

Golden apple snail, *Pomacea canaliculata* meal as protein source for rabbitfish, *Siganus guttatus* culture

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Abstract. The golden apple snail (GAS) was introduced in the Philippines in 1980s as food for human and farm animals and later turned into a destructive invasive species especially in rice fields. The feasibility of the golden apple snail as an alternative protein source has been tested for tilapia, shrimp and prawn. This study determined the growth performance of rabbitfish, *Siganus guttatus* using GAS meal as protein source in terms of growth parameters, survival rates (SR), feeding efficiency and economic viability of formulated feeds used. The experiment consisted of four treatments (T₁. control diet; T₂. test diet with 15% CP; T₃. test diet with 30% CP; T₄. test diet with 45% CP) that was replicated three times over a period of 120 days. Each cage was stocked with 60 pcs fry and set up in a randomized completely block design. This study revealed that utilization of GAS meal with 45% crude protein (CP) as protein source for *S. guttatus* can replace or substitute fish meal for more than two months at a cheaper cost. For better results of formulated feeds, pre-mixed vitamins and minerals must be added.

Key Words: golden apple snail meal, alternative protein source, *Siganus guttatus* culture, feeds, economic viability, growth performance, Romblon, Philippines.

Introduction. Rabbitfish *Siganus guttatus* is considered as good species for mariculture in view of its desirable production traits, high demand and increasing value (Saoud et al 2008; Tabugo et al 2012). *Siganus guttatus* is one of the species that can attain large size, withstand overcrowding and tolerate low dissolved oxygen (DO) levels (Carumbana & Luchavez 1979; Ayson et al 2014).

Improvement in feeds, feeding strategies and clear understanding of nutritional requirements can help improve productivity and profit (Allan 2004). At present, feed is the largest single costly item, as it constitutes more than 50% of the operational cost in production (El-Sayed 2004). It is critical that feeds should be economically-and environmentally-sustainable. Among feed ingredients, fish meal is the major contributor to feed cost. With the higher feed demand in aquaculture, poultry, and livestock, the supply of fish meal is expected to decline due to shortage of world production (El-Sayed 2004). This has stimulated the evaluation of a variety of alternative dietary protein sources for partial or total replacement of fish meal protein in aquaculture feeds (Felix Brindo 2008).

Replacement of fish meal in practical diets without reducing the performance would result in more profitable production. Therefore, this aspect should be studied further to expand the list of appropriate plant and animal protein sources to replace fish meal. One of the alternative animal protein sources that can replace fish meal is golden apple snail, *Pomacea canaliculata* (Jintasatporn et al 2004).

The golden apple snail (GAS), locally known as "golden kuhol" is a freshwater gastropod native to South America. It was introduced in the Philippines between 1982 and 1984 (Philippine Rice Research Institute 2001). A few years after its introduction, GAS became a major pest in all rice ecosystems of the Philippines including the UNESCO Heritage Site of Ifugao Rice Terraces (Dancel & Joshi 2000). Nevertheless, GAS is a good

source of protein for human (Rejesus et al 1988) and animal diets because it contains 54% (Bombero-Tuburan et al 1995; Jintasataporn et al 2004) to 62% protein (Serra 1997; University of Agriculture and Forestry Laboratory 2004) on a dry weight basis. Additionally, GAS meal is deemed cheaper than fish meal (Hertrampf & Piedad-Pascual 2000). Few studies have been conducted utilizing GAS as feed ingredient for aquaculture (El-Sayed 2004; Jintasataporn et al 2004) and in poultry and livestock production (Serra 1997).

This study was conducted to evaluate the performance of GAS as protein source for rabbitfish, *S. guttatus* to reduce or replace fish meal as protein component of formulated feed.

Material and Method

Feed formulation and analysis. GAS was used as protein source of the formulated feeds. Matured GAS characterized by tight brown shell and creamy white to pinkish or orange flesh were collected from a rice field in Pandan, Santa Fe, Romblon (Figure 1). The materials were washed, boiled, cooked, removed from the shells, dried, ground, and passed through 1 mm x 1 mm fine mesh net.

