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Early feeding frequency improves biological performance, glycogen and triglyceride levels in Jullien's golden carp (*Probarbus jullieni*)

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Abstract

This study aimed to evaluate the effects of feeding frequency on Jullien's golden carp (*Probarbus jullieni*) during their initial feeding period. The effects of four different feeding frequencies, treatment 1 (Trt-1), 2 (Trt-2), 3 (Trt-3) and 4 (Trt-4) meals per day were assessed on growth performance, feed utilization, survival rate and whole body composition. The larvae fish, initial weight 2.16 ± 0.01 mg were fed with a commercial powder feed for 40 and 70 days after hatching (DAH). The effect on growth performance, including final body weight (FBW), weight gain (WG), average daily weight gain (ADG), specific growth rate (SGR), and feeding conversion ratio (FCR) were assessed. At 40 DAH after feeding, the larvae were at Trt-2, Trt-3, and Trt-4 meals per day showed significantly higher FBW, WG, ADG, and SGR than those fed at Trt-1 meal per day ($p<0.05$). Moreover, the FCR of groups at Trt-3 and Trt-4 was better than those of group at Trt-1 and Trt-2 ($p<0.05$). There were significant increases in growth rate and feed efficiency in larvae fed Trt-3 and Trt-4 groups compared to Trt-1 and Trt-2 groups over the 70 DAH ($p<0.05$). The results also showed that both Trt-3 and Trt-4 groups had higher whole body glycogen and triglyceride levels than Trt-1 and Trt-2 groups ($p<0.05$). Based on the findings of this study, it can be concluded that feeding three meals per day to the larvae was optimal for growth performance and feed efficiency for Jullien's golden carp.

Keywords: Feeding frequency, Jullien's golden carp, Optimal feeding

1. Introduction

The main costs of intensified aquaculture are associated with feeding the fish. Feed management can help aquaculture become more profitable by reducing these costs. The size and quality of the food, the rate of feeding, the spacing between feedings, and the method of distribution are all important aspects of feeding-related tasks [1]. The critical part of feeding frequency, considered one of the most important factors, has piqued the interest of aquaculture researchers [2]. Adjusting the feeding frequency can offer several advantages, including improved growth rates, lower feed conversion ratios, and higher survival rates. Additionally, it may lead to reduced feed waste, improved water quality, and/or less size variability [3]. Moreover, inadequate feeding intervals have been shown to negatively effect on fish, leading to reduced growth, increased cannibalism, oxidative damage, and weakened immunity, all of which result in significant economic losses [4-6]. Given the economic importance of fish, extensive research has been conducted to determine the optimal feeding schedules for various species [7].

Numerous research studies have investigated the feeding requirements and optimal feeding intervals for various commercial fish species, such as the dark barbel catfish (*Pelteobagrus vachelli*) [8], zebrafish (*Danio *rerio*) [9] and cobia (*Rachycentron canadum*) [10]. According to research on African catfish (*Clarias gariepinus*) fingerlings, feeding frequency substantially positively impacted growth and survival when fish were fed twice daily [11]. It was also observed that *C. gariepinus* fry fed five or six rations per day (15% body weight per day) exhibited growth advantages compared to those fed at lower frequencies [12]. On the other hand, an abnormally low feeding frequency results in reduced growth and survival rates in fish, leading to greater size variation and increased incidences of cannibalism [13,14]. Additionally, determining the ideal feeding schedule for African catfish could help reduce cannibalism, which is the primary cause of mortality during the rearing of this species [15]. In Nile tilapia (*Oreochromis niloticus*), an omnivorous model fish, early feeding has been shown to increase whole body glycogen and triglyceride levels, indicating a significantly higher intake [16]. Consequently, the response of fish to feeding, the application of feed, and the timing of daily feedings greatly impact the stages of natural development [11,15].

Jullien's golden carp (*Probarbus jullieni*) a significant commercial species of Thai river carp found in the Mae Klong and Chao Phraya River basins. With its large body size, high market value, and superb flavor, this fish is highly sought after in fisheries. Additionally, its listing in Appendix I as an endangered species under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has attracted the attention of scientists and conservationists [17,18]. Thailand has successfully bred this species to support its use to support its use as an ornamental or food fish as well as its release into the wild in accordance with conservation biology. Meanwhile, current culture models have struggled to increase production volume and reduce production costs, particularly feed costs, which account for more than 50-60% of expenses in intensive culture [16]. Feeding regimes are therefore considered one of the most crucial methods for reducing feed costs by preventing under or overfeeding, which can lower growth, feed conversion efficiency, and degrade water quality through excess waste accumulation. Specific requirements must be met to achieve high productivity [19,20].

