EFFECTS OF DIETS WITH VARIOUS PROTEIN CONCENTRATIONS ON GROWTH, SURVIVAL AND METAMORPHOSIS OF *RANA TIGERINA* AND *R. CATESBEIANA*

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ABSTRACT

Culture of Rana tigerina *and* R. catesbeiana *was carried out. The objective of this investigation is to study the effects of diets varying in protein concentrations on frogs at various stages. Tadpoles were fed with diets containing 35%, 39%, 43% and 47% protein while froglets were fed with diets containing 21%, 25%, 29% and 33% protein. Both* R. tigerina *and* R. catesbeiana *tadpoles fed with diet containing 35% protein concentration achieved the best results with regard to feed conversion (2.00 in both species of frogs). For froglets, diets containing 21-33% protein resulted in relatively similar feed conversion values(2.3-2.5 in* R. tigerina *; 1.7-1.9 in* R. catesbeiana*).*

INTRODUCTION

Feeding is one of the most important problems encountered in commercial culturing of frogs. Usually the frog feed can be divided into three types according to the stages of the frog: food for tadpoles, for froglets and for adult frogs. A variety of feed has been used in feeding amphibian larvae[1-3] The food for tadpoles usually consists of artemia, boiled egg yolk, boiled fish, rice bran, boiled lettuce and steamed egg.

The food for froglets consists of flies' worms. Culturing of flies' worms can be done by using a wooden tray containing animals' wastes mixed with rotten fish as a culture medium. After eggs hatch into worms, froglets will feed on the emerging worms. The food for adult frogs are insects which are naturally preferred bait. In addition, Priddy and Culley[4] suggested that tadpoles, fish and crawfish were the best food for growing frogs. In large commercial farms, pelleted fish food and puppy food can be used as feed for growing frogs.

For *Rana catesbeiana* or bullfrog tadpoles and adults, different formulation of artificial feed has been introduced[5-10]. The standard diet formulation of Culley *et al.*[5] consisted of shrimp meal, fish meal, soy and yeast protein, rice bran, whey, fish oil, fish solubles, vitamin premix, linoleic acid, sodium hexametaphosphate and Kelgin as binder. The formulation of Fontanello *et al.*[7] consisted of crude protein of vegetable (soybean, wheat and maize meals) or animal (fish and meat meals and powdered milk).

Effects of additive substances in diet on the development of frogs had also been studied. Marshall *et al.*[11] reported that calcium in diet (0.5-0.9%) was required for the normal development of skeleton in *R. catesbeiana* larvae. In addition, diets containing 2-8% vitamin C were reported to reduce the incidence of scoliosis in larvae of *R. catesbeiana*[12]. The ingredients consisted of fish meal, shrimp meal, soybean meal, yeast protein and defatted rice bran, and the additives contained vitamin mix, calcium phosphate monobasic and coated ascorbic acid[6].

From current literature and local practice, it is believed that there has not yet been any systematic scientific investigation on the types of food, especially pelleted feed, that are most economically and practically suitable for frogs at various stages. Therefore, the aim of the present study is to establish the most suitable feed formulation for *R. tigerina* and *R. catesbeiana* of various ages.

MATERIALS AND METHODS

Artificial pelleted feed

The basic formulae for artificial pelleted feed in frog culture was modified from catfish pelleted feed[13]. Different formulae were developed to obtain various total protein contents of 21%, 25%, 29%, 33%, 35%, 39%, 43% and 47% (Table 1).

Fish meal, rice bran, soybean meal and glutinous rice flour were mixed with horizontal mixer; then, water was added (approximately 25% of the total weight of the ingredients). The ingredients were mixed immediately with vitamin C, vitamins and minerals, binders (rice flour), sodium benzoate and fish oil. Next, the ingredients were poured into the pelleting machine. The pellets consisted of three sizes with different diameters, i.e., 0.2 mm for tadpoles, 0.5 mm for froglets and 0.7 mm for adult frogs. The pellets were put in the oven (60°C) for two hours, packed and stored at room temperature.

Effects of various protein concentrations on growth, survival and metamorphosis of tadpoles

Round concrete tanks (0.80 m in diameter) with the water depth of 0.10 - 0.20 m were used for rearing tadpoles of *R. tigerina* and *R. catesbeiana*. The water temperature was 25-30°C with the pH of 7.1 - 7.5. One hundred tadpoles of each species were placed in each tank. In each species, there were four experimental groups of tadpoles fed with different formulae of

Table 1 Different formulae of artificial pelleted feed with total protein contents of 21%,25%, 29%, 33%, 35%, 39%, 43% and 47%.

