



Effects of protein levels of commercial diets on the growth performance and survival rate of rabbitfish (*Siganus guttatus*) at the nursing stage

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KEYWORDS

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Protein levels

Growth performance

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ABSTRACT

This study aimed to determine the effect of a commercial diet's protein level on the fry-to-fingerling stage. Thirty days-old fries having the initial length and weight of 18.25 ± 0.15 mm fish⁻¹ and 0.036 ± 0.50 g fish⁻¹ respectively have been used in this study. Diet having three protein levels i.e. 30% (trial 1 as control), 35% (trial 2), 40% (trial 3), and 45% (trial 4), respectively, have been used to evaluate the effect of protein, and each trial has been repeated three times. During the study, stocking density was allocated to 1000 fish per composite tank with a volume of 1 m³. After 30 days of rearing, the weight of fingerlings in trial 1 reached up to 1.50 ± 0.02 g fish⁻¹ and it was recorded as 1.52 ± 0.01 g for trial 2, these two were lower than that of trials 3 and 4, where fingerling weight was reported 1.69 ± 0.01 and 1.58 g fish⁻¹ respectively and obtained the best weight compared to others. The length of fingerlings at the end of the experimental period was also changed in different trials and it was recorded 47.12; 46.92; 50.97; and 48.89 mm fish⁻¹ for trail 1, 2, 3, and 4 respectively, among the tested combinations lower fingerlings length was recorded for trial 2 (35% CP), but it is not significantly different for trial 1 and 2 and a significant difference ($P < 0.05$) was reported for trail 2, 3, and 4. The survival rate of fingerlings ranged from 67.27 to 72.33%. Meanwhile, the herd distribution coefficient variation (CVW) in the treatment using 40% protein (trial 3) was the highest at 72.33% ($p < 0.05$). The results of the study can be concluded that the level of protein has a significant effect on the various growth parameters of fingerlings.

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1 Introduction

Rabbitfish is a brackish water cultured fish species with a high economic value in Tam Giang - Cau Hai lagoon systems, Vietnam. According to Tram and Ngoan (2013), rabbitfish has a digestible protein ratio that is distinct in the different types of feed (e.g., whole soybean meal, shrimp head, peanut meal, and oil meal), which reached more than 45% in practice, farmers usually used the protein diet around 35% protein for a commercial culture of this species (Hieu et al. 2018) but they found it is not suitable for the better survival and growth performance. The species has a comprehensive food plan and can use a variety of foods such as seaweed, phytoplankton, large algae, zooplankton, Copepoda, organic residues, and other low-energy feed (Parazo 1990). However, under farming conditions, rabbitfish and other species (*S. canaliculatus* and *S. spinus*) can accept many types of food (Carumbana and Luchavez 1979). At the fingerling stage, rabbitfish requires a high amount of protein that is approximately 30 - 55%, and an energy level of 3,832 - 4,000 kcal/kg, (Parazo 1990); therefore, species that have dietary protein and lipid levels of 40% and 6%, respectively resulted in the best growth performance and feed utilization efficiency (Salem et al. 2021). In aquaculture, the feed cost reached up to 70 - 80% of the total input cost because of the high protein requirements, and due to this more investment is needed for the diets of *S. guttatus* particularly, during the fry to fingerling nursing stage, which is a crucial stage. Protein level and diet for fish in the fingerling stage is a practical choice from the beginning of this period. Therefore, this study aimed to determine the protein required of rabbit fish at an early stage. In this study, the effect of the different dietary proteins were examined, and determined the influence of the protein levels on growth and survival rate, lower feed cost, and suitable industrial diets of the species during the fry to fingerling stage.