In this study, we aimed to determine the optimal daily feeding frequency for Jullien's golden carp by measuring growth, feed efficiency, and survival rates. The findings will provide new insights that can promote improved growth of this species and serve as a benchmark for industrial-scale fish nurseries, leading to better aquaculture outcomes for this species.

2. Materials and methods

Effects of early feeding frequency on growth performance, and the use of glycogen and triglyceride for feeding Jullien's golden carp were carried out at Muban Chombueng Rajabhat University, Ratchaburi, Thailand.

2.1 Fish larvae preparation

Striped carp broodstock were obtained from the Ratchaburi Inland Aquaculture Research and Development Center, located in Ratchaburi, Thailand. The fertilized eggs were produced from female broodstock by injecting a combination of buserelin acetate (15 and 25 µg/kg fish for the initial and secondary injections respectively) and domperidone (10 mg/kg fish for the both injections). Prior to semen collection, mature male broodstock were induced with a single injection of buserelin acetate (10 µg/kg fish) and domperidone (10 mg/kg fish). The fertilized eggs were incubated in a fiberglass tank incubator (2.00 m diameter x 0.60 m water depth) at 20.0°C to 22.0°C (6-8 December 2022) with constant aeration to prevent suffocation. Three thousand larvae were then transferred to a rearing cement pond (1.00 m x 4.00 m with 0.40 m water level). The newly hatched larvae were not fed until their yolk sac was depleted. The fish larvae at 3-9 days after hatching (DAH) were subsequently fed with artemia (EG artemia, INVE Aquaculture) and powdered feed nursery diet (NRD)1/2, INVE; 60% crude protein, 10% crude fat, 1% crude fiber and 8% moisture; size 150-200 µm) three times per day (09:00 a.m. 01:00 p.m. and 06:00 p.m.) until they were visibly stationary. Recirculation at a flow rate of 4 L/min and continued aeration were employed to maintain water quality during the feeding strategies.

2.2 Experimental design and feeding management

The experiment was conducted in three replicates following a completely randomized design (CRD) with 50 fish per replication. After acclimatized (10 DAH), four treatments of feeding frequency-1 (Trt-1), frequency-2 (Trt-2), frequency-3 (Trt-3) and frequency-4 (Trt-4) meals per day as shown in Table 1 were tested in triplicate (4 x 3 total 12 ponds) for 60 days, and their effects were evaluated in term of growth performance and physiological parameters (Figure 1). Ten days' old larvae with initial weight of 2.16 ± 0.01 mg were randomly distributed to 12 glass tanks (0.45 m x 0.90 m, W x L; depth 0.40 m water volume 0.16 m^3). A total number of 600 individuals were uniformly allocated at a stocking rate of 50 fish per tank. The fish larvae were fed to satiation (10% total body weight per day) with the powder commercial diet (Orange start 1/2 contain 50% crude protein, 10% crude fat, 2.5% crude fiber and 10% moisture; size 100-200µm). Every day, the water exchange rate was approximately 30% of the total water volume, conducted using a siphon pipe with minimal disturbance to the fish. Air stone was used to submerge an air blower in an aeration system. The number of dead fish was recorded daily to ascertain the survival rate, and the growth performance was observed. The diurnal cycle was set according to the natural hours of light.