Ingredients	Formulae for froglets (g)				Formulae for tadpoles (g)			
	I	II	III	IV	V	VI	VII	VIII
Fish meal	14.6	19.87	25.13	30.4	33.03	38.29	41.74	43.56
Rice bran	30.4	25.13	19.87	14.6	11.97	6.72	3.26	1.45
Soybean meal	14.6	19.87	25.13	30.4	33.03	38.29	41.74	43.56
Glutinous rice flour	30.4	25.13	19.87	14.6	11.97	6.72	3.26	1.45
Fish oil	5	5	5	5	5	5	5	5
Vitamin C	3	3	3	3	3	3	3	3
Vitamins and minerals	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Binders	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sodium benzoate	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Total protein content	**21%**	**25%**	**29%**	**33%**	**35%**	**39%**	**43%**	**47%**

pelleted feed containing 35%, 39%, 43% and 47% protein. Tadpoles were fed once in the evening. The quantity of feed was 10-30% of body weight or indicated by demand. The tanks were supplied with oxygen from air pump. The water was changed every other day.

Each experiment was replicated three times with 100 tadpoles per replicate. All of the tadpoles in each replicate were counted and weighed once every two weeks until they reached metamorphosis (six weeks for *R. tigerina*; 12 weeks for *R. catesbeiana*). Dead tadpoles were removed and the numbers recorded every day. Food conversion data were gathered at the beginning of seventh day.

Effects of various protein concentrations on growth and survival of froglets

After metamorphosis, froglets of *R. tigerina* and *R. catesbeiana* were transferred into the concrete ponds, 2 x 2 x 1.2m in size with water depth of 0.03-0.2m. The water temperature generally varied between 25-30°C, with the pH of 7.1 - 7.5 and relative humidity range was 80-100%. One hundred froglets were placed in each pond. In each species, the froglets were divided into four groups and fed with different formulae of pelleted feed containing 21%, 25%, 29% and 33% protein.

To prevent the froglets from harmful diseases, pelleted feed were mixed with antibiotics (chlortetracycline HCl or aureomycin) and fed to the froglets for five consecutive days. Froglets were fed once in the evening. The quantity of feed was 5-20% of body weight or indicated by demand. The water in the pond was changed every other day and the ponds were cleaned once a week. Selection of size was done once a week and froglets of different sizes were put in different ponds to prevent cannibalism. Dead froglets were removed and numbers recorded every day.

Each experiment was replicated three times with 100 froglets per replicate. All of the froglets in each replicate were counted and weighed once a month until they were sexually mature (12 months for *R. tigerina*; 15 months for *R. catesbeiana*). Food conversion data were gathered at the beginning of seventh day after froglet culture.

Food conversion

Food conversion data were obtained by weighing the food before and after feeding. The weights of tadpoles, froglets or frogs were taken prior to feeding. The feed was weighed daily as described to determine food consumption. Food conversion values were obtained by dividing the total food consumed wet weight by the total gain of each group. A dietary protein somatic index was obtained by dividing the dry weight of protein consumed by the total food consumed wet weight by the total gain of each group. This value is an index of protein consumption related to growth. Analyses of diets were performed using Standard Association of Official Agriculture Chemists[14] method to determine composition percents.

RESULTS

Growth, survival and metamorphosis of tadpoles

Growth The tadpoles of *R. tigerina* and *R. catesbeiana* were fed with different formulae of pelleted feed of varying protein contents (35%, 39%, 43%, 47%). Growth curves summarized in Figs. 1 and 2 show the average body weights of the tadpoles fed on various diets. They suggested that higher protein levels promoted better growth. Results from the comparison of average body weights of tadpoles throughout the experiment indicated that at the end of metamorphosis, tadpoles receiving higher dietary protein (43%, 47%) increased their body

weights more than those of the two lower protein groups (35%, 39%). After metamorphosis, the diet with 43% protein promoted the best tadpole growth (1.95 g in *R. tigerina*; 4.72g in *R. catesbeiana*).

Daily consumption did not differ between groups throughout the experimental period. The amount of food given was the same in all groups, although there were differences in the amount of protein taken in by the tadpoles (Tables 2,3). The results obtained from the food consumed daily (% body weight) showed that as the level of protein increased, the tadpoles consumed less. The values for the indices of nutritive used and feed conversion are shown in Tables 2 and 3. Feed conversion values tended to decline while percentages of protein increased. Thus, feed conversion for tadpoles fed with diet of 35% protein (2.00) was the best for both *R. tigerina* and *R. catesbeiana.*

Survival The results indicated similarities of percentages of survival in tadpoles fed with diets varying in protein concentrations (Figs.3,4). The average percentages of survival were very high in both species of frogs except for that of *R. tigerina* tadpoles fed with diet containing 43% protein (Fig.3).

Metamorphosis Metamorphosis occurred in all groups (Table 4). In *R. tigerina*, on the third week, the tadpoles developed hind-legs. Then, on the fourth week, some became froglets. On the fifth week, 89% of tadpoles fed with diet containing 47% protein became froglets. The lower percentages of metamorphosis occurred in tadpoles fed with diets containing 39% protein (80%), 43% protein (70%) and 35% protein (54%). By the sixth week, 99% of tadpoles fed with diet containing 47% protein became froglets. The lower percentages of metamorphosis occurred in tadpoles fed with diets containing 39% protein (93%), 35% protein (84%) and 43% protein (80%) (Table 4). Diets with lower protein content tended to result in slower rate of metamorphosis.

