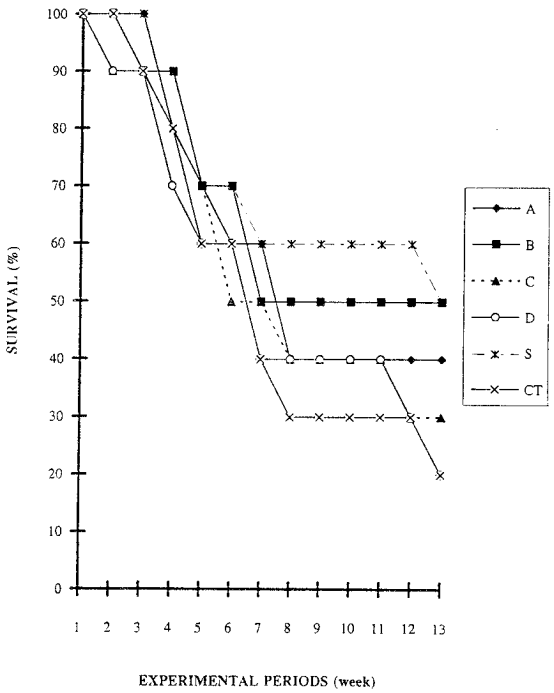

Fig. 7. Average survival rate (%) of *M. rosenbergii* treated with various concentrations of sapogenins by injection.

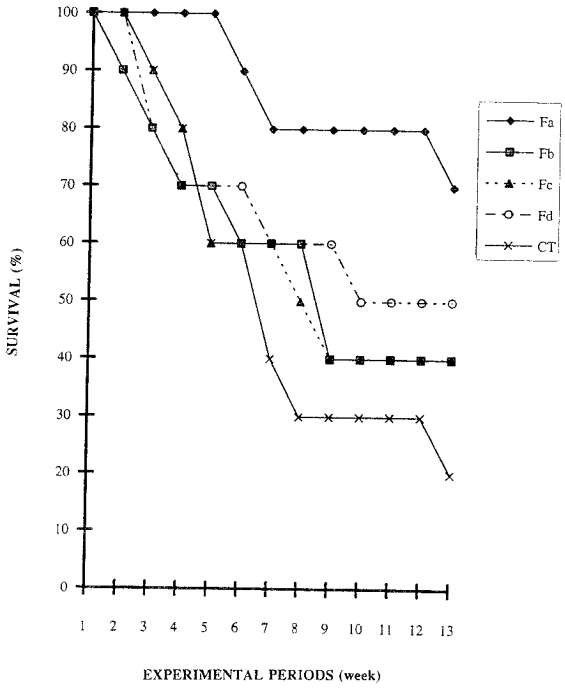

Fig. 8. Average survival rate (%) of *M. rosenbergii* fed with different concentrations of sapogenins mixed in the diet.

The sapogenin diet treatment showed that prawns could metabolize sapogenins to shorten molt duration and promote growth although they did not promote length increase, feed consumption rate and food conversion rate. Nevertheless, prawns exposed to sapogenins at higher concentrations exhibited good survival rates in the unnatural aquarium environments used. This was comparable to the survival rates in recirculating ponds with fresh diet (53.2%.)[9]

From the 2 treatment studies here, it was indicated that sapogenins in diet gave better growth and a shorter molt period than sapogenins by injection. Sapogenins in the diet at 10.0 ug/kg dry weight of feed gave shortest molt period, and the best growth rate. Hence, it can be recommended that sapogenins mixed in the prawn diet would improve the yield in the giant freshwater prawn aquaculture.

When tests were completed, analysis of residues in the prawn muscle was undertaken. No residues of the sapogenins; tigogenin and hecogenin were found.

ACKNOWLEDGMENTS

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REFERENCES

1. Ditsayaboot, P. and T. Sappinant. 1990. Study on industrial waste recycling, disposal and steroid production from *Agave sisalana*. Report for Thailand Institute of Science and Technology Research, Bangkok, 17pp.
2. Vickery, M.L. and B. Vickery. 1981. Secondary plant metabolism, In The Acetate Mevalonate Pathway (M.L. Vickery and B. Vickery, editors). The MacMillan Press Ltd., London, p 113-153.
3. Department of Agriculture Economics Research. 1992. Freshwater fishery product consumption assessment in Thailand, Ministry of Agriculture and Cooperatives, Bangkok, 47 pp.
4. Peebles, J.B. 1977. A rapid technique for molt staging in live *Macrobrachium rosenbergii*. *Aquaculture* **18**, 173-180.
5. Imai, S., T. Toyosato, M. Sakai, Y. Sato, S. Fujioka, E. Murata and M. Goto. 1969. Screening results of plants for photoecdysone. *Chem. Pharm. Bull. Japan*. 17, 335-339.
6. Piyatiratitivorakul, S., K. Sripirom, P. Menasveta and A. W. Fast. 1994. Effect of steroid hormones on ovarian development and molting of pond-reared giant tiger prawn, *Penaeus monodon* Fabricius. The Third Asian Forum (Chou, L.M., Munro, A.D., Lam, T.J., Chen, T.W., Cheong, I.K.K., Ding, J.K., Hooi, K.K, Khoo, H.W., Phang, V.P.E., Shim, K.F. and Tan, C.H. editors). Asian Fisheries Society, Manila, Philippines, 928-931.
7. Lowe, M.E., B.H.S. Horn and M.N. Galbraity. 1968. The role of crustecdysone in molting crayfish. *Experientia* **24**, 518.
8. Flint, R.W. 1972. Effect of eyestalk removal and ecdysterone infusion on molting in *Homarus americanus*. *J. Fish. Fes. Board. Can.* **29(8)**,1229-1233.
9. Phrommanont, P. and T. Sahawacharintr. 1968. Study on biology and culture of the giant freshwater prawn (*Macrobrachium resenbergii*, de Man). Songkhla Marine Fishery Station, Fishery Exploration Unit, The Department of Fishery, Bangkok, 33 pp.