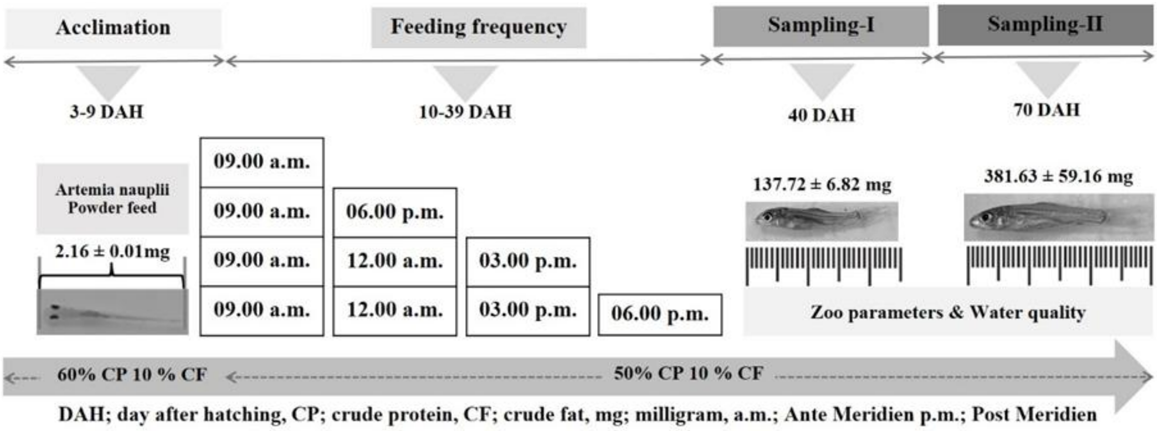

Figure 1 Experimental plan of optimal early powder feeding frequency reared under controlled environmental conditions of Jullien's golden carp (*P. jullieni*). The early feeding was acclimated for 7 days (3-9 Day after hatching; DAH), larvae were subjected to Artemia and commercial powder feed (60% crude protein + 10% crude fat). Subsequently, all experimental fish were fed a commercial diet for 40 DAH and 70 DAH (50% crude protein + 10% crude fat).

Table 1 Feeding times of Jullien's golden carp fed at different daily feeding frequencies.

Treatment	Frequency	Time of feeding
Trt-1	Frequency-1	09.00 a.m.
Trt-2	Frequency-2	09.00 a.m. and 06.00 p.m.
Trt-3	Frequency-3	09.00 a.m. 12.00 a.m. and 03.00 p.m.
Trt-4	Frequency-4	09.00 a.m. 12.00 a.m. 03.00 a.m. and 06.00 p.m.

2.3 Water quality parameters

During the period of the trial, the air temperatures, water temperatures, dissolved oxygen and pH were monitored daily by using a digital oximeter (HI98194 HANNA), to measure pH with pH meter (HI98107 HANNA). While the levels of total ammonia nitrogen (NH_3/NH_4) and nitrite (NO_2) were qualitatively checked every three days by a commercial test kit as described by protocol (Monitor Test Kit conforms to AWWA method For Aquaculture and Aquarium). Throughout the experiment periods, daily average air temperatures were recorded within 33.7 ± 2.3°C.

2.4 Sampling collection and analysis

At initial 10 DAH (Ten larvae per tank, in total 120 larvae) 40 DAH and 70 DAH (Ten fries per tank, in total 120 fries) were randomly sampled for analysis. The fish endured anesthesia with clove oil (40 mg/L) 12 hours after feeding [16]. Growth parameters were performed on five fish of each experimental time to evaluate the effect of different feeding frequency times. Nutrient compositions were generated using six fish from sampling time at 40 DAH and 70 DAH (Six fries per tank, in total 72 fries) to evaluated compositions. After bleeding, whole fish were collected and stored at -20 °C for the analysis of glycogen and triglyceride. Glycogen and triglyceride were monitored for responses from nutrient consumption on 10 DAH (initial) 40 DAH (short-time) and 70 DAH (long-time). The amounts of glycogen and triglyceride were calculated, relative to the amount of standard (glycogen and triglyceride) in each sample. All experiments were run in biological duplicates from every experimental unit.

2.5 Growth parameters and feeding regium

Biological performance at 10 DAH 40 DAH and 70 DAH, all experimental fish were anesthetized with clove oil 40 mg/L according to [16]. A digital balance (unit in mg) was performed to record the fresh body weight. The total length was measured with a vernier meter (1 mm precision) and weight with a digital scale (0.01 mg precision). All experimental data, including final body weight (FBW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), and survival rate (SR), were analyzed to evaluate the significant differences in feeding frequency. Additionally, commercial feed was provided to the experimental fish ad libitum for two weeks. To assess feed consumption efficiency, feeding was then initiated at 10% of the total body weight per day.