In *R. catesbeiana*, the tadpoles developed hind-legs on the fourth week (Table 5). Then, on the ninth week, some became froglets. On the tenth week, 96% of tadpoles fed with diet containing 39% protein became froglets. By the twelfth week, 100% of tadpoles fed with diet containing 39% became froglets. The lower percentages of metamorphosis occurred in tadpoles fed with diets containing 43% protein (99%), 47% protein (97%) and 35% protein (78%) (Table 5).

Growth and survival of froglets

Growth Froglets of *R. tigerina* and *R. catesbeiana* were fed with different formulae of pelleted feed varying in protein concentrations (21%, 25%, 29%, 33%). The average body weight values from the growth curves showed that protein was necessary early in the first 3-4 months. After that, the average body weights were quite different. The results from the comparison of average body weights of froglets throughout the experiment indicated that froglets fed with diet of 29% protein showed the best growth (200.70g in *R. tigerina*; 330.60 g in *R. catesbeiana*) (Figs. 5,6). The highest weight gain per day (0.52 g in *R. tigerina*; 0.70 g in *R. catesbeiana*) was also observed in froglets fed with diet of 29% protein (Tables 6,7). The weights of froglets increased rapidly during the first 3-5 months.

Daily consumption did not differ among groups throughout the experimental period, although there were differences in the amount of protein taken in by the frogs (Tables 6,7). The results obtained from the food consumed daily (% body weight) showed that different groups of frogs consumed different amount of food (Tables 6,7). Feed conversion values are also different in both *R. tigerina* and *R. catesbeiana* froglets fed with diets containing varying

Table 2 Weight, growth responses and food utilization for 28 days in *R. tigerina* tadpoles fed with diets varying in protein concentrations.

	Protein concentration			
	35%	**39%**	**43%**	**47%**
Mean initial wt.(g)	0.44	0.55	0.65	0.56
Mean final wt.(g)	1.23	1.53	1.95	1.80
Gain/day(g)	0.03	0.04	0.05	0.04
Gain/day as % body wt.	6.82	7.27	7.69	7.14
Consumed/day(g dry wt.)	0.06	0.06	0.05	0.06
Cons/day as % body wt.	13.64	10.91	7.69	10.71
Feed conversion	2.00	1.50	1.00	1.50

$$\text{Gain/day (g)} = \frac{\text{mean (f) - mean (i)}}{\text{number of day}}$$

$$\text{Gain/day as \% body wt.} = \frac{\text{gain/day(g)x100}}{\text{mean (i)}}$$

$$\text{Cons/day as \% body wt.} = \frac{\text{cons/day (g) x 100}}{\text{mean (i)}}$$

$$\text{Feed conversion} = \frac{\text{cons/day (g)}}{\text{gain/day (g)}}$$

Table 3 Weight, growth responses and food utilization for 63 days in *R. catesbeiana* tadpoles fed with diets varying in protein concentrations.

	Protein concentration			
	35%	**39%**	**43%**	**47%**
Mean initial wt.(g)	0.29	0.29	0.29	0.29
Mean final wt.(g)	3.63	4.13	4.72	4.71
Gain/day(g)	0.05	0.06	0.07	0.07
Gain/day as % body wt.	17.24	20.69	24.14	24.14
Consumed/day(g dry wt.)	0.10	0.09	0.09	0.09
Cons/day as % body wt.	34.48	31.03	31.03	31.03
Feed conversion	2.00	1.50	1.29	1.29

Table 4 Metamorphosis in *R. tigerina* tadpoles fed with diets varying in protein concentrations.

Age (week)	% metamorphosis											
	35%			39%			43%			47%		
	Froglets	Hind-legs	Tadpoles	Froglets	Hind-legs	Tadpoles	Froglets	Hind-legs	Tadpoles	Froglets	Hind-legs	Tadpoles
1	0	0	100	0	0	100	0	0	100	0	0	100
2	0	0	100	0	0	100	0	0	100	0	0	100
3	0	100	0	0	99	1	0	100	0	0	100	0
4	1	99	0	8	90	1	14	70	0	7	93	0
5	54	43	0	80	18	1	70	10	0	89	11	0
6	84	13	0	93	0	1	80	0	0	99	0	0

Table 5 Metamorphosis in *R. catesbeiana* tadpoles fed with diets varying in protein concentrations.

Age (week)	% metamorphosis											
	35%			39%			43%			47%		
	Froglets	Hind-legs	Tadpoles	Froglets	Hind-legs	Tadpoles	Froglets	Hind-legs	Tadpoles	Froglets	Hind-legs	Tadpoles
3	0	0	100	0	0	100	0	0	100	0	0	100
4	0	100	0	0	100	0	0	100	0	0	100	0
5	0	100	0	0	100	0	0	100	0	0	100	0
6	0	97	0	0	100	0	0	100	0	0	100	0
7	0	95	0	0	100	0	0	99	0	0	98	0
8	0	95	0	0	100	0	0	99	0	0	97	0
9	6	89	0	55	45	0	24	75	0	70	27	0
10	31	64	0	96	4	0	69	30	0	92	5	0
11	57	38	0	97	3	0	92	7	0	96	1	0
12	78	17	0	100	0	0	99	0	0	97	0	0

Table 6 Weight, growth responses and food utilization for 353 days in *R. tigerina* froglets fed with diets varying in protein concentrations.