The ingredients used in the study were readily available in the locality. Formulation of feeds were conducted at Romblon State University, College of Fisheries (RSU-CF) laboratory and was adapted from the process used by Allan (2004) wherein bulk ingredients were mixed together while the binder was cooked. The diets were formulated using varying proportions of corn meal, rice bran and protein from GAS. A uniform amount of bread flour, and olive oil were used in all three diets (see Table 1). No premix vitamins and minerals were added due to non-availability of such ingredients. Representative samples of the three test formulated diets weighing 50 g were brought to the SEAFDEC-AQD Laboratory for complete proximate analysis (moisture, crude protein, crude fat, crude fiber and ash) to determine the nutrient contents of the feeds using standard method of AOAC (2000) and done in duplicate. Nitrogen-free extract was determined by subtraction. The estimated energy was computed based on standard physiological fuel values of 9 kcal g⁻¹ lipid and 4 kcal g⁻¹ protein and carbohydrate (DOST-FNRI 1997). No proximate analysis was conducted on the raw materials because of the existence of established data (Hertrampf & Piedad-Pascual 2000; Millamena et al 2002).

Table 1
Composition of formulated diets using GAS meal at different levels of protein for *S. guttatus* using trial error method and proximate analysis of experimental diets (% of dry matter)

Ingredients (g/100g)	Control*	Percentage of GAS meal as protein source for <i>S. guttatus</i>		
		15%	30%	45%
GAS meal	-	15	47	75
Corn meal	-	38	22	5
Rice bran	-	30	14	3
Bread flour	-	15	15	15
Olive oil	-	2	2	2
Total	-	100	100	100

*The composition of control feed (commercial feed) is not available for public.

Experimental site. The feeding trial was conducted in the RSU-CF Aquazest Project located at Pandan, Santa Fe, Romblon, Philippines in 12°9'32.15" N and 121°59'17.40" E (Figure 1). The site is about one kilometer away from Santa Fe. The area was used from time to time for mariculture projects of the university wherein different species of fish were experimentally reared. The water depth of the cultured area was 2.2 meters during low tide.

