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## EFFECT OF ANIMAL AND PLANT PROTEIN DIETS ON GROWTH AND FECUNDITY IN ORNAMENTAL FISH, *BETTA SPLENDENS* (REGAN)

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### Abstract

The effects of different levels of animal and plant protein (10%, 15%, 25%, 35% and 45%) on growth and fecundity in Siamese fighting fish, *Betta splendens*, were tested over a period of 154 days. Fish fed 35% animal or plant protein had the highest mean body wet weight, growth rate, gonad weight and fecundity than any other tested level. Animal protein induced gonad development earlier than plant protein. Female *B. splendens* fed the 35% animal protein diet had a 79% higher gonad weight (87 mg wet weight) than those fed the diet with the same level of plant protein (49 mg wet weight). Spawning trials showed that 35% animal or plant protein diets produced the highest number of eggs and the highest hatching rate compared to the other diets; hence, 35% animal or plant protein is considered the optimum level for *B. splendens*. Females fed the 35% animal protein diet laid 1044 eggs in three spawnings in contrast to 846 eggs produced by fish fed the 35% plant protein diet. The necessity of incorporating an optimum level of animal protein in the diet for maximum reproductive performance in *B. splendens* is discussed.

### Introduction

The production of any aquaculture species can be economical only when its qualitative and quantitative feed requirements are known, making the formulation of nutritionally balanced low-cost diets possible (Tacon et al., 1983; Hashim et al., 1992; James et al., 1993). The quantity and composition of

dietary protein are known to affect fish reproduction (Shim et al., 1989). Female fish need adequate protein for egg/embryo development and reproduction. Protein is also needed for the formation of follicles and other ovarian tissues, and for the growth and development of embryos. The absence of an adequate

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quantity of protein can affect the survival of the offspring.

The Siamese fighting fish *Betta splendens* is oviparous and feeds on live foods, chiefly tubifex worms, *Daphnia*, small aquatic insects and larvae of mosquitoes and *Chironomus* in their natural habitat. However, it accepts pellets and frozen diets also. The energy sources for this species are proteins, carbohydrates and fats, like those of other animals (Black et al., 1966; Degani et al., 1985, 1986). Many studies have been carried out on the nutrition and growth of cultivable fishes (Degani et al., 1985, 1986, 1989; Hashim, 1994; Catacutan and Coloso, 1995; Sumagaysay and Borlongan, 1995). Less attention has been paid to the growth and nutrition of tropical fishes (Shim et al., 1989; Degani and Gur, 1992). And no study has been published on the optimum level of protein in the nutrition of *Betta splendens*. Hence, the present study was undertaken to study the effect of different levels of dietary animal and plant proteins on growth, gonad weight and fecundity in *Betta splendens*.

#### Materials and Methods

**Experimental fish.** The Bettas belong to the Anabantidae family. The species *Betta splendens* is known as the Siamese fighting fish. Aquarium breeding has produced many forms of gorgeous colors and flowing fins from the short-finned, dirty-brown native stock from Thailand. Siamese fighters are peaceful when kept with other fishes, but two males in the same tank will fight viciously. If a male does not get a breeding response from a female, it will ruthlessly tear her fins and kill her. The male builds a bubble nest on the water surface during spawning and both male and female gather the eggs and press them into the bubble nest. The males incubate the eggs for 36-40 hours and the fry grow very rapidly. They attain sexual maturity at the age of 60-75 days and females lay about 300-400 eggs, depending on the nature of their diet. They grow to a maximum of 7 cm and come to the surface water to take atmospheric air using their labyrinth organ.

**Fish and maintenance.** Seven hundred

and fifty active 30-day-old juvenile *B. splendens* ( $0.07 \pm 0.002$  g;  $14.80 \pm 0.50$  mm) were collected from three laboratory bred brooders. They were divided into ten groups, each group was offered one of ten diets with different levels (10%, 15%, 25%, 35% or 45%) of either animal or plant protein. Triplicates were maintained for each treatment. Each group consisted of 25 individuals of a similar body weight and was reared in a circular cement tank (height 1.75' x diameter 1.50', capacity 110 l) containing 50 l of water.

**Feeding and water exchange.** During the experimental period of 154 days, fish were fed *ad libitum* once a day in a feeding tray for two hours after which unconsumed feed was removed and dried in a hot air oven at 80°C. The water was clean unchlorinated well water. Its quality was monitored biweekly (temperature  $28 \pm 1^\circ\text{C}$ ; pH  $7.8 \pm 0.05$ ; salinity  $0.13 \pm 0.01\text{‰}$  and DO 4.04 ppm). The tanks were drained twice a week and replenished with fresh water to remove the accumulated feces at the bottom.

**Growth and gonad measurements.** Growth of the experimental fish was measured at intervals of 28 days by weighing all the individuals in the tanks. The mean body weight (g) was calculated by dividing the total wet weight of the test animals by the total number of animals in the aquarium at that time. The 'sacrifice method' (Maynard and Loosli, 1962) was adopted to estimate the gonad growth of the animals. Two females from each replicate tank were sacrificed, the ovaries were dissected out and weighed. The gonadosomatic index (GSI) was computed following Dahlgren (1979): gonadosomatic index = (weight of gonad/weight of fish) x 100.

Fish, feed samples, unconsumed feed and ovaries were weighed in an electrical monopan balance to an accuracy of 0.1 mg. The following formulae were used to estimate the feeding rate, growth rate and conversion efficiency. Both rates were expressed as mg per g live fish per day; conversion efficiency was expressed as a percent. Feed consumed = total feed offered - amount of unconsumed feed; feeding rate = feed consumed/(initial wet weight of fish x duration); weight gain = final

dry weight - initial dry weight; growth rate = weight gain / (initial wet weight of fish x duration); feed conversion efficiency = (weight gain/feed consumed) x 100.