Data on fish feed weight were collected to adjust the weekly feeding quantity by dividing it into specified times and calculating the volume per period. Uneaten feed was siphoned off 1 hour after feeding, dried at 60°C until reaching a constant weight, and used to calculate the feed conversion ratio (FCR). Based on these data, the following parameters were estimated [21,22].

[1] Weight gain (WG) = final body weight (mg) - initial body weight (mg)
[2] Average daily gain (ADG) = (final body weight (mg) - initial body weight (mg)) / times (day)
[3] Specific growth rate (SGR) = 100 x [(ln final body weight (mg) - ln initial body weight (mg))/ times (days)]
[4] Feed conversion ratio (FCR)= dry feed fed (mg) /wet weight gain (mg)
[5] Survival rate = 100 × [(Original number of fish- Finishing number of fish)/ Original number of fish]

2.6 Glycogen and Triglyceride compositions

Glycogen and Triglyceride compositions were evaluated during experiment at 10 DAH (Ten larvae per tank, in total 120 larvae) 40 DAH (Six fries per tank, in total 72 fries) and 70 DAH (Six fries per tank, in total 72 fries) after feeding frequency. The whole body glycogen were determined (10, 40 and 70 DAH) according to the hydrolysis technique described by [23], with modifications [21,22]. Triglyceride analysis was conducted by homogenizing the samples in liquid nitrogen, followed by a second homogenization in 0.5 mL of 5% IGEPAL solution in deionized water. The samples (whole body) then underwent two repetitions of heating to 90°C for 5 minutes, followed by cooling to room temperature. The reaction mixture was centrifuged at 10,000 x g and 4 °C for 10 min, and the supernatant were then collected to eliminate any insoluble material. Upon dilution of the supernatant with deionized water, the triglycerides were quantified using glucerol-3 phosphate oxidase-sodium N-ethyl-N-(3-sulfopropyl)-m-anisidine method [24].

2.7 Statistical analysis

All of the set data was examined by one-way analysis of variance (ANOVA) using the R application, and all of the study’s data was examined using a 95% confidence level (R version 4.0.3 (2020-10-10)). When significant differences were found among the groups, Tukey's range test was applied in order to rank the different treatments. Throughout the experiment, the effects and differences were declared to be significant at $P < 0.05$.

3. Results

Table 2 exhibits the effects of feed frequency on growth performance in Jullien’s golden carp. FBW, WG, ADG, and FCR were all affected by early feeding frequencies. At 30 days of the experimental period (40 DAH), the larvae fed at 2 (Trt-2), 3 (Trt-3) and 4 (Trt-4) meals per day showed significantly higher FBW, WG, ADG, and SGR than those fed Trt-1 meal per day (P<0.05). The feed conversion ratio at feeding at Trt-3 and Trt-4 meals per day better than Trt-1 and Trt-2 meals per day (P<0.05). Feeding frequencies for 70 days Trt-3 and Trt-4 meals per day fry showed differences in growth rate and feed efficiency over that of Trt-1 and Trt-2 meals per day (P<0.05). The FBW, WG, ADG, SGR and FCR of feeding Trt-3 and Trt-4 meals per day, which had a high feed efficiency at more than Trt-1 and Trt-2 meals per day (P<0.05). The survival rate of Jullien’s golden carp larvae was not affected by different feeding frequencies meals per day (P>0.05) (Table 2).

Table 3 shows the effects of the frequency of feeding on the physiological changes in the body. An increase in whole body glycogen and triglyceride were detected from different feeding frequencies, whole body glycogen and triglyceride were more elevated in fish fed at Trt-3 and Trt-4 meals per day than at Trt-1 and Trt-2 meals per day in both experimental periods (P<0.05). At 40 DAH, the accumulation of glycogen at Trt-3 and Trt-4 meals per day was higher than at Trt-1 and Trt-2 meals per day (P<0.05), with the direction of increase in line with the triglycerides at Trt-3 and Trt-4 meals per day which had a higher triglyceride level (P<0.05). Moreover, 70 DAH at Trt-3 and Trt-4 meals per day, whole body glycogen and triglyceride levels were still more than at Trt-1 and Trt-2 meals per day (P<0.05). Glycogen accumulation at Trt-4 meals per day had the highest levels (P<0.05), while triglycerides at Trt-3 and Trt-4 meals per day had higher levels compared to any meals per day (P<0.05) (Table 3).There were no effects of feeding frequencies on the water quality parameters including water temperature, Dissolved oxygen, pH, Ammonia and Nitrate in both experiments time for 40 and 70 DAH feeding frequencies times meals daily (P>0.05) (Table 4). Table 1 Table of water quality measurements and quality standards.