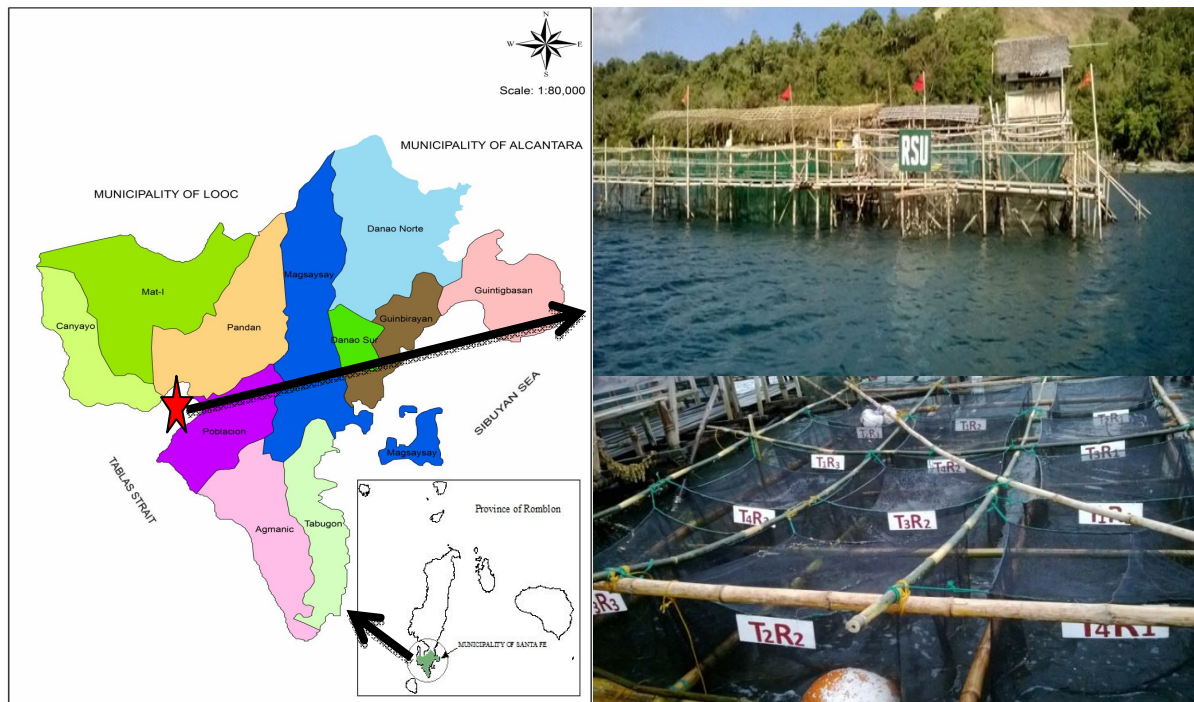


Figure 1. Location of the grow-out culture at Santa Fe, Romblon, Philippines.

Experimental fish and grow-out culture. A three-day old *S. guttatus* fry were purchased from Southeast Asian Fisheries Development Centre-Aquaculture Department, Tigbauan, Iloilo. The fish were acclimatized and conditioned for one month before stocking. Fingerlings that weighed 2.1 g in average body weight (BW) and measured 3.1 cm in average total body length (BL) were used. The feeds were given thrice a day at 06:00 h, 12:00 h, and 16:30 h. The feeding rate ranged from 3-10% of the body weight. Sampling of the stock and inspection of net cages were done every 15 days. Daily monitoring of water parameters was done. Water pH was monitored using a Mettler Toledo pH meter; salinity was determined using an Atago refractometer; temperature and DO were read using a Lutron, YK22DO digital DO meter-combined. The study was conducted for four months, from July to October 2015.

Experimental setup. A floating cage composed of 12 bamboo compartments (with dimensions of 1 m x 1 m x 1.5 m) was constructed using polyethylene material no. 17 (mesh size 1 mm) and polyethylene rope (no. 8). The experimental set-up was arranged through Randomized Completely Block Design (RCBD) (Figure 2). Each treatment was stocked with 60 fingerlings of *S. guttatus*. There were the following variants:

- treatment I (T_1) - control (commercial feeds);
- treatment II (T_2) - formulated feeds (protein concentrate, PC-15% dry matter);
- treatment III (T_3) - formulated feeds (PC-30% dry matter);
- treatment IV (T_4) - formulated feeds (PC-45% dry matter).

Statistical analysis. Data were treated using Analysis of Variance (ANOVA) to detect the statistical differences among treatments means following the procedures of Gomez & Gomez (1984). When there were significant differences detected at 5% and 1% levels, the data were analyzed further for Least Significance Different Test to determine the prescribed level of significance between any pair of treatment means (Gomez & Gomez 1984).

T ₂ R ₁	T ₃ R ₁	T ₁ R ₁	T ₄ R ₁
T ₁ R ₂	T ₄ R ₂	T ₃ R ₂	T ₂ R ₂
T ₂ R ₃	T ₁ R ₃	T ₄ R ₃	T ₃ R ₃

Figure 2. Randomization of the experimental set-up using a table of random numbers. This was carried out for each block. The object of blocking was to have units in a block as uniform as possible so that the observed differences will be largely due to treatments.

Results

Nutritional quality of diets and economic viability. Three diets containing different levels of protein (15%, 30% and 45% CP) were formulated using trial and error method. The computed crude protein of test diets using trial and error method was approximately the same with results of proximate analysis except for T₄. The formulated diets containing different levels of protein were priced lower compared with control diet or T₁ (commercial feed). The prices were: T₂ with a computed price of Php 19.80/kg, followed by T₃ at Php 23.00/kg; T₄ priced at Php 26.40/kg and T₁ worth Php 35.00/kg, respectively. Therefore, as the level of protein increases, the price of the diet also increases in the case of the three formulated diets (Table 2).