**Spawning.** After attaining sexual maturity, two males and two females were randomly selected from each replicate and used for the spawning studies. One male *B. splendens* was paired with one female in a plastic trough containing 5 l of water. A few plant tufts were placed on the surface under which the female found shelter against the highly aggressive male. The male built a foam nest, made of bubbles covered with saliva, on the water surface or under the plant tufts. The female brooders laid eggs within 24-30 hours. Both the male and female collected the falling eggs and squeezed them into the nest. The number of eggs laid by the female was counted by using a sterilized needle, without causing much disturbance. Fecundity is the number of eggs laid in a single spawn by an oviparous female fish (McFadden et al., 1965). After spawning, the male drove the female away and guarded the developing eggs very intensively. The fry hatched after 36-40 hours of incubation. The male betta was removed as soon as the fry hatched. The number of fry hatched and the number of unhatched eggs were also counted. The hatching rate was calculated by dividing the number of eggs hatched by the number of eggs laid and multiplying it by 100. The experiment ended on day 154 after the completion of three spawnings. The remaining animals in the experimental tanks were removed and sacrificed for gonad and growth estimations.

**Experimental diets and composition.** The diets were prepared following the square method (Hardy, 1980). The compositions of the diets are given in Tables 1 and 2. The dry ingredients were blended to make a homogeneous mixture. Then the diets were mixed with an aliquot of boiled water and cooked by steam for 15-20 minutes. After moderate cooling, pellets (2 mm size) were prepared with a hand operated pelletizer and dried in sunlight. After drying, diets were stored separately in a refrigerator until use. The protein and lipid contents of the experimental diets were deter-

mined in a spectrophotometer following Lowry et al. (1951) and Bragdon (1951), respectively. The moisture content was analyzed by drying in an electric hot air oven at 100°C. The mineral content was estimated following the method of Paine (1964). Nitrogen free extract (NFE) was calculated by subtracting the protein, lipid and mineral contents from the dry weight of the feed samples.

**Statistical analysis.** Two-way ANOVA was used to detect significant effects of the dietary protein level and rearing period on feeding parameters of the *B. splendens*. Student's *t* test was applied to determine the significance of mean values between the experimental groups. Regression analysis was carried out based on the method of least square (Zar, 1974).

## Results

The mean body weight, selected food utilization parameters, gonad weight and fecundity increased with the increase in protein level up to 35% and declined thereafter. The fish fed animal protein diets elicited better responses than those fed plant protein diets. The mean wet body weights of the fish fed animal protein diets at the 10%, 15%, 25%, 35% and 45% protein levels were 0.82, 0.94, 1.24, 1.47 (25% vs 35%:  $t = 6.22$ ;  $p < 0.01$ ) and 1.31 g (35% vs 45%:  $t = 5.71$ ;  $p < 0.01$ ), respectively, on day 154 (Fig. 1). A similar trend was obtained in fish fed plant protein. On day 84, the growth rates for fish fed animal protein at the 10%, 15%, 25%, 35% and 45% levels were 3.02, 3.38, 4.72, 5.82 and 5.62 mg per g live fish per day, respectively (Table 3) and for fish fed plant protein were 2.36, 3.30, 3.73, 4.76 and 4.55 mg per g live fish per day (Table 4). Feeding rates and conversion efficiencies were similar to growth rates. ANOVA showed that the differences in feeding and growth rates and in conversion efficiency were highly significant ( $p < 0.01$ ). The fish fed 35% animal and plant protein diets had the highest growth rates, and fish fed animal protein diets performed better than those fed plant protein diets.

Female *B. splendens* fed 35% animal or plant protein had significantly heavier gonads

Table 1. Ingredients (g) and proximate chemical composition (%) of experimental pellet feeds (animal protein diet).

<i>Ingredient</i>	<i>Protein level (%)</i>				
	10	15	25	35	45
Fishmeal	13	83	224	365	500
Squid meal	4	28	75	122	167
Maida	838	744	556	368	188
Egg yolk	85	85	85	85	85
Sunflower oil	43	43	43	43	43
* Vitamin/mineral mix	17	17	17	17	17
<i>Proximate composition (%)</i>					
Dry matter	92.40	92.63	92.10	92.55	92.06
Moisture	7.60	7.37	7.90	7.45	7.94
Crude protein	9.89	15.25	25.40	35.69	45.46
Crude fat	7.10	7.39	7.62	7.22	7.54
Nitrogen free extracts	71.61	65.66	55.66	45.66	35.33
Ash	11.40	11.70	11.32	11.43	11.67

<i>* Vitamin/mineral mix</i>	
<i>Compound</i>	<i>g per kg diet</i>
Vitamin A	40,000 IU
Vitamin D3	8000 IU
Vitamin B2	0.016
Vitamin E	6.0 units
Vitamin K	0.008
Calcium pantothenate	0.02
Nicotinamide	0.08
Vitamin B12	0.048
Choline chloride	1.2
Calcium	6.0
Manganese	0.22
Iodine	0.008
Iron	0.06
Zinc	0.12
Copper	0.016
Cobalt	0.004

Table 2. Ingredients (g) and proximate chemical composition (%) of experimental plant protein diet.

Ingredient	Protein level (%)				
	10	15	25	35	45
Soybean meal	22	136	363	589	690
Groundnut oil cake	6	33	91	149	172
Bajra	302	255	160	66	24
Ragi	302	255	160	66	24
Maida	302	255	160	66	24
Sunflower oil	48	48	48	48	48
* Vitamin/mineral mix	18	18	18	18	18
<i>Proximate composition (%)</i>					
Dry matter	91.00	91.50	92.64	91.48	91.60
Moisture	9.00	8.50	7.36	8.52	8.40
Crude protein	10.24	15.39	24.49	35.70	45.10
Crude fat	6.55	6.80	6.50	6.20	6.52
Nitrogen free extracts	70.76	65.21	56.91	46.30	35.98
Ash	12.45	12.60	12.10	11.80	12.40

\* See Table 1.

than the females receiving other protein levels (Table 5). On day 112, the gonad (87 mg) in fish that consumed 35% animal protein weighed 79% more than the gonad (48.5 mg) in those that consumed the same level of plant protein. A similar trend was obtained for GSI. In all groups fed plant protein diets, no gonads were developed on day 56. However, fish fed 25-45% animal protein had gonads on day 56.