2 Materials and Methods

2.1 Materials and animals

Twelve thousand fries were used in this study, before starting the study these fries were acclimatized under laboratory conditions. Once these fries were acclimatized, they were assigned to three tanks, and 1,000 individuals were selected for each tank, with 4 protein levels i.e. 30 percent as control, while 35, 40, and 45% CP were used as a trial, each treatment replicated three times. These fries were supplied from the brood stock station. They had an average weight of 0.04 g fish⁻¹ and an average body length of 18.25 mm fish⁻¹ at 30 AHD (after hatchery days) and nurtured the fish in each composite tank with a volume of 1 m³ (water volume = 800 L or 80% of water), the experiment used commercial feed sources with different crude protein contents as mentioned above and balanced for nutritional values the same in trials.

2.2 Experimental set-up

The initial water quality in tanks was uniform and suitable for rearing fish, the water quality is as follows: temperature 28-29°C, salinity 29 – 30‰, pH 7.8-8.5; dissolved oxygen (DO) > 5 mg L⁻¹; and stocking density 1,000 fish per tank. The nursing period for fries was 30 to 60 days, and in this period all the fries become fingerlings for delivery to the cultural community. The experiment was arranged on three trials with three replicates, and each practice used industrial diets with three distinct levels of protein trials 1, 2, 3, and 4 as 30; 35; 40; and 45%, respectively. The amount of feed consumed in this experiment was 10% during the first ten days and 9% during the next 10 days. After reaching 8% body weight, the fish were fed 3 times per day.

2.3 Water management and variables in tanks

Fluctuation in water factors such as pH, water temperature, dissolved oxygen (DO), total NH₄/NH₃, and salinity were checked twice a day during the nursery stage. For this, a machine of 9 indicators was used (Machine Automatic Stainless Steel Aluminum Cooper Lampshade contact supplier by sensors).

2.4 Variables and methods

The growth performance of fish was determined at the time of initial weight and stocking (30 days) and weighed at the end (60 days). For this, 30 fish were collected from each tank to determine the weight using a hand-held electronic scale APTP453 (Japan). The length was determined using a graduated ruler and the survival rate at the end of the experiment.

2.5 Variable calculations and accounted

Growth performance indices in terms of percent survival rate, daily weight gain (DG, g day⁻¹), and length (DLG, g day⁻¹) were directly measured, while weight gain (g), length gain (mm), and coefficient of variation (%) were determined by following the equation of weight and length gain given by Rahman et al. (2009) and Yousif et al. (2005).

Weight gain (g) = (final body weight – initial body weight)

Length growth (mm) = (final body length – initial body length)

Daily weight gain (g day⁻¹) = (final body weight – initial body weight)/30 days;

Daily length growth (mm day⁻¹) = (final body length - initial body length)/30 days;

CV (%) = (δ/M) *100 (δ, standard deviation; M, the average value of the weight).

Survival rate (%) = [(Initial number of fish) – (final number of fish)]/initial number *100

2.6 Statistical analysis

The mean value (M) ± standard error (SE, m) was calculated. Data were analyzed using a one-way analysis of variance by ANOVA, T-test through Microsoft Office Excel™ 2010, and SPSS ver. 16.0 software was applied to determine significant differences among dietary CP levels; finally, Tukey's HSD ranking test was used when significant differences for the T-test ($p < 0.05$).

3 Results

3.1 Environmental variables

Aquatic animals are thermogenic, and their body temperature changes according to the fluctuations in the living environment, they can adjust their body temperature according to the living environment. Therefore, environmental water factors directly affect fish growth and survival. The results of changes in water environmental factors during the nursery of *Siganus guttatus* are presented in table 1. Results of the study are showing less fluctuation in water environmental indicators during the nursery

period of the fish and all the studied parameters are relatively stable and suitable for the development of *S. guttatus* under general and marine conditions.