	21%	25%	29%	33%
Mean initial wt.(g)	19.90	18.50	19.70	17.30
Mean final wt.(g)	179.00	193.10	202.80	180.30
Gain/day(g)	0.45	0.49	0.52	0.46
Gain/day as % body wt.	2.26	2.65	2.64	2.66
Consumed/day(g dry wt.)	1.13	1.13	1.13	1.13
Cons/day as % body wt.	5.68	6.11	5.74	6.53
Feed conversion	2.51	2.31	2.17	2.46

Table 7 Weight, growth responses and food utilization for 443 days in *R. catesbeiana* froglets fed with diets varying in protein concentrations.

	21%	25%	29%	33%
Mean initial wt.(g)	23.10	22.80	20.40	16.80
Mean final wt.(g)	313.40	312.50	330.60	322.30
Gain/day(g)	0.66	0.65	0.70	0.69
Gain/day as % body wt.	2.86	2.85	3.43	4.11
Consumed/day(g dry wt.)	1.15	1.23	1.23	1.23
Cons/day as % body wt.	4.98	5.39	6.03	7.32
Feed conversion	1.74	1.89	1.76	1.78

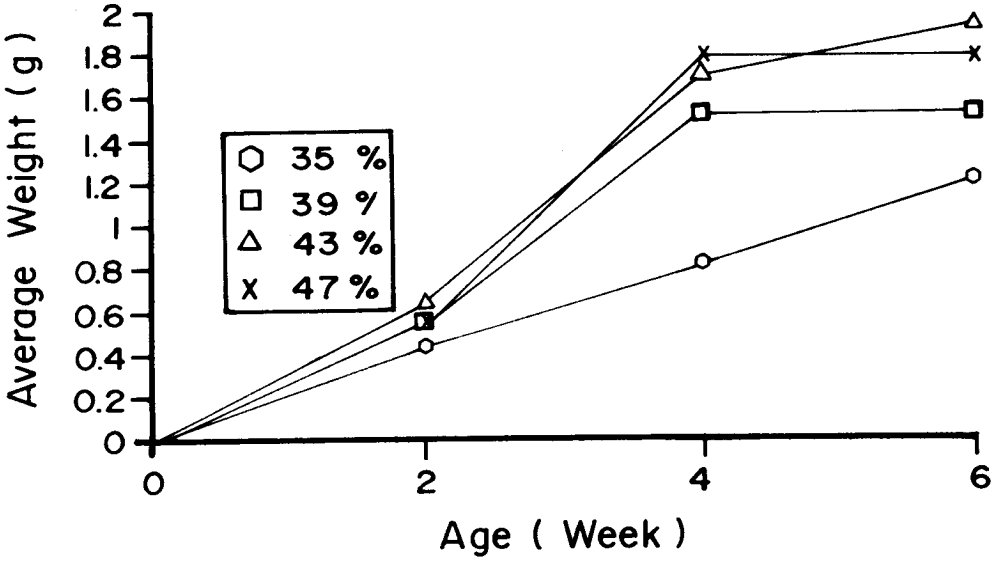

Fig.1 Growth curves of *R. tigerina* tadpoles fed with diets containing 35%, 39%, 43% and 47% protein.