Fish fed the 35% animal and plant protein diets spawned more eggs (Fig. 2) and the hatching rate of their eggs was highest amongst all other diets with the same source of protein (Table 6). Comparatively, fish fed 35% animal protein laid significantly ( $t = 22.83$ ;  $p < 0.01$ ) more eggs (1044) than the females fed 35% plant protein (846), indicat-

ing that an animal protein diet is better than a plant protein diet.

### Discussion

The present study reveals that 35% protein levels in the test diets resulted in faster growth in terms of mean body weight in *B. splendens*. Similar results were obtained in dwarf gourami *Colisa lalia* (Shim et al., 1989). Degani and Gur (1992) found that mean body weight of the pearl gourami *Trichogaster leerii* increased significantly with the increase in protein levels in the 13-32% range than in the 32-49% range. *B. splendens* reared on 35% animal or plant protein had the highest feeding rate, growth rate and conversion efficiency and, therefore, this level is considered optimum. The experimental results are in agree-

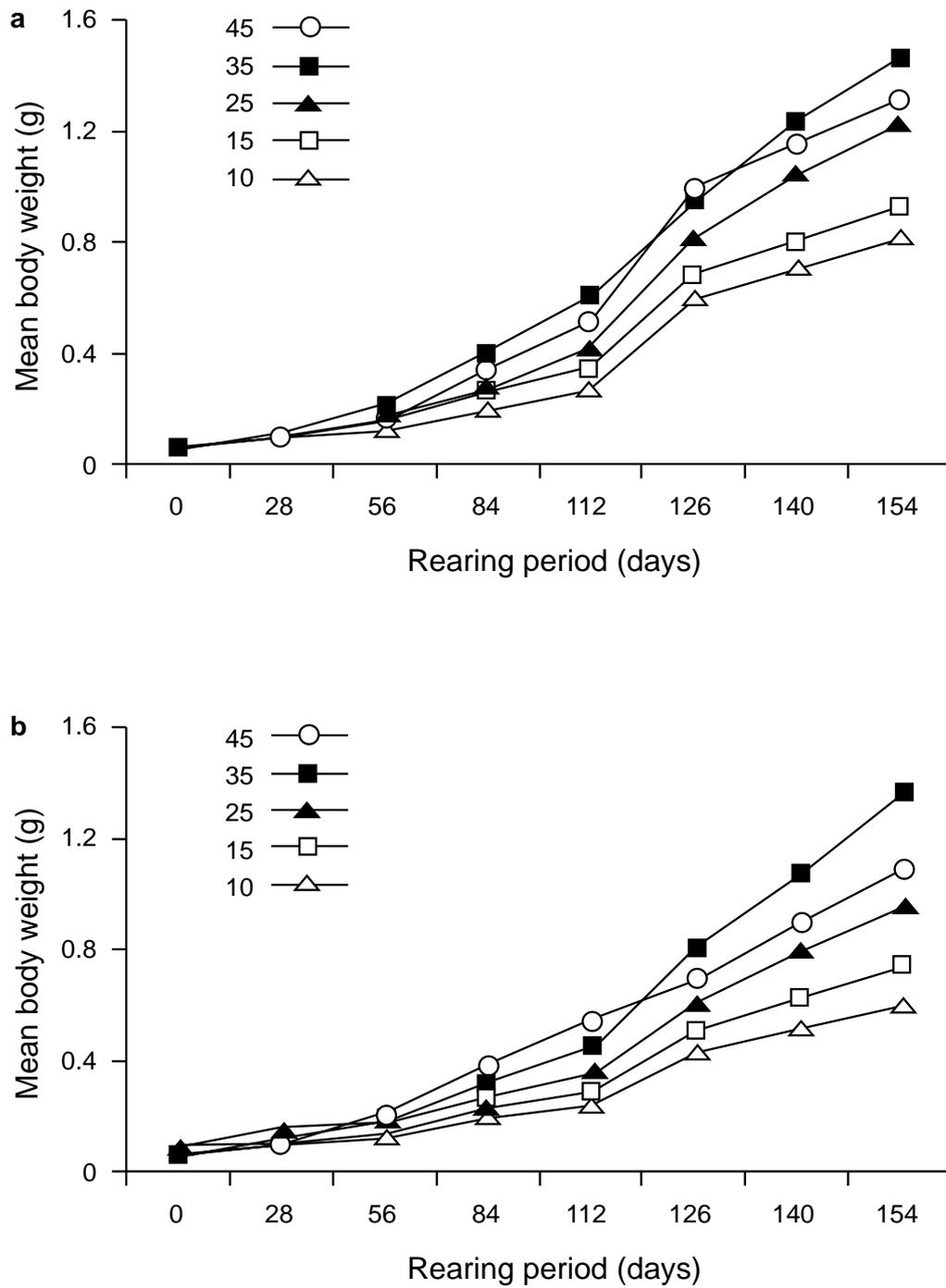


Fig 1. Impact of animal (a) and plant (b) protein diets on mean body weight in *Betta splendens*.

Table 3. Effect of various levels of animal protein on feeding rate (mg/g live fish/day), growth rate (mg/g live fish/day) and feed conversion efficiency (%) in Siamese fighting fish *Betta splendens* reared for 154 days. Values are means ( $\pm$ SD) of three replicates.