Table 2
Proximate analysis of experimental diets (% of dry matter) and economic viability

Proximate analysis	Formulated diets			
	T ₁ *	T ₂	T ₃	T ₄
Crude protein (%)	28-31	14.55	30.60	42.02
Crude fat (%)	6-9	18.03	11.86	12.74
Crude fiber (%)	6-7	4.42	2.69	0.89
Ash (%)	16	4.92	8.78	11.24
Nitrogen-free extract (%)	24-30	58.09	46.00	33.12
Moisture (%)	13	6.63	7.85	7.95
Estimated energy (kcal kg ⁻¹) ²		357.64	351.04	349.09
Total feed given (Kg)	19.20	10.58	13.48	16.12
Price/Kg (Php)	35.00**	19.80	23.00	26.40
Total price	671.83	209.52	310.13	425.62

*The proximate analysis of the control diet is based on the content printed on the sack;

**The price of the commercial feed is based on the retail price in the market.

Growth performance, feed efficiency and survival rates. For the four-month culture period, T₁ had the final average body weight (BW±SE) of 81.25±2.79 g compared to 61.52±1.74 g (T₄), 49.49±1.13 g (T₃), and 38.12±1.86 g (T₂), respectively (Table 3). Analysis of variance (ANOVA) showed that there were significant differences ($p < 0.05$) obtained as early as 15th day of culture; this finding was consistently obtained from the 45th day until harvest. Results of Least Significant Difference (LSD) Test showed that T₄ was significantly different ($p < 0.05$) from T₁ and T₂ on 15th day; while, T₁ was observed to be significantly different from T₂ and T₃ on the 45th day and onward. T₁ and T₄ were found significantly different on the 75th, 90th, 105th, and 120th days (Table 3).

Table 3

Growth performance (body weight - BW, and body length - BL) of *S. guttatus* fed with GAS at varying levels of protein

Treatment	Average body weight ($g \pm SE$) and total body length ($cm \pm SE$) at days of culture (DOC)									
	Initial	15	30	45	60	75	90	105	120	
1	BW	2.1	3.78±0.24 ^c	5.89±0.74 ^{ns}	8.61±0.56 ^a	13.47±1.08 ^a	26.93±2.17 ^a	41.63±2.50 ^a	56.82±3.04 ^a	81.25±2.79 ^a
	BL	3.1	4.91±0.11 ^{ns}	6.09±0.22 ^{ns}	7.06±0.31 ^a	8.13±0.28 ^a	9.33±0.17 ^a	11.50±0.32 ^a	12.10±0.06 ^a	13.73±0.15 ^{ns}
2	BW	2.1	3.83±0.17 ^{bc}	4.06±0.11 ^{ns}	6.11±0.28 ^c	8.26±0.38 ^c	14.04±0.65 ^{bc}	19.65±0.91 ^d	26.66±1.24 ^c	38.12±1.86 ^c
	BL	3.1	5.12±0.11 ^{ns}	5.34±0.18 ^{ns}	6.10±0.06 ^c	6.90±0.32 ^c	7.70±0.15 ^d	8.37±0.12 ^c	9.37±0.07 ^c	10.13±0.15 ^{ns}
3	BW	2.1	4.17±0.0 ^{abc}	4.83±0.28 ^{ns}	7.22±0.23 ^{bc}	10.06±0.19 ^{bc}	17.97±0.51 ^b	26.96±0.76 ^c	35.67±0.81 ^{bc}	49.49±1.13 ^{bc}
	BL	3.1	5.02±0.07 ^{ns}	5.62±0.1 ^{ns}	6.33±0.08 ^{bc}	7.41±0.04 ^{bc}	8.53±0.24 ^c	9.73±0.15 ^b	10.97±0.03 ^b	12.47±0.18 ^{ns}
4	BW	2.1	4.44±0.28 ^a	5.22±0.39 ^{ns}	7.61±0.11 ^{ab}	12.03±1.0 ^{ab}	22.85±1.89 ^{ab}	34.28±2.84 ^b	44.56±3.69 ^b	61.52±1.74 ^b
	BL	3.1	5.23±0.21 ^{ns}	5.98±0.1 ^{ns}	6.64±0.07 ^{abc}	7.62±0.04 ^{ab}	8.83±0.13 ^b	9.93±0.19 ^b	11.33±0.24 ^b	12.83±0.2 ^{ns}