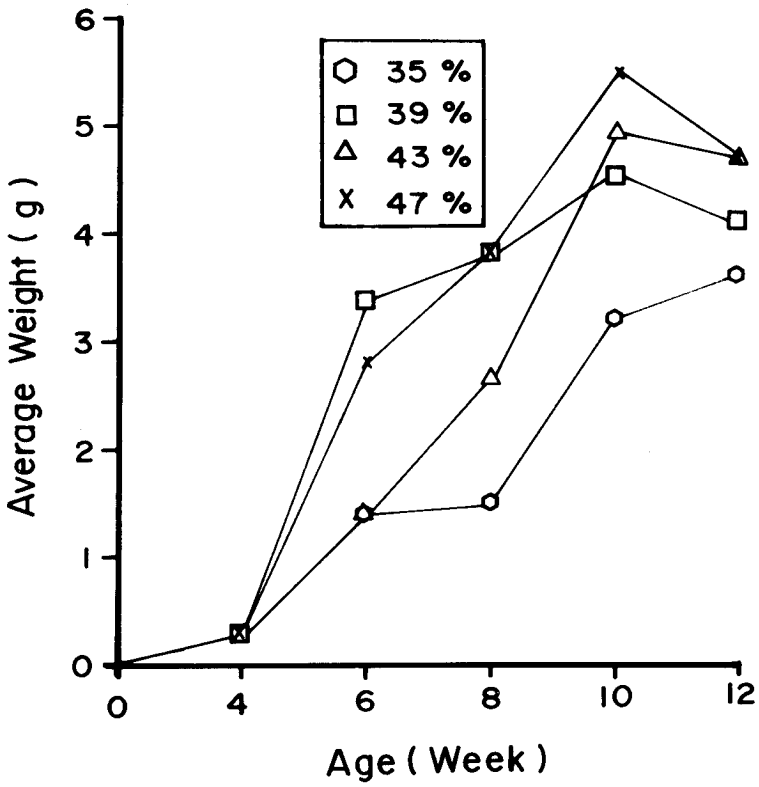

Fig.2 Growth curves of *R. catesbeiana* tadpoles fed with diets containing 35%, 39%, 43% and 47% protein.

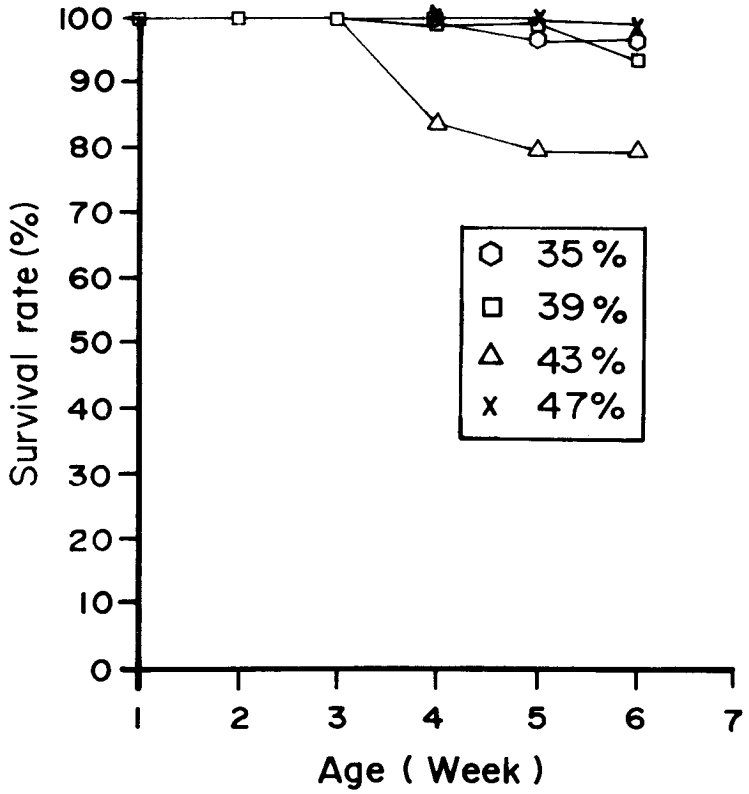

Fig.3 Percentages of survival in *R. tigerina* tadpoles fed with diets containing 35%, 37%, 43% and 47% protein.

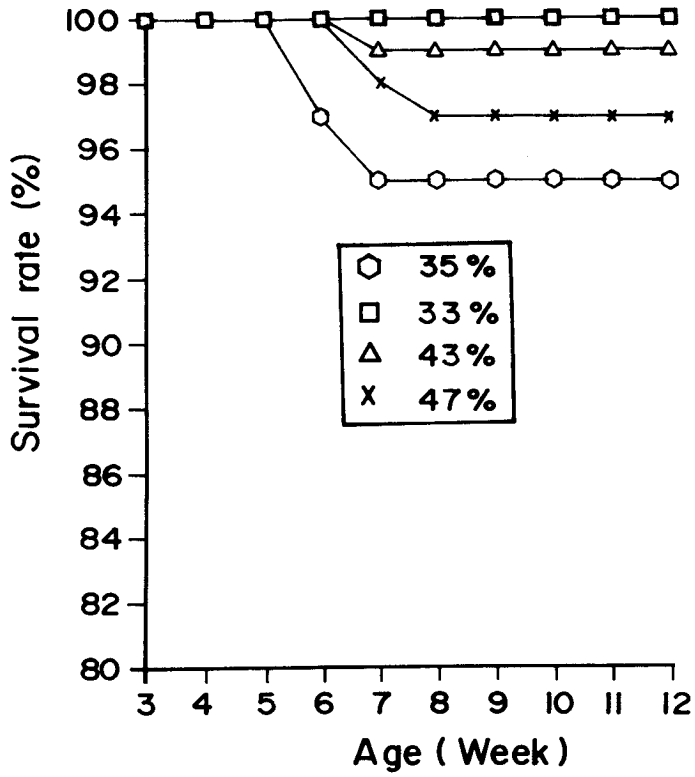

Fig.4 Percentages of survival in *R. catesbeiana* tadpoles fed with diets containing 35%, 37%, 43% and 47% protein.