Day	Protein level (%)				
	10	15	25	35	45
<i>Feeding rate</i> <sup>1</sup>					
28	19.90 $\pm$ 0.6	20.74 $\pm$ 1.6	23.67 $\pm$ 2.4	28.69 $\pm$ 1.3	25.07 $\pm$ 1.7
56	22.48 $\pm$ 0.8	22.80 $\pm$ 2.3	26.60 $\pm$ 1.8	33.24 $\pm$ 1.8	30.66 $\pm$ 2.3
84	19.94 $\pm$ 0.5	21.17 $\pm$ 1.7	24.77 $\pm$ 2.0	31.83 $\pm$ 0.7	27.70 $\pm$ 2.5
112 <sup>4</sup>	17.43 $\pm$ 1.1	18.57 $\pm$ 0.9	21.46 $\pm$ 1.9	29.13 $\pm$ 1.3	24.01 $\pm$ 1.9
126	23.86 $\pm$ 2.3	26.29 $\pm$ 2.3	27.81 $\pm$ 2.3	28.10 $\pm$ 2.3	26.62 $\pm$ 2.4
140	18.72 $\pm$ 1.5	18.42 $\pm$ 1.3	21.01 $\pm$ 1.7	22.28 $\pm$ 1.6	20.36 $\pm$ 1.7
154	16.21 $\pm$ 1.1	16.23 $\pm$ 1.4	17.69 $\pm$ 1.4	18.03 $\pm$ 1.3	17.30 $\pm$ 1.2
<i>Growth rate</i> <sup>2</sup>					
28	2.50 $\pm$ 0.17	3.56 $\pm$ 0.14	4.43 $\pm$ 0.28	8.13 $\pm$ 0.15	5.33 $\pm$ 0.35
56	3.53 $\pm$ 0.26	4.11 $\pm$ 0.23	5.42 $\pm$ 0.47	7.16 $\pm$ 0.36	6.50 $\pm$ 0.23
84	3.02 $\pm$ 0.14	3.38 $\pm$ 0.35	4.72 $\pm$ 0.34	5.82 $\pm$ 0.22	5.62 $\pm$ 0.67
112 <sup>4</sup>	2.86 $\pm$ 0.33	3.10 $\pm$ 0.17	3.76 $\pm$ 0.19	5.06 $\pm$ 0.18	4.41 $\pm$ 0.28
126	6.75 $\pm$ 0.48	7.60 $\pm$ 0.48	8.42 $\pm$ 0.43	9.72 $\pm$ 0.61	8.19 $\pm$ 0.66
140	3.08 $\pm$ 0.35	2.75 $\pm$ 0.23	4.63 $\pm$ 0.26	5.72 $\pm$ 0.58	2.62 $\pm$ 0.21
154	2.62 $\pm$ 0.17	2.82 $\pm$ 0.33	3.05 $\pm$ 0.18	3.29 $\pm$ 0.44	2.28 $\pm$ 0.11
<i>Feed conversion efficiency</i> <sup>3</sup>					
28	12.56 $\pm$ 1.34	17.41 $\pm$ 1.61	18.72 $\pm$ 0.75	28.35 $\pm$ 0.93	21.28 $\pm$ 1.75
56	15.70 $\pm$ 1.68	18.05 $\pm$ 1.34	20.39 $\pm$ 1.48	21.54 $\pm$ 1.48	21.22 $\pm$ 1.38
84	15.12 $\pm$ 0.91	15.95 $\pm$ 1.26	19.06 $\pm$ 1.69	18.27 $\pm$ 1.67	19.58 $\pm$ 1.27
112 <sup>4</sup>	16.40 $\pm$ 1.53	16.69 $\pm$ 1.35	17.54 $\pm$ 1.30	17.36 $\pm$ 1.07	18.34 $\pm$ 1.18
126	28.28 $\pm$ 2.14	28.89 $\pm$ 2.01	30.28 $\pm$ 2.35	34.58 $\pm$ 2.77	30.47 $\pm$ 2.67
140	16.46 $\pm$ 1.81	14.92 $\pm$ 1.18	22.04 $\pm$ 1.79	23.65 $\pm$ 2.10	12.89 $\pm$ 1.78
154	16.25 $\pm$ 1.73	17.39 $\pm$ 1.36	17.24 $\pm$ 1.44	18.27 $\pm$ 1.56	13.17 $\pm$ 1.25

<sup>1</sup> Feeding rate = feed consumed/(initial wet weight of fish x days)

<sup>2</sup> Growth rate = weight gain/(initial wet weight of fish x days)

<sup>3</sup> Feed conversion efficiency = (weight gain/feed consumed) x 100

<sup>4</sup> Spawning commenced

Table 4. Effect of various levels of plant protein on feeding rate (mg/g live fish/day), growth rate (mg/g live fish/day) and feed conversion efficiency (%) in Siamese fighting fish *Betta splendens* reared for 154 days. Values are means ( $\pm$ SD) of three replicates.