3.2 Growth rate at different protein levels

The results presented in table 2 showed the daily fish gain after 30 days of nursing, using the commercial diets with different protein levels. Among the carried out trials highest average growth in fingerling weight was reported in trial 3 (1.69 g fish⁻¹), this was followed by the fish in trial 4 (1.58 g fish⁻¹), trial 2 (1.52 ± 0.11) and trial 1 (1.50^a ± 0.12) but these three are not significantly different. Similar trends were observed in daily weight gain and it was reported highest in trial 3 (0.055g/fingerling/day) while the least daily weight gain was reported in trial 1 (0.048g/fingerling/day).

The average growths concerning the length of fish from 30 to 60 days old have been presented in table 3. Results of the study revealed that fish length increased with the increased protein percentage from 40 and 45 % CP. Further results of the study suggested that the length of fish ranged from 47.12, 46.92, 50.97, and 48.89 mm fish⁻¹ for the trials 30, 35, 40, and 45% CP

Table 1 Effect of used protein diet on various water indicators during the nursery stage of fingerling

Variables	Time	Trials % CP (M ± m), n = 30			
		30	35	40	45
pH	Morning	7.80 ± 0.01	7.85 ± 0.02	7.90 ± 0.12	7.89 ± 0.12
	Afternoon	8.29 ± 0.05	8.31 ± 0.07	8.20 ± 0.11	8.24 ± 0.10
Temperature (°C)	Morning	28.19 ± 0.53	28.09 ± 0.73	27.66 ± 0.63	28.26 ± 0.75
	Afternoon	32.45 ± 0.52	32.55 ± 0.62	32.39 ± 0.51	32.67 ± 0.55
DO (mg L ⁻¹)	Morning	4.40 ± 0.03	4.50 ± 0.04	4.41 ± 0.08	4.33 ± 0.06
	Afternoon	5.35 ± 0.09	5.45 ± 0.10	5.39 ± 0.05	5.44 ± 0.03
Salinity (%)	Morning	29.12 ± 0.41	29.02 ± 0.31	29.33 ± 0.27	29.00 ± 0.22
NH ₄ /NH ₃ (mg L ⁻¹)	Morning	0.57 ± 0.02	0.67 ± 0.03	0.72 ± 0.08	0.81 ± 0.05

Mean ± SE value followed by the different letters in the same horizontal row are significantly different at $p > 0.05$; the above-given data are the mean value (M) of thirty replicates

Table 2 Effect of different protein diets on weight gain

Variables	Trials % CP (M ± m), n = 30			
	30	35	40	45
Initial weight (g fish ⁻¹)	0.04 ^a ± 0.045	0.04 ^a ± 0.045	0.04 ^a ± 0.050	0.04 ^a ± 0.050
Final weight (g fish ⁻¹)	1.50 ^a ± 0.12	1.52 ^a ± 0.11	1.69 ^b ± 0.14	1.58 ^c ± 0.18
Weight gain (g fish ⁻¹)	1.45 ^a ± 0.02	1.48 ^a ± 0.02	1.65 ^b ± 0.06	1.55 ^c ± 0.05
DG (g day ⁻¹)	0.048 ^a ± 0.05	0.049 ^a ± 0.05	0.055 ^b ± 0.07	0.051 ^c ± 0.07

Mean ± SE value followed by the different letters in the same horizontal row are significantly different at $p > 0.05$; the above-given data are the mean value (M) of thirty replicates

Table 3 Effect of various CP levels on studied Length variables

Variables	Trials % CP (M ± m), n = 30			
	30	35	40	45
Initial length (mm fish ⁻¹)	18.25 ^a ± 0.29	18.25 ^a ± 0.22	18.25 ^a ± 0.15	18.24 ^a ± 0.05
Final length (mm fish ⁻¹)	47.12 ^a ± 0.50	46.92 ^a ± 0.60	50.97 ^b ± 0.35	48.89 ^c ± 0.43
Length (mm fish ⁻¹)	29.87 ^a ± 0.58 ^a	29.67 ^a ± 0.98	34.72 ^b ± 0.60	30.12 ^c ± 0.47
DLG (mm day ⁻¹)	0.99 ^a ± 0.09	0.98 ^a ± 0.03	1.15 ^b ± 0.02	1.00 ^c ± 0.02