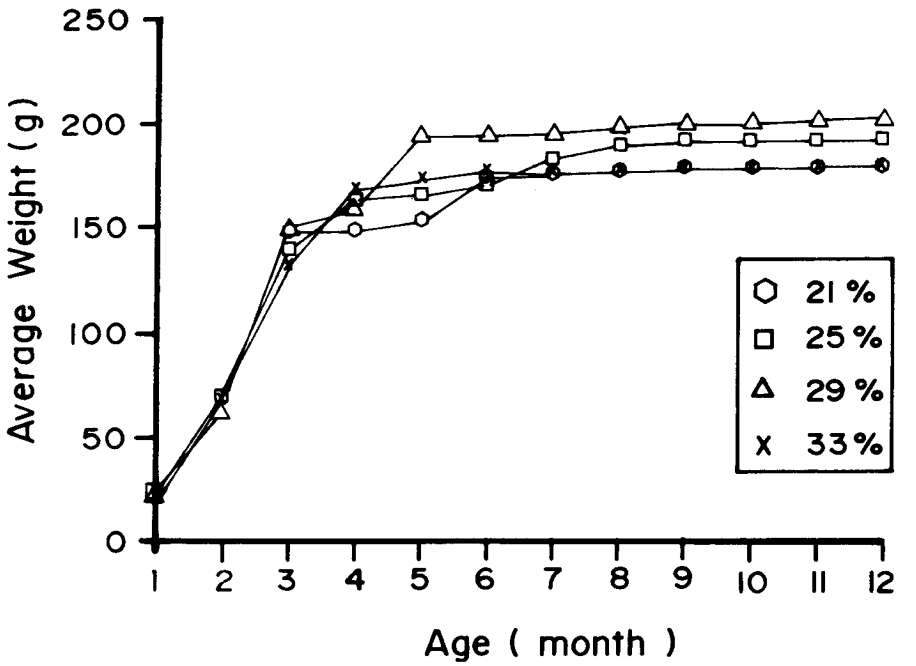

Fig.5 Growth curves of *R. tigerina* froglets fed with diets containing 21%, 25%, 29% and 35% protein.

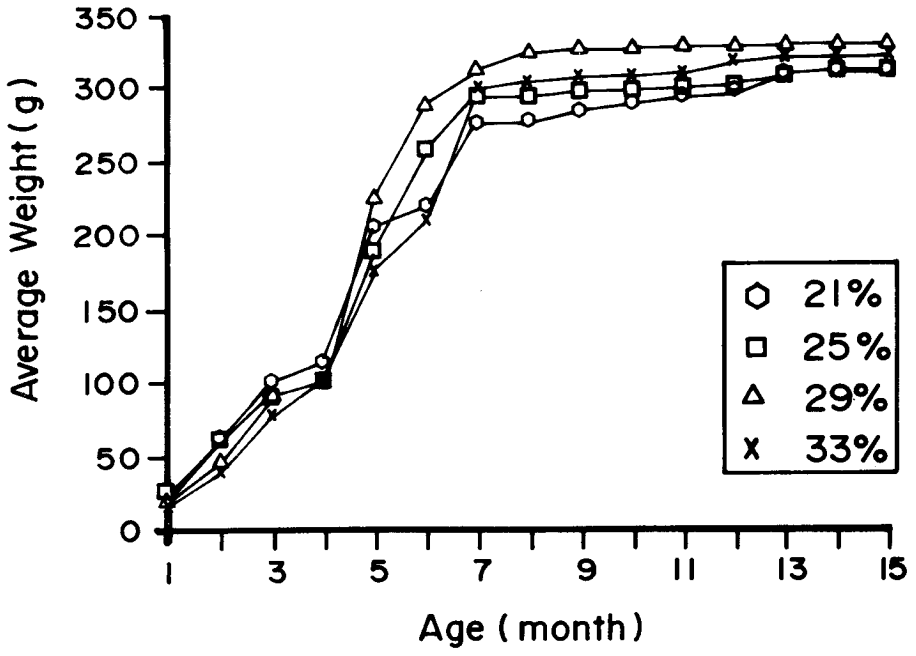

Fig.6 Growth curves of *R catesbeiana* froglets fed with diets containing 21%, 25%, 29% and 35% protein.