Day	Protein levels (%)				
	10	15	25	35	45
<i>Feeding rate*</i>					
28	10.73 $\pm$ 0.81	12.97 $\pm$ 0.75	14.29 $\pm$ 0.52	19.43 $\pm$ 0.86	17.26 $\pm$ 0.68
56	16.02 $\pm$ 1.12	17.07 $\pm$ 1.36	19.66 $\pm$ 1.58	24.00 $\pm$ 1.42	22.38 $\pm$ 1.72
84	16.72 $\pm$ 1.24	19.00 $\pm$ 1.64	19.10 $\pm$ 1.73	26.15 $\pm$ 1.50	22.44 $\pm$ 1.48
112*	14.42 $\pm$ 0.83	16.31 $\pm$ 1.11	17.08 $\pm$ 1.26	21.42 $\pm$ 1.03	20.68 $\pm$ 1.36
126	25.32 $\pm$ 0.00	26.92 $\pm$ 2.23	28.35 $\pm$ 2.38	29.72 $\pm$ 1.54	28.63 $\pm$ 2.04
140	22.78 $\pm$ 0.00	23.38 $\pm$ 2.50	24.48 $\pm$ 1.66	25.52 $\pm$ 0.87	24.08 $\pm$ 1.79
154	19.63 $\pm$ 0.00	20.09 $\pm$ 1.43	22.11 $\pm$ 1.57	24.12 $\pm$ 1.61	23.73 $\pm$ 2.13
<i>Growth rate*</i>					
28	1.16 $\pm$ 0.02	1.77 $\pm$ 0.13	2.20 $\pm$ 0.16	4.35 $\pm$ 0.18	2.91 $\pm$ 0.23
56	2.39 $\pm$ 0.05	2.94 $\pm$ 0.19	3.36 $\pm$ 0.24	4.93 $\pm$ 0.22	4.50 $\pm$ 0.38
84	2.36 $\pm$ 0.04	3.30 $\pm$ 0.24	3.73 $\pm$ 0.10	4.76 $\pm$ 0.15	4.55 $\pm$ 0.18
112*	2.47 $\pm$ 0.07	2.52 $\pm$ 0.15	2.99 $\pm$ 0.26	3.53 $\pm$ 0.20	3.38 $\pm$ 0.24
126	5.20 $\pm$ 0.13	5.80 $\pm$ 0.34	6.89 $\pm$ 0.47	8.80 $\pm$ 0.56	7.99 $\pm$ 0.37
140	3.42 $\pm$ 0.15	4.76 $\pm$ 0.28	5.18 $\pm$ 0.50	5.93 $\pm$ 0.44	4.94 $\pm$ 0.25
154	2.60 $\pm$ 0.21	3.05 $\pm$ 0.17	3.55 $\pm$ 0.29	4.63 $\pm$ 0.37	3.93 $\pm$ 0.34
<i>Feed conversion efficiency*</i>					
28	10.73 $\pm$ 0.86	13.62 $\pm$ 1.44	15.41 $\pm$ 1.21	22.38 $\pm$ 1.42	16.88 $\pm$ 1.62
56	14.91 $\pm$ 1.26	17.21 $\pm$ 0.81	17.10 $\pm$ 1.36	20.55 $\pm$ 0.74	20.12 $\pm$ 1.48
84	14.09 $\pm$ 1.17	17.33 $\pm$ 1.66	19.51 $\pm$ 0.67	18.24 $\pm$ 1.28	20.28 $\pm$ 2.14
112*	17.06 $\pm$ 1.02	15.47 $\pm$ 1.19	16.13 $\pm$ 1.10	16.49 $\pm$ 1.06	16.34 $\pm$ 1.43
126	20.54 $\pm$ 1.86	21.54 $\pm$ 2.26	24.31 $\pm$ 2.16	29.60 $\pm$ 2.09	27.89 $\pm$ 2.38
140	15.00 $\pm$ 0.75	20.37 $\pm$ 1.74	21.15 $\pm$ 1.73	23.26 $\pm$ 2.14	20.51 $\pm$ 1.29
154	13.19 $\pm$ 1.23	15.71 $\pm$ 1.34	16.05 $\pm$ 1.13	19.18 $\pm$ 1.20	16.55 $\pm$ 1.67

\* See Table 3.

Table 5. Effect of different levels of animal and plant protein on ovary weight and gonadosomatic index\* in *Betta splendens*. Values are means ( $\pm$ SD) of six observations.

Day	Protein level (%)											
	10		15		25		35		45			
	Gonad wt (mg)	GSI (%)	Gonad wt (mg)	GSI (%)	Gonad wt (mg)	GSI (%)	Gonad wt (mg)	GSI (%)	Gonad wt (mg)	GSI (%)	Gonad wt (mg)	GSI (%)
<i>Animal protein diet</i>												
56	-	-	-	-	2.86	1.23	7.01 $\pm$ 0.01	2.22 $\pm$ 0.04	3.96 $\pm$ 0.07	1.35 $\pm$ 0.03	-	-
84	1.50 $\pm$ 0.01	0.61 $\pm$ 0.01	4.00 $\pm$ 0.03	1.34 $\pm$ 0.10	12.50 $\pm$ 0.14	3.81 $\pm$ 0.27	23.00 $\pm$ 0.15	5.72 $\pm$ 0.04	17.00 $\pm$ 0.13	4.69 $\pm$ 0.04	-	-
112	7.00 $\pm$ 0.60	2.20 $\pm$ 0.02	14.00 $\pm$ 1.09	4.04 $\pm$ 0.12	27.00 $\pm$ 2.23	5.89 $\pm$ 0.18	87.00 $\pm$ 5.31	17.02 $\pm$ 1.02	38.50 $\pm$ 2.84	8.22 $\pm$ 0.38	-	-
<i>Plant protein diet</i>												
56	-	-	-	-	-	-	-	-	-	-	-	-
84	1.00 $\pm$ 0.04	0.48 $\pm$ 0.00	4.00 $\pm$ 0.24	1.63 $\pm$ 0.23	7.00 $\pm$ 0.34	2.43 $\pm$ 0.04	16.00 $\pm$ 1.04	4.45 $\pm$ 0.10	12.50 $\pm$ 0.88	3.69 $\pm$ 0.26	-	-
112	3.50 $\pm$ 0.12	1.50 $\pm$ 0.03	9.50 $\pm$ 0.31	3.08 $\pm$ 0.05	19.50 $\pm$ 1.26	3.77 $\pm$ 0.76	48.50 $\pm$ 2.87	11.47 $\pm$ 0.13	29.00 $\pm$ 2.16	6.94 $\pm$ 0.48	-	-