Means with different superscripts (read by column) show that there were highly significant statistical differences after analysis of variance (ANOVA) and Least Significant Difference (LSD) Test.

In the test diets, the fish in T₄ grew significantly faster than those in T₂ on the 15th to 120th days but only on the 90th day statistical difference was detected in T₃. The total body length (BL) as a growth indicator was highest in T₁ (13.73±0.15 cm), followed by T₄ (12.83±0.2 cm), T₃ (12.47±0.18), and T₂ (10.13±0.15), respectively (Table 3). Results showed that there were significant differences (p < 0.05) in treatment means from 45 to 60 days; and highly significant differences (p < 0.01) on the 75th to 105 days but no statistical difference detected on the 15th, 30th, and 120th days. Results further showed that T₁ was found to be significantly different from T₂ and T₃ on the 45th, 60th, 75th, 90th, and 105th days. A very close look at values per se showed that T₄ had significant increase over T₂ from the 60th to the 105th days, but had no statistical difference over T₃.

After 120 days of culture (DOC), the daily weight gain (DWG) ranged from 0.29±0.02 to 0.67±0.02 g, while specific growth rate (SGR) was better in T₁ (3.05±0.03) compared to T₄ (2.81±0.02), T₃ (2.63±0.02), and T₂ (2.42±0.04), respectively. Analysis on DWG and SGR revealed that T₁ was significantly differed among other treatments. However, protein efficiency ratio (PER) was observed significantly higher in T₂ (3.88±0.17) compared to all treatments. On the other hand, feed conversion ratio (FCR) was obtained better in T₁ (1.36±0.02), followed by T₄ (1.55±0.03), T₃ (1.63±0.04), and T₂ (1.72±0.04), respectively. Values showed that significant difference found in T₁ from other treatments.

The highest survival rates (SR) after 120 days were seen in the T₁ (99.44±0.56), followed by the diets T₃ and T₄ (97.22±0.45, 97.22±1.11), and the T₂ diet (95.00±0.96). These values were no significant statistical differences (p > 0.05) among each other (Table 4).

Table 4

Growth increment, feeding efficiency and survival of *S. guttatus* fed with GAS at varying levels of protein

Parameters	T1	T2	T3	T4
DWG	0.67±0.02 ^a	0.29±0.02 ^d	0.40±0.01 ^c	0.50±0.01 ^b
SGR	3.05±0.03 ^a	2.42±0.04 ^d	2.63±0.02 ^c	2.81±0.02 ^b
PER	2.38±0.09 ^b	3.88±0.17 ^a	2.05±0.04 ^b	1.43±0.05 ^c
FCR	1.36±0.02 ^a	1.72±0.04 ^{bc}	1.63±0.04 ^b	1.55±0.03 ^b
SR	99.44±0.56 ^{ns}	95.00±0.96 ^{ns}	97.22±0.45 ^{ns}	97.22±1.11 ^{ns}

Means with different superscripts show that there were highly significant statistical differences after ANOVA and LSD Test.