Mean ± SE value followed by the different letters in the same horizontal row are significantly different at $p > 0.05$; the above-given data are the mean value (M) of thirty replicates

Table 4 Effect of various CP diets on the survival rate and herd variation coefficient (CV_w)

Variables	Trials % CP (M ± m), n = 30			
	30	35	40	45
Survival rate (%)	67.53 ^a ± 2.80	68.53 ^a ± 1.80	72.33 ^b ± 3.13	67.27 ^c ± 4.76
CV (%)	5.41 ^a ± 1.25	5.31 ^a ± 1.25	5.10 ^b ± 0.88	7.42 ^c ± 2.64

Mean ± SE value followed by the different letters in the same horizontal row are significantly different at $p > 0.05$; the above-given data are the mean value (M) of thirty replicates

respectively. The study also indicates that the body length growth increased by 0.99, 0.98, 1.15, and 1.00 mm per fingerling per day. The results of this study are in agreement with the findings of Andam et al. (2016) who reported a 23.90 mm increase in the *S. guttatus* during the fingerling period.

Body length growths (BLG) of the 4 trials were 0.99; 0.98; 1.15, and 1.00 mm day⁻¹ for 30, 35, 40 and 45% CP, respectively. The average increasing lengths of the body per day was around 12 – 15% and also have significant differences between trials 2, 3, and 4.

3.3 Survival rate and coefficient of variation

The survival rate and flock division coefficient variation (CV) were important results for the rearing studies. The results of the rearing *S. guttatus* fingerling stage are shown in table 4. There were no significant differences were reported in the survival rate of the fingerlings in the diets with different protein content, and diets with 30, 35, 40 and 45% CP having survival rates of 67.53, 68.55, 72.33, and 67.27%, respectively, which were significantly different ($p > 0.05$) for trials 2, 3, and 4.

4 Discussions

4.1 Water variables

The average pH in the morning and afternoon ranged from 7.85 to 8.31 and are in agreement with the findings of previous studies that suggested the water pH range 6.5-9.0 is suitable for the growth of tropical fish and pH lower or higher than this affects the growth and reproduction of fish. Water temperature also plays an important role in aquaculture and the temperature range from 27.66

to 32.55 °C, is suitable for the species that live in warm water ranges from 25 to 32 °C as mentioned by Carumbana and Luchavez (1979) and Boyd (1998). Further, dissolved oxygen (DO) ranged from 4.33-5.45 mg L⁻¹, salinity ranged from 28 to 29‰, and total ammonia (NH₄/NH₃) ranged from 0.67 to 0.81 mg L⁻¹ are suitable water characteristics for fish culture (Rahman et al., 2009; Andam et al. 2016). In general, the water environmental factors recorded in this study are within the threshold of good growth and development of fish, and in this study levels of 30, 35, 40, and 45% of dietary protein percentages have not affected the water environmental quality for fish culture, therefore, water control was evaluated every day by leaning of tank bottom, as mentioned the water control by Parazo (1990) and Salem et al. (2021).

4.2 Daily gain and lengths

In a study on mullet (*Liza subviridis*) from the fry to fingerling stage (density 1,000 fish m⁻³) fed with protein levels from 35 to 45%, the growth performance of fish between the daily weight growth (DG) was significantly different ($p < 0.05$) and reported by Viet et al. (2010) for different protein diets were not significant. Various previous studies on different fish species have shown that fish growth is reduced when fishes are fed on diets that have too high a protein content. In addition, increasing the weight and length is also an important factor to evaluate the development in the fingerling stage. The growth rate of fingerlings at 60 days as shown above indicated the supplement has more protein content up to 40% beneficitation for the nursery of fries to fingerlings. Studies by Tu et al. (2014) also used a protein diet at 35% CP, while research by El-Dakar et al. (2011); Andam et al. (2016); Thiet et