Table 2 Performance of Jullien's golden carp fed with different frequencies for 30 (age 40 DAH) and 60 days (age 70 DAH).

Feeding time (meal/day)	IBW (mg)	FBW (mg)	WG[1] (mg)	ADG[2] (mg/day/fish)	SGR[3] (%/day)	FCR[4]	SR[5] (%)
40 DAH							
Trt-1	2.16 ± 0.02	129.33 ± 1.10[c]	127.17 ± 1.09[c]	4.24 ± 0.04[c]	13.64 ± 0.03[c]	2.19 ± 0.02[a]	96.67 ± 1.15
Trt-2	2.15 ± 0.01	137.93 ± 0.99[b]	135.78 ± 0.98[b]	4.53 ± 0.03[b]	13.87 ± 0.01[b]	2.11 ± 0.03[ab]	95.33 ± 1.15
Trt-3	2.16 ± 0.00	139.67 ± 1.79[b]	137.51 ± 1.79[b]	4.58 ± 0.06[b]	13.90 ± 0.04[b]	2.10 ± 0.04[c]	94.67 ± 3.06
Trt-4	2.17 ± 0.01	145.87 ± 0.42[a]	143.70 ± 0.43[a]	4.79 ± 0.01[a]	14.03 ± 0.03[a]	2.14 ± 0.02[ab]	96.67 ± 1.15
P-value	0.672	<0.001	<0.001	<0.001	<0.001	0.025	0.482
70 DAH							
Trt-1		315.47 ± 9.29[c]	313.31 ± 9.29[c]	5.22 ± 0.15[c]	8.31 ± 0.05[c]	2.27 ± 0.08[a]	96.67 ± 1.44
Trt-2		348.33 ± 6.91[b]	346.18 ± 6.92[b]	5.77 ± 0.12[b]	8.48 ± 0.04[b]	2.15 ± 0.03[ab]	95.83 ± 1.44
Trt-3		425.60 ± 5.55[a]	423.44 ± 5.55[a]	7.06 ± 0.09[a]	8.81 ± 0.02[a]	2.09 ± 0.03[c]	96.67 ± 1.44
Trt-4		437.13 ± 1.79[a]	434.97 ± 1.80[a]	7.25 ± 0.03[a]	8.85 ± 0.01[a]	2.08 ± 0.02[c]	95.83 ± 1.44
P-value		<0.001	<0.001	<0.001	<0.001	0.004	0.482

Trt-1 Frequency-1 09.00 a.m
Trt-2 Frequency-2 09.00 a.m. and 06.00 p.m.
Trt-3 Frequency-3 09.00 a.m. 12.00 a.m. and 03.00 p.m.
Trt-4 Frequency-4 09.00 a.m. 12.00 a.m. 03.00 a.m. and 06.00 p.m.
Abbreviations: DAH-day after hatching; IBW-Initial body weight; FBW-Final body weight; WG-Weight gain; SGR-Specific growth rate; FCR-Feed conversion ratio; SR-Survival rate. Values (Mean ± SD) in the column followed by different letters are different according to Tukey's range test (P<0.05).

Table 3 Whole body glycogen and triglyceride of Jullien's golden carp fed with different frequencies for 30 (age 40 DAH) and 60 days (age 70 DAH).

Feeding time (meal/day)	Initial		40 DAH		70 DAH	
	Glycogen (mg/g tissue)	Triglyceride (mg/g tissue)	Glycogen (mg/g tissue)	Triglyceride (mg/g tissue)	Glycogen (mg/g tissue)	Triglyceride (mg/g tissue)
Trt-1	7.26 ± 0.77	4.15 ± 0.32	14.19 ± 0.92[b]	6.74 ± 0.40[c]	23.41 ± 1.02[c]	12.54 ± 0.61[c]
Trt-2	7.05 ± 0.15	4.17 ± 0.23	14.15 ± 0.45[b]	6.61 ± 0.93[c]	23.11 ± 0.42[c]	13.94 ± 0.61[b]
Trt-3	7.29 ± 0.30	3.84 ± 0.40	18.27 ± 0.14[a]	8.25 ± 0.67[b]	36.85 ± 2.09[b]	18.84 ± 0.27[a]
Trt-4	7.33 ± 0.63	3.95 ± 0.38	19.09 ± 0.33[a]	9.93 ± 0.40[a]	40.05 ± 1.00[a]	18.55 ± 0.61[a]
P-value	0.941	0.588	<0.001	<0.001	<0.001	0.001