Water quality monitoring. Results of daily monitoring of water parameters were plotted and presented in Figure 3. The salinity ranged from 19.48 to 33.95‰ (average/week). Highest salinity of 40‰ was recorded on the first week of July; lowest salinity of 5‰ was noted during first week of September. Among the water parameters, salinity was observed most affected by the rainy season (June-October). Water temperature ranged from 28.50 to 30.59°C during the culture. The DO of the water ranged between 5.37 and 6.43 mg L⁻¹; while water pH ranged from 6.78 to 9.78. The water depth in the site ranged from 2 m (low tide) to 4 m (high tide).

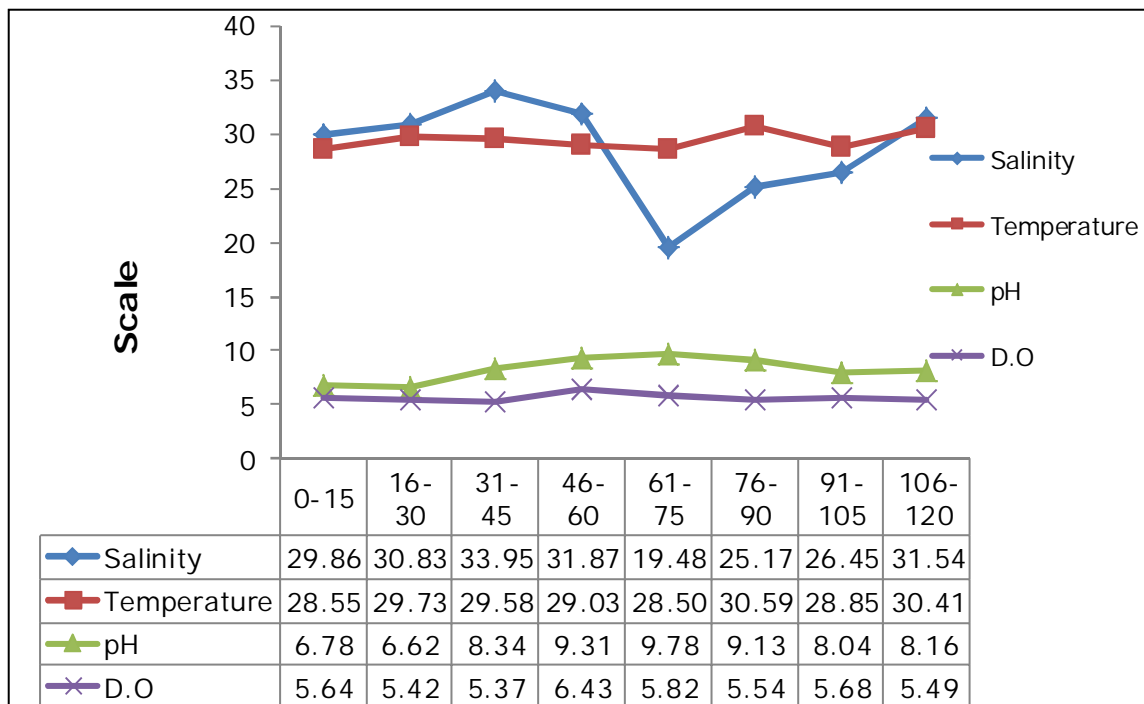


Figure 3. Results of water quality monitoring for 120-day culture period.

Discussion. GAS meal can be a good alternative source of protein for siganid due to higher crude protein which was comparable to fish meal (Bombero-Tuburan et al 1994; Jintasataporn et al 2004) and the price of GAS meal was relatively cheaper than fish meal (Hertrampf and Piedad-Pascual 2000). Thus, formulated feed with GAS meal was priced lower compared to commercial feed.

The growth of *S. guttatus* fed with different levels of GAS meal was lower than commercial feed (38.12 to 61.52 g and 81.25 g, respectively) but there is no significant difference observed in the first 75 days of culture period. This indicates that utilization of GAS meal as protein source can substitute for or replace fish meal for two and a half months. Jintasataporn et al (2004) found out that GAS can totally substitute for fish meal in the diet of the giant freshwater prawn, *Macrobrachium rosenbergii* but not for more than two months. GAS could be a better alternative protein source for fish but not for crustaceans.