\* Gonadosomatic index = (weight of gonad/weight of fish) x 100

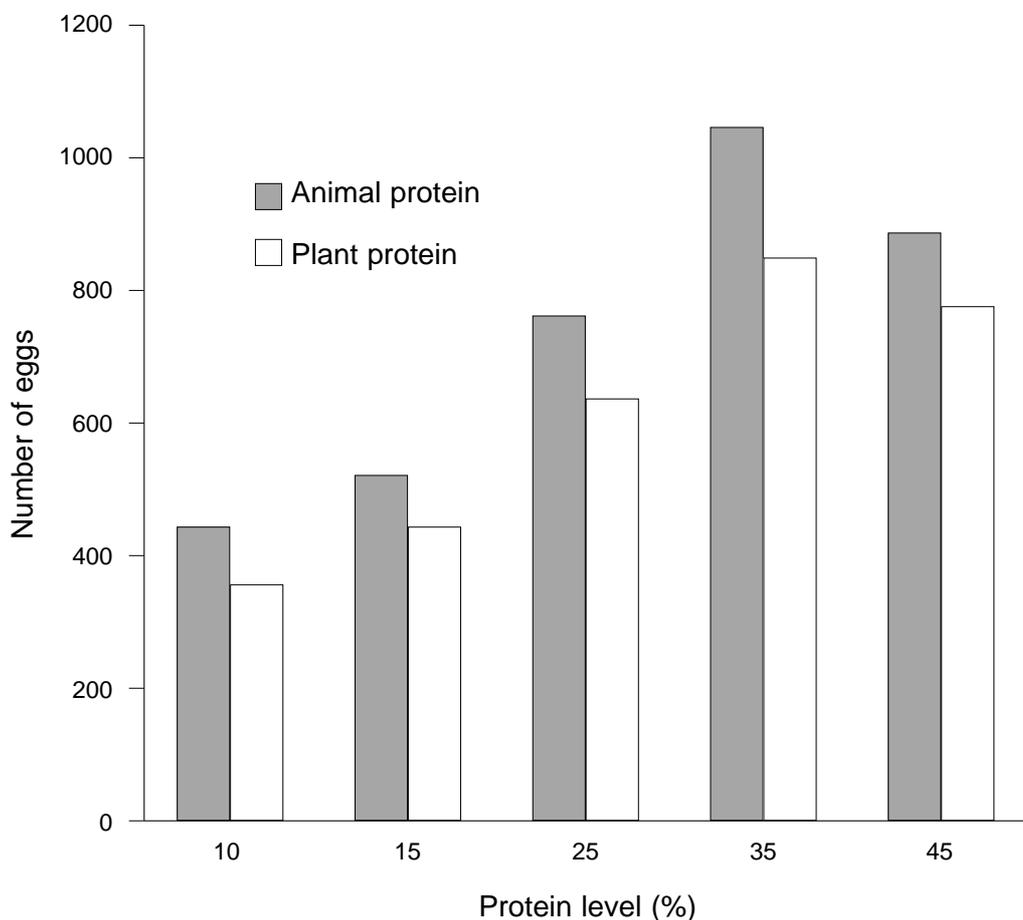


Fig. 2. Impact of animal and plant protein diets on number of eggs laid per female (total of three spawnings).

ment with those of Shim et al. (1989) who reported that 35% protein was the optimum diet, resulting in the highest increase in mean body weight and length in dwarf gourami *Colisa lalia*. *B. splendens* appears to have similar protein requirements as the pearl gourami *T. leerii* (Degani and Gur, 1992), carp (Santiago and Reyes, 1991) and catfish (Chuapoehek, 1987), all of which require 32-36% dietary protein for maximum growth. The low protein levels of 10-25% produced the lowest growth rate of *B. splendens* and this agrees with the findings of Shim et al. (1989)

in gourami and Degani and Gur (1992) in pearl gourami.

A sufficient intake of energy and protein are essential for maximum growth (Lee and Putman, 1973; Jauncey, 1982). It seems that protein retention was higher in fish that consumed high protein diets, suggesting that a portion of the digested protein was used as energy for maintenance and the remaining energy was diverted for somatic or gonad tissue growth. In fish that consumed a low protein diet, the protein retention was lower because a major amount of the consumed

Table 6. Impact of different levels of animal and plant protein on fecundity and hatching rate in *Betta splendens*. Values are means ( $\pm$ SD) of six observations.