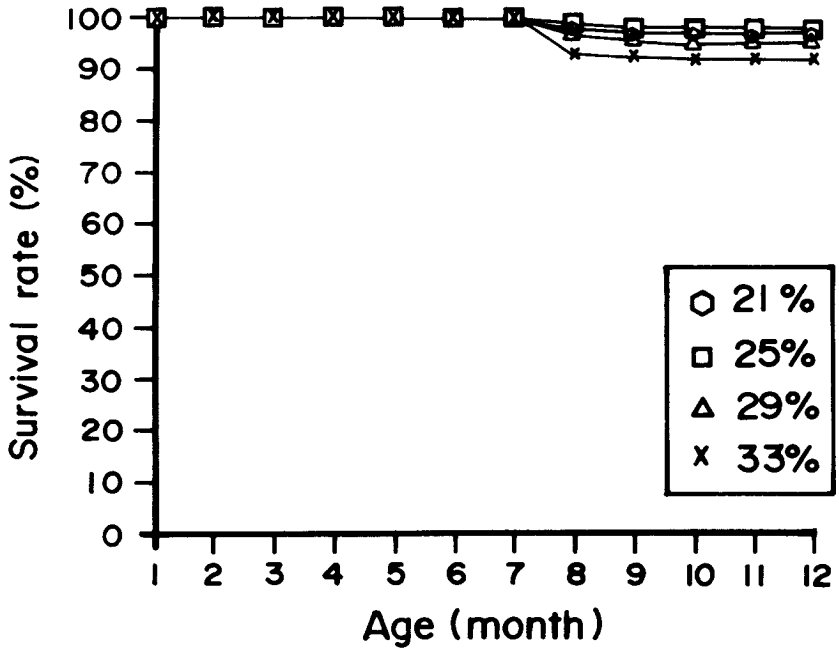

Fig.7 Percentages of survival in *R. tigerina* froglets fed with diets containing 21%, 25%, 29% and 35% protein.

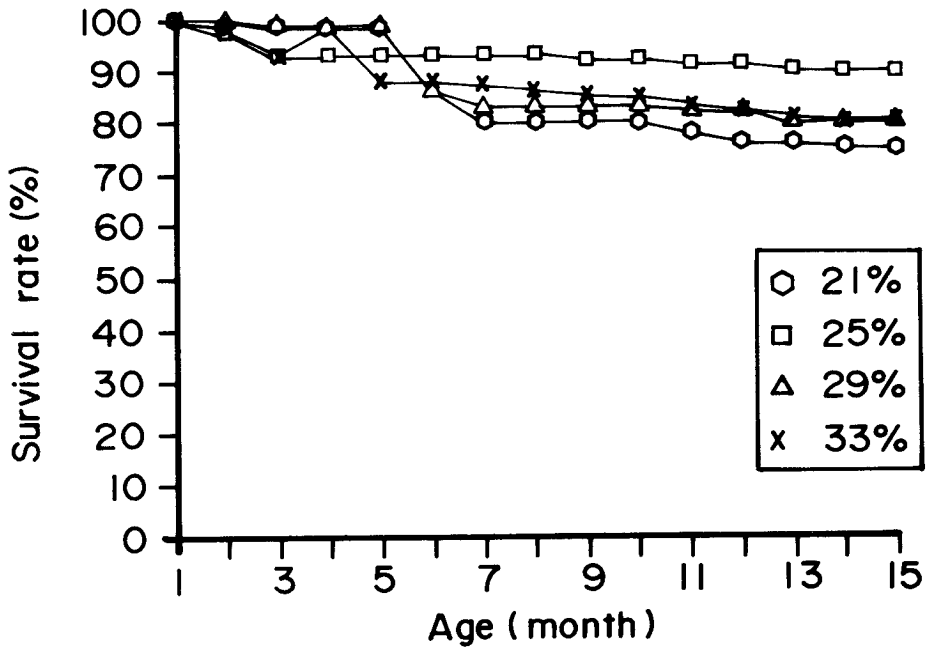

Fig.8 Percentages of survival in *R. catesbeiana* froglets fed with diets containing 21%, 25%, 29% and 35% protein.

protein concentrations. In *R. tigerina*, the diet of 21% protein promoted the best feed conversion value (2.51) while the diet of 25% protein promoted the best feed conversion value for *R. catesbeiana* (1.89).

Survival In *R. tigerina*, the percentages of survival of froglets are not different significantly among the experimental groups (Fig.7). The highest percentage was found in froglets fed with diet of 25% protein (99%). However, in *R. catesbeiana*, the percentages of survival are different (Fig.8). The highest percentage was also found in froglets fed with diet of 25% protein (93%).

DISCUSSION

It is often necessary to develop special feed formulation for frogs since conventional feed may rapidly deteriorate and contaminate the environment when added to the water. Artificial pelleted feed of three sizes (0.20, 0.52, 0.71 mm) were used for tadpoles, froglets and adult frogs in this study. Mandelli *et al.*[15] studied the effect of particle size of the feed on weight gain in intensively reared tadpoles of *R. catesbeiana* and showed that pelleted feed of 0.21 mm in diameter was most suitable for tadpole stage. In addition to the size of the pelleted feed, other factors have to be taken into consideration. Pelleted feed should stay solid above the water for 18-24 hours so that frogs can consume at longer time. The artificial feed should contain only 25% of water. It was recommended that the moisture in the feed should not exceed 75%[16]. At a higher level of water content, the digestive activity of the tadpoles would increase greatly. Hence, the food was less consumed. Mohanty and Dash[17] registered a slow growth rate and a delay in metamorphosis in *Rana tigrina* larvae fed on vegetables or meat. Culley and Sotiaridis[16] considered that these food stuffs (lettuce, liver, etc.), which were so frequently used on the non-commercial rearing of amphibians would never meet the nutritional requirements of tadpoles. It is agreed that larvae of the genus *Rana* are omnivorous - detrivorous[18]. Currently, results obtained with fresh diets showed that they were not suitable for the intensive culture of anuran larvae[16,17,19].