Trt-1 Frequency-1 09.00 a.m
Trt-2 Frequency-2 09.00 a.m. and 06.00 p.m.
Trt-3 Frequency-3 09.00 a.m. 12.00 a.m. and 03.00 p.m.
Trt-4 Frequency-4 09.00 a.m. 12.00 a.m. 03.00 a.m. and 06.00 p.m
Abbreviations: DAH-day after hatching; mg-milligram; g-gram
Values (Mean ± SD) in the column followed by different letters are different according to Tukey's range test (P<0.05).

Table 4 Water quality variable of Jullien's golden carp fed at different frequencies for 30 (age 40 DAH) and 60 days (age 70 DAH).

Feeding time (meal/day)	Water (°C)	DO (mg/L)	pH	Total ammonia nitrogen (ppm)	Nitrate (ppm)
40 DAH					
Trt-1	27.37 ± 0.10	4.98 ± 0.12	8.08 ± 0.05	0.20 ± 0.03	0.21 ± 0.01
Trt-2	27.42 ± 0.06	4.96 ± 0.02	8.07 ± 0.10	0.20 ± 0.03	0.22 ± 0.01
Trt-3	27.39 ± 0.09	4.85 ± 0.15	8.08 ± 0.04	0.24 ± 0.01	0.20 ± 0.01
Trt-4	27.39 ± 0.09	4.88 ± 0.07	8.09 ± 0.13	0.24 ± 0.01	0.20 ± 0.01
P-value	0.881	0.398	0.994	0.054	0.244
70 DAH					
Trt-1	27.51 ± 0.25	5.46 ± 1.44	8.09 ± 0.08	0.23 ± 0.00	0.18 ± 0.03
Trt-2	27.52 ± 0.20	4.56 ± 0.10	8.08 ± 0.04	0.24 ± 0.01	0.18 ± 0.03
Trt-3	27.55 ± 0.15	5.01 ± 0.76	8.11 ± 0.05	0.24 ± 0.01	0.21 ± 0.00
Trt-4	27.55 ± 0.15	4.48 ± 0.04	8.11 ± 0.05	0.25 ± 0.03	0.22 ± 0.01
P-value	0.994	0.466	0.883	0.363	0.106

Trt-1 Frequency-1 09.00 a.m
Trt-2 Frequency-2 09.00 a.m. and 06.00 p.m.
Trt-3 Frequency-3 09.00 a.m. 12.00 a.m. and 03.00 p.m.
Trt-4 Frequency-4 09.00 a.m. 12.00 a.m. 03.00 a.m. and 06.00 p.m
Abbreviations: DAH-day after hatching; °C- degree Celsius; DO-Dissolved Oxygen; ppm-parts per million
Values (Mean ± SD) in the column followed by different letters are different according to Tukey's range test (P<0.05).

4. Discussion

Feeding management affects fish farming to increase growth rate as well as feed efficiency that points to proper management without compromising production performance. Optimal water quality (water temperature, dissolved oxygen, pH, ammonia, and nitrate) values in feeding with varying frequency per meal demonstrate the suitability of culture conditions directly affecting growth [12, 25-27]. Previous studies of different feeding frequencies for Amazonian ornamental (*Heros severus*) and African catfish (*Clarias gariepinus*) showed that the water qualities of all treatments for both studies were within the level of recommended ranges for the culture of the fish [12,25]. In this study water quality at different feeding frequencies did not affect changes in water quality for rearing. These results confirm that the feeding and cleaning management of the experimental units were efficient and did not damage the water quality.