Day	Protein level (%)														
	10			15			25			35			45		
Spawn no.	No. eggs spawned	Hatching rate (%)	No. eggs spawned	Hatching rate (%)	No. eggs spawned	Hatching rate (%)	No. eggs spawned	Hatching rate (%)	No. eggs spawned	Hatching rate (%)	No. eggs spawned	Hatching rate (%)	No. eggs spawned	Hatching rate (%)	
<i>Animal protein diet</i>															
I	117 $\pm$ 12.6	84.5 $\pm$ 6.3	72.5 $\pm$ 4.2	139 $\pm$ 17	107 $\pm$ 12	77.3 $\pm$ 4.8	214 $\pm$ 18	179 $\pm$ 12	83.6 $\pm$ 3.8	297 $\pm$ 15	269 $\pm$ 14	90.6 $\pm$ 3.4	269 $\pm$ 20	242 $\pm$ 16	90.0 $\pm$ 5.8
II	139 $\pm$ 15.8	115 $\pm$ 8.9	82.4 $\pm$ 6.5	161 $\pm$ 15	139 $\pm$ 16	86.3 $\pm$ 7.3	234 $\pm$ 23	222 $\pm$ 10	94.7 $\pm$ 5.8	333 $\pm$ 28	322 $\pm$ 26	96.7 $\pm$ 5.2	305 $\pm$ 18	282 $\pm$ 19	92.6 $\pm$ 4.3
III	179 $\pm$ 19.7	160 $\pm$ 15.6	89.4 $\pm$ 5.2	215 $\pm$ 20	193 $\pm$ 18	90.0 $\pm$ 6.5	310 $\pm$ 28	285 $\pm$ 12	92.1 $\pm$ 6.7	414 $\pm$ 24	407 $\pm$ 19	98.3 $\pm$ 5.9	372 $\pm$ 24	360 $\pm$ 26	96.9 $\pm$ 3.6
Total	435 $\pm$ 16.0			515 $\pm$ 17.3			758 $\pm$ 33.0			1044 $\pm$ 32.8			884 $\pm$ 30.6		
<i>Plant protein diet</i>															
I	91.5 $\pm$ 6.8	55.5 $\pm$ 4.5	60.7 $\pm$ 4.1	127 $\pm$ 10.3	92 $\pm$ 6.6	72.4 $\pm$ 5.8	187 $\pm$ 12.8	148 $\pm$ 8.8	79.1 $\pm$ 5.2	241 $\pm$ 12.4	219 $\pm$ 9.7	91.1 $\pm$ 3.9	228 $\pm$ 18.4	195 $\pm$ 13.6	85.5 $\pm$ 5.6
II	115 $\pm$ 12.4	70.5 $\pm$ 8.0	61.3 $\pm$ 6.4	141 $\pm$ 12.4	115 $\pm$ 9.3	81.2 $\pm$ 6.9	195 $\pm$ 16.5	161 $\pm$ 13.7	82.3 $\pm$ 6.7	264 $\pm$ 15.8	225 $\pm$ 12.3	85.4 $\pm$ 6.7	239 $\pm$ 21.3	217 $\pm$ 18.4	90.8 $\pm$ 6.2
III	145 $\pm$ 15.6	110 $\pm$ 9.6	76.1 $\pm$ 5.9	174 $\pm$ 11.8	158 $\pm$ 10.4	91.1 $\pm$ 9.5	252 $\pm$ 22.3	224 $\pm$ 16.7	88.9 $\pm$ 7.3	341 $\pm$ 20.6	322 $\pm$ 18.3	94.6 $\pm$ 7.3	306 $\pm$ 24.3	272 $\pm$ 9.8	88.7 $\pm$ 4.7
Total	352 $\pm$ 18.0			442 $\pm$ 11.3			634 $\pm$ 17.8			846 $\pm$ 16.6			773 $\pm$ 22.0		

protein was used for maintenance and a meager amount was left for conversion to flesh or gonad tissue, resulting in a lower growth rate. This was confirmed by the growth rates and ovary weights. Protein beyond the optimum level produced a lower growth rate in *B. splendens*. The lower growth rate obtained in fish fed the 45% protein diets may be due to increased energy expenditure on protein catabolism and increased production of ammonia. Such an effect of varying dietary protein levels on the rate of growth is similar to that reported for the grass carp (Dabrowski, 1977) and tilapia (Jauncey, 1982).

The female *B. splendens* fed the 35% protein diets had a greater gonad weight and GSI than the females fed the 10% protein diets. This finding is similar to those of Santiago et al. (1985) on *Oreochromis niloticus* and Shim et al. (1989) on *C. lalia*. The present investigation reveals that there is an optimal dietary protein level (35%) for the maximum development of the ovary and fecundity for *B. splendens* and too high (45%) or too low (10%) a protein level is less favorable for reproduction.

Many factors influence fecundity in female fish. Among them, size, age, physical condition, reproductive history and nutritional state are important (Woodhead, 1960). Low dietary protein in *B. splendens* reduced egg production, however, egg hatchability was not affected much. A similar observation was made in the rainbow trout (Washburn et al., 1990). Maximum growth, egg production and hatchability were observed in fish fed 35% animal and plant protein diets. Hence, 35% protein is considered the optimum level for *B. splendens*. If female fish receive sufficient protein in their diet, they can continuously produce more viable eggs and larvae and this was evidently confirmed in the present study. Similar findings on the effect of dietary protein on fish oocytes were observed in the gourami (Shim et al., 1989) and the red sea bream (Watanabe et al., 1984).

The present results showed that the experimental fish fed animal protein had significantly better growth and fecundity than those fed plant protein. This might be due to

the presence of appetite stimulators such as fish and squid meals in the animal protein diets. The lower growth indices and fecundity in the fish fed plant protein can probably be attributed to amino acid imbalance, especially methionine and lysine deficiencies and/or the presence of anti-nutritional factors (Wee, 1991). Degani et al. (1989) found that the African catfish *Clarias gariepinus* grew better on a diet composed of fishmeal than on one containing soybean meal. Haiqing and Xiqin (1994) reported that the specific growth rate increased with a diet containing more fishmeal than plant protein sources in the bream *Megalobrama skolkovii*. Research work on the energy balance of *Macropodus cupanus* (Mathavan and Christopher, 1980) and *Labeo rohita* (Haniffa et al., 1987) fed a combination of algal and animal food revealed that the feeding rate was highest in fish fed an exclusively animal feed and declined steadily as the proportion of plant feed increased.

#### References

- Black E.C., Bosomworth N.J. and G.E. Docherty**, 1966. Combined effect of starvation and severe exercise of glycogen metabolism of rainbow trout *Salmo gairdneri*. *J. Fish. Res. Bd. Can.*, 23:1461-1463.
- Bragdon J.H.**, 1951. Colorimetric determination of blood lipids. *J. Biol. Chem.*, 190:513.
- Catacutan M.R. and R.M. Coloso**, 1995. Effect of dietary protein to energy ratios on growth, survival and body composition of juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, 131:125-133.
- Chuapoehuk W.**, 1987. Protein requirements of walking catfish *Clarias batrachus* (Linnaeus) fry. *Aquaculture*, 63:215-219.
- Dabrowski K.**, 1977. Protein requirements of grass carp fry (*Ctenopharyngodon idella*). *Aquaculture*, 12:63-73.
- Dahlgren B.T.**, 1979. The effects of population density on fecundity and fertility in the guppy, *Poecilia reticulata* (Peters). *J. Fish Biol.*, 15:71-91.
- Degani G. and N. Gur**, 1992. Growth of juvenile *Trichogaster leerii* (Bleeker, 1852) on diets with various protein levels. *Aquacult. Fish. Manag.*, 23:161-166.