al. (2017) used much higher protein levels (49.9 and 55.5%) at the fingerling stage. Parazo (1990) and Manh et al. (2015) found that while using a diet containing 40% CP and 3832 kcal/kg energy for *S. guttatus* a better growth rate was achieved. The results of this study showed that a protein content of 40% CP had the best growth rate. Another study by Hieu et al. (2018) found these proteins contents levels in diets also showed the highest value of growth rate. The survival rate in this study was higher than that reported by Andam et al. (2016) when using a diet with a protein level of 30 – 40%, which was mentioned as the best growth of the species at 40% CP in the diet. However, the species in the brackish water of Tam Giang lagoon had different characteristics, there was no successful result by artificial reproduction yet and there were still more biological mysteries.

The length of fish during of nursing period from 30 to 60 days is an important fingerling criterion. In this study, best length development was achieved with a dietary level of 40% CP duration 30 days of the nursery, and these results are in agreement with the findings of Izquierdo and Fernandez – Palacios (2019) and Salem et al. (2021) also indicated the length of fish at this stage will be influenced by growth when enough protein content is available, so the discovering a type of protein for this species as soybean meal, is good because at this stage these fingerlings preferred more plant protein than animals.

4.3 Survival rates

Survival results of the current study compared with Hieu et al. (2018) those who fed orange fin loach (*Botia modesta*) with different protein contents ranged from 25 to 55% and it did not affect the survival rate and it was reported between 96.70 to 99.30%. In a further study, Khanh et al. (2020) reported a 100% survival rate for (*T. blochii*) and Thiet et al. (2017) reported a 97.70% survival when reared with different protein contents. A study on the protein levels at 40 and 45% CP also showed a high growth efficiency and optimal survival rate in mullet (*L. subviridis*) at the fingerling stage (Viet et al. 2020). When trying to determine the best growth rate in another marine species based on the fed protein level, studies mentioned by Xu et al. (2019) found that the 35 and 40% level of protein was the most effective. Meanwhile, the coefficient of variation for *T. blochii* using industrial feed (44% CP) reached 0.24% was higher than the treatment combined between industrial feed and trash fish was 0.19%, and the treatment only used trash fish was 0.15% ($p < 0.05$) (Khanh et al. 2020). A commercial feed with 40% crude protein content is suitable for the nursing rabbitfish's fingerling stage, helping the fish to optimal growth and a survival rate of 72.33%, which corresponded to a coefficient variation of 5.10%. In addition to the study of protein levels in commercial feeds, further studies are needed on the respective energy levels to evaluate the effect of the role of energy and protein levels in the feed on the growth of fish.

Conclusion

Nursery of rabbitfish from fry to fingerlings, we can use commercial feed with crude protein of 40% for the best result of growth and survival rate. Although this species was considered a difficult alternative to live food and feedstuff for larvae, fry, and fingerling stages, even many practices were not successfully work in artificial reproduction.

Ethical certification and consent to participate

Ethical certification and consent to participate Certificate Reference Number: HUVNO019 Date 10th March 2022 The Animal Ethics Committee (AEC), I hereby make an ethical approval concerning the commitment of the Principle Researcher and supervisors for the project titled: "Effects of protein levels in commercial feed on growth and performance of Rabbitfishsh (*Siganus guttatus*) from 30 days of fry to fingerling stage"

Consent to publication

The Committee agreed to permit researchers to publish the article in any journal.

Competing interests

Not applicable

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Author contributions

Tran Vinh Phuong, Nguyen Anh Tuan, Nguyen Duy Quynh Tram, Nguyen Duy Thuan, and Ngo Thi Huong Giang collected data and maintained the experiments, Nguyen Quang Linh is leading the research groups and ideas and all conditions, major writing and revised and funding support, Tran Nguyen Ngoc collected data and contributed to experiments.

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