From the experiment, the artificial pelleted feed in frog culture was modified from catfish pelleted feed which was quite similar to the standard diet formulation[5]. Vitamin C is an important ingredient in the diet formula. Previous studies indicated that scoliosis occurred in larvae of *R. catesbeiana* because of vitamin C deficiencies[12]. However, the skeleton deformities did not appear in *R. tigerina* and *R. catesbeiana* tadpoles in the present study. Thus, diets varying in protein concentrations containing 3% vitamin C might reduce the incidence of scoliosis in frog larvae. This percentage agreed with that of Leibovitz *et al.*[12]

In nutritional studies, the evaluation of the indices of nutritive use is important. The values of feed conversion and dietary protein concentration of *R. tigerina* and *R. catesbeiana* obtained in this study are quite similar to those of the other studies. *R. tigerina* and *R. catesbeiana* tadpoles provided with diet of 35% protein achieved the best results with regard to feed conversion. Marschall[20] found that a content of 35-43% protein in diet for *R. catesbeiana* larvae was sufficient to achieve efficient growth, allowing metamorphosis to take place. These percentages agreed with those of Culley *et al.*[21] (35%), Lima and Agostinho[22] (40%), Chim-Figueiredo and Gallassini[23] (40%), Fontenelle *et al.*[24] (33-40%) and Monteiro *et al.*[25] (40%). High dietary protein levels are counterproductive because animals must expend energy to metabolize excess protein, and unless compensated for by fat and carbohydrates, they must use protein as an energy source, reducing the performance of protein growth purposes[26]. Moreover, the proportion of other macronutrients (lipids and carbohydrates) present in the diet influences the use of the protein[27]. Up to a certain point, fat improves growth considerably and causes

a saving in proteins[28].

In this kind of study, it is important to determine the appropriate quantities of feed that should be given to the animals because the amount of food consumed determines the primary availability of nutrients and energy. In the present study, the quantity of food daily offered to tadpoles of *R. tigerina* and *R. catesbeiana* was 20% body weight. In other experiments, the quantity of food daily offered to *R. catesbeiana* larvae varied from 3% to 13% body weight[29,30]. In this experiment, the feed intake of *R. tigerina* larvae was from 8% to 14%, but the feed intake of *R. catesbeiana* larvae was from 30% to 33% during late metamorphosis. Culley *et al.*[21] found that daily consumption of *R. catesbeiana* larvae prior to metamorphic climax was at 7% body weight. However, Lima and Agostinho[22] registered the daily consumption to be only 3% or 4%.

Studies carried out with *R. catesbeiana* larvae fed with diets containing protein levels of 20-70% indicated that significant differences observed in daily consumption were related to protein concentrations[20]. Feed intake increased as protein percentage decreased. Similar correlation was also observed in the present study. This fact is related to the lower average weights of the larvae fed with poorer diets, therefore, the larvae were able to regulate the quantity of feed consumed according to dietary protein levels. In fishes, feed intake is conditioned mainly by available energy in the diet[31]. Protein level alone has relative importance, taking into account that lipids and carbohydrates also act as energy sources[32].

In the froglet stage, it seems that there is no correlation between feed conversion and protein concentration. In *R. tigerina*, diets containing 21%, 25% and 33% protein resulted in relatively similar feed conversion values (2.51, 2.31 and 2.46, respectively). In *R. catesbeiana*, diets containing 21%, 25%, 29% and 33% also resulted in relatively similar feed conversion values (1.74, 1.89, 1.76 and 1.78, respectively). Sarwono[33] reported that in rearing *R. catesbeiana,* the frog weighed at 5-10 g after four months could reach 100-150g each as a consumption size with feeding conversion ratio of 1-2.

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บทคัดย่อ

ในการทดลองเลี้ยงกบนา (*Rana tigerina*) และกบบูลฟรอก (*R. catesbeiana*) ด้วยอาหารเม็ดที่มีปริมาณโปรตีนต่างๆ กัน กล่าวคือ อาหารเม็ดที่เลี้ยงลูกอ๊อดมีปริมาณโปรตีน 35%, 39%, 43% และ 47% ส่วนอาหารเม็ดที่เลี้ยงลูกกบมีปริมาณโปรตีน 21%, 25%, 29% และ 33% ผลการทดลองแสดงว่าอาหารเม็ดที่มีโปรตีน 35% เหมาะในการเลี้ยงลูกอ๊อดของกบทั้งสองสปีชีส์ เพราะในอัตราการแลกเนื้อที่ดีที่สุดสำหรับระยะลูกกบนั้น อาหารเม็ดที่มีโปรตีน 21% เหมาะสำหรับการเลี้ยงลูกกบนา ส่วนลูกกบบูลฟรอกนั้น อาหารเม็ดที่มีโปรตีน 25% เหมาะสมที่สุด อาหารเม็ดเหล่านี้ให้อัตราการแลกเนื้อที่ดีที่สุดเช่นเดียวกัน