- Degani G., Horowitz A. and D. Levanon,** 1985. Effects of protein level, impurities, diet, density and ammonia and the level on growth of European eel *Anguilla anguilla* (L). *Aquaculture*, 46:193-200.
- Degani G., Viola S. and D. Levanon,** 1986. Effect of dietary carbohydrate source on growth and body composition of European eel *Anguilla anguilla* (L). *Aquaculture*, 52:97-107.
- Degani G., Zvi Y.B. and D. Levanon,** 1989. The effect of different protein levels and temperatures on feed utilization, growth and body composition of *Clarias gariepinus* (Burchell 1822). *Aquaculture*, 76:293-30.
- Haiqing S. and H. Xiqin,** 1994. Effects of dietary animal and plant protein ratios and energy levels on growth and body composition of bream (*Megalobrama skolkovii*) fingerlings. *Aquaculture*, 127:189-196.
- Haniffa M.A., Murugesan A.G. and A.T. Fleming,** 1987. Influence of plant and animal food on the food utilization of the freshwater carp *Labeo rohita* (Ham.) *Curr. Sci.*, 56:846-848.
- Hardy R.,** 1980. Fish feed formulation. pp. 233-239. In: *Fish Feed Technology*. ADCP/REP/80/11, FAO, UN, Rome.
- Hashim R.,** 1994. The effect of mixed feeding schedules of varying dietary protein content on the growth performance of *Channa striatus* fry. *Asian Fish. Sci.*, 7:149-155.
- Hashim R., Ahyaudin A. and N.A.M. Saat,** 1992. Improvement of growth and feed conversion of hybrid catfish (*Clarias gariepinus* x *C. macrocephalus*) fry fed with diets supplemented with live *Tubifex*. *J. Aqua. Trop.*, 7:239-248.
- James R., Muthukrishnan J. and K. Sampath,** 1993. Effects of food quality on temporal and energetics cost of feeding in *Cyprinus carpio* (Pisces: Cyprinidae). *J. Aquat. Trop.*, 8:47-53.
- Jauncey K.,** 1982. The effects of varying dietary protein levels on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture*, 27:43-54.
- Lee D.J. and C.B. Putman,** 1973. The response of rainbow trout to varying protein/energy ratios in test diet. *J. Nutr.*, 103:916-922.
- Lowry O.H., Rosebrough N.J., Farr A.L. and R.J. Randall,** 1951. Protein measurements with folin-phenol reagent. *J. Biol. Chem.*, 193:265-275.
- Mathavan S. and M.S.M. Christopher,** 1980. Studies on food utilization in *Macropodus cupanus*. *Matsya*, 6:23-29.
- Maynard A.L. and K.S. Loosli,** 1962. *Animal Nutrition*. McGraw Hill, New York. 533 pp.
- McFadden J.T., Cooper E.L. and J.K. Anderson,** 1965. Some effect of environment on egg production in brown trout (*Salmo trutta*). *Limnol. Oceanogr.*, 10:88-95.
- Paine R.T.,** 1964. Ash and caloric determinations of sponge and ophisthobranchs tissues. *Ecology*, 45:384-387.
- Santiago C.B. and O.S. Reyes,** 1991. Optimum dietary protein level for growth of bighead carp (*Aristichthys nobilis*) fry in a static water system. *Aquaculture*, 93:155-165.
- Santiago C.B., Aldaba M.B., Abuan E.F. and M.A. Laron,** 1985. The effects of artificial diets on fry production and growth of *Oreochromis niloticus* breeders. *Aquaculture*, 47:193-203.
- Shim K.F., Landesman L. and T.J. Lam,** 1989. Effect of dietary protein on growth, ovarian development and fecundity in the dwarf gourami *Colisa lalia* (Hamilton). *J. Aquat. Trop.*, 4:111-123.
- Sumagaysay N.S. and I.G. Borlongan,** 1995. Growth and production of milkfish (*Chanos chanos*) in brackishwater ponds: Effects of dietary protein and feeding levels. *Aquaculture*, 132:273-283.
- Tacon A.G.J., Stafford E.A. and C.A. Edwards,** 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. *Aquaculture*, 35:187-199.
- Washburn B.S., Frye D.J., Hung S.S.O., Doroshov F.S. and F.S. Conte,** 1990. Density effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 90:179-195.
- Watanabe T., Arakawa T., Kitajima C. and S. Fujita,** 1984. Effects of nutritional quality of brood stock diets on reproduction of red sea bream. *Bull. Jpn. Soc. Sci. Fish.*, 50:495-501.

**Wee K.L.**, 1991. Use of non-conventional feed stuff of plant origin as fish feeds - is it practical and economically feasible? pp.13-32. In: S.S. De Silva (ed.). *Fish Nutrition Research in Asia, Proc. 4<sup>th</sup> Asian Fish Nutrition Workshop*. Spec. publ. no. 5. Asian Fish. Soc., Manila, Philippines,

**Woodhead A.D.**, 1960. Nutrition and reproductive capacity in fish. *Symp. Proc. Zool. Soc. London*, 19:23-28.

**Zar J.M.**, 1974. *Biostatistical Analysis*. Prentice Hall, New Jersey. 260 pp.