The success of the production system depends to a great extent on how the fish are fed during their larval stage. Many studies have confirmed that increasing feed frequency can increase survival rates, limit size variation, improve stress tolerance and increase feed efficiency [28,10]. The present study showed that increasing feeding times could improve growth rates and feed efficiency rather than reducing the number of feeding times per day. Also, the survival rate was not affected by feeding frequency at either short or long times. There were no effects of feeding frequency on the survival rates of herbivorous and omnivorous fish [26,27]. Some carnivorous fish have cannibalistic behavior that affects the survival of fish in the experiment [12]. Feeding frequency had a positive effect on growth and feed efficiency, which are more effectively accentuated when there is significant size heterogeneity among fish [29,30]. The study on non-variation of Jullien golden carp proper management of feeding levels and rearing conditions. Furthermore, the fact that feeding frequency has no effect on survival rate could possibly be explained by an appropriate stocking density and high-quality water [27]. Additionally, even though the feed was provided to Jullien's golden carp larvae at a high frequency, the high quality of the feed may also account for the survival results noticed in this study. A previous study on *P. jullieni* juvenile fed three times a day feeding had more positive response in terms of growth performance and feed efficiency than those fed one and two meals [31]. While statistics on water quality allow for the evaluation of environmental conditions and nutrient buildup that may influence growth, this remains an issue to be considered in line with previous studies [31]. The growth performance of fish fed twice and three times daily was similar and higher than that of fish fed only once per day in Nile tilapia (*Oreochromis niloticus*). However, these changes did not affect the muscle quality or carcass composition of the fish [32]. In contrast, in river puffer (*Takifugu obscurus*) cultured in zero-exchange mixed biofloc community systems, there were no significant differences in final body weight, daily feed consumption, or weight gain among fish fed two, three, or five meals per day. Additionally, as feeding frequency increased, the fish's crude protein and fat content increased significantly [33]. This indicates that the growth and development of fish larvae are supported by an appropriate feeding frequency, which varies according to the environmental conditions, stage of development, and fish species. At higher feeding frequencies, larvae may have more opportunities to obtain sufficient food, leading to a faster growth rate [26]. A comparable study examined the significance of feeding frequencies of three and four meals per day, showing positive growth and feed efficiency in *P. jullieni* when fed three meals per day, which was found to be the most effective for rearing this species at an early stage. These findings can also guide the development of nursery models for *P. jullieni* to boost on-farm production efficiency, thereby increasing the species' value [31].

In addition, three and four daily feedings of hybrid sunfish larvae (*Lepomis cyanellus* × *L. macrochirus*) to satiation resulted in significantly higher growth than fish fed only once a day [34]. Moreover, in large yellow croaker (*Pseudosciaena crocea*) larvae, a higher feeding frequency appeared to result in better specific growth rates [35]. The efficiency of feed also correlated with the impact of feeding frequency on fish growth. As a result, larvae require more frequent feedings since their digestive systems are still developing. The study's findings indicate that increasing feeding frequency improves growth performance and feeding efficiency in early *P. jullieni* larvae. It is evident that early feeding of *P. jullieni* larvae under an intensive culture method may be advised, as three meals per day were optimal for growth performance, including average daily weight gain, specific growth rate, feed conversion ratio, and feed efficiency.

To confirm the analysis of fish diet reception, we ensured that Jullien's golden carp received the early powder feed well at 40 days after hatching (DAH) and 70 DAH during the rearing period. Our results showed higher whole-body glycogen and triglyceride contents with more frequent feeding, which was significantly improved with increased feeding frequency. Similarly, it has been reported that the body composition of *C. gariepinus* fry and fingerlings was influenced by different feeding frequencies, with higher protein and lipid content recorded in those fish fed twice a day [11]. Complementary research on fry and fingerlings has documented an increase in body fat with increased feeding frequency, which aligns with our findings that, at an optimal feeding frequency, an adequate food supply helps to lower energy requirements and facilitates the accumulation of fat in tissue [19,36,37].

5. Conclusion

Based on the findings of this study, it is recommended that Jullien's golden carp be fed three meals per day at 10% of the larval fish's body weight as an effective method for improving growth performance. These results will help enhance early-stage culture practices for the species, leading to a profitable impact on production systems through proper feed optimization.

6. Ethical declarations

All experimental procedures involving fish were approved by the Animal Care and Use Committee's Ethics Committee at Muban Chombueng Rajabhat University accepted all protocols pertaining to the study's experimental animals (approval no. AS6607005).

7. Acknowledgements

Not show in the process.

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