In addition, study of Bombero-Tuburan et al (1995) on the use of snail meal as substitute for fishmeal in tiger shrimp, *Penaeus monodon* showed that growth performance, feeding efficiency and higher production was attained from combination of snail meal and maize or cassava compared to maize and golden snail alone. While, Cagauan & Doria (1989) observed that Nile tilapia, *Oreochromis niloticus* fingerlings fed 100% golden snail meal showed a higher growth rate of about 0.84 g/week.

Other researchers obtained different growth patterns in different culture variations. In the present study, the BW gains were 2.03-4.62 g/week, while Carumbana and Luchavez (1979) observed BW increases of 3.07 g/week. In their study, Zhao et al (2013) found that BW was better at 10 ppt, even with a 1.5 g/week increased for 6 weeks using commercial diet. Some authors who conducted studies with *S. guttatus* in fish cages ended up with weight gains/week of 5.20 g for three months (Horstman 1975) from an initial weight of 12 g. In the study of Tahil (1978) attains an increase in BW of 0.75 g/week for six months from an initial weight of 15.49 g; while Ponce (1983) found that BW gains 4.1 g/week for two months culture from an initial weight of 4.55 g.

In the present study the BL increased by 4.1 mm to 6.2 mm/week. The study by Carumbana & Luchavez (1979) obtained a BL increased from 5.89 mm to 10.9 mm/week for two months. In the experiment by Zhao et al (2013), they stocked 20 fish/tank and obtained an 8.8 mm for six weeks culture period.

The lower growth of *S. guttatus* fed with GAS meal might be attributed to the lack of vitamins and minerals in the formulated diets and the digestibility of the GAS meal. Vitamins and minerals are important nutrients in the diet of fish for normal growth, reproduction and health (NRC 1993). According to Jintasataporn et al (2004), the protein digestibility of GAS meal was 86.36%, slightly lower than fish meal with 88.69 %. Based on the NRC (1993), the digestion coefficient of protein rich feedstuff ranged from 75 to 95%. The decrease of protein digestibility was probably caused by the fibrous protein structure of GAS and the high percentage of ash with 18.33% (Hertrampf & Piedad-Pascual 2000).

Result of the present study shows that SR of *S. guttatus* was 95 to 99.44%. Similar findings were reported at different culture conditions. Zhao (2013) showed that 100% SR can be achieved in different salinities in indoor tanks over a six week period. Previously, Parazo (1989) varied the levels of protein and energy and obtained 96-100% SR. Consequently, in her later study (Parazo 1991) on different artificial diets with higher crude protein the SR was lower (60-70%). Carumbana & Luchavez (1979) using *Enteromorpha* and supplements came out with 83-100% SR. However, in using *Enteromorpha* only, all fish died. In SEAFDEC (1995) a study conducted for 5-8 months in floating cages, the SR was generally high at 70-80%. Abalos (2015) conducted a study on *S. guttatus* in floating net cages at various stocking density and feeding scheme for six months obtained SR of 90 to 96%. On the other hand, Visca et al (2017) reported 80-95.33% SR of *S. canaliculatus* in fixed and floating net cages for two months.

Water quality is important factor in growth and survival of *S. guttatus*. The obtained values of the physico-chemical parameters (25 to 30°C, 30 to 37‰, 6.3 to 8.3 pH, and 7.0 to 7.4 mg L⁻¹) obtained are tolerated by *S. guttatus* during the culture (Carumbana & Luchavez 1979).

Conclusions. Fish fed with commercial pellets were found to grow faster and had higher survival rates than those fed with formulated diets. However, formulated diets were more economically-viable than the commercial feed used. It was observed that GAS meal with 45% crude protein can replace fish meal as protein source for *S. guttatus* for more than two months. For better results of the formulated feeds, premix vitamins and minerals must